

Poultry Science

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Abstracts of the Poultry Science Association Annual Meeting

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Student Competition: Metabolism and Nutrition: Feed Additives

1 Probiotic supplementation reduces intestinal permeability and alters splenic immune cell populations without altering performance in healthy broilers. Krysten Fries-Craft*, Meaghan Meyer, and Elizabeth Bobeck, *Iowa State University, Ames, Iowa, United States.*

Growing interest in the functionality of the intestinal microbiota and limitations on in-feed antibiotic growth promoters have created a niche for probiotic use in poultry. Feeding probiotics to enrich beneficial community members can potentially alter microbial communication with the host immune system and mediate intestinal inflammatory responses to impact performance. Responses to probiotic supplementation may vary due to inclusion level, bacterial composition, and individual host health status. Overall health in response to probiotic supplementation has been described, but specific physiological changes remain unclear. The objective of this study was to evaluate performance, intestinal permeability, and splenic immune cell populations in unchallenged broilers fed 2 different probiotics at 0.05 or 0.1% dietary inclusion. A total of 960 1d-old Ross 708 broilers were housed in 80 floor pens (12 birds/pen) and assigned to 1 of 5 diets for 42 d, divided into 14d starter, grower, and finisher periods. Dietary treatments consisted of a corn-soybean meal basal diet without probiotics (control) or containing 0.05 or 0.10% of LactoCare® or LactoPlan®, respectively (Nutraferma Inc., Sioux City, IA). Feed intakes were monitored and body weights were taken at the start and end of each period to calculate FI, ADG, and FCR. On d28, birds from 4 pens/treatment were orally gavaged with FITC-dextran ± feed restriction, and serum was collected after 1h to detect fluorescence as a measure of intestinal permeability. Spleens from 8 birds/treatment were collected and leukocyte populations were analyzed by multi-color flow cytometry. Statistical analysis was performed using PROC MIXED (SAS 9.4) with fixed effects of probiotic type, inclusion, and type × inclusion and significance denoted at $P \leq 0.05$. Probiotic type, inclusion, or the interaction did not impact broiler performance. An inclusion rate of 0.10% maintained intestinal integrity during feed restriction regardless of probiotic type, but birds fed 0.05% LactoPlan® displayed increased intestinal permeability ($P < 0.05$). Feeding LactoCare® resulted in a 15.3% increase in CD3⁺T cells compared with control and LactoPlan® diets, regardless of inclusion level ($P < 0.0001$). Birds fed LactoCare® had 3.9% more splenic innate immune cells (monocytes/macrophages) and 3.2% more lipid antigen-presenting cells (CD1.1⁺) than those fed LactoPlan®, regardless of inclusion ($P < 0.0001$). Birds fed 0.10% LactoCare® had the highest percentages of splenic leukocytes (CD45⁺) and T cells ($P < 0.0001$). These results indicate that probiotic supplementation changed intestinal permeability and splenic immune cell populations in healthy broilers without negatively impacting overall performance.

Key Words: broilers, probiotics, immunity, performance

2 Evaluation of tributyrin in diets varying in lipid source and concentration on broiler live performance and nutrient utilization. Brooke Bodle*¹, Stefan Vaessen², Sami Dridi¹, and Samuel Rochell¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²Perstorp AB, Perstorp, Sweden.

Tributyrin (TB) is a glyceride ester of butyrate that has the potential to improve broiler performance and intestinal development. As a triglyceride, tributyrin efficacy may be influenced by dietary lipid source and concentration. Therefore, an experiment was conducted to investigate the interactive effects of TB and dietary lipids on broiler performance

and apparent ileal nutrient (AID) and energy digestibility (IDE). Dietary treatments consisted of a factorial arrangement of 2 lipid sources (poultry fat or soy oil) × 2 lipid concentrations (standard and high) × 2 tributyrin concentrations (with or without 500 mg/kg tributyrin). For the high lipid diets, lipid supplementation was increased from 1.89% to 3.77% equating to a 100 kcal/kg increase in AME for the soy oil diets. Dietary treatments were fed in a single feeding phase throughout the 21 d experiment. Titanium dioxide was used as an indigestible marker in the feed for determination of AID and IDE following collection of ileal digesta at 21 (5 reps) or 22 (4 reps) d post-hatch. Each treatment was replicated with 9 cages of 8 Cobb 500 male broilers. From 0 to 21 d post-hatch, broilers fed soy oil had ($P = 0.039$) lower FCR than birds fed poultry fat. In addition, broilers fed the higher lipid concentration had lower ($P < 0.001$) FCR than broilers fed the standard lipid concentration. However, lipid concentration × TB interactions were observed ($P < 0.05$) for both BWG and FI, where TB inclusion reduced these measurements in broilers fed the standard lipid diets but increased them in broilers fed the high lipid diets. Increasing dietary lipid concentration from standard to high increased AID of nitrogen ($P < 0.001$) and fat ($P < 0.001$) and IDE ($P < 0.001$). In addition, a lipid source × concentration interaction was observed whereby increasing the dietary concentration of poultry fat resulted in greater improvements in AID of nitrogen ($P = 0.002$), fat ($P = 0.018$), and IDE ($P < 0.001$) for broilers than did increasing the dietary concentration of soy oil. A 2-way interaction between fat source and TB was also observed where TB increased AID of nitrogen ($P < 0.001$) and IDE ($P < 0.001$) in broilers fed soy oil, but reduced these measurements in broilers fed poultry fat. In conclusion, the effects of TB supplementation on growth performance and nutrient digestibility appear to be influenced by dietary lipid source and concentration.

Key Words: tributyrin, poultry fat, soy oil, digestibility energy, broiler

3 Evaluation of two strains of *Bacillus amyloliquefaciens* and two strains of *Bacillus licheniformis* direct fed microbials as viable alternatives to antibacterial treatment as measured by production traits in broilers. Ariel Diggan*¹, Rodrigo Lopez¹, Darin Bennett², Sean Griffin³, and Katy Tarrant¹, ¹Fresno State, Fresno, California, United States, ²California Polytechnic State University, San Luis Obispo, California, United States, ³Osprey Biotechnics, Sarasota, Florida, United States.

To meet the growing demand for no antibiotics ever (NAE) broilers, producers require alternative methods of promoting poultry health and performance. Direct fed microbials are becoming a popular feed additive to achieve production goals. The objective of this study was to compare the performance of broilers raised on 6 different diet, including 2 *Bacillus amyloliquefaciens* strains (A and B), 2 *B. licheniformis* strains (C and D), one Bacitracin Methylene Disalicylate (BMD) diet, and one negative control (NC) diet. Nine replications of each diet were given to Ross 708 broilers totaling 54 test groups. Each test group consisted of 14 straight-run birds housed in 1.51 m² pens and vaccinated at DOT 0 for coccidiosis, for a total of 756 subjects. At d 42, live weight was recorded in grams for each individual. Mean live weight, feed conversion, and necrotic lesion scores comparing the BMD and NC diet with *Bacillus* diets A, B, C, and D were analyzed using Tukey's HSD in JMP v.13.0.0. At d 42, the NC diet birds had the smallest body weight (2875 ± 65). Body weight of birds fed BMD diet averaged 89 g more than birds from NC. As compared with the NC, *Bacillus* average weights compared with NC were the following: A at -164 g, B at +62 g, C +95

g and D+125 g. All *Bacillus* diets reported equal or numerically lower FC scores compared with BMD. *B. licheniformis* C reported the lowest FC, 5 points (3%) lower than NC. *B. amyloliquefaciens* B (1.59) and *B. licheniformis* C (1.57) had the best adjusted feed conversion scores, compared with the BMD (1.60). Necrotic lesions scores did not vary significantly between the diets. *B. licheniformis* probiotics did as well as, or numerically better than, the BMD and NC in all traits measured, while the feed conversion of *B. amyloliquefaciens* preformed numerically better than the BMD and NC. Results from this study indicate that *B. licheniformis* and *B. amyloliquefaciens* strains should be considered useful vehicles for NAE broiler production, and merit further study.

Key Words: broiler, *Bacillus amyloliquefaciens*, *Bacillus licheniformis*, BMD, direct fed microbials

4 In feed *Bacillus subtilis* 29784 benefits broiler live performance similarly to antibiotic growth promoters. Patricia de Carvalho*¹, Douglas Maria¹, André Favero², Liris Kindlein¹, Pablo Ibarro¹, Wanderley Quintero-Filho³, and Sergio Vieira¹, ¹University of Rio Grande do Sul - UFRGS, Porto Alegre, Rio Grande do Sul, Brazil, ²DSA, Garibaldi, Brazil, ³Adisseo, São Paulo, Brazil.

In feed probiotics have been used in animal production for a long time. However, their development became more pronounced as antibiotic growth promoters (AGP) have been banned or voluntarily discontinued. The aim of the current study was to evaluate the efficiency of a *Bacillus subtilis* strain (DSM 29784) on performance responses of broiler chickens. Broilers in the number of 1,375 male broiler chicks were placed in floor pens (1.65 X 1.65 m), with 5 treatments and 11 replications of 25 birds. Particular in this house is that the pens are built inside of a commercial house populated with broilers placed at the same moment. Treatments were in feed additives: negative control (NC) without additive; Enramycin at 10 ppm; *B. subtilis* DSM 29784 (Alterion® NE50) at 500 g/Ton (1x10⁸ UFC/kg of feed); in feed Probiotic A: *B. subtilis* at 500g/Ton and Product B, commercial symbiotic product (Multistrain probiotic + prebiotic) at 200 g/Ton. Data were analyzed using ANOVA and, when significant, means were compared by Tukey test at 5% probability. In general, the in feed *B. subtilis* 29784 significantly increased BWG compared with the NC ($P < 0.05$) whereas all additives, except for product B, improved FCR compared with NC at d 7 ($P < 0.05$). At d 14 and thereafter, *B. subtilis* 29784 and Enramycin increased BWG compared NC ($P < 0.05$) and along with Product A improved FCR ($P < 0.05$). Adding *B. subtilis* 29784 to the feeds led to improvements in BWG and FCR throughout the study when compared with the NC. A similarity in these responses occurred with Enramycin at 10 ppm and Product A, alternatively at different moments. However, a differential mode of action could not be established for the products when presenting benefits since no further evaluations were conducted. Since the study was performed in a typical commercial environment, “challenges” that could have suppressed growth are supposedly those giving room for the effects of the additives used.

Key Words: *Bacillus subtilis*, enramycin, in feed probiotic, performance

5 Effect of allicin supplementation on relative weight of organs of the immune system, ratio of right to total ventricle weight and productive performance of broiler chickens. Leonardo Osio-Orihuela*³, Jaime León Landeros³, Elvia Martínez-Cruz³, Arturo Pro-Martínez¹, Luis Salinas-Jiménez³, José Reyes-Bello³, Edgar Ahedo-Quedo³, Raúl Argüello-García², Eliseo Sosa-Montes³, Benito Bello-Olivera³, and Fernando González-Cerón³, ¹Colegio de Post-graduados, Campus Montecillo., México, México, Mexico, ²Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional, México, México, Mexico, ³Universidad Autónoma Chapingo, Texcoco, Mexico, Mexico.

Broiler productive performance results from multiple factors including nutritional profile, feeding strategy, intestinal health, activity of the immune system, etc. Allicin is a bioactive compound of garlic that could have beneficial effects on health of birds and improve productive performance. The objective of the present study was to evaluate the effect of different doses of allicin on relative weight of immune system organs, the ratio of right to total ventricle weight (RVR) and productive performance of broiler chickens. A total of 168 one-d-old male chickens (Ross 308) were randomly assigned to 3 treatments (8 pen replicates per treatment and 7 birds per pen): T1 or Control, 0 mg of allicin/kg of body weight (BW); T2, 0.5 mg of allicin/kg of BW from 21 to 49 d of age; T3, 1 mg of allicin/kg of BW from 21 to 49 d of age. Allicin was administrated by esophageal route using a pediatric catheter (5-Fr caliber). All birds were fed with the same starter (1 to 21 d) and grower-finisher (22 to 49 d) diets. BW and feed conversion ratio (FCR) were monitored weekly during the 7-wk trial. At 35, 42 and 49 d of age, 8 birds per treatment were randomly slaughtered and the relative weight of thymus (TMRW), spleen (SPRW) and bursa of Fabricius (BFRW) were determined. RVR was also recorded. Data were subjected to an ANOVA using the MIXED procedure of SAS software. No differences ($P > 0.05$) were detected among treatments on TMRW (0.20 to 0.30 ± 0.03%), SPRW (0.13 to 0.15 ± 0.01%), BFRW (0.18 to 0.24 ± 0.02%) and RVR (0.17 to 0.21 ± 0.01) at 35, 42 and 49 d of age. Likewise, BW was not different ($P > 0.05$) among treatments during the time allicin was administered (4 wk, 1044 to 1097 ± 34 g; 5 wk, 1345 to 1414 ± 25 g; 6 wk, 1776 to 1796 ± 29 g; 7 wk, 2326 to 2425 ± 75 g). A similar pattern ($P > 0.05$) was observed in FCR once allicin was given (4 wk, 2.22 to 2.59 ± 0.16; 5 wk, 2.82 to 2.95 ± 0.24; 6 wk, 2.04 to 2.41 ± 0.10; 7 wk, 2.26 to 2.96 ± 0.24). It is concluded that allicin does not influence relative weight of thymus, spleen, bursa of Fabricius, the ratio of right to total ventricle weight and it does not improve productive performance of broiler chickens.

Key Words: allicin, immune system, right ventricle ratio, performance, broilers.

Student Competition: Metabolism and Nutrition: Nutrition I

6 Data mining tool agrees with statistical models on the effect of corn particle size and mineral source on turkey performance. Karlinton Flores*, Jesse Grimes, and Adam Fahrenholz, *North Carolina State University, Raleigh, North Carolina, United States.*

Organic or inorganic mineral sources (MS) have as a goal to provide the required minerals at the lowest, but optimal, dietary level using least cost by either affecting the cost per kg or digestibility of the premix. The corn particle size (CPS) in the feed has the potential to save feed milling cost by adding the corn after pelleting and increasing CPS with bird age in feed phases until market age. The objective of this effort was to compare Machine Learning (ML), Data mining (DM) and statistical models as tools to analyze both MS and CPS effects on turkey body weight (BW) and feed intake (FI) classifiers. Twelve hundred Nicholas Select male poults were randomly assigned to 48 concrete-floor pens. The experimental design was a completely randomized block design with a 2×2 factorial arrangement of 2 sources of minerals (organic vs inorganic) and 2 levels of corn particle size (coarse corn vs fine corn). Birds' BW, weekly body weight gain (BWG), FI, feed conversion ratio (FCR), gut pH, gut leakage (FITC-D), cloacal temperature, density, and others were used as variables for the ML analysis and were analyzed in SAS 9.4 in a mixed model. A total of 38 variables were used in ML and DM analysis. BW and FI at 18 weeks of age were classified as low, objective and high based on a 5% for BW and 3% for FI margin of the Aviagen male objectives for ML analysis. Support vector machine (SMO), neural networks, and decision trees (J48) were used as the algorithms for ML with a 20-fold validation system using WEKA 3.8. SMO yield 85.42% correctly classified BW at 18 weeks of age with an optimal of 14 variables, where the mineral source and corn particle size were critical variables in the model accuracy. In comparison, SMO correctly classified 68.75% of the FI classifications with an optimal number of variables of 9 variables, where neither corn particle size nor mineral source was critical for the model. These results concur with the statistical analysis for both FI and BW. In conclusion, both statistical and ML models agree with the data analysis results. These ML and DM tools could be applied to turkey research and production systems by analyzing multiple variables and be compared with statistical models.

Key Words: machine learning, data mining, corn particle size, mineral source

7 Evaluation of different particle size analysis methods for ground corn. Gerardo Abascal-Ponciano*, Allan Calderon, Joshua Flees, Danny Patino, Samuel Leiva, Jorge Sandoval, Marc Presume, Kevin Ordonez, Luis Avila, Wilmer Pacheco, and Charles Starkey, *Auburn University, Auburn, Alabama, United States.*

Particle size (PS) reduction is a common feed manufacturing process and is important for animal feed production on a global scale. The objectives were to evaluate the performance of a roller mill and evaluating PS testing methods. Corn was milled using a 2-pair roller mill (Roskamp Champion Series 900–12) at 8 different settings: from 3 to 2 to 6–6 (top pair-bottom roller pair setting, 50.8 μm to 152.4 μm). The PS of 3 replicate samples per setting were analyzed using the ANSI/ASAE S319.4 "Method of determining and expressing fineness of feed materials by sieving" standard using 13 sieves and an electric sieve shaker (ANSI) and a hand-shaken 3-sieve field method developed by Kansas State University (KSU3S). Data were analyzed using the GLIMMIX procedure of SAS V9.4. Least squares means were separated using the

PDIFF option at $P \leq 0.05$. Particle mean diameter (PS or DgW) ranged from 693 to 3,343 μm from setting 3–2 to 6–6 when measured with ANSI and from 584 to 1,702 μm when the KSU3S method was used. Modification of the formulas used in the ANSI method were made to calculate PS using the KSU3S method and resulted in PS ranging from 640 to 3,196 μm (AUM3S). As expected, using the ANSI method, PS decreased as the bottom rollers were adjusted 25.4 μm ($P < 0.0001$). The KSU3S method produced similar PS values to the ANSI method for only 2 of the 8 roller settings ($P < 0.0001$). However, the AUM3S method was accurate for 6 of the 8 settings and similar to the ANSI method, PS decreased as the bottom rollers moved in 25.4- μm increments ($P < 0.0001$). Additionally, both the ANSI and AUM3S methods allow for calculation of standard deviation (Sgw), surface area (cm^2 per g), and particles per g. With the ANSI method, there were no differences in Sgw among roller mill settings, while the AUM3S method resulted in different Sgw among settings ($P < 0.0001$). When surface area was determined using the ANSI method, there were differences among all settings except 5–5 and 6–6 ($P < 0.001$). The AUM3S method produced a similar pattern in surface area but differed from the ANSI method for settings 3–2, 3–3, and 4–3 ($P < 0.001$). Using ANSI, no differences were observed among settings for particles per g ($P > 0.05$). However, using the AUM3S method, particles per g did differ among settings ($P < 0.001$). The AUM3S differed from ANSI at settings 3–2 and 3–3 ($P < 0.001$). In conclusion, the different roller mill settings produced different PS when the bottom pair of rollers were adjusted 25.4 μm at a time. Modification of the KSU3S PS method (AUM3S) produced more accurate PS results for ground corn compared with the standard ANSI PS method, while also providing the additional measurements of Sgw, surface area, and particles per gram.

Key Words: feed, particle size, roller mill, sieve, surface area

8 Effect of particle size on near infrared reflectance spectroscopy (NIRS) nutrient analysis of ground corn. Samuel Leiva*, Jorge Sandoval, Joshua Flees, Allan Calderon, Gerardo Abascal-Ponciano, Kevin Ordonez, Danny Patino, Luis Avila, Marc Presume, Wilmer Pacheco, and Charles Starkey, *Auburn University, Auburn, Alabama, United States.*

Near infrared reflectance spectroscopy (NIRS) is a technology widely used for the determination of nutrient composition of feedstuffs. NIRS is a useful tool that can provide immediate nutrient values for formulators to ensure that feeds are manufactured to specification. However, to achieve proper readings from NIRS, samples should be handled consistently and ground to a uniform and consistent particle size for each evaluation. Particle size (PS) of the sample may affect NIRS nutrient estimates of corn. The objective of this experiment was to evaluate the effect of corn PS on the accuracy and ability of NIRS to provide proper nutrient values. Corn was milled using a 2-pair roller mill (Roskamp Champion Series 900–12) set at 8 different roller pair settings. The roller pair spacing ranged from 50.8 μm at setting 2 to 152.4 μm at setting 6. Setting 3–2, 3–3, 4–3, 4–4, 5–4, 5–5, 6–5, 6–6 generated samples with mean PS of 693, 882, 968, 1,750, 1,877, 2,862, 2,911, and 3,343 μm , respectively. NIRS was used to determine dry matter, ash, crude fat, crude protein, and crude fiber. In addition, a rotor grinder (Retsch ZM 200) with a 500-mesh screen was used to grind corn to 267 μm , which served as a control for the study based upon recommendations from NIRS equipment companies. Three replicate samples per setting were analyzed using a Bruker MPA II NIR spectrometer and the spectra

were analyzed using Adissee PNE software. Data were analyzed with the GLIMMIX procedure of SAS V9.4 and least squares means were separated using the PDIF option at $P \leq 0.05$. For ash content, differences were observed between PS, with the 3,343 μm sample being the closest to the 267 μm corn value. Samples with PS of 693 (0.86%), 882 (0.89%) and, 968 (0.84%) μm produced ash values below those of larger PS samples ($P < 0.0001$). At a PS of 3,343 μm , protein content (8.08%) was not different ($P = 0.28$) when compared with 267 μm (8.22%). However, all other PS (693 to 2911) had reduced ($P < 0.0001$) protein percentages (6.67% to 7.56%). NIRS results for fat content for samples ground using the roller mill were underestimated ($P < 0.0001$) from 0.36% to 0.59% for PS 693 μm to 3,433 μm when compared with the 267 μm control sample. In contrast, samples from corn ground using the roller mill resulted ($P < 0.0001$) in overestimations of dry matter when compared with the 267 μm control rotor ground samples (0.83% to 3.06%). This could be due to moisture loss during the use of the rotor grinder. While it may take some additional time and effort to properly prepare samples for NIRS analysis, these data suggest that it is important to do so because PS of corn samples affects the ability of the NIRS to produce accurate proximate nutrient analysis readings.

Key Words: NIRS, corn, particle size, nutrient analysis

9 Effect of ground corn particle size and soybean oil addition on angle of repose and flow time. Danny Patino*, Edman Mendez, Jorge Sandoval, Joshua Flees, Allan Calderon, Gerardo Abascal-Ponciano, Kevin Ordonez, Luis Avila, Marc Presume, Wilmer Pacheco, and Charles Starkey, *Auburn University, Auburn, Alabama, United States*.

Particle size (PS) reduction is an important component of feed manufacturing that impacts pellet quality, mixability, feed flowability, and animal performance. However, reducing particle size too fine can result in reduced flowability of the ground corn. Measuring particle size using funnels, to obtain an angle of repose (AOR) as well as a flow time (FT), can differ greatly depending upon the opening of the funnel used. The objective of this this experiment was to analyze how fractionation and increasing added concentrations of soybean oil (SBO) affected flowability of ground corn for the measurements of AOR and FT using funnels with different diameter openings (FDO). A complete block randomized design experiment with an $8 \times 6 \times 3$ factorial treatment arrangement was conducted with 3 replicates per treatment. Corn was ground using a 2-pair roller mill (Roskamp Champion Series 900–12) using 8 different settings: 3–2, 3–3, 4–3, 4–4, 5–4, 5–5, 6–5, and 6–6, where the roller pair spacing ranged from 50.8 μm at setting 2 to 152.4 μm at setting 6. The settings generated samples with mean PS of 693, 882, 968, 1,750, 1,877, 2,862, 2,911, and 3,343 μm , respectively. The ground corn produced from each roller mill setting was mixed with SBO to achieve 0, 2, 4, 6, 8, and 10% added fat. The flowability of any material is dependent on a set of parameters, one of which is the angle of repose (AOR). Therefore, the angle of repose was used as an indication of flowability and was analyzed on triplicate samples of each PS and SBO combination using the ASTM/C 1444 “Standard Test Method for Measuring the Angle of repose.” For this study, 3 different FDO used were: 13 mm, 18 mm, and 31 mm, and were placed at a height of 30 cm. One-hundred g of each sample was allowed to flow through each funnel and allowed to freely form a cone. The AOR and the FT were measured 3 times for each PS and SBO and FO combination. Data were analyzed using the GLIMMIX procedure of SAS (V9.4). Means were obtained different when $P < 0.05$. An interaction was observed among the fixed effects of PS,

SBO and FDO for both AOR and FT ($P < 0.0001$). Samples with 0% added SBO had decreasing AOR as FDO increased with the exception of samples with a PS of 3,343 ($P < 0.0001$). FT decreased as the FDO increased from 13 to 18 mm; however, this was not consistent when FDO changed from 18 to 31 mm. As PS increased, additions of 2 or 4% SBO increased AOR, while addition of 6% SBO decreased AOR, and 8 or 10% SBO addition increased AOR ($P < 0.0001$). In conclusion, PS, SBO, and FDO all have important implications for flowability.

Key Words: particle size, funnel, flow time, fat addition

10 Assessment of angle of repose as a tool to measure the flowability of different particle size ground corn with and without added soybean oil. Marc Presume*, Jorge Sandoval, Gerardo Abascal-Ponciano, Allan Calderon, Danny Patino, Kevin Ordonez, Luis Avila, Samuel Leiva, Joshua Flees, Wilmer Pacheco, and Charles Starkey, *Auburn University, Auburn, Alabama, United States*.

Flowability of ingredients is an important factor in feed manufacturing that can impact batching time, mixability, and conveyance throughout the system. Flowability in ground corn is often affected by factors such as particle size (PS), cohesive forces between particles, relative humidity, temperature, and bulk density. One method to assess the flowability of a material is to measure the angle of repose (AOR), which is the angle formed between the horizontal and the slope of a pile. The AOR is inversely proportional to flowability. The objective of this study was to assess the effects of PS and soybean oil (SBO) addition on AOR, and thus flowability, of ground corn. This randomized complete block experiment with an 8×6 factorial treatment structure with 3 replicates per treatment was conducted. Corn was ground using a 2 pair roller mill (Roskamp Champion Series 900–12) at 8 different roller settings to produce samples with mean PS of 693, 882, 968, 1,750, 1,877, 2,826, 2,911, and 3,343 μm . The roller pair spacing ranged from 50.8 μm at setting 2 to 152.4 μm at setting 6. After milling, SBO was added to sample of each PS to achieve 0, 2, 4, 6, 8, and 10% fat. A transparent box with a 5.08-cm opening was used to determine AOR and 200-g of sample was poured into the box using a funnel with a 31.75-mm opening. Then, the right and the left angles of the pile were measured with a protractor to determine the AOR for each sample. Data were analyzed using GLIMMIX procedure of SAS (V.9.4) and means were separated using the PDIF option at $P \leq 0.05$. An interaction was observed between PS and SBO additions ($P < 0.0001$). For samples containing no added SBO, the AOR did not differ for any PS ($P < 0.0001$). In every case, increasing additions of SBO increased the AOR ($P < 0.0001$). For corn with PS of 882, 968, 1,750, 1,877, 2,911, and 3,343 μm , the addition of 2% and 4% SBO, AOR was similar but was differed for 693 and 2,862- μm PS corn ($P < 0.0001$). Increasing the SBO addition from 4% to 6% did not alter AOR for corn PS ranging from 882 to 2,862 μm , but AOR was impacted for 693, 2,911, and 3,343- μm PS corn ($P < 0.0001$). Differences were observed when SBO additions increased from 6 to 8% for PS 882, 1,750, 1,877, 2,862, 2,911, and 3,343 μm , but not for PS 693 and 968 μm ($P < 0.0001$). Increasing SBO addition from 8 to 10% increased AOR at every corn PS except 3,343 μm ($P < 0.0001$). In conclusion, corn PS and SBO addition interact to impact AOR. A consistent increase in AOR was observed as SBO additions increased. With no added SBO, the AOR was similar among corn samples with PS from 693 to 3,343 μm ($P > 0.05$). Overall, based on their lower AOR, flowability was enhanced in samples with smaller PS and less SBO.

Key Words: angle of repose, flowability, particle size, oil addition, roller mill

11 Effect of particle size and oil addition on near infrared spectroscopy (NIRS) nutrient analysis of ground corn. Jorge Sandoval*, Samuel Leiva, Joshua Flees, Allan Calderon, Gerardo Abascal-Ponciano, Kevin Ordonez, Danny Patino, Luis Avila, Marc Presume, Wilmer Pacheco, and Charles Starkey, *Auburn University, Auburn, Alabama, United States.*

Near infrared reflectance spectroscopy (NIRS) can be used to estimate quality aspects and nutrient composition of feed. The objective here was to evaluate the effect of ground corn particle size (PS) with increasing additions of soybean oil (SBO) on the ability of NIRS to provide accurate nutrient estimates. Corn was milled using a 2-pair roller mill (Roskamp Champion Series 900–12) with 8 different roller settings. The roller pair spacing ranged from 50.8 μm at setting 2 to 152.4 μm at setting 6. Setting 3–2, 3–3, 4–3, 4–4, 5–4, 5–5, 6–5, 6–6 generated samples with mean PS of 693, 882, 968, 1,750, 1,877, 2,862, 2,911, and 3,343 μm , respectively. SBO was mixed with the ground corn to achieve samples of each PS with 2, 4, 6, 8, and 10% SBO. Based on recommendations from NIRS equipment companies, corn was also ground to 267 μm with a rotor grinder (Retsch ZM 200) with a 500-mesh screen to serve as a control. Three replicate samples from each combination of PS and SBO inclusion were analyzed using a Bruker MPA II NIR spectrometer and Adisseo PNE software to estimate dry matter (DM), ash, crude fat (CFAT), crude fiber (CFIB), and crude protein (CP). Data were analyzed with the GLIMMIX procedure of SAS V9.4 and means were separated

using the PDIFF option at $P \leq 0.05$. Dry matter was higher for 267- μm PS samples, likely due to increased heat and surface area allowing for increased evaporative moisture loss. For the 267- μm control, DM increased as SBO increased ($P \leq 0.0001$). For PS 693 to 3,433 μm , DM first increased up to either the 4 or 6% SBO additions and then decreased through 10% ($P \leq 0.0001$). Ash increased as SBO increased for each PS ($P \leq 0.0001$). As expected, regardless of PS, increasing additions of SBO increased CFAT ($P \leq 0.0001$). However, the increases were not equal to the actual added SBO and only ranged from 0.5 to 1.5%. CFIB was lower for the 267- μm control samples with 2 to 4% SBO addition, increased for 693 through 2,862 μm PS samples with 4 to 10% SBO, and was inconsistent for 2,911 and 3,343 μm PS samples ($P \leq 0.0001$). CP ranged from 5.95 to 12.53%. For the 267- μm control, the CP increased as SBO increased ($P \leq 0.0001$). For 693 to 968 μm PS samples, CP decreased ($P \leq 0.0001$) with 2 to 4% added SBO and then increased with 6, 8, and 10% SBO. Samples with 2,911 and 3,343 μm PS had more consistent CP values with the increasing SBO additions having little effect. In conclusion, sample PS and added SBO interacted to negatively impact the ability of NIRS to provide accurate nutrient values. Overall, these results demonstrate the importance of properly preparing samples by grinding them to 267 μm before NIRS analysis as well as properly training the NIRS for samples with oil additions.

Key Words: NIRS, near infrared reflectance spectroscopy, particle size, feed manufacturing, oil addition

Student Competition: Metabolism and Nutrition: Vitamins and Minerals I

12 Effects of the *in ovo* injection of vitamin D₃ and 25-hydroxyvitamin D₃ on the small intestine morphology and immunity of broilers challenged with coccidiosis. Saman Fatemi¹, Katie Elliott¹, Abdulmohsen Alqhtani¹, Abiodun Bello¹, and Edgar Peebles*², ¹University of Alberta, Edmonton, Alberta, Canada, ²Mississippi State University, Mississippi State, Mississippi, United States.

Broiler small intestine morphology and immunity have been shown to be improved by the use of supplemental 25-hydroxyvitamin D₃ (25OHD₃) during a coccidiosis challenge. But these effects have not been investigated in response to the injection of either vitamin D₃ (D₃) or 25OHD₃. Therefore, the objectives of this study were to determine the effects of the *in ovo* administration of D₃ and 25OHD₃ on the small intestine morphology and inflammatory response of broilers challenged by coccidiosis. Live embryonated Ross 708 broiler hatching eggs were randomly assigned to one of the following 5 *in ovo* injection treatments at 18 d of incubation (doi): 1) non-injected; 2) diluent; diluent containing either 3) 2.4 µg D₃, 4) 2.4 µg 25OHD₃, or 5) 2.4 µg D₃ + 2.4 µg 25OHD₃. A 50 µL solution volume was injected into each egg using an Inovoject multi-egg injector. At hatch, 4 male chicks were randomly assigned to each of 80 battery cages in each of 2 rooms with 8 replicates per treatment group. Only *in ovo*-injected treatments were challenged with a 20x live coccidial vaccine dosage at 14 d of age (doa). At 14 and 28 doa, blood samples were collected from 8 birds per treatment for determination of serum IL-1β and nitric oxide (NO) levels. From the same birds, 2 cm of the middle of the duodenum, jejunum, and ileum were excised for histomorphological analysis. Duodenum, jejunum, and ileum villus length (VL), crypt depth (CD), villus length to crypt depth ratio (RVC), and villus surface area (VSA) were measured. All data were analyzed as a one-way ANOVA using SAS 9.4. No significant differences were observed among treatments for immunity measurements before and after challenge. At 14 doa, *in ovo* injection of 25OHD₃ alone resulted in higher duodenal RVC ($P = 0.001$) in comparison to all other treatments. At 28 doa, *in ovo* injection of 25OHD₃ alone resulted in greater duodenal RVC ($P = 0.001$) in comparison to diluent, D₃ alone, and the D₃ + 25OHD₃ combination treatments. These results indicate that the *in ovo* injection of 25OHD₃ at 2.4 µg has a potential to improve the small intestine morphology of broilers during a coccidiosis challenge. The improvement could be due to a greater rate of absorption and longer half-life of 25OHD₃. In addition, the *in ovo* injection of 25OHD₃ may result in a higher expression of 1-α-hydroxylase which converts 25OHD₃ to the active form of vitamin D in the chicken intestine, with a subsequent increase in intestinal development. Further research is required to determine effects of the *in ovo* injection of vitamin D sources on the expression of 1-α-hydroxylase in the small intestine of broilers.

Key Words: *in ovo* injection, vitamin D sources, coccidiosis, small intestine morphology, immunity

13 Dietary administration of 25-hydroxycholecalciferol, a vitamin D₃ metabolite, increases tibial mass by suppression bone absorption in meat duck. Huaiyong Zhang*¹, Feng Zeng¹, Mei Ding¹, Ping Bai¹, Ping Wang¹, Yue Xuan¹, Wei Su¹, Bing Yao³, Gregory Fraley², and Ying Zhang¹, ¹Animal Nutrition Institute, Chengdu, Sichuan, China, ²Biology Department, Holland, Michigan, United States, ³DSM (China) Ltd., Shanghai, China.

The aim of this study was to evaluate the effect of 25-hydroxycholecalciferol (HyD) on tibial mass of meat duck. In experiment (Expt.)

1, response to the effect of dietary HyD on tibial mass in a standard nutrient density diet. 256 1-d-old duck was equally divided into 0 or 69 µg HyD/kg diet with National Research Council (NRC, 1994) vitamin regimen for 35 d. Data were subjected to one-way ANOVA and statistical significance was detected at $P < 0.05$. Dietary HyD notably increased the mineral content, microarchitecture, and mechanical properties of the tibia, which accompanied by a significantly increased calcium (Ca) concentration, and a remarkably decreased bone absorption markers in serum ($P < 0.05$). Supplementation HyD reduced the osteoclast number ($P < 0.05$) and the ratio of receptor activator for nuclear factor κB ligand and osteoprotegerin, as well as blocked the expression of *cathepsin K* ($P < 0.05$). Subsequently, Expt. Two were conducted to estimate the effect of HyD on tibia mass in a low nutrient density (LND) diet with 2 vitamin regimens. 512 duckling was randomized to 0 or 69 µg/kg HyD and NRC or China Agricultural industry standards (NY/T, 2012) (2 × 2 factorial design). The LND diet included 10.25 MJ/kg metabolic energy, 13.28% crude protein, 0.7% Ca and 0.35% non-phytate phosphorus. The results were analyzed using 2-way ANOVA. NY/T vitamin regimen inhibited bone turnover and consequently increased bone trabecular area and mineral deposition compared with NRC vitamin premix ($P < 0.05$). Supplementation HyD to NRC but not NY/T vitamin regimen remarkably enhanced tibial mineral content and microarchitecture, and decreased serum bone absorption and increased Ca concentration ($P < 0.05$). Collectively, these findings indicated that no matter what nutrient density, HyD treatment increased tibia mass in NRC vitamin regimen by suppressing bone resorption for meat duck. In addition, when fed the LND diet, the NY/T vitamin regimen made a favorable effect on the tibia mass of duck by decreasing bone turnover rate.

Key Words: 25-hydroxycholecalciferol, vitamin, bone turnover, tibia mass, meat duck

14 Economically important production traits and enteric health of broilers fed free or lipid matrix-encapsulated vitamin and trace mineral elements at recommended and reduced levels. Olivia Wedegaertner*¹, Elle Chadwick¹, Robert Beckstead¹, Elizabeth Santin², Jean-Christophe Bodin², Jean Fontaine², and Peter Ferket¹, ¹North Carolina State University, Raleigh, North Carolina, United States, ²JEFO Nutrition, Inc, Saint-Hyacinthe, Quebec, Canada.

Protection of vitamin and mineral elements (VME) in a lipid encapsulated matrix for controlled release could enhance bioactivity and permit lower dietary inclusion levels by limiting chemical or microbial exposure without adversely affecting growth, welfare, enteric health or value of poultry products. Our objective was to determine the effect of reduced inclusion levels of free and lipid matrix encapsulated VME on production traits and enteric health. Two 2 X 2 factorial designed experiments were conducted in which free or lipid matrix encapsulated VME were included at 100% of Aviagen recommended levels or at 60% or 70% reduced levels in trials 1 and 2, respectively. Reduced levels of free VME are not commercially feasible due to the risk of activity losses during feed manufacturing and storage. In trial 1, Ross 708 male broilers were randomly assigned to 4 dietary treatments (25 birds/pen, 2 pens/trt). Feed and individual bird weights were recorded on D7, 14, 21, 28 and 42. Samples for 28d and 42d jejunum histology and 42d cecal 16s RNA analysis was collected from 3 birds per pen. Based on trial 1 results replicates were increased for trial 2. In trial 2 Ross 708 male and female broilers were assigned to 4 treatments (32

birds/pen, 6 pens/trt). Feed and bird weights were recorded on D14, 28 and 42. On D49 birds from trial 2 were processed; carcass and cut up weights, white striping, wooden breast, drip loss, paw quality, and shank, skin and breast meat color (Minolta Colorimeter) were recorded. Data were analyzed by factorial ANOVA. No treatment effects were observed on growth performance in trial 1. Encapsulation reduced jejunum villi surface area ($P < 0.05$) at 28d and 42d regardless of level, suggesting less mucosa inflammation. Encapsulation resulted in higher Firmicutes:Bacteroidetes ratios of cecal contents than free VME. In trial 2 there were no effects observed on the growth of females or on carcass yield or muscle myopathies of males. In the males, D0–42 feed intake was reduced 3.5% by VME encapsulation without significant effects on 42d BW, which improved 0–42d FCR in comparison to free VME (1.57 vs 1.66). A 70% reduction in the dietary level of free VME resulted in the highest incidence of hock and paw lesions. In contrast encapsulated VME improved the quality and yellowness values of paws and breast skin, and significantly reduced breast meat drip loss after 3, 5 and 7 d of refrigerated storage ($P < 0.01$). Lipid matrix encapsulated VME improve growth performance, enteric health, and market value of poultry products regardless of dietary level, but encapsulation also remediates the risk of lower dietary VME inclusion levels that would otherwise be infeasible as free premix forms.

Key Words: vitamins, minerals, lipid encapsulation matrix, microbiome, poultry product quality

15 Not Presented

16 Growth performance and carcass characteristics of broilers fed diets varying in supplemental copper concentrations from 29 to 53 days of age. Stephanie Philpot^{*1}, Kurt Perryman², and William Dozier, III¹, ¹Auburn University, Auburn, Alabama, United States, ²Micronutrients, Indianapolis, Indiana, United States.

Previous research has established that feeding broilers high dietary Cu concentrations (>100 mg/kg) can provide growth promoting benefits. However, information is sparse about effects of feeding high concentrations of Cu to broilers from 2.8 to 4.0 kg of BW. An experiment was conducted to determine the effects of supplemental Cu concentrations on growth performance and carcass characteristics of Ross × Ross 708 broilers from 29 to 53 d of age. Male chicks were randomly distributed to 60 floor pens (22 birds/pen, 0.1 m²/bird). Birds were provided with common starter and grower diets containing 135 mg/kg of Cu from 1 to 29 d of age. Corn, soybean meal, and DDGS were the primary ingredients. At 29 d of age, pens were equalized for bird number and assigned to 1 of 5 dietary treatments (12 replicate pens per treatment). Experimental treatments contained either 0–0, 135–0, 270–0, 135–135, or 270–270 mg/kg of Cu in the form of tribasic Cu chloride (Micronutrients USA, LLC) in the finisher 1 (29 to 41 d of age) and finisher 2 (41 to 53 d of age) periods. Diets were formulated to be identical in nutrient composition except for supplemental Cu concentration. Body weight gain, feed intake, feed conversion ratio, and mortality were determined at 29, 41, and 53 d of age. At 54 d of age, 14 birds per pen were processed and front-half deboned to determine total breast meat (pectoralis major and minor muscles) weight and yield. Additionally, fillets (pectoralis major muscles) were scored for white striping and wooden breast. Data were analyzed using a one-way ANOVA. No significant differences ($P \geq 0.08$) were observed for growth performance characteristics. Broilers

fed diets containing 270 mg/kg of Cu in the finisher 1 and finisher 2 diets had higher total breast yield than the broilers fed no supplemental Cu from 29 to 53 d of age (28.1% vs. 27.6%, $P = 0.017$). This represented an increase in fillet weight ($P = 0.003$) of 32 g compared with birds fed diets containing no supplemental Cu from 29 to 53 d of age and the birds fed 135 mg/kg of supplemental Cu from 29 to 41 d of age. Severity of white striping or wooden breast was not affected ($P \geq 0.08$) by Cu supplementation. These data indicate that high concentrations of dietary Cu may increase breast meat weight and yield of broilers processed at 54 d of age with no adverse effects on meat quality.

Key Words: antibiotic free, broiler, copper, debone, yield

17 Zinc amino acid complex supplementation mitigates negative effects of cyclic heat stress on intestinal integrity of broilers.

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Performance and intestinal integrity are knowingly affected by thermal stress. Zinc supplementation has shown potential to mitigate these detrimental effects by enhancing tight junction proteins' expression and maintaining mucosal small intestine structure. The objective of the study was to determine the effect of a zinc amino acid complex (ZnAA; Availa[®]Zn) on intestinal integrity of broilers submitted to heat stress. Ninety-six 21-d old Cobb male broilers were randomly allotted to 4 dietary treatments (0, 20, 40 and 60 ppm of zinc) and 2 environments, resulting in a 4 × 2 factorial scheme. The environmental conditions consisted of thermoneutral (TN) and chronic cyclic heat stress (HS). The unsupplemented basal corn-soybean meal diet contained 30 ppm of Zn. Birds were fed experimental diets from 1d up to 21d, and from 21d to 42d housed in 3 wired-cages/treatment (4 birds/cage). This experiment was repeated over time, resulting in 12 replicates. FITC-d permeability assay, intestinal histomorphometry and expression of claudin-1, occludin and zonula occludens-1 by Western blot were performed at 42d. Data were subjected to 2-way ANOVA in a 4 × 2 factorial scheme, with ZnAA level and environmental conditions as the main effects and their interactions. The repetition over time was considered a random effect. Polynomial contrasts were used to determine linear and quadratic responses to dietary ZnAA levels, within HS and TN groups or over main effect. Heat stress reduced villus height in jejunum and ileum ($P < 0.05$). In jejunum, TN showed higher crypt depth compared with HS broilers ($P < 0.05$). HS birds presented decreased villus height: crypt depth ratio compared with TN birds, on ileum ($P < 0.05$). An interaction between ZnAA supplementation and environment was observed for villus height in jejunum ($P < 0.05$). A positive linear effect ($P = 0.071$) was observed for HS birds, while no significant effect was observed for TN birds. For occludin and ZO-1, interaction between environment and ZnAA level was observed ($P < 0.06$). For both proteins, a positive quadratic response was observed for broilers exposed to heat stress as ZnAA increased. It means that the intermediate levels of supplemental zinc (20 and 40 ppm) enhanced the protein expression, resulting in greater intestinal cohesion. FITC-d results showed that zinc supplemented broilers presented lower levels of the marker on the blood for HS broilers ($P < 0.05$), indicating a less permeable gut. Altogether, HS reduced gut integrity, while ZnAA supplementation mitigated negative effects caused by heat stress.

Key Words: mineral nutrition, zinc, stress, gut permeability

Student Competition: Processing and Products

18 Detection of woody breast condition in commercial broiler carcasses using image analysis. Juan Caldas-Cueva*, Andy Mauro-moustakos, and Casey Owens, *University of Arkansas, Fayetteville, Arkansas, United States.*

Image analysis could be an objective and rapid method to identify woody breast (WB) myopathy and benefit the global poultry industry. The aim of this study was to determine if there are conformation changes that can be used to detect WB characteristics in commercial broiler carcasses across strains, gender, and ages using image analysis method. A total of 900 images of male (n = 450) and female (n = 450) broiler carcasses from 3 commercial strains and 5 ages (6, 7, 8, 9 and 10 weeks) were captured before evisceration. Whole breast fillets were hot deboned and scored for WB severity based on tactile assessment. Broiler carcass images were processed and analyzed using ImageJ software. Parameters for carcass conformation were M0: breast length; M1: breast width in the cranial region; M2: vertical line from the tip of keel to 1/5th of breast length; M3: breast width at the end of M2; M4: angle formed at the tip of keel and extending to outer points of M3; M5: area of the triangle formed by M3 and lines generated by M4; M6: area of the breast above M3; M7: M6 minus M5. In addition, 4 ratios [M8 (M3/M1), M9 (M3/M2), M10 (M7/M5), and M11 (M1/M0)] were considered. Spearman correlation coefficients were estimated for WB severity scores and image measurements. Binary nominal logistic regression was carried out to evaluate and select suitable prediction models for WB condition. Regardless of strain, sex and age, M11 (M1/M0), M9 (M3/M2), and M4 (angle at keel) had the highest correlation to WB score ($r \geq 0.65$; $P < 0.01$) followed by M3, M1, M7, M6, M5, M8, M10, and M2 respectively. Overall, the simplest and most adequate validated prediction model ($P < 0.01$) included M1, M2, and M3 measurements, which showed high accuracy estimating WB condition [Gen. $R^2 = 0.61$, AUC = 0.94, misclassification rate (MR) = 8.52% and sensitivity (ST) = 71.11%]. Alternatively, M4 and M6 measurements as well as ratios M9 and M11 could be considered as predictors. Regardless of strain and gender, the most adequate model to predict WB condition in broiler carcasses from 6 and 7 weeks included M1 and M9 (Gen. $R^2 \geq 0.62$, AUC ≥ 0.96 , MR $\leq 7.78\%$, and ST $\geq 54.55\%$), whereas the model for 8 to 10 weeks included M1, M2, and M3 (Gen. $R^2 \geq 0.58$, AUC ≥ 0.91 , MR $\leq 15.56\%$, and ST $\geq 63.83\%$). These data support the feasibility of the use of image analysis to predict WB condition in broiler carcasses regardless of the strain, sex and age. The potential integration of these image measurements into commercial in-line, non-destructive and non-contact vision grading systems would allow processors to identify broilers with WB to potentially sort, provide information downstream to deboning operations, and/or provide information upstream to live production.

Key Words: wooden breast, image analysis, processing, in-line carcass sorting, meat quality

19 Evaluating broiler breast and tenderloin meat tenderness using various versions of the Muellenet Owens Razor Shear. Clay Maynard*, Amelia Chapman, and Casey Owens, *University of Arkansas, Fayetteville, Arkansas, United States.*

Broiler meat quality is important to consumers, in particular breast meat tenderness. Tenderness can be assessed indirectly with instrumental methods. The Muellenet Owens Razor shear method (MORS) was developed to assess tenderness without requiring added sample cutting, thus simplifying the process compared with traditional methods.

A blunted version (BMORS) was since used to better assess tougher meat, and recently, a larger incisor blade (IMORS) has been used by the food service industry for quality control. However, no data is available in literature on how the IMORS relates to current methods, nor is there any data related to tenderness of tenderloins. The purpose of this study was to assess effectiveness of MORS, BMORS, and IMORS to evaluate shear properties of broiler breast fillets and tenderloins deboned at various times postmortem (PM). In 2 trials, 600 broilers were processed in a commercial processing plant and deboned at 2h, 4h, 6h, or 8h PM. Tenderloins (n = 480) were also collected at the same time using the same debone treatments. Right fillets and tenderloins were cooked to 76C and used for shear evaluation using MORS (0.5 mm razor edge blade), BMORS (0.5 mm blunted blade) and IMORS (1.5 mm blunted blade) on a TA.XTPlus (Texture Technologies Corp.). Data was subjected to ANOVA and correlation coefficients among methods were determined. In most cases for broiler breast fillets, the fillets deboned at 2h had higher ($P < 0.05$) shear values than fillets deboned at 4, 6, or 8 h PM, which were not different ($P > 0.05$). The exception was for IMORS energy where the fillets deboned at 2h were similar ($P > 0.05$) to fillets deboned 4 and 6h pm but higher ($P < 0.05$) than 8h. Overall, using blunted versions of MORS resulted in higher force/energy values (IMORS > BMORS > MORS). Force and energy were highly correlated ($r = 0.92$) for all methods. MORS energy was highly correlated to both BMORS energy ($r = 0.71$) and IMORS energy ($r = 0.75$) while force measurements were slightly less correlated (MORS force to BMORS force, $r = 0.63$, and to IMORS force, $r = 0.66$). BMORS force and energy were highly correlated to IMORS force and energy ($r = 0.71$ and $r = 0.78$, respectively). For tenderloins, MORS force and energy decreased ($P < 0.05$) as deboning time increased with each debone period. Data from this study suggest that all versions of the MORS method are adequate for assessing poultry meat tenderness and that tenderloin tenderness (via shear) is impacted by deboning time. However, future studies should assess the effectiveness of these methods in distinguishing differences of meat that is considered tough and to assess the relationship of the IMORS to sensory attributes.

Key Words: tenderness, breast, tenderloins, MORS, shear

20 Effect of trans-cinnamaldehyde in scalding water on broiler drumsticks contaminated with Salmonella Heidelberg. Claire Peichel*¹, Divek V. T. Nair¹, Shijinaraj Manjankattil², Annie Donoghue³, and Anup Kollanoor Johny¹, ¹University of Minnesota, Minneapolis, Minnesota, United States, ²University of Minnesota, Saint Paul, Minnesota, United States, ³USDA, Fayetteville, Arkansas, United States.

Salmonella is a major foodborne pathogen that causes gastrointestinal illness in humans worldwide. Contaminated poultry products play a significant role in the transmission of the pathogen to humans. This study determined the antibacterial effect of trans-cinnamaldehyde (TC) against *Salmonella* Heidelberg (SH) on broiler drumsticks during the scalding step of poultry processing. Drumsticks were used as a model to simulate carcass skin and meat. Drumsticks were inoculated with either low (~3.0 log₁₀ cfu/g) or high (~4.5 log₁₀ cfu/g) concentrations of SH and were immersed in treatment water containing 0.5% TC, 1% TC, 0.05% peracetic acid (PAA), 0.5% TC + 0.05% PAA or 1% TC + 0.05% PAA at 54oC for 2 min (USDA-recommended time-temperature combination for scalding). Drumsticks inoculated with SH and without any antimicrobial treatment served as the positive control (PC). After

scalding, the drumsticks were homogenized in 350 mL Phosphate Buffered Saline for 30s to obtain surviving SH populations. Populations of SH on drumsticks and in the scalding water were enumerated by surface plating and enrichment methods. We also determined the efficacy of the scalding treatments against SH survival on drumsticks during chilled storage. For this study, the drumsticks were inoculated with 4.5 log₁₀ cfu/g SH and immersed in scalding water containing 1% TC, 0.05% PAA or 1% TC + 0.05% PAA. After scalding, the drumsticks were immersed in chilling water without any antimicrobials for 30 min, packed and stored at 4°C for 48 h. Surviving SH populations were determined immediately after chilling and after 48h of storage. Finally, the effect of the treatments on the surface color of drumsticks was evaluated. All experiments were repeated 6 times and data were analyzed using the PROC-MIXED procedure of SAS. Results revealed that PAA and its combination with TC (0.5 or 1%) resulted in the significant reduction of SH at low (1.7 to 2.4 log₁₀ cfu/g reduction) and high inoculum (2.1 to 3.1 log₁₀ cfu/g reduction) levels of SH ($P < 0.05$). The same treatments inactivated SH to non-detectable levels from the scalding water whereas 2.0- (low inoculum study) and 4.0- (high inoculum study) log₁₀ cfu/ml SH survived in PC ($P < 0.05$). The scalding treatments were also effective in inhibiting SH on the drumsticks compared with the respective controls during chilled storage. The treatments did not affect the surface color of the drumsticks ($P > 0.05$). The study reveals that TC could be an alternative antimicrobial for SH control in poultry processing. Studies exploring the antimicrobial effect of TC on SH-contaminated carcasses in the presence of organic matter are underway (MAES #MIN-16-120; USDA NNF #2016-38420-25285).

Key Words: processing, salmonella, drumsticks, trans-cinnamaldehyde, organic

21 Shelf life of eggs coated with whey protein. Carolina Haubert Franceschi*, Paula Gabriela Pires, Brenda Santaiana Prato, Aline Fernanda Rodrigues Leuven, Katia Maria Cardinal, and Ines Andretta, *Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.*

Whey protein has been studied as an edible coatings for different foods. These coatings act as barriers against moisture and oxygen to prevent rapid deterioration and increase the product shelf life. In the current study, the effect of using whey protein coatings on the quality of eggs was evaluated during 6 weeks. One hundred and 68 non-fertile eggs from an Isa Brown laying line were used after washing under tap water. Egg quality was assessed in uncoated eggs (control treatment) and eggs coated once or twice with a solution containing 5% whey protein. The coated eggs were immerse manually in the whey protein solution. Then, the eggs were stored at room temperature (20°C) for 6 weeks. This higher temperature was used to simulate the reality of egg production in Brazil. Twelve eggs of each treatment were taken at day zero to represent a fresh egg and posteriorly taken in weekly intervals for determination of weight loss. Egg quality variables (Haugh unit - HU, pH, and yolk index - YI) were analyzed of 12 eggs at day zero and at the end of the experimental period. Statistical analysis was performed using the SAS PROC GLM method ($P < 0.05$). Results indicated that whey protein coatings (single or double application) were able to reduce ($P < 0.001$) the loss of egg mass in comparison to uncoated eggs at the 6th week of storage (7.44, 4.35, and 4.28%, and, respectively for uncoated or eggs coated once or twice). The use of the coatings preserved the internal quality of the eggs for up to 6 weeks longer than uncoated eggs. The HU and YI were higher in coated eggs ($P < 0.001$). Uncoated eggs had

the worst ($P < 0.001$) HU (54.02), albumen pH (9.25), and YI (0.27) after 6 weeks of storage, compared with coated eggs. Additionally, the double application (HU: 62.12, pH: 8.91, YI: 0.31) did not improve the protection for egg quality maintenance compared with the single application (HU: 63.25, pH: 8.89, YI: 0.30) as both treatments showed similar results. In conclusion, when no refrigeration is used, the use of coatings based on whey protein influences the internal quality of eggs during storage and may be a promising technology to extend the eggs shelf life.

Key Words: eggshells, Haugh unit, protein coatings, storage, yolk index

22 Automated deboning trajectory models for handling natural variability in processing. Louise Zhuang*, Stephanie Richter, Konrad Ahlin, and Wayne Daley, *Georgia Tech Research Institute, Atlanta, Georgia, United States.*

Automated systems to handle natural morphological variability during poultry processing have been difficult to engineer. This has led to the prevalence of manual operations; however, market needs are beginning to outpace available labor. These problems can be alleviated with improved automation in second and further processing, especially when supplemented with a system to streamline design. The objective of this work was to create a deterministic approach to generate deboning trajectories for chickens based on their external morphologies that could be tested and evaluated through actual implementation. To illustrate and test the concept, a wing cut trajectory model was developed due to its importance in determining overall deboning efficiency. The development pipeline consisted of CT scan acquisition and analysis, virtual reality (VR) trajectory determination and evaluation, and robotic implementation. The VR environment was first populated with biologically accurate chicken models created from CT scans. Using 28 training bird models of varying sizes, 2 key control trajectory points for a wing deboning cut were placed on both sides of each chicken: the tip of the scapula (ST) and the estimated center of the scapulohumeral ligament (SH) and teres minor (TM) supporting the shoulder joint. The trajectory model was created using a least-squares fit between the reference points and trained control points to create a trajectory from ST to SH and TM. The trajectories were then visually simulated in the VR environment. To analyze the accuracy of the simulated trajectory models, the calculated control points for 10 test birds were compared with the desired trajectory point locations. The mean distance between the desired and calculated ST point was 3.0 mm with a standard deviation of 1.1 mm, and the mean distance for the SH and TM point was 2.8 mm with a standard deviation of 0.9 mm. The real-world performance was also tested by sending the model data to a robotic arm for use on real chicken carcasses. The results appeared to be within the errors cited earlier, but more testing is needed to fully characterize practical performance. Likewise, trajectory models created based on size also appeared to improve prediction accuracy. The capability of the trajectory model to determine accurate cut locations both in simulation and in real life indicates that this method of developing deboning trajectories is viable and can be used to enhance the flexibility of automated deboning to account for natural variation and to increase meat yield. In the future, a full implementation of this system on more trajectory types can be tested using sensor-equipped robots and utilized to advance the automation of poultry processing.

Key Words: robotics, deboning trajectories, automated processing, virtual reality (VR)

Student Competition: Animal Well-Being and Behavior

23 A unique form of environmental enrichment motivated broiler physical activity and maintained tibia quality. Meaghan Meyer*, Anna Johnson, and Elizabeth Bobeck, *Iowa State University, Ames, Iowa, United States.*

Today's commercial broiler chicken is a highly feed efficient, fast-growing genetic hybrid that is afflicted by leg lameness compounded by a large proportion of time spent inactive. Adequate biologically relevant environmental enrichment that stimulates natural behavior is lacking in commercial broiler barns. Therefore, a novel laser enrichment device was designed to project 2 randomly moving red dots across the floor of 2 adjoining pens with the objective to motivate broiler physical activity and ultimately improve performance by driving birds toward the feeder. Twelve-hundred Ross 308 broilers were housed in pens of 30 for a 6-wk grow-out where half of the birds, considered laser enriched birds, received enrichment treatment daily (4 "laser periods"/day at 05:30, 11:30, 17:30, and 23:30) and the other half served as controls. Seven pens from each treatment ($n = 14$) were video recorded d0–8 and once weekly wk 2–6. Five randomly selected focal birds/camera pen ($n = 70$) were marked with animal-safe food coloring on d0 and observed for welfare and behavior measures. Videos categorized broiler behavior in the home pen (%), latency to feed (s), and walking distance (cm). On d42, the 70 focal birds were euthanized for tibia quality measures. Data were tested for normality and analyzed using a one-way ANOVA (Proc Mixed or Proc Glimmix, SAS Version 9.4). Laser enriched birds showed less inactivity on d2 (peak of 45.32% decrease), 3, 4, 5, 6, and 8, and on wk 1 and 5 ($P \leq 0.05$). Focal birds in enriched pens spent more time at the feeder on d0, 1, 2, 5 (peak of 10.21% increase), and 8, and on wk 1 and 5 ($P \leq 0.05$). In the first 9 d, 70.89% of laser enriched birds went to the feeder at least once during laser periods or in < 5 min following laser turn off. Over wk 1–6, 60.54% of birds visited the feeder at least once during or within 5 min following laser periods. Distance walked during laser periods was increased in enriched pens on d0–8, with a 646.49 cm increase on d1, and on wk 2–5, with a 367.54 cm increase on wk 2 ($P \leq 0.05$). There were no differences in tibia quality measures due to enrichment treatment ($P > 0.05$). In summary, the novel device tested for the first time in this study increased physical activity, walking distance, and time at the feeder with no negative impacts on tibia quality.

Key Words: broiler, environmental enrichment, welfare, behavior, bone quality

24 Evaluation of three platform enrichments in a commercial broiler house. Rodrigo Lopez*¹, Shawna Weimer², and Katy Tarrant¹, ¹*Fresno State, Fresno, California, United States*, ²*University of Maryland, College Park, Maryland, United States.*

Consumer demand regarding standards of care, welfare, and production methods are increasingly important for livestock producers. To meet consumer demands, the US is on a path to increase levels of welfare. Structures added to the environment act as welfare enrichments, but establishing scientific understanding of their impact is critical. This study aims to evaluate platform parameters as they relate to frequency of use as a guide for selecting platform designs in a conventional broiler house. Three replicates of wooden platform prototypes were examined with the following designs: 1) slatted ramps with a vertical angle of 10, 20,

and 30° (AG), 2) 0.91 m x 0.41 m rectangles raised to a height of 5 cm, 10 cm, 15 cm, and 20 cm (RA), and 3) 1.52 m x 1.52 m pyramids with 1, 2, and 3 levels of steps raised 5 cm per step (PY). Platforms were placed alternately placed centrally in the house equal distances from the water and feed line. Platform usage was recorded with cameras positioned above the platforms in a commercial broiler house. Conventional broilers had unrestricted access to the platforms. Platform usage was recorded daily as snapshots intervals at 5 time points d7–22: 3:30 AM, 9:30 AM, 1:30 PM, 5:30 PM, 9:30 PM. Birds were considered to be using the platform if in contact with the platform. The platform usage frequency was calculated as (number of birds using the platform)/(the maximum number of birds that could occupy the platform based the surface area). Frequencies were evaluated as the percentage of usage based on the surface area of the platform. Categories were designated as None (0%), Low ($> 33\%$), Medium (33–66%), and High ($< 67\%$) usage based on the available space. Usage among platform types were analyzed across days using JMPv.11 using a Pearson chi-squared test. Significance was evaluated at $P \leq 0.05$. There were no differences in usage between the PY platform designs at any of the time points evaluated. With the exception of 4 d (d7, d8, d19, d20), there was a difference in the usage of the AG platforms. The largest angle at 30° had a lower frequency of usage compared the smaller angles ($P < 0.05$). The 20 cm RA platforms had a larger frequency of None and Low uses on d8 compared with all other sized RA platforms ($P < 0.001$). On d18, d21, and d22, the 20 cm RA platforms had more High and Medium uses than other RA platform designs ($P < 0.05$). Interestingly, when adjusted for available surface area, usage of the PY platforms was the lowest compared with all other platform types. Data show that the platforms with smaller surface area and lower ramp angles were preferred in conventional birds from d7–22. Considering these design variables are critical when developing enrichments for conventional broiler facilities.

Key Words: broiler, platform, enrichment

25 Evaluating the effects of stocking density on welfare and performance of broilers. Chandler Keck*, Chelsea Phillips, and Chris Ashwell, *North Carolina State University, Raleigh, North Carolina, United States.*

As consumer emphasis on animal welfare increases, so has the attention and research on welfare parameters. One parameter of interest is stocking density. At the 2018 average market weight of 2.84 kg, the National Chicken Council advocates a maximum stocking density of 41.5 kg/m². The European Commission, however, requires special notification, documentation and additional regulations to use stocking densities higher than 33 kg/m². This difference begs the question, are there welfare or performance implications across this range? The objective of this study was to evaluate the effects of stocking density on welfare and performance of broilers at final target densities at 42 d of 24.4 kg/m²(A), 29.3 kg/m²(B), 34.2 kg/m²(C), and 39.1 kg/m²(D). For this study, 1,800 Ross 708 eggs were obtained and incubated at industry standards. At hatch, chicks were sexed (only males used), tagged, vaccinated for Marek's, Newcastle, and Infectious bronchitis, and randomly assigned to treatments (trt). To mimic industry conditions, used-litter, industry standard lighting, temperature and 3 phase feeding programs were used.

A randomized block design was used with 20 broilers per pen and 7 pens per treatment with varying square footage, but identical water and feed access. Blood collection (Corticosterone), welfare assessments (foot pad and gait scores), and pen group weights were performed weekly. Any mortality was replaced with extra birds to maintain target density but extra birds were never sampled. Three birds per pen were injected with sheep red blood cells (SRBCs) at 27d and blood was collected at day of injection, 7, and 19d. At 42d, body weight, breast weight, muscle, liver, and spleen samples were taken from 3 birds per pen for molecular analyses. Litter samples were taken pre and post-trial. All data was analyzed using one-way ANOVA of JMP 13 and p -value < 0.05 as the threshold. There was a significant difference in body weight from wk 2–5 with trt C having the highest weight. A significant difference in footpad scores throughout the trial was observed with trt D having the highest (worst) score. Gait scores also had a significant difference at wk 3 with trt D having the highest (worst) score. Differences in the litter were noted with trt C having the highest moisture content. At 7d post injections, there was a significant difference in antibody response to SRBCs with trt A having the highest response. There were no differences found in breast weight, breast yield, feed conversion ratio, mortality, or blood corticosterone levels. Based on these results, the variation of stocking density within 24–39kg/m² does not affect production but has effects on welfare parameters such as pad score and immune response.

Key Words: stocking density, welfare, broilers

26 Effects of a perforated floor system in the area of food and water supply on animal welfare and production performance in broiler housing systems. Carolin Adler*, Sonja Hillemacher, Wolfgang Büscher, and Inga Tiemann, *Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany.*

Alternative floors in broiler production systems have been subject of intensive research. Furthermore, effects of housing systems on animal-based welfare parameters are gaining public interest. Animal-based parameters can be divided into health status and animal behavior. Little experimental information exists about the effects of perforated plastic floors on animal behavior compared with conventional litter housing. Our study investigates the effect of perforated vs. littered floors on the animal's behavior and performance of fast-growing Ross 308 broilers. Conventional litter was used in both groups, but litter under the food and water supply chain was replaced by a perforated plastic floor system. This system reduces the animal's contact to at least half of the excrement-litter-mixture and offers an elevated area at the same time. The study was performed with 500 broilers per group ($n = 2$) until 32 d of age. Beside the floor system, conditions were identical (case-control study). Animal welfare was measured weekly using the avoidance distance test (ADT) and the novel object test (NOT) of the Welfare Quality® Assessment protocol for poultry (2009). Production performance data were also collected weekly, focused on the animals' weight. An univariate analyses of variance (ANOVA) was used for statistical evaluation. Results show that production performance was not affected by the floor system ($F(1) = 0.034$, $P = 0.854$). Based on further analyses, ADT scores of the 2 flocks were significantly different ($F(1) = 7.79$, $P = 0.006$) indicated by more animals near the experimenter in the perforated floor group. Also the results for the NOT showed significant differences between the 2 floor systems ($F(1) = 10.77$, $P = 0.001$) with more animals investigating the object in the perforated floor group. Our findings indicate an improvement in the animal-human-relationship as well as a reduction of neophobia. Our results implicate that animals had less stress and fear when kept on perforated floors. Compared with the full litter house, the perforated floor positively influenced animal welfare.

This effect is assumed to be due to the enrichment of the husbandry concept which results in an increased opportunity to perform natural behavior in different functional areas.

Key Words: broiler production, animal welfare, animal behavior, flooring type, perforated floor

27 Evaluation of on-farm euthanasia of broiler breeders utilizing captive bolt, electrocution, mechanical cervical dislocation, and carbon dioxide. Ranjit Boyal*¹, Leonie Jacobs², Richard Jeff Buhr³, Caitlin Harris⁴, Nicole Bartenfeld Josselson³, Sam Cowles⁵, Ileana Berganza¹, and Dianna Bourassa¹, ¹*Auburn University, Auburn, Alabama, United States*, ²*Virginia Tech, Blacksburg, Virginia, United States*, ³*USDA-ARS-USNPRC, Athens, Georgia, United States*, ⁴*University of Georgia, Athens, Georgia, United States*, ⁵*Aviagen, Albertville, Alabama, United States.*

Using manual cervical dislocation to euthanize broiler breeders can be challenging due to their size and the strength required by the operator, which can impact animal welfare. The objective was to investigate the efficacy of euthanasia with alternative methods of carbon dioxide (CO₂), Turkey Euthanasia Device (TED), Koechner Euthanizing Device (KED), and electrocution in achieving rapid unconsciousness and death in broiler breeders. Euthanasia methods were applied to both males and female broiler breeders (4.4, 3.9 kg). A subset of birds ($n = 17$) were equipped with cutaneous electrodes to monitor heart rate (electrocardiogram). To improve safety and ease of use for a single operator, all methods were performed with birds placed in a mobile bird euthanasia apparatus (MBEA). For CO₂ euthanasia the head was enclosed in a small portable device for 4 min ($n = 64$). TED was applied at the base of the comb ($n = 69$). KED was placed at the base of the skull, perpendicular to the neck, after which the handles were pressed together ($n = 59$). Electrocution was applied at 110 V AC for 15 s to induce cardiac fibrillation ($n = 70$). Effectiveness was assessed based on kill success after one attempt, broken skin (presence/absence), external blood loss (presence/absence), and durations of persisting nictitating membrane reflex, gasping (rhythmic opening/closing), and musculoskeletal movements. Nonparametric data were analyzed using Chi-squared and duration data using the GLM procedure of SAS with means separated by Tukey's HSD test. There were no differences in kill success with TED and electrocution consistently reliable (100%) followed by CO₂ and KED (98, 97%). Broken skin and blood loss were more frequent in TED (93, 97%) than in KED (72, 68%, $P = 0.0032$, $P < 0.0001$), with both TED and KED resulting in more frequent skin damage and blood loss than CO₂ (0, 6%, $P < 0.0001$) and electrocution (0, 3%, $P < 0.0001$). The induced nictitating membrane reflex duration for KED (89 s) was longer than TED, CO₂, and electrocution (1, 0, 0 s, $P < 0.0001$). Gasping durations after KED application persisted significantly longer (158 s) than TED, CO₂, and electrocution (4, 0, 0 s, $P < 0.0001$). KED and TED (210, 204 s) resulted in significantly longer durations of body movement when compared with CO₂ and electrocution (4, 4 s, $P < 0.0001$). Heart rate was depressed (< 180 bpm) within 88 s for all euthanasia methods. Heart beat cessation occurred more quickly for electrocution (0 s) than TED, CO₂, and KED (427 to 659 s, $P < 0.0001$). All methods were successful with CO₂ and electrocution resulting in minimal skin damage and blood loss. Euthanasia by electrocution resulted in the shortest duration of physiological and behavioral responses, followed by CO₂, TED, and KED.

Key Words: broiler breeder, euthanasia, animal welfare, electrocardiogram

28 Comparison of broiler and turkey physiological responses associated with acute heat stress and elevated CO₂ associated with emergency depopulation practices. Dellila Hodgson*¹, Kenneth Anderson¹, Krista Eberle-Krish¹, Michael Martin², Sanjay Shah², Ramon Malheiros¹, Shelly Nolin¹, and Kimberly Livingston¹, ¹North Carolina State University, Raleigh, North Carolina, United States, ²North Carolina Department of Agriculture and Consumer Services, Raleigh, North Carolina, United States.

In the face of an outbreak, poultry producers need depopulation methods that can be executed quickly to end the suffering of diseased poultry and prevent the spread to healthy flocks. APHIS defines depopulation as “the destruction of a large number of animals with as much consideration to the welfare of the animal as practical.” Due to the risk of virus amplification, USDA-APHIS prescribes depopulation of a HPAI presumptive positive flock within 24 h. Current Approved methods for depopulation are CO₂ and water-based foam. If depopulation with the approved methods cannot be achieved within 24 h, alternative methods that can be approved for use may be ventilation shutdown plus (VSD+). However, which depopulation method used is made on a premise-by-premise basis, considering resources, epidemiology, and State/USDA-APHIS concurrence on the method. The stress response of turkeys and broilers was evaluated using 3 different depopulation methods, Ventilation Shut Down (VSD), Ventilation Shut Down + Heat (VSDH), Ventilation Shut Down + CO₂ (VSDCO₂) The initial phase provided the required time of death (TOD) for each species. Blood was collected before and at TOD to determine changes in corticosterone (CORT) levels and at 5 points beginning at time 0 for phase 2. Data was analyzed as a 2-way ANOVA using SAS 9.4. Initial phase determinations indicated that TOD was shortest in both the turkeys and broilers for VSDCO₂ at 23 and 25 min. VSD took the longest for both turkeys and broilers respectively (270 min and 130 min). VSDH had an intermediate TOD at 86 min for turkeys and 75 min for broilers. Despite having similar CORT levels before the method, turkeys had higher CORT levels than the broilers ($P < 0.05$) regardless of method. CORT levels did not differ among the methods for broilers. However, turkeys subjected to VSD had the lowest CORT levels at 1.55 ng/ml whereas VSDH and VSDCO₂ had greater levels at 8.26 and 6.30 ng/ml. In phase 2, CORT was highest in the VSDCO₂ methods for both the turkeys and broilers. However, the turkeys peaked at 18 min while broiler peaked at the end (28 min). VSD had the lowest CORT levels in both the turkeys and broilers throughout the time points. In conclusion, VSDCO₂ results in the highest CORT levels in both turkeys and broilers; however, the TOD is much shorter when compared with VSD or VSDH.

Key Words: corticosterone, depopulation

29 Does wavelength in a photoperiod program impact broiler fear and stress levels? Bruna Maria Remonato Franco*¹, Tory Shynkaruk¹, Bryan Fancher², Nick French², Scott Gillingham², and Karen Schwean-Lardner¹, ¹University of Saskatchewan, Saskatoon, Saskatchewan, Canada, ²Aviagen Group, Huntsville, Alabama, United States.

Light emitting diode (LED) lamps are of interest for broiler production since they are inexpensive to run, have a long life and provide monochromatic colors. The effect of varying wavelengths was examined using 3 light treatments (L) (blue (BL, 455nm), green (GL, 510nm) and white (WL)) on broiler welfare and production; behavior traits will be discussed in this abstract. A total of 14,256 broilers, of 2 sexes (Sx) and 2 strains (Ross EPMx708 and Ross YPMx708; St) were housed in 2 blocked trials, in 9 rooms (3 replicates per lighting treatment per trial). Each independent environmentally controlled room was divided

into 12 pens (2x2.3m), allowing Sx and St to be housed separately. Stocking density was of 31kg/m² (62 males or 70 females/pen). Commercial diets (starter, grower and finisher) were formulated based on Aviagen recommendations. Light was provided at 23L:1D for d1 and decreased 1h daily until it reached 16L:8D by d5 (with dawn and dusk transitions of 15min). Intensity of light on the first week varied between trial blocks 1 and 2 (trial1 – 9.6 clux; trial2 – 14.3 clux) but was maintained equal between rooms/trial; intensity was reduced to 9.6 clux on d8. To assess fear, tonic immobility testing (TI) was performed on d23 (14 birds per lighting treatment x strain x sex, 1 bird per pen) followed by a novel object study at d30 (3 pens per LxStxSx per trial) and a response to observer test on the same day (9 pens per LxStxSx per trial). Stress levels were examined by collecting blood and determining the heterophil to lymphocyte (H:L) ratio at d22 for 9 birds per LxStxSx per trial. Data were analyzed as a 3x2x2 factorial design with lighting treatment nested within room (Proc Mixed - SAS 9.4). Differences were considered significant when $P < 0.05$. TI revealed a lower latency to rise in BL birds ($P = 0.001$) then GL and WL (71.79, 140.11, 150.41 s respectively), indicating reduced fear. A lower percentage ($P = 0.01$) of BL birds moved as a result of an observer's presence compared with birds raised in GL and WL (3.31%, 6.89%, 6.22% respectively) and an interaction was noted between strain and sex, where a higher percentage of Y-708 males moved compared with Y-708 females. The average time birds took to approach a novel object did not differ between light treatments ($P = 0.12$). Birds raised under BL had a lower H:L ratio (0.312) compared with birds raised under WL (0.467; $P = 0.03$). With respect to sex, males had a lower H:L ratio compared with females ($P = 0.001$), and no strain differences were noted. Overall, the results show that raising birds under BL leads to lower stress and a reduction in fear, suggesting that blue lighting in particular play an interesting role on emotional states and welfare in poultry.

Key Words: wavelength, LED, broiler, behaviour, welfare

30 Not Presented

31 Comparison of red, blue, and green monochromatic LED lights to white LED lights on Pekin duck stress, behavior, and growth. Gabrielle House*, Eric Sobotik, Austin Stiewert, Jill Nelson, and Greg Archer, *Texas A&M University, College Station, Texas, United States.*

Ducks and other poultry are known to be sensitive to light spectrum, intensity, flicker, and photoperiod. Limited research has been conducted on how light spectrum affects Pekin duck production and welfare. The objective of this study was to evaluate the effects of 4 monochromatic light spectrums on Pekin duck stress, behavior, and growth. Pekin ducks ($n = 768$) were reared in environments illuminated either by red (RD), blue (BL), green (GR), or white (WH) monochromatic LEDs using a 16L:8D photoperiod. Stress susceptibility was measured using plasma corticosterone (CORT; $n = 160$), heterophil to lymphocyte ratio (HL), and physical asymmetry of 3 bilateral traits (ASYM; $n = 480$). Behavioral fear response was determined using tonic immobility (TI; $n = 480$) and inversion (INV; $n = 480$) tests. Physical and production parameters were measured using body weight, pen feed conversion ratio (FCR; $n = 64$) and gait score (GS; $n = 384$). To determine the effects of light spectrum on eye development, left and right eye dimensions (weight, length, and width; $n = 160$) were measured. Averages and differences between left and right eyes were calculated. Data were analyzed using GLM, and $P < 0.05$ was considered a significant difference. A LSD post hoc test was used for mean separation. Higher CORT levels were

present in RD and BL ducks (8984.90 pg/mL) than in GR and WH ducks (5818.20 pg/mL, $P < 0.01$). HL counts were higher in RD and BL ducks (0.56) compared with GR and WH ducks (0.38, $P < 0.05$). BL ducks (2.55 mm) were more asymmetrical than RD, GR, and WH ducks (0.97 mm, $P < 0.01$). The number of flaps during INV was higher in BL and GR ducks (5.36 flaps) than in WH and RD ducks (4.41 flaps, $P < 0.02$). Duration of INV flapping was longer in BL and GR ducks (1.90 s) than in WH and RD ducks (3.09 s, $P < 0.02$). Eye weight was lower in RD and BL ducks (1.49 g) compared with GR and WH ducks (1.55 g, $P < 0.01$). Left and right eye weight differences were higher in WH and GR ducks (0.07 g) compared with RD and BL ducks (0.05 g, $P < 0.03$). Narrower eyes were present in RD and GR ducks compared with BL and WH (9.44, 9.50, 9.69, and 9.72 g, respectively, $P < 0.02$). No differences were observed in weight, FCR, GS, flapping intensity, or TI ($P > 0.05$). The results of this study indicate that duck stress susceptibility and eye development can be altered by light spectrum, and that blue monochromatic LED light may be detrimental to duck welfare.

Key Words: duck, lighting, behavior, stress, welfare

32 Use of probiotic bacteria to modulate the kynurenine pathway and feather pecking behavior in laying hens. Claire Mindus^{*1}, Nienke van Staaveren¹, Haylee Champagne¹, Paul Forsythe⁴, Johanna M. Gostner², Joergen B. Kjaer³, Wolfgang Kunze⁴, Dietmar Fuchs², and Alexandra Harlander¹, ¹University of Guelph, Guelph, Ontario, Canada, ²Innsbruck Medical University, Innsbruck, Austria, ³Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Celle, Germany, ⁴St. Joseph's Healthcare, Hamilton, Ontario, Canada.

Kynurenine is the most prevalent metabolic pathway, after protein synthesis, of the amino acid tryptophan (TRP). Additionally, TRP is the sole precursor of serotonin (SER), a neurotransmitter involved in mood disorders and cognition. Altered levels of SER also have been linked with feather pecking (FP) behavior when laying hens pluck and pull out feathers of their conspecifics. Additionally, birds that express high levels of FP showed lowered levels of Lactobacillaceae in their gastro-intestinal microbiome compared with birds expressing low levels of FP. Furthermore, research in mammals showed potential for lactic bacteria to modulate the kynurenine metabolism with positive effects on anti-social behaviors. This study examined the impact of a specific Lactobacillus probiotic bacteria supplementation on plasma concentrations of TRP, KYN and on severe feather pecking (SFP) behavior in pullets. Eighty-six white Leghorns (19 weeks of age) were distributed into pens of 7 birds and assigned to a probiotic supplementation (PRO, 6 pens) or control supplementation (CON, 6 pens). During 5 weeks, PRO birds orally received a daily dose of 5×10^9 Lactobacillus bacteria dissolved in 1 mL of water and CON birds received 1 mL of water. Blood samples were collected before and after the supplementation treatment. The pens were video-recorded (5 min/day, 3 d before treatment and 9 d during treatment) and analyzed for SFP by a blinded observer. Generalized linear mixed models were used to assess the effect of the supplementation on concentrations of TRP, KYN and SFP. This experiment was approved by the Animal Care Committee of the University of Guelph (AUP 3206). TRP significantly decreased over time in both treatments, but TRP breakdown to KYN was significantly less pronounced in PRO treatment ($P < 0.05$). There was a higher proportion of birds classified as "severe peckers" in the PRO compared with the CON treatment ($P = 0.05$), likely due to a numerically higher proportion of these birds already observed in the PRO treatment at baseline. However, despite this finding, the amount of SFP bouts and the proportion of birds classified as "severe peckers" did not increase over the 5 weeks of treatment (P

> 0.05) suggesting that PRO treatment might have helped counter the spread of FP. These results indicate that the bacteria could be a useful tool to modulate the kynurenine pathway in laying hens. The decrease of TRP breakdown to KYN in the PRO treatment, which may illustrate an increased TRP availability to the brain for SER production, may explain, at least partly, the countering of the expected spread of SFP within the PRO treatment.

Key Words: chicken, feather pecking, probiotic, bacteria, tryptophan

33 Influence of ramps on keel bone fractures, hen distribution, and welfare in an aviary system. Sarah MacLachlan^{*1}, Ahmed Ali¹, Ariane Stratmann², Michael Toscano², and Janice Siegford¹, ¹Michigan State University, Okemos, Michigan, United States, ²University of Bern, Bern, Switzerland.

Commercial laying hen housing is shifting from traditional cages to non-cage housing styles, such as the aviary. The aviary has gained popularity because it allows for more species-typical behavior in laying hens. While aviaries offer resources to improve welfare, they are also associated with problems such as keel bone fracture (KBF) that may occur when a bird collides with structures in the aviary system. KBF is a sign of poor hen welfare and can affect a hen's productivity. One possible way to reduce KBF is to add ramps into an aviary system, allowing hens to walk between levels rather than jump or fly, to decrease injuries. The objective of this study was to evaluate the impact of the addition of ramps in rearing and laying aviaries on KBF, welfare quality scores, and bird distribution. LSL pullets were raised in 2 treatments; 4 pens (550 birds/pen) were raised with wire mesh ramps to aid movement between aviary tiers (RR) and 4 pens (550 birds/pen) were raised without ramps (RO). At 17 weeks of age, all birds were moved to the laying facility, which contained 16 aviary pens with 220 birds/pen. Half the pens ($n = 8$) were supplemented with wire mesh ramps (LR) and the other half were not (LO). Within each laying treatment group, 4 pens were populated with RR hens and 4 pens were populated with RN hens, creating 4 treatment combinations (RRLR, RRLO, ROLR, ROLO). From each pen, 15 focal hens were selected for radiographic imaging of their keel bones. The same 15 hens had images taken at 21, 36, 45, and 60 WOA and the images were subsequently scored for KBF severity. Focal hens were also scored for feather coverage and footpad quality at 36 and 60 WOA using the Welfare Quality® assessment protocol. Bird distribution was observed at 19–20, 23–24, and 30–31 WOA. Data were analyzed using generalized linear mixed models in R software. Hens who received ramps in lay (RRLR and ROLR) had less KBF than those who did not receive ramps in the laying aviaries (36WOA $P < 0.001$, 45WOA $P < 0.005$, 60WOA $P < 0.001$). At 60 WOA, hens in the RRLR treatment had greater feather coverage than those in ROLR and RRLO treatments ($P = 0.008$). Birds in the RRLR treatment had better foot pad condition overall than those in treatments without ramps in lay (RRLR-RRLO: $P = 0.044$, RRLR-ROLO: $P = 0.013$). Ramps did not have an effect on bird distribution in the aviary. Our results indicate that providing ramps to birds in laying aviaries has an effect on welfare parameters such as footpad condition, feather coverage and KBF but does not influence bird distribution. Future research will examine hens' use of ramps to transition among levels in aviaries as a possible explanation for reduced incidence of KBF.

Key Words: laying hen, aviary, ramps, health, behavior

34 Influence of strain and inter-bird distance on dust bathing in laying hens. Tessa Grebey^{*}, Ahmed Ali, Janice Siegford, and

Mikkena Ludka, *Michigan State University, East Lansing, Michigan, United States.*

The egg industry is implementing aviary systems designed to enhance the welfare of laying hens by providing litter areas that encourage ethologically important behaviors such as dust bathing. Guidelines recommending resource allocations on a per hen basis are intended to allow the expression of these behaviors. However, the dynamic movement of hens and the effect of conspecifics on hens' ability or willingness to perform behaviors are not usually considered. Our objective was to investigate the influence of the number of hens on the litter, number of hens simultaneously dust bathing (DB), and inter-bird distances (IBD) on the space used to DB and duration of DB bouts across 4 strains of laying hens (Hy-Line Brown [HB], Bovans Brown [BB], DeKalb White [DW] and Hy-Line [W36]). Hens were videoed DB on litter from 11:30–14:30 at 28 weeks of age. We analyzed 128 hens (32 hens/strain). Images of focal hens at the start, middle, and end of DB bout were captured and duration of each bout noted. Area of the focal hen and IBD between the focal and nearest surrounding hens were recorded from each image using ImageJ software. We analyzed differences among strains using GLMM and relationships between variables using multi variables regression analysis. On average, brown hens needed more space to DB than white hens (HB 1125.26; BB 1146.51 vs DW 962.65;

W36 943.39 cm²; $P < 0.01$). Generally, more white hens were on the litter at one time than brown hens (43 DW, 41 W36 vs 28 HB, 31 BB; $P < 0.01$), and more white hens DB at once than brown hens (11 DW, 19 W36 vs 4 HB, 4 BB; $P < 0.01$). While DB, brown hens had a larger average IBD than white hens (HB 13.99, BB 15.11 vs DW 8.39, W36 7.85 cm; $P < 0.01$) and larger minimum IBD (HB 6.76, BB 7.35 cm vs DW 1.63, W36 1.79 cm; all $P < 0.01$). Brown hens had shorter DB durations than white hens (HB 7.37, BB 9.00 min vs DW 13.91, W36 15.16 min; $P < 0.01$). In white strains, the space used per hen to DB decreased if either the number of hens on litter increased (DW 0.85; W36 0.79 cm; $P < 0.05$) or the minimum IBD decreased (DW 3.66, W36 2.98 cm; $P < 0.01$). For brown hens, duration of DB bouts decreased if the number of hens on the litter increased (HB 0.87, BB 0.95 min; $P < 0.01$), the number of other hens DB increased (HB 0.75, BB 0.69 min; $P < 0.02$), or if the minimum IBD decreased (HB 2.39, BB 2.31 min; $P < 0.01$). Our results suggest that DW and W36 white hens minimize their DB area in response to smaller IBD and more hens using the litter at once. In the same situation, BB and HB brown hens shorten their DB bouts, potentially terminating bouts before fulfilling their needs. These variations in DB behavior among strains should be considered when planning and stocking laying hen aviaries.

Key Words: dust bathing, aviary, laying hens, welfare, behavior

Student Competition: Genetics and Genomics

35 Evaluation of the broiler pectoralis major and liver transcriptome of mRNA via artificial intelligence in association with the muscle myopathy woody breast. Chelsea Phillips*, Matthew Livingston, Kimberly Livingston, and Chris Ashwell, *North Carolina State University, Raleigh, North Carolina, United States.*

The muscle myopathy wooden breast (WB) has recently appeared in broiler production and has a negative impact on meat quality. WB is described as hard/firm consistency found within the pectoralis major (PM). In the present study, we use machine learning from our PM and liver transcriptome data set to capture the complex relationships that are not typically revealed by traditional statistical methods. Gene expression data was evaluated between the PM and liver of birds with WB and those that were normal using the Waikato Environment for Knowledge Analysis, "WEKA" version 3.8.3. Two separate machine learning algorithms were performed to analyze the data set including the sequential minimal optimization (SMO) of support vector machines (SVMs) and Multilayer Perceptron (MLP) of Artificial Neural Network (ANN). Machine learning algorithms were compared with identify genes within a gene expression data set of approximately 16,000 genes for both liver and PM, which can be correctly classified from birds with or without WB. Two cross validation strategies were used to evaluate the classification performance, a percentage split and a stratified hold-out method. The performance of both machine learning algorithms SMO and MLP was determined using percent correct classification during the cross validations, which indicated the likelihood each individual replicate could accurately be placed into the group with WB or normal based on the gene expression data provided. Kappa statistic and ROC score were recorded. Any kappa statistic greater than 0 indicated that the machine learning classifier is performing better than random chance along with a ROC score of greater than 0.500. By evaluating the WB transcriptome data sets by 5x cross validation using MLP, the expression of 10 genes based on entropy from PM were able to correctly classify if the individual bird was normal or exhibited WB 100% of the time. These top 10 genes were nearly all protein coding and potential biomarkers. When PM gene expression data was evaluated between normal birds and those with WB using the WEKA SMO function of SVMs they were correctly classified 95% of the time using 450 of the top genes sorted based on entropy as a preprocessing step. The kappa statistic and ROC score were optimal at 0.8571 and 0.9, consecutively. When evaluating the 450 attributes that were 95% correctly classified using SMO through Ingenuity Pathway Analysis (IPA) there was an overlap in top molecules identified through MLP. This information has revealed many molecules and pathways making up a complex molecular mechanism involved with the progression of woody breast.

Key Words: woody breast, machine learning, support vector machines, artificial neural network, transcriptome

36 Molecular cloning, tissue distribution and the expression of cystine/glutamate exchanger (xCT, SLC7A11) in different tissues during development in broiler chickens. Janghan Choi*¹, Weiqi Li¹, Brayden Schindell¹, Liju Ni², Shangxi Liu¹, Xiaoya Zhao¹, Joshua Gong³, Martin Nyachoti¹, and Chengbo Yang¹, ¹*University of Manitoba Animal Science, Winnipeg, Manitoba, Canada*, ²*Shanghai Lab-Animal Research Center, Shanghai, China*, ³*Guelph Research and Development Centre, Guelph, Ontario, Canada.*

The cystine/glutamate exchanger (xCT, SLC7A11) is a component of the system X_c⁻ amino-acid antiporter that can export glutamate and import cysteine into cells. The xCT amino acid exchanger has received a lot of attention because cysteine is an essential substrate for the synthesis of glutathione (GSH), an endogenous antioxidant in cells. The objective of this research was to clone the full-length cDNA of chicken xCT and to investigate the gene expression of xCT in different tissues, including intestinal segments of broiler chickens during development. The full-length cDNA of chicken xCT (2703 bp) was obtained from the jejunum by reverse transcription-polymerase chain reaction (RT-PCR) and then sequenced. Homology test showed that chicken xCT has 80.4%, 80.2% and 71.2% homology at the nucleotide level with human, cattle and rat, respectively. Likewise, amino acid sequence analysis showed that chicken xCT protein is 86.4%, 79.3% and 75.6% homologous with human, cattle and rat, respectively. Phylogenetic analysis indicated that chicken xCT gene shares closer genetic relationships with human and cattle than with rat. The chicken xCT protein has 12 transmembrane helices, 6 extracellular loops and 5 intracellular loops. The mRNA of xCT was detected in all tissues including intestinal segments. In terms of intestinal segments, the mRNA expression of xCT was significantly higher ($P < 0.05$) in the colon than that in the jejunum and ileum. A linear pattern of changes of the xCT mRNA abundance in the duodenum ($P < 0.05$) was found during development. There were linear and cubic patterns of changes ($P < 0.05$) of the xCT mRNA abundance in the colon during development. These results indicate that xCT is widely expressed both in the intestinal segments and other organs which are not associated with nutrient absorption. Further investigation is needed to characterize the functional relevance of xCT activity in oxidative stress and inflammation in the small intestine of broiler chickens.

Key Words: amino acid, broiler chickens, cystine/glutamate transporter (SLC7A11), development, gene expression

37 The joint contributions of dominant and maternal effects on negative heterosis in early growth of chickens. Chunning Mai*, Congjiao Sun, Chaoliang Wen, Zhiyuan Xu, Sirui Chen, and Ning Yang, *China Agricultural University, Beijing, China.*

Heterosis or more narrowly hybrid vigor is an important genetic phenomenon and has been widely utilized in animal and plant breeding. However, the negative heterosis has also been observed in plants and animals. Compared with the systematic studies of positive heterosis, the phenomenon of negative heterosis is largely ignored in the genetic studies and breeding programs, and the genetic mechanism of this phenomenon is still poorly understood. Here, we performed a reciprocal crossing experiment with 2 distinct chicken, Cornish and Rhode Island White, to generate purebred progenies and reciprocal crosses. The body weights of each chickens (n = 632) were measured weekly from hatch to 8 weeks of age. At the end of 6 weeks of age, we randomly slaughtered 64 females and 44 males from the 4 groups, and measured their carcass traits. Meanwhile, we conducted RNA-sequencing on breast muscle of reciprocal hybrids and parental lines to determine the underlying mechanism of negative heterosis. Our results showed that the negative heterosis of body weight and carcass traits was more common than the positive heterosis in reciprocal crosses at the early growth stage. Among the carcass traits, muscle mass exhibited the largest negative heterosis. A large number of differentially expression genes (DEGs) were identified between reciprocal crosses and parental lines. We classified these DEGs into 3 categories: additivity, dominance and overdominance.

Dominance was the major gene expression mode in reciprocal crosses, and the dominant genes were significantly associated with the biological process of oxidative phosphorylation. Then, we detected the ATP content and ATPase activity of breast muscle for 4 groups, and found that birds with lower breast muscle yield had the lower ATP content, but higher ATPase activity. This result validated the important role of oxidative phosphorylation in negative heterosis. In addition, allele-specific expression analyses revealed that maternally biased genes in reciprocal crosses were significantly enriched in GO terms relating to ubiquitination and de-ubiquitination, which participated in the degradation of proteins in an ATP-dependent manner. Muscle is the largest portion in the body weight, and protein accounts for a large proportion of the breast muscle. Thus, these findings revealed that dominant and maternal effects contribute collectively to the negative heterosis of growth traits in early growth of chickens. The combined analyses for genome-wide gene expression models and allele-specific expression is an efficient strategy that could be helpful for the following studies on genetic and molecular mechanism of heterosis in other species.

Key Words: negative heterosis, dominant effects, maternal effects, gene expression, chicken

38 Preliminary results from genome wide association study of wooden breast in commercial broilers. Juniper Lake*^{1,2}, Jack Dekkers², Sandra Velleman³, Erin Brannick¹, and Behnam Abasht^{1,2}, ¹University of Delaware, Newark, Delaware, United States, ²Iowa State University, Ames, Iowa, United States, ³The Ohio State University, Wooster, Ohio, United States.

Wooden breast is a myopathy of commercial broiler chickens involving venous inflammation (phlebitis), lipid accumulation, oxidative stress, myofiber degeneration, regeneration, and fibrillar collagen deposition primarily in the cranial part of the pectoralis major. Affected birds present clinically with palpably firm breast muscle, reduced wing mobility, and impaired gait. Although the etiology of the disease is still poorly understood, the myopathy disproportionately affects birds with rapid growth, high feed efficiency, and large breast muscle yield. The aim of this study was to identify DNA variants associated with wooden breast and white striping scores in approximately 1200 Cobb500 broilers raised in a commercial setting to 7 weeks of age as 2 hatches from the same breeding population. Genotyping by sequencing yielded 561,020 single nucleotide polymorphisms with mean call rate of 0.895, mean co-call rate of 0.820, and mean sample depth of 7.51 at detected variants. Relatedness matrices were constructed from SNP data and used to estimate heritability. Additional experiments were conducted on sub-groups of birds to study the relationship between wooden breast and live performance, carcass characteristics, blood parameters, and blood metabolite profiles. Blood analysis performed with the i-STAT hand-held blood analyzer on samples taken from the brachial vein of 103 live birds revealed significant differences in blood gases between affected and unaffected chickens, with affected chickens exhibiting higher partial pressure of CO₂, total CO₂, bicarbonate, and base excess, and lower partial pressure of O₂, oxygen saturation, and pH. Affected chickens in this same group of 103 birds also possessed a significantly larger pectoralis major muscle and whole left wing relative to body weight. Hypercapnia and hypoxemia in affected chickens suggest greater metabolic demand and insufficient gas exchange. Preliminary data analysis with regard to the genome-wide association study and metabolomic profiling is ongoing.

Key Words: wooden breast, white striping, GWAS, meat quality, pectoralis major

39 High impact genetic changes associated with rapid growth rate and differential gene expression in commercial broilers.

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Determining genotypes associated with muscle disorders found commonly in fast-growth broilers is a priority for selection regimens in the poultry industry. In this study, we identified genes, gene expression patterns, and underlying SNPs likely to have a large impact on growth rate. We compared genotypes and phenotypes between fast growth and slow growth breeds at 2 time points (d 11 and d 42). We sampled 30 birds per time point and breed, resulting in a total sample size of 120 birds. For each individual bird, we collected breast tissue and extracted Total RNA. We prepped libraries for RNA-seq using the Lexogen Quant Seq 3' RNA-seq kit. 72 libraries were externally sequenced on an Illumina NextSeq. We performed quality assurance and filtering on raw sequences used in statistical analyses. We obtained counts of the reads mapped with Htseq-count, and used EdgeR to identify differentially expressed genes (FDR-adjusted $P < 0.05$) using TMM normalization. Following identification of genes displaying differential expression, we filtered genes based on growth rate. Finally, we used Ensembl's Variant Effect Predictor to characterize mutations and identify high impact SNPs impacting these differentially expressed genes. Overall, we found 26 genes implicated matching our analysis criteria, on differential gene expression and high-impact mutations (FDR-adjusted $P < 0.05$). These genes code for proteins necessary for musculoskeletal structure formation, oxidative stress response, and immune function. Within this gene list, we identified 1 high impact SNP in the MHC gene "ENS-GALG0000004594," shared among all fast-growth individuals. This transition mutation alters the stop codon, and likely impacts final protein structure. Slow growth and fast growth breeds show unique sets of differentially expressed genes (DEG) between d 11 and d 42. Our study showed that a handful of these DEG was also harboring high impact variants. This analysis highlights the importance of cis-regulated DNA variants in driving growth rate and associated conditions in broilers.

Key Words: growth rate, genomics, mutation, quantitative trait, SNPs

40 Not Presented

41 Assessing the effects of gut microbiota and host genetics on feed efficiency in chickens. Chaoliang Wen*¹, Congjiao Sun¹, Wei Yan¹, Jiangxia Zheng¹, Congliang Ji², Dexiang Zhang², and Ning Yang¹, ¹China Agricultural University, Beijing, China, ²Guangdong Wen's Nanfang Poultry Breeding Co. Ltd., Xinxing, China.

Feed accounts for the largest portion of the overall costs in the poultry production. Improving feed efficiency (FE) is an extremely important goal in the modern broiler industry. While artificial selection on FE over the recent decades has resulted in dramatic improvements in chickens, FE may also be improved through the regulation of gut microbiota, as the microbiota plays an important role in animal health and performance. The extent to which gut microbiota influence FE has yet to be determined. Therefore, the objective of this study was to estimate the effects between the gut microbiota and host genetics and on FE in broilers using extended quantitative genetic methods. A total of 206 males from one hatch were reared in a half-open poultry facility with free access to water and corn-soybean-based diets. The individual body weight and feed intake were continuously recorded with the automated electronic feeder during the fast-growing period from 56 to 76 d of age. The feed residual intake (RFI) was estimated for each broiler based on linear regression. At 78 d

of age, all chickens were slaughtered, the luminal content and mucosal surfaces of each gut segment (including the duodenum, jejunum, ileum and cecum) were collected, and droppings were gathered from the cloaca. We performed whole-genome sequencing of the hosts and 16S rRNA gene sequencing of the microbiota. The SNP and microbiota data were then used to systematically evaluate the contribution of the host genetics and diverse gut microbial community to RFI using a linear mixed model. Our results showed that the variation of RFI explained by the host genetics was 0.32, indicating that the host genetics have considerable effects on FE. In analogy to the heritability, microbiability (m^2) could quantify the effect of gut microbial abundance on host phenotypes. The m^2 of RFI estimated for the cecal (0.29) and duodenal (0.12) microbiota were higher than that estimated for other anatomical sites (0.01 for the jejunum, 0 for the ileum and 0.09 for the feces). As the function of the duodenum is nutrient digestion and absorption, and the cecum is an important site for the microbial fermentation of undigested nutrients, it is likely that the microbiota in these sections may influence the nutrient utilization efficiency. Our results demonstrated that both host genetics and gut microbiota contribute to RFI. We further compared microbiota between high-RFI and low-RFI individuals by Wilcoxon rank-sum test and identified several microorganisms whose abundances were significantly associated with RFI. Therefore, we could develop approaches for altering the gut microbiota to improve feed efficiency in chickens apart from selective breeding.

Key Words: host genetics, gut microbiota, residual feed intake, microbiability, chicken

42 Evaluation of the suitability of cloacal swabs to characterize gut microbiota in chicken. Travis Williams* and Giri Athrey, *Texas A&M University, Bryan, Texas, United States.*

Background: Alternative methods like the use of fecal sampling have been investigated in multiple species, including poultry, however recent research demonstrates qualitative similarities with quantitative differences. We hypothesized that cloacal swabs, a non-lethal sampling method, are a suitable method to characterize the cecal, large intestine, and cloacal microbiota profiles. To test our hypothesis, 20 broilers

were opportunistically sampled from an ongoing experiment. Sampling included the collection of a cloacal swab followed by the euthanasia of the animal (AUP IACUC 2016–0064) and the collection of the large intestine (with content) and cecal content from each individual. Methods: The large intestine was split longitudinally, exposing the lumen contents and the epithelial mucosal layer. The combination of lumen content and mucosal scrapings were collected to provide a broad scale sampling of microbiota in the large intestine. Cloacal swab material was suspended into an extraction solution containing TBS, EDTA, and Tween-20. All resultant samples were then subjected to DNA extraction using the DNazol reagent followed by a sodium acetate purification. Purified samples were then amplified for 16srRNA-V4 hypervariable region. PCR products were sequenced on an Illumina MiSeq platform with sequencing parameters of 2x250bp paired-end reads per sample by the Genome Sequencing and Analysis Facility in Austin, TX. Resultant data were analyzed using the MOTHUR pipeline and multiple packages in the R statistical platform. We used both weighted UNIFRAC and Permanova approaches to test for β diversity differences between pairs of sampling methods (i.e., cecal content to cloacal swab). Results: Of the 20 originally sampled and sequenced birds, 9 complete trios of cecal content, large intestine, and cloacal swab passed the sample quality parameters. Tests of β diversity with weighted UNIFRAC showed that 3 sample groups were significantly different (P-values < 0.001) for each comparison. α diversity was assessed using the Inverse Simpson to estimate richness of the sampling site. A Wilcoxon Rank Sum test was performed on this estimator to test if the distributions differ by a shift of 0, with all comparisons yielding nonsignificant P-values > 0.05. In summary, through 16srRNA analysis of a trio of samples taken from the lower GI tract we observed a distinction of cecal content. These results show α diversity and richness of microbiota within samples (birds) were not different (as expected), but when considering diversity and abundance (β diversity), the 3 groups were significantly different. Our results show that cloacal swabs are not a suitable substitute to measure the β diversity of GI tract microbiota.

Key Words: microbiota, bioinformatics, gut health, microbiome, microbiota diversity

Student Competition: Physiology and Reproduction

43 Effect of reduced incubational temperature on broiler posthatch body temperature and growth. Lauren Lindsey^{*1}, Katie Elliott¹, Seyed Fatemi¹, Peter Ishola¹, Patrick Gerard², and Edgar Peebles¹, ¹Mississippi State University, Mississippi State, Mississippi, United States, ²Clemson University, Clemson, South Carolina, United States.

Modern strain broiler hatching eggs, experiencing increased embryonic metabolic rates, may be incubated at higher than optimal temperatures. This experiment was conducted to compare the effects of incubational temperature treatment on posthatch chick body temperature and growth. From 0 to 12 d of incubation (doi), Ross 708 broiler hatching eggs were incubated under standard conditions in a common incubator. On 12 doi, 2 replicate incubators were set at either 37.5 (standard) or 35.6°C (reduced) temperature treatments. All 30 eggs in each of the 4 incubators received air cell transponder implants and remained at the 12 doi set temperatures until all viable embryos hatched. At hatch, air cell transponders were transferred to their corresponding hatchling by s.c. insertion in the dorsal region of the neck. Between 21.0 and 22.5 doi, approximately 3 male and 3 female chicks from each of the 2 replicate incubators in each incubation temperature treatment group were placed in each of 6 replicate pens (blocks) in an RCB design to test for the main and interactive effects of posthatch age, treatment, and sex. Transponder and litter temperature readings were taken daily at 7 a.m., 12 p.m., and 5 p.m. with a wireless probe and infrared thermometer, respectively. Posthatch data collection began at 22.5 doi, and measurements taken on the same bird over time were accounted for in the data analysis. Body weight and length measurements were taken weekly until 3 wk of age (woa), and sex was confirmed by necropsy at 3 woa. Embryo mortality was not affected by incubation temperature treatment. Across the 3 woa posthatch period, mean chick body temperature was 0.11°C higher in the standard than in the reduced incubation treatment group ($P = 0.0053$), with posthatch age interacting with treatment ($P = 0.0009$). Chick BW (posthatch age *treatment* sex, $P = 0.0172$) was greater in the standard treatment group at 2 and 3 woa for males, but only at 3 woa for females. Chick body length (BL) and BW to length ratio (BWTLR) were greater in males than females (BL, $P = 0.0037$; BWTLR, $P < 0.0001$) and greater in those incubated at the standard temperature (BL, $P = 0.0007$; BWTLR, $P < 0.05$). In conclusion, in conjunction with a significant elevation in chick body temperature through 3 wk posthatch, the standard incubational temperature resulted in improved posthatch broiler growth in comparison to the reduced incubational temperature.

Key Words: body temperature, broiler, incubation temperature, performance, transponder

44 Impact of *in ovo* injected *Lactobacillus* species on hatchability, growth performance and broiler immune status. Claudia Castañeda^{*}, Julio Miotto, Li Zhang, Kelley Wamsley, Chris McDaniel, and Aaron Kiess, Mississippi State University, Mississippi State, Mississippi, United States.

The inclusion of probiotics in broiler feed has improved their growth performance and overall health status. However, an earlier administration of these probiotics could promote benefits that protect the chick from the moment it hatches. Therefore, the objective of this study was to determine if *in ovo* injecting 3 different species of *Lactobacillus* impacts hatchability, growth performance, and histo-immunological status. On d18 of incubation, 4 *in ovo* treatments (T) were applied: T1 = Marek's

vaccine (MV) without probiotic, T2 = MV+ *L. animalis*, T3 = MV+ *L. reuteri*, and T4 = MV+ *L. rhamnosus*. All probiotics were provided at ~10⁶ cfu/50µl dose. Injected eggs were transferred to 3 hatchers per T. At hatch, % hatch of transfer, chick weight, and hatch residue analysis was conducted. A total of 720 male broiler chicks, representing 18 chicks per pen with a total of 10 replicate pens per T were moved to a grow-out facility for a 42d grow-out. On d14 a 20X dose of B52 Coccivac® was used for a coccidiosis challenge. Performance characteristics (BW gain, feed intake, and FCR) were collected on d0, 14, 21, and 28. Spleen, bursa and blood samples were collected on d 0, 14, and 21 for immunophysiological analysis. Hatch data were analyzed using a completely randomized design; growth performance and immune parameters were analyzed using a randomized complete block design with a split plot over time. Means were separated using Fisher's protected LSD when $P \leq 0.05$. No differences in hatchability or live performance characteristics were detected ($P > 0.05$). A trend was apparent for chick weight where T2 chicks were lighter than all other T ($P = 0.076$). Lesions were present in all birds however not different among T. A T by day interaction was observed for bursa follicle area where on d21, T2 and T4 had larger bursa follicle areas than T1 and T3 ($P = 0.02$). Spleens were found to be heavier on d21 for T3 when compared with all other T on that day ($P = 0.02$). Two out of the 10 cytokines evaluated, demonstrated T by day interactions. Pentraxin 3, an anti-inflammatory cytokine, was at lower levels on d14 in T2 than all other T; however, by d21, levels were highest in T3, with no difference among all other T ($P = 0.04$). On d0, a pro-inflammatory cytokine (IL16) was higher in T4 compared with T3. Levels of IL 16 changed by d14, where T1 had higher levels than T2 and T3, but by d21 no difference among T were observed ($P = 0.02$). In conclusion, T3 appears to stimulate immune-physiological parameters more effectively than the other *Lactobacillus* treatments, which could reduce the incidence of disease in poultry. Research that defines how *L. reuteri* stimulates the immune system still needs to be explored further.

Key Words: broilers, *in ovo*, *Lactobacillus*, coccidiosis, immunity

45 Effects of dietary protein source and litter condition on mitotically active cell and macrophage densities in the duodenum of young broilers. A. Keel^{*}, Allan Calderon, Oscar Tejada, Jessica Starkey, and Charles Starkey, Auburn University, Auburn, Alabama, United States.

The small intestine plays an essential role in growth performance by serving as the site of nutrient absorption and a line of defense against ingested pathogens. These functions are supported by mitotically active cells, which proliferate to continuously renew epithelial cells, and macrophages, which can destroy pathogens and recruit other immune cells to fight infection. The objective of this study was to investigate the effects of dietary protein source and litter condition on the cell densities of mitotically active (proliferative) cells and macrophages in the duodenum of broiler chickens. To accomplish this, a randomized complete block design experiment with a 3 × 2 factorial treatment structure was conducted. The 3 different dietary protein sources fed were soybean meal (SBM), 50% poultry by-product and 50% feather meal (PFM), and porcine meat and bone meal (MBM). Broilers were reared on either new litter (NL; fresh pine shavings) or used litter (UL; litter 3 flocks were previously reared on). On d 0, Yield Plus x Ross 708 female broiler chicks (Aviagen, Huntsville, AL) were randomly allotted to 1 of 6 treatments and placed in an environmentally-controlled, raised floor pen facility. Broilers received *ad libitum* access to water

and crumbled corn and soybean meal-based starter diets. On d 3, 8, 11, 15, and 21, 6 birds per treatment from 6 different blocks (total n = 36 each day) were randomly selected for duodenal sample collection. One h before sample collection, broilers were injected intraperitoneally with 5'-bromo-2'-deoxyuridine (BrdU) to label mitotically active cells. Duodenal sample were frozen in liquid nitrogen and subsequently analyzed using cryohistology, immunofluorescence staining, and digital fluorescence microscopy procedures to determine the density of mitotically active (BrdU+) cells and macrophages (cells per mm²). Data were analyzed using the GLIMMIX procedure of SAS (V9.4) and least squares means were separated using the PDIFF option at $P \leq 0.05$. Neither dietary protein source or litter condition influenced proliferative or macrophage cell densities on d 11 or 21. However, broilers reared on UL had a greater mitotically active cell density than those reared on NL on d 3 (1,481 v. 1,202 \pm 78; $P = 0.0126$) and d 15 (1,540 v. 1,298 \pm 77; $P = 0.0292$). On d 8, broilers fed MBM had an increased macrophage density compared with broilers fed PFM and SBM (781 v. 682 v. 686 \pm 31; $P = 0.0401$). Overall, these data indicate that dietary protein source and litter condition may affect small intestinal physiology throughout development. Additional investigation is necessary to further characterize the underlying mechanisms involved.

Key Words: broiler chicken, duodenum, macrophage, bromodeoxyuridine, intestinal cell proliferation

46 The dietary inclusion of formalin, formic acid, and 1-monoglycerides of short and medium chain fatty acids on gut morphology and body weight of Cobb 700 broilers. Dana Dittoe*¹, Kristina Feye¹, Joshua Jendza², Adam Lattin¹, Guillermo Tellez¹, Casey Owens¹, Michael Kidd¹, and Steven Ricke¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²BASF Corp., Florham Park, New Jersey, United States.

Commonly, formalin is added to commercial broiler diets to prevent contamination of feed with pathogens such as *Salmonella* and to subsequently improve broiler performance. However, the use of formalin is dangerous to the health of those making and handling the feed; thus, safer alternatives such as formic acid must be explored. Therefore, the objective of the current study was to compare the gut morphology and body weight of Cobb 700 broilers when fed diets containing formalin, formic acid, or 1-monoglycerides of short and medium chain fatty acids over a 42 d growout period. Upon hatch, d 0, 864 male broilers were vaccinated for coccidiosis and placed in 48 floor pens (5 treatments, 10 replicates, 18 birds per pen) and supplied feed and water ad libitum. Birds were fed 3 phases of diets (d 0 to 14, d 14 to 28, d 28 to 42) prepared by the University of Arkansas feed mill and contained the following 5 experimental treatments: the basal diet (NC), the basal diet with the inclusion of formalin (0.5% formaldehyde), basal diet with formic acid (0.25 and 0.5% Amasil NA), and the basal diet with the inclusion of 1-monoglycerides of short and medium chain fatty acids (0.25% SILO Health 104L). On d 14, 28, and 42, during feed change, 2 birds per pen were randomly selected and humanely euthanized to collect samples for gut morphology. Data were analyzed using a random complete block design in JMP 14.0 and means were separated using Dunnett's comparison with 0.5% formalin set as the control and a significance level of $P \leq 0.05$. The current experiment demonstrated that birds fed diets containing formalin were smaller (301 g) than those fed all other diets on d 14 (384, 363, 379, and 370 g, $P < 0.05$). In addition, birds fed diets containing formalin had smaller and lighter gastrointestinal tracts (GIT) and GIT higher pH than other treatments ($P < 0.05$). However, by d 28 and 42, differences in body weight, GIT length, weight, and pH were less pronounced in broilers fed diets containing formalin. Therefore,

the dietary inclusion of 0.05% formaldehyde delayed the development of the GIT, resulting in shorter and lighter organ weights relative to those fed diets containing NC or Amasil NA (0.25 and 0.5%) on d 14. However, by d 28, differences in the GIT segment length, weight, and pH became less pronounced and less consistent due to broilers achieving GIT maturity. In conclusion, the dietary inclusion of 0.25 and 0.5% Amasil NA in Cobb 700 broiler diets is an effective dietary alternative capable of improving GIT morphology and subsequent body weight in comparison to the inclusion of 0.05% formaldehyde.

Key Words: broilers, formaldehyde, formic acid, gut morphology, gut pH

47 Blood chemistry changes as the severity of wooden breast and white striping myopathies increase in broiler chickens.

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Recently studies have shown that broiler chickens that have severe wooden breast may also be hypoxic. Therefore, data from 6 different studies evaluating wooden breast and blood chemistry were analyzed together to determine if there is a consistent change in blood chemistry as wooden breast and white striping severity increased. All broiler chicks were male Ross 708 ranging in age from 29 to 49 d of age. Blood chemistry was determined using the iStat analyzer with CG8+ cartridges. Blood was collected in lithium heparin vacutainers and run within 5 min of blood collection. Birds were then processed and scored on a 1 to 4 point ordinal scale for both wooden breast and white striping. Data were compiled and analyzed with JMP 14.1. Total carbon dioxide (TCO₂) and base excesses (BE_{ecf}) were greatest in birds that had severe wooden breast scores of 4 ($P < 0.01$), while those same birds had the lowest saturated oxygen (SO₂). Similar patterns were also observed with white striping myopathies with TCO₂ and BE_{ecf} increasing with the severity of striping scores ($P < 0.002$). Partial pressure of carbon dioxide (PCO₂), K⁺ and insoluble calcium also increased with white striping, whereas blood pH decreased with white striping scores. In conclusion, changes in blood chemistry may give producers an indication of the severity of wooden breast and white striping in broiler chickens.

Key Words: wooden breast, white striping, blood chemistry

48 In-ovo culturing of turkey (*Meleagris gallopavo*) ovarian tissue to assess overall viability and germ cell development.

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Cryobanking of ovarian tissue and semen appears to be the most cost-effective option for the long-term preservation of turkey genetics. Before cryobanking ovaries for future revival, it is imperative to first establish that fresh ovaries can survive transplantation and develop normally. To demonstrate this, 8 turkey ovaries were collected from poults at 1, 3, 5, 7 and 15 d post-hatch, with 4 from each age group being cultured *in-ovo* for 6 d, via xenografting to the chorioallantoic membrane, before being fixed. With the other 4 being immediately fixed, representing pre-culture ovaries. Another set of 4 ovaries were collected at 9, 11, 13 and 21 d of age and fixed, representing normal *in vivo* development. After fixation, ovaries were sectioned (5 μ m) and stained (H&E) for imaging. The viability of tissue was assessed visually on whole grafts, with cell viability being measured on sections via the TUNEL assay. Size and density

of pre-follicular germ cells and primordial follicles were measured to assess developmental progression. Cultured (C) ovaries were compared with both pre-culture (PC) and *in vivo* (IV) ovaries. For example, 7-d old ovaries, *in-ovo* cultured for 6 d (C13) were compared with both pre-culture 7-d old ovaries (PC7) and *in vivo* 13-d old ovaries (IV13). A 2-way ANOVA was used to determine the effect of treatment (PC, C or IV) or the interaction with age, followed by a “simple effects” test to determine differences within ages. Visually, all grafts re-vascularized. Analyses via TUNEL showed minimal late-stage apoptotic or necrotic cells within all treatments. For germ cell development, pre-follicular germ cells and primordial follicles were overall smaller ($P \leq 0.001$) in C than IV ovaries. For pre-follicular germ cell density, only C7 was different compared with its IV counterpart, having a lower ($P \leq 0.001$) density than IV7. Whereas, C13 was close to having ($P = 0.08$) a higher density than IV13, being more indicative of normal germ cell development within the culture system. For C9, C11 and C13, densities of primordial follicles increased ($P \leq 0.001$) during culture, although densities remained below ($P \leq 0.05$) their IV counterparts (IV9, IV11 and IV13). In contrast, the density of C21 primordial follicles decreased ($P \leq 0.001$) during the culture period compared with PC15. Based on our results, the initial age of ovaries did not affect viability during culture; however, age did affect germ cell development. Therefore, the transplantation of 1- and 15-d old turkey ovaries should be avoided, due to marginal developmental success, instead, 7 d old ovaries appear to be the most suitable for transplantation.

Key Words: Culturing, In-Ovo, Germ Cell, Ovary, Turkey

49 Epithelial cell adhesion molecule (EpCAM) expression in peripheral blood cells for the diagnosis of ovarian cancer in Leghorn chickens. Lalitha Gopalan* and Ramesh Ramachandran, *The Pennsylvania State University, State College, Pennsylvania, United States.*

Ovarian cancer is the most fatal gynecological cancer as the disease is asymptomatic and typically diagnosed only in the advanced stage. Leghorn chickens spontaneously develop ovarian cancer and therefore can be used as an animal model to develop tools for early diagnosis of the disease. Epithelial cells are shed from the ovarian tumor into blood circulation during various stages of cancer and such circulating tumor cells (CTC) play a major role in cancer metastasis. Epithelial cell adhesion molecule (EpCAM), a cell surface tumor antigen, is highly expressed in the CTC of human subjects with epithelial carcinoma. The hypothesis for the present study is that EpCAM is highly expressed in the CTC in the chicken model of ovarian cancer. Leghorn hens (4–5 years-old) were routinely subjected to per-rectal ultrasonography to determine the ovarian morphology and for diagnosis of ovarian cancer. Based on the ultrasonography, animals were classified into 3 groups: Group 1 = animals showing tumor nodules with or without ascites ($n = 23$); Group 2 = animals showing hyperechoic ovarian stroma with cystic spaces and abnormal size/number of follicles ($n = 12$); and Group 3 = animals showing normal ovarian morphology ($n = 16$). A blood sample (5mL) was collected from the wing vein all the animals and CTC were separated along with peripheral blood mononuclear cells using Ficoll-Paque™ gradient centrifugation. Total RNA was extracted from CTC and reverse transcribed to prepare cDNA. The relative abundance of EpCAM mRNA normalized to β -actin mRNA levels in CTC was determined by qPCR using 7500/7500 Fast Real-time PCR system. Data analysis was conducted using the SAS (SAS Institute, NC, USA). The mRNA abundance between the 2 groups was compared using student's *t*-test. A probability level of $P < 0.05$ was considered to be significant. EpCAM mRNA abundance in CTC was found to be 34.03-fold higher

in Group 1 (cancerous) animals compared with Group 3 (control); ($P < 0.01$). Out of 16 animals in Group 3 (control), 10 animals did not have detectable levels of EpCAM and therefore the levels of EpCAM in peripheral blood cells of the remaining 6 animals were used for comparison with Group 1 or 2. Although EpCAM mRNA abundance in Group 2 animals were not statistically different from Group 3 animals, a wide range of downregulation or upregulation of EpCAM expression (0.15 to 4.44- fold change) was observed and therefore, further studies are required to investigate the significance of variation. However, our studies prove that animals with ovarian cancer with or without overt symptoms display an overabundance of EpCAM in the circulating blood cells. Future studies will refine this methodology for diagnosing ovarian cancer at an earlier stage.

Key Words: chicken, ovarian cancer, circulating tumor cells(CTC), epithelial cell adhesion molecule (EpCAM), ultrasonography

50 Not Presented

51 How does energy intake affect the molecular mechanism of energy balance in broiler breeder pullets at the onset of lay? S. H. Hadinia*¹, Gregoy Bedecarrats², and Martin Zuidhof¹, ¹University of Alberta, Edmonton, Alberta, Canada, ²University of Guelph, Guelph, Ontario, Canada.

To control energy balance the hypothalamic melanocortin system is composed of 2 populations of neurons, one set that expresses neuropeptide Y (NPY) and a second set that expresses proopiomelanocortin (POMC) that both have important roles in feed intake regulation. It has been proposed that stimulation of NPY neurons mediates increased feed intake whereas stimulation of the POMC neurons causes energy intake to decrease. It was previously shown that leptin suppressed feed intake in broiler chickens. Leptin along with its receptor (LEPR) plays an anorexigenic (loss of appetite) role in the body. The LEPR is expressed within NPY and POMC neurons in the hypothalamus and has a role in energy balance. In poultry, sexual maturation is mainly regulated by stimulatory gonadotropin releasing hormone (GnRH-I) in the hypothalamus. In poultry, the current understanding of the molecular mechanisms impacted by ME intake (MEI) is limited. Thus, to understand energy balance and sexual maturation in broiler breeder pullets the effect of MEI was evaluated on the mRNA levels of NPY, POMC, LEPR, and GnRH-I in the hypothalamus of Ross 308 broiler breeder pullets at the onset of lay. From 22 to 26 wk of age, 140 pullets were equally assigned to 2 treatments: 1) a low ME diet (2,807 kcal/kg) fed restricted according to the breeder-recommended BW target (Low MEI), and 2) a high ME diet (3,109 kcal/kg) fed unrestricted (High MEI). Day length was increased from 8L:16D to 14L:10D (30 lx) at the start of the experiment. Daily palpation was used to detect onset of lay via the presence of a hard-shelled egg in the shell gland. Total RNA was extracted from 80 hypothalamus samples ($n = 8$ per treatment, per wk) and was used for qRT-PCR assays. Data were analyzed using the MIXED procedure and Pearson correlation coefficients for the relationship between the expression of genes estimated using the CORR procedure in SAS. Pullets with High MEI had a 36% and 57% increase respectively in POMC and GnRH-I mRNA levels compared with pullets with Low MEI ($P < 0.05$). However, MEI did not affect the mRNA levels of NPY and LEPR genes ($P > 0.05$). The overall expression of the POMC and GnRH-I genes were positively correlated ($r = 0.31$; $P = 0.04$). The onset of lay for pullets in the High MEI treatment was accelerated as 100% of birds had laid by 26 wk of age, compared with 30% in the Low MEI treatment ($P < 0.001$). Positive correlation of POMC with GnRH-I provides a new insight for

POMC function. We hypothesize that POMC may play a role in linking the energy status to the initiation of reproduction in broiler breeders, with higher MEI resulting in increased POMC mRNA levels which in turn results in advancing the onset of lay in broiler breeder pullets.

Key Words: caloric restriction, gene expression, energy balance, meat-type chicken, sexual maturation

52 Metformin alters granulosa cell FSH-responsiveness in preovulatory and prehierarchical follicles in broiler breeder hens.

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Broiler breeder hens have poor reproductive efficiency due to rapid growth and increased adiposity, resulting in severe ovarian dysfunction. Egg production remains suboptimal due to excessive and aberrant follicle recruitment, often leading to non-viable, double or internal ovulations. In preliminary studies, dietary metformin supplementation increased egg production in broiler breeder hens, but the underlying mechanisms of action via which metformin acts on the ovary is unknown. In the present study, we hypothesize that treatment with metformin inhibits FSH-mediated signaling pathways rendering granulosa cells from the most recently recruited (9–12 mm) and prehierarchical (6–8- and 3–5mm) follicles steroidogenically incompetent. Cobb 500 broiler breeder hens were raised according to the COBB Breeder Management Guidelines. Ovaries were harvested from hens around 40 weeks of age ($n = 16$) and individual follicles dissected out for granulosa cell extraction. Granulosa cells collected from individual follicles were grouped by follicle diameter (3–5-, 6–8- and 9–12 mm) and were pooled from 4 individual birds to form one biological replicate. Granulosa cells were treated with 0, 1, 10 or 20 mM of metformin, challenged after 30 min with recombinant-human follicle stimulating hormone (rhFSH) and incubated for a total of 4 h. Gene transcripts analyzed via qPCR ($n = 3$) included FSHR, VIPR1, STAR, CYP11A1 and HSD3B. In granulosa cells isolated from 9 to 12- and 6–8 mm follicles, the addition of metformin significantly decreased the levels of FSHR, VIPR1, STAR, CYP11A1 and HSD3B mRNA in a dose dependent manner ($P < 0.001$). In granulosa cells from 3 to 5 mm follicles, FSHR, STAR and CYP11A1 mRNA levels were significantly decreased with the addition of 10 and 20 mM of metformin ($P < 0.001$). Treatment with 20 mM of metformin significantly decreased HSD3B mRNA levels ($P < 0.01$) but there was no effect on VIPR1 mRNA expression in any of the treatment groups. Next, we wanted to analyze the effects of metformin on known FSH-signaling pathways. Proteins analyzed via Western blots ($n = 4$) included ERK, Akt, ACC and AMPK. In granulosa cells isolated from 9 to 12 mm follicles, metformin significantly decreased the abundance of phospho-ERK in a dose dependent manner, with 20 mM having the greatest effect ($P < 0.001$). Metformin treatment (20 mM) also significantly decreased phospho-ERK abundance in granulosa cells isolated from 3 to 5- and 6–8 mm follicles ($P < 0.05$). Further statistical analyses pending on Akt, ACC and AMPK. All results were analyzed using one-way ANOVA ($P \leq 0.05$) and Tukey's HSD. Results obtained thus far suggest that metformin treatment affects steroidogenic pathways in broiler breeder ovarian follicles.

Key Words: folliculogenesis, steroidogenesis, metformin, broiler breeder, ovary

53 Shotgun proteomics reveal increased expression of RELAXIN-3 during follicle cyclic recruitment in the hen ovary.

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Chicken ovarian pre-recruitment follicles 1 to 8 mm diameter are arranged in a size hierarchy, and viable follicles are recruited to the preovulatory stage in a sequential fashion through the process of cyclic recruitment. Published evidence suggests that pre-recruitment follicles acquire an increased responsiveness to follicle stimulating hormone (FSH) with increasing size. We hypothesized that this differential responsiveness is due to differences in protein expression within the granulosa cell layer (GC) of the follicle. GC layers from the most recently recruited (GC9) follicle and from each of the 4 largest pre-recruitment follicles (GC1–4), plus theca tissue from the most recently recruited (TH9) and largest pre-recruitment (TH1) follicles were collected from ovaries of 34-week-old hens ($n = 4$). A shotgun proteomics analysis was conducted using isobaric tags for relative and absolute quantification labeling, followed by 2-dimensional liquid chromatography and tandem mass spectrometry. The non-parametric Mann-Whitney test for a 2 sample or the Kruskal-Wallis test ANOVA by rank was performed where appropriate. The Benjamini-Hochberg procedure was used to control for the False Discovery Rate (5%). Of 1535 proteins identified, none were determined to be differentially expressed between TH9 and TH1. A pairwise comparison between GC9 and GC1, GC2, GC3 or GC4 identified 1, 5, 5, and 6 differentially expressed proteins, respectively, including yolk and cholesterol transport proteins (vitellogenin 1, 2, 3 and apolipoprotein B). In addition, GC differentiation mediators transforming growth factor β 1 and microRNA-21 pathways were predicted to be activated at recruitment, while a mediator of apoptosis, oncostatin M, was predicted to be inhibited. We also report, for the first time, expression of the neuropeptide, RELAXIN-3 (RLN3), in GC. Expression of *RLN3* and its receptors, *RXFPI* and *RXFP3*, was measured using quantitative polymerase chain reaction from ovarian cortex, atretic follicles, plus GC and TH from the most recently recruited follicle (9–12 mm), 8 large pre-recruitment follicles (6–8 mm), pooled 4 mm, and pooled 2 mm pre-recruitment follicles ($n = 4$ hens). One-way ANOVA was used to compare mRNA expression in tissues. *RLN3* mRNA expression was highest in GC9 and GC1 ($P < 0.0001$), while its receptors, *RXFPI* and *RXFP3*, were highest in TH and cortex, respectively ($P < 0.0001$). Overall, cyclic recruitment is associated with changes in protein expression predominantly within follicle GC, and a potential role for *RLN3* in follicle recruitment and the initiation of GC differentiation warrants further investigation.

Key Words: proteomics, follicle, ovary, granulosa, RELAXIN-3

54 Are the differences in sexual maturation observed between layers with different degrees of genetic selection mediated via the pituitary gland? Charlene Hanlon*, Kayo Takeshima, and Gregory Bedecarrats, *University of Guelph, Guelph, Ontario, Canada.*

Breeding goals for commercial laying hens continue to improve numerous traits, with emphasis remaining on the advancement of sexual maturation. As age at first egg (AFE) decreases with selection pressure, hens tend to spontaneously lay before photostimulation, traditionally used to activate the hypothalamic-pituitary gonadal (HPG) axis. We hypothesize that selection for earlier maturation has altered the control of the HPG axis with metabolic cues possibly interfering with photoperiodic signals at all levels. This study was conducted to compare activation of the pituitary gland between a heavily selected commercial strain (Lohmann LSL-lite; L), a mid-point selected strain (heritage White Leghorn Shavers; S), and a non-selected strain (Smoky Joes; SJ). Pullets were randomly assigned by strain to cages ($n = 2/\text{cage}$) from 12 weeks of

age (woa). Photoperiod remained at 10h until step-up photostimulation at 18woa (1h/week to 16h light). Pituitary glands were collected from birds at 12, 17, 20, and 25woa ($n = 4/\text{breed}$ per time point). After RNA extraction and cDNA synthesis, levels of FSH- β subunit and estrogen receptor- α (ER- α) mRNA were determined by semiquantitative RT-PCR with GAPDH and β -Actin as housekeeping genes. Data was analyzed using the 2(- $\Delta\Delta\text{Ct}$) method, normalized to 12woa per breed. In addition, body weight (BW) was recorded and blood samples were taken before sacrifice to measure estradiol (E_2) concentrations by ELISA. Statistical analysis used MIXED procedure (SAS) with Tukey's adjustment. As previously reported, E_2 levels peaked at 19woa for L and S, while this peak was delayed to 21woa in the SJ ($P < 0.001$), confirming photostimulation was not the primary cue responsible for initiating ovarian activation in selected strains. Furthermore, FSH- β mRNA levels, linked to the organization of the follicular hierarchy, also appeared to slightly increase between 12 and 17woa for the L and S (2.9 and 6.9-folds, respectively), peaking at 20woa (12.1-fold, $P = 0.014$ for L; 15.7-fold, $P = 0.07$ for S). Conversely, FSH- β levels in SJ only increased at 20woa (23.9-fold, $P < 0.01$), following photostimulation. Meanwhile, it was determined while ER- α was present in the pituitary, expression did not significantly change throughout maturity. Interestingly, levels of FSH- β mRNA and E_2 were correlated ($r = 0.90$, $P < 0.05$), and so were E_2 and BW ($r = 0.55, 0.78, 0.74$ for L, S, SJ, respectively, $P < 0.05$). Together, the earlier increase in FSH- β mRNA observed at 17woa for L and S, and the strong correlation of BW and E_2 supports our hypothesis that selected strains of layers are influenced by metabolic status, rather than relying exclusively on photostimulation as a primary cue of sexual maturation.

Key Words: sexual maturation, FSH, estradiol, HPG axis, laying hens

55 Surgical disruption of the nucleus of the hippocampal commissure provides evidence that it plays a role in the hypothalamo-pituitary-adrenal axis. Michael Kidd*, Hakeem Kadhim, Seong Kang, and Wayne Kuenzel, *University of Arkansas, Fayetteville, Arkansas, United States.*

The Nucleus of the Hippocampal Commissure (NHpC) in previous studies has been linked to the hypothalamo-pituitary-adrenal (HPA) axis regarding its regulation in the avian stress responses induced by both restraint and feed deprivation stressors. The objective of the study was to apply electrolytic lesions to the NHpC, effectively disrupting corticotropin releasing hormone (CRH) neurons, which are essential to HPA activity in vertebrates. "Cobb 500" male broilers (BW 300–350g, 10–14 d of age) were used in this experiment and split into 2 groups; lesioned birds (LES) ($n = 10$) and sham operation controls (sCON) ($n = 6$), which are birds subjected to surgery including placement of the electrode within the brain, but no electrical current was applied. Birds were given a 4-d recovery period post-surgery. On d 5 post surgery, a 2-h food deprivation stress was applied. Blood, brain and anterior pituitary samples were promptly taken at 2 h of food deprivation. Brain samples from NHpC and PVN were punched and stored in trizol for qRT PCR. Gene expression of CRH and corticotropin releasing hormone receptor 1 (CRHR1) in the NHpC were measured as well as CRH and CRHR1 in the paraventricular nucleus (PVN), the major hypothalamic nucleus involved in the stress response. CRHR1 and proopiomelanocortin mRNA (POMC) were measured in the anterior pituitary. All data were analyzed using a one-way ANOVA. CRH and CRHR1 in the NHpC were found significantly down due to lesion placement ($P < 0.0001$) in LES in comparison to sCON. In the PVN, CRHR1 was found significantly downregulated ($P < 0.05$) in LES, however, CRH in the PVN was found significantly upregulated ($P < 0.01$) in LES in comparison to sCON. In the anterior pituitary, CRHR1 was found significantly upregulated

($P < 0.001$) in LES. In contrast, POMC in the anterior pituitary was found significantly downregulated ($P < 0.0001$) in LES in comparison to sCON. Evidence suggests that lesioning the NHpC including its CRH neurons effectively disrupts the food restriction related HPA stress response due to anterior pituitary downregulation of POMC and upregulation of CRHR1. Although PVN upregulation of CRH is present, the elevated PVN upregulation could be due to a compensatory response caused by the lack of normal NHpC CRH expression due to surgical disruption. Even with upregulated expression of CRH in the PVN, the downregulation of POMC and upregulation of CRHR1, illustrate a lack of HPA activation expected from an intact NHpC. In conclusion, lesioning NHpC CRH neurons disrupts HPA activity during food deprivation stress adding direct evidence that the NHpC plays a role in HPA activity. (Supported by an Arkansas Biosciences Institute Grant).

Key Words: food deprivation, lesioning, stress, corticotropin releasing hormone, paraventricular nucleus

56 Effect of different basal culture media and sera combinations on primary broiler chicken muscle satellite cell differentiation and heterogeneity. Joshua Flees*, Cierla McQuire Sams, and Jessica Starkey, *Auburn University, Auburn, Alabama, United States.*

Broiler chicken satellite cells (SC) isolated from *pectoralis major* (PM) muscles provide a useful in vitro model to study muscle development and growth in broilers. There is little consistency in the literature regarding optimal culture conditions for terminal differentiation (DIFF) of SC into nascent myofibers termed myotubes. Previously, we reported the effects of 3 basal culture media (BCM) and 2 sera combinations on SC proliferation (PROLIF). This randomized complete block experiment with a $3 \times 2 \times 2$ factorial treatment arrangement (3 BCM x 2 PROLIF sera x 2 DIFF sera) was designed to determine the effects of both PROLIF and DIFF media on myotube formation and the heterogeneity of unfused myogenic cells. Parallel plates of SC were cultured in PROLIF media consisting of either McCoy's 5A (MCCOY), High Glucose Dulbecco's Modified Eagle's medium (HGMEM), or Low Glucose DMEM (LGMEM) media containing either 15% chicken serum (CS) or a combination of 5% horse serum (HS) + 10% CS (HSCS) at 40°C with 5% CO₂. Ninety-six h post-plating, 3 independent replicates of 2 parallel plates of SC isolated from 22-d-old, male broiler PM plated at 1.66×10^6 cells per well on 24-well plates coated with 0.1% gelatin were switched to DIFF media consisting of the same BCM with either 3% HS or 3% CS sera. After 96 h in DIFF media, cultures were immunofluorescence (IF) stained to detect cells expressing the myogenic regulatory factors, myogenic differentiation factor 1 (MYOD) and paired box 7 (PAX7) as well as myosin heavy chain (MHC), a myotube marker. After IF staining, a DAPI nuclear stain was applied. Nikon Elements software was used to capture and analyze 2 images per well (8 images per treatment). Densities of unfused MYOD+, PAX7+, and MYOD+:PAX7+ cells per mm² were also determined. Data were analyzed using the GLIMMIX procedure of SAS (V9.4). Well served as the experimental unit and means were separated using the PDIFF option at $P \leq 0.05$. SC cultured in DMEM produced cultures with MHC+ myotubes, while those cultured in MCCOY BCM failed to differentiate into myotubes ($P \leq 0.0001$). SC cultured in DMEM+HSCS PROLIF media and then CS DIFF media had the highest MHC+ myotube nuclear density ($P = 0.0007$) and MYOD+ myonuclei compared with those from all other treatments ($P = 0.0108$). When DIFF in CS, SC grown in LGMEM + HSCS PROLIF media had fewer myotube nuclei ($P = 0.0007$) and MYOD+ myonuclei compared with HGMEM + HSCS PROLIF ($P = 0.0108$). SC cultures grown in DMEM + HSCS and differentiated in CS also maintained the highest density of unfused PAX7+ cells (P

≤ 0.0001). In conclusion, cells cultured in HGD MEM+HSCS during PROLIF and then switched to CS for DIFF best supported broiler SC DIFF into MHC+ myotubes.

Key Words: muscle satellite cell, broiler chicken, cell culture, media, serum

Student Competition: Metabolism and Nutrition: Nutrition II

57 Evaluation of different methods for particle size analysis of milled corn with added soybean oil. Allan Calderon*, Gerardo Abascal-Ponciano, Luis Avila, Joshua Flees, Samuel Leiva, Kevin Ordenez, Danny Patino, Marc Presume, Jorge Sandoval, Wilmer Pacheco, and Charles Starkey, *Auburn University, Auburn, Alabama, United States.*

Particle size (PS) of grain is an important component of feed manufacturing that impacts pellet quality, mixability, feed flowability, and animal growth performance. The objective of this randomized complete block design experiment with an $3 \times 8 \times 6$ factorial treatment arrangement was conducted to evaluate the effectiveness of 3 different PS analysis methods on ground corn of 8 different PS with increasing additions (6) of soybean oil (SBO). Corn was ground with a 2-pair roller mill (Roskamp Champion Series 900–12) at 8 different roller settings: 3–2, 3–3, 4–3, 4–4, 5–4, 5–5, 6–5, and 6–6, where the roller pair spacing ranged from 50.8 μm at setting 2 to 152.4 μm at setting 6. These settings generated samples with mean PS of 693, 882, 968, 1,750, 1,877, 2,862, 2,911, and 3,343 μm , respectively. After milling, the ground corn was mixed with SBO to achieve 0, 2, 4, 6, 8, and 10% added fat. Three repetitions of each of the combinations of PS and SBO mixture were analyzed for PS using the 3 methods: 1) ANSI/ASAE S319.4 “Method of determining and expressing fineness of feed materials by sieving” 13-sieve method, 2) a 3-sieve hand-shaken method developed by Kansas State University for field testing of PS (KSU3S), and 3) a modification of the KSU3S method in which the formulas from the ANSI/ASAE method were adapted for use with 3-sieves (AUM3S). Data were analyzed using the GLIMMIX procedure of SAS (v9.4). Least squares means were separated with the PDIF option and considered significantly different when $P \leq 0.05$. A 3-way interaction among PS, SBO, and method was observed ($P \leq 0.0001$). The PS values for the standard ANSI method were from 604 to 3,730 μm , the KSU3S PS values ranged from 584 to 1,730 μm , and the AUM3S values ranged from 640 to 3,335 μm . With the ANSI method, PS values were reduced with 2% SBO compared with 0% SBO ($P \leq 0.0001$); however, the PS then increased as SBO additions increased ($P \leq 0.0001$). Above 2,862- μm PS, SBO additions did not alter PS values ($P \leq 0.0001$) Using the AUM3S method, for 693 to 968- μm PS samples, PS was not affected by addition of 0, 2, 4, and 6% added SBO ($P \leq 0.0001$). The AUM3S method produced PS results more similar to the standard ANSI method than the KSU3S method. Our results indicate that as the PS of ground corn increased more differences were observed among the different SBO concentrations when measuring PS. Additionally, inclusions of SBO below 6% did not impact the ability of these methods to measure PS in milled corn at the lower PS, whereas larger PS appears to increase the deviation among the methods. Therefore, it is important to utilize accurate PS analysis methods that provide more consistent values for corn of different PS.

Key Words: particle size, feed, fat addition, sieve, roller mill

58 Evaluation of the Aviagen Turkeys shaker sieve to determine particle size for ground corn with increasing additions of soybean oil. Luis Avila*, Gerardo Abascal-Ponciano, Allan Calderon, Samuel Leiva, Joshua Flees, Kevin Ordenez, Danny Patino, Marc Presume, Wilmer Pacheco, and Charles Starkey, *Auburn University, Auburn, Alabama, United States.*

Ground corn is typically the greatest inclusion ingredient of poultry feeds. Therefore, particle size (PS) determination and its variability

are important. Particle size of grain is an important component of feed manufacturing that impacts pellet quality, mixability, feed flowability, and animal performance. Aviagen Turkey’s shaker sieve (AVS) is a field tool used to determine pellet quality and estimate PS. The objective of this experiment was to evaluate the ability of AVS to determine PS and geometric standard deviation (STDEV) of ground corn with added soybean oil (SBO) compared with the ANSI/ASAE S319.4 mechanic 13-sieve (13S), and the hand-shaken Kansas State University 3-sieve method (3S). A randomized complete block experiment with an $8 \times 6 \times 3$ factorial treatment structure was conducted. Corn was ground using a 2-pair roller mill (Roskamp Champion Series 900–12) with 8 different settings: 3–2, 3–3, 4–3, 4–4, 5–4, 5–5, 6–5, and 6–6, where the roller pair spacing ranged from 50.8 μm at setting 2 to 152.4 μm at setting 6. The settings generated samples with mean PS of 693, 882, 968, 1,750, 1,877, 2,862, 2,911, and 3,343 μm , respectively (CGR). The ground corn was mixed with SBO to achieve 0, 2, 4, 6, 8, and 10% added fat. The AVS was agitated by hand for 1 min and the 3S was agitated by hand for 1.5 min as per the recommendations provided. For the 13S method, an electric sieve shaker was used for 10 min. Three replicate samples from each CGR and SBO combination were analyzed with each of the 3 PS methods (PROC). STDEV could only be calculated for the 13S and AVS methods. Data were analyzed using the GLIMMIX procedure of SAS (V9.4) and means were declared different when $P \leq 0.05$. All fixed effects (PS, SBO, and PROC) produced differences in PS values ($P \leq 0.0001$). In addition, an interaction among CGR, SBO, and PS PROC was observed ($P \leq 0.0001$). The AVS method was different from ANSI for samples 693 through 2,862 μm regardless of percentage SBO addition ($P \leq 0.0001$). However, for PS 2,911 and 3,433, the AVS method provided values similar to the ANSI standard but only for SBO additions between 2 and 6% ($P \leq 0.0001$). Inversely, the 3S method was similar to the 13S method results at PS 693 and 882 with 0 to 6% added SBO but was different for PS of 968 and above regardless of percentage of SBO additions ($P \leq 0.0001$). The STDEV value produced by the AVS method were consistently different from the ANSI method regardless of PS or SBO addition ($P \leq 0.0001$). In conclusion, distinct differences in PS PROC were observed and resulted in different PS values when compared with ANSI standard method dependent upon corn PS.

Key Words: particle size, sieve, feed, physical quality, shaker

59 Corn drying temperature, particle size, and amylase supplementation affect broiler live performance. Hernan Cordova-Noboa¹, Edgar Oviedo¹, Andres Ortiz¹, Yilmar Matta¹, Juan Hoyos¹, Gherly Buitrago¹, Juan Martinez¹, Jose Yanquen¹, Lina Peñuela², Jose-Otavio Sorbara³, and Aaron Cowieson³, ¹*North Carolina State University, Raleigh, North Carolina, United States*, ²*Universidad del Tolima, Ibagué, Tolima, Colombia*, ³*DSM Nutritional Products, Kaiseraugst, Switzerland.*

Broiler live performance may be influenced by drying temperature of corn and results could depend on particle size after grinding. Supplementation with an exogenous amylase could improve performance parameters, but responses to enzymes are also affected by grain particle size. An experiment was conducted to assess the effects of hard corn kernel dried at 2 temperatures (35 and 120°C), ground coarse or fine (differing ~400 μm d_{gw}), with or without the supplementation (0, 133, and 266 g/ton) of an exogenous amylase on live performance and gastrointestinal organ development of Cobb 500 male broilers raised to 3.3 kg. Twelve dietary treatments resulted from a $2 \times 2 \times 3$ factorial arrangement of corn

drying temperature, particle size, and amylase supplementation that were considered as main effects. A total of 1,920 male-chicks were randomly placed in 96 floor pens (20 chicks/pen). Data were analyzed using a 3-way ANOVA in a randomized complete block design. At 14d, interaction effects of drying temperature with particle size ($P < 0.05$) and enzyme supplementation ($P < 0.05$) were observed on BW gain and BW. Amylase supplementation (133 and 266 g/ton vs. 0 g/ton) resulted in heavier chickens (512 and 512 vs. 501 g, respectively), but only when feeding the diets containing corn dried at 35°C. Results obtained at 28d showed that amylase supplementation improved ($P < 0.01$) in 40g BW and up to 3 points of FCR only in chickens fed coarse-corn based diets. At 40d, broilers fed corn dried at 120°C presented better FCR ($P = 0.08$) compared with broilers fed diets with corn dried at 35°C (1.533 vs. 1.542 g:g). Coarse-corn based diets resulted in 44g lighter ($P < 0.01$) broilers (3,284 vs. 3,328 g) and worst (1.544 g:g) FCR ($P < 0.05$) than fine-corn (1.531 g:g) based diets. Organ development at 42d indicated that relative intestinal length was increased ($P < 0.05$) by supplementing amylase at 266 g/ton compared with supplementing with 133 g/ton. An interaction effect ($P < 0.01$) was detected for ceca relative length. In broilers fed only fine-corn based diets, the supplementation with amylase (133 and 266 g/ton) resulted in larger ceca (5.83 and 5.99 cm/kg, respectively) compared with chickens receiving non-supplemented feed (5.51 cm/kg), indicating more hindgut fermentation. Moreover, chickens fed coarse-corn based diets had heavier gizzard ($P < 0.001$) and liver ($P < 0.05$) compared with broilers that ate fine-corn based diets. Interestingly, an opposite response ($P < 0.05$) was observed for proventriculus. In conclusion, corn-drying temperature and particle size affected gastrointestinal organ development and live performance. Live performance was improved by amylase supplementation in the diets with corn dried at 35°C.

Key Words: corn drying temperature, particle size, broiler performance, amylase, organ development

60 Effect of conditioning temperature on broiler performance, nutrient digestibility and processing yield. Martha Rueda Lastres^{*1}, Andrea Rubio³, Charles Starkey¹, Franco Mussini², and Wilmer Pacheco¹, ¹Auburn University, Auburn, Alabama, United States, ²DuPont Industrial Biosciences, Cave Springs, Arkansas, United States, ³North Carolina State University, Raleigh, North Carolina, United States.

During pelleting, mash is conditioned with steam to raise its moisture and temperature to increase production rate and pellet quality. However, high conditioning temperatures can negatively affect the availability of thermolabile nutrients such as vitamins, enzymes, and amino acids. This study was conducted to evaluate the effect of conditioning temperature on broiler performance, processing yield and nutrient digestibility from 1 to 49 d of age. A total of 1120 Ross x Ross 708 broilers were randomly distributed among 4 treatments with 10 replicates per treatment and 28 birds/pen. Treatments consisted of 4 conditioning temperatures; 71, 77, 82 and 88°C. A common starter diet was fed as crumbles from 1 to 14 d of age, whereas experimental grower and finisher feeds were fed as whole pellets from 15 to 49 d of age. Titanium dioxide was added as an indigestible marker (0.5%) during the grower phase to determine nutrient digestibility. At 28 d of age, 4 birds per pen were euthanized and ileal digesta was collected for nutrient digestibility analyses. Feed consumption and BWG were determined at 14, 28 and 49 d of age. Feed conversion ratio (FCR) was adjusted by adding the weight of the mortality to the BW of live birds in each pen. At 50 d of age, 10 birds per pen were processed to determine chilled carcass weights and on d 51, carcasses were deboned to determine meat yield. Data were statis-

tically evaluated using ANOVA procedure and means were separated by Tukey's HSD test. Increasing conditioning temperature from 71 to 88°C resulted in higher ($P < 0.05$) pellet quality and moisture content of finished feeds, while reducing ($P < 0.05$) percentage of fines. Feed consumption, BWG, and FCR were not affected by conditioning temperatures. However, broilers fed diets conditioned at 77°C had higher fat digestibility ($P < 0.05$) compared with broilers fed diets conditioned at 88°C. In addition, birds fed diets conditioned at 82°C had higher ($P < 0.05$) tender weight compared with birds fed diets conditioned at 71 and 77°C. These results demonstrate that conditioning at high temperatures has an unfavorable effect on fat digestibility, while not affecting broiler performance. Although pellet quality was increased and level of fines decreased, a longer cooling time should be considered, as conditioning temperature increases to reduce moisture content in finished feeds.

Key Words: conditioning, digestibility, feed quality, thermolabile nutrients

61 Evaluation of differential scanning calorimetry for analysis of starch gelatinization in multi-component animal feed. Lindsey Spencer*, Luis Avila, Danny Patino, Allan Calderon, Wilmer Pacheco, Emefa Monu, Oladiran Fasina, Leonard Bell, and Charles Starkey, Auburn University, Auburn, Alabama, United States.

Typical commercial animal diets are formulated with high concentrations of ground corn (GC) and contain large amounts of native starch to provide energy for growth. Thermal processes are used to gelatinize starch in animal feed to increase digestibility. Pelleting is a common thermal process utilized for the manufacture of food for production animals. Research has shown that calorimetry procedures are acceptable for determining the percentage of starch gelatinization in purified starch samples; however, few research studies have been conducted utilizing calorimetry procedures on multi-component animal feed. The objective of this experiment was to evaluate the effectiveness of differential scanning calorimetry (DSC) to analyze the degree of starch gelatinization (DG) in a complete animal feed. Replicate samples of cooked and uncooked corn starch (CS), cooked and uncooked GC, uncooked meal and pelleted animal feed (PAF) were analyzed at 160 and 200°C. The CS and GC samples were cooked using an autoclave (15 min at 121°C), while the PAF samples were processed using a conditioner and pellet mill at 95°C for 240 s. Samples were weighed into hermetic pans at a 1:2 ratio with 10 mg of the dry sample and 20 µL of deionized water and allowed to equilibrate overnight. DSC thermogram peaks were analyzed using TA Universal Analysis 2000 software to determine changes in enthalpy (J per g). Data were analyzed using the GLIMMIX procedure of SAS (V9.4) and least squares means were separated using the PDIF option at $P \leq 0.05$. Enthalpy (H_1) differed among cooked starch sources ($P = 0.0080$) and DSC temperature ($P = 0.0429$). No significant interaction was observed between starch source and DSC temperature ($P = 0.2166$). Calculated DG of CS was 71.3%, whereas the DG for GC and PAF could not be calculated as the uncooked enthalpy (H_0) for both sources was greater than the cooked enthalpy (H_1). Based on these results, the DSC is not a suitable method to assess DG in multi-component animal feed. The combination of proteins, lipids, and other carbohydrate fractions do not appear to allow for the use of DSC as an alternative to traditional enzymatic wet chemistry determination of the DG of starch in complex animal feed. However, when utilizing the AOAC 996.11 method, DG differed among cooked starch sources ($P = 0.016$). For AOAC 996.11, DG was calculated by subtracting the total starch content of cooked samples on an "as-is" basis from the average total starch in uncooked samples; DG (g per 100 g) was able to be calculated for both cooked CS

and cooked GC. Therefore, DSC should not be used as an alternative method to AOAC 996.11 in complex animal feed.

Key Words: starch gelatinization, DSC, enthalpy, animal feed manufacturing

62 Effects of wheat cultivars and feed processing method on starch damage and its impact on apparent metabolizable energy, starch and protein digestion in broiler chickens. Yousef Khanfas* and Rex Newkirk, *University of Saskatchewan, Saskatoon, Saskatchewan, Canada.*

Wheat is typically classified into 2 classes based on the hardness of the endosperm. Hardness can affect digestibility due to mechanical damage to starch during processing and can be measured as starch damage. The objective of this study was to evaluate starch damage in wheat caused by feed processing (grind and pellet) and determine its impact on starch and protein digestion and apparent metabolizable energy (AMEn) in broiler chickens. The experiment was a RCBD in a 2 × 4 factorial design (2 wheat classes × 4 levels of processing intensity; mash, cold pellet, hot pellet and extreme pellet). Statistical significance was considered at $P \leq 0.05$. In a digestibility trial, 280-d-old male Ross 708 broiler chicks were fed test diet from 7 to 28 d of age and were assigned to 8 treatments with 7 replicates of 4 birds per bioassay cage. The level of starch damage (as a % of total starch) was affected by the interaction between main effects ($P = 0.003$). For low intensity processing (mash) more starch damage was found for hard wheat (9.29%) than soft wheat (4.19%). The difference in damaged starch decreased as the intensity of processing increased with no differences between wheat types for extreme intensity treatment (9.11 vs 9.43%; soft and hard). Starch digestion in anterior jejunum was higher in extreme pelleted diets in both wheat types and was affected by feed processing ($P = 0.004$). However, particularly with hot pellet soft wheat was superior in all segments of small intestine (SI) compared with hard wheat. AMEn in mash and cold pellet diets were higher in hard wheat compared with soft wheat (2967 versus 3248 and 2985 versus 3126 kcal/kg DM). AMEn of soft wheat was significantly increased compared with hard wheat with hot pellet (3160 vs 3015 kcal/kg DM). Apparent intestinal protein digestibility of soft wheat in mash diet was higher in all parts of SI compared with hard wheat ($P < 0.05$). With pellet process, protein digestion of hard wheat was increased and superior than soft wheat in most of SI segments. In summary, starch damage occurred in both wheat types through feed processing, increasing AMEn and starch digestion while protein digestion was increased by pellet process only in hard wheat in broiler chickens.

Key Words: wheat, starch-damage, feed processing, digestion, AMEn

63 Effects of feed form on the development of the proximal part of the gastrointestinal tract and growth performance of three broiler strains from 0 to 21 days of age. J. Archs*, A. Rybicka, G. Fondevila, L. Cámara, D. Morcuende, and Gonzalo Mateos, *UPM, Madrid, Madrid, Spain.*

We studied the effects of feed form on the development of the upper part of the gastrointestinal tract (GIT) and growth performance of broilers from 1 to 21 d of age. The experiment included 6 treatments arranged as a 2 × 3 factorial with 2 feed forms (mash vs. crumble) and 3 broiler lines [2 fast-rate growing strains and one medium-rate growing strain]. The birds received a common diet based on corn and SBM with 2,950 kcal AMEn/kg and 1.22% SID lys. Each treatment was replicated 6 times and the experimental unit was a pen floor with 17 birds for all traits. Feed intake (FI), BW gain (BWG), and feed conversion ratio

(FCR) were determined weekly and cumulatively. At 7 and 21 d of age 2 broilers per cage were slaughtered by CO₂ asphyxiation to measure GIT traits. The gizzard and the crop were excised, clamped and weighed. Then, the organs were emptied of digesta content and weighed again. The absolute (g) and relative (% BW) weight, the pH, and the moisture content of the digesta of both organs were recorded. Data were analyzed as a completely randomized design with feed form and bird type as main effects, using the MIXED procedure of SAS. The fast-growing strains had better BWG and FCR ($P < 0.001$) than the medium growth strain at all ages, with differences in FI being the most important reason for the improvement observed. Feed form affected growth performance of the birds at all ages. Birds fed crumbles had higher BWG ($P < 0.001$) and better FCR ($P < 0.001$) than birds fed mash. In absolute values, the weight of the GIT was greater for the fast-growing than for the medium growing strain but an opposite result was observed in relative terms. One of the fast-rate growing strain showed a greater gizzard weight than the other. Mash feeding increased the empty gizzard weight ($P < 0.001$) in absolute and relative terms. Mash feeding increased the DM of the crop content ($P < 0.05$), and decreased the weight of empty crop ($P < 0.001$) and moisture content of the crop ($P < 0.001$). The pH the crop and gizzard decreased with mash feeding ($P < 0.05$). In summary, feeding crumbles improved growth performance in all broiler lines. The weight of the crop increased with crumbles where an opposite effect was detected for the gizzard.

Key Words: broiler performance, broiler strain, crumble feeding, gastrointestinal tract, mash feeding

64 Influence of the origin of the bean on the ileal digestibility of crude protein and amino acids of soybean meals in 21 day-old broilers. Lewis Aguirre*, L. Cámara, O. Chandi, G. Fondevila, J. Ben Mabrouk, and Gonzalo Mateos, *UPM, Madrid, Madrid, Spain.*

An experiment was conducted to study the influence of the origin of the beans on the apparent (AID) and standardized (SID) ileal digestibility of CP and amino acids (AA) of commercial high-protein soybean meals (SBM) in broilers from 18 to 21 d of age. The SBM samples were obtained from identity preserved beans from USA, Brazil (BRA), and Argentina (ARG) origin. The samples ($n = 24$ per each country) were collected by specialized quality control personnel in the country of origin of the beans or at the arrival of the vessels to Europe. The SBM were analyzed for main components (CP, crude fiber, neutral detergent fiber, sucrose, oligosaccharides, and mineral content) and protein quality indicators [TI activity, KOH solubility, PDI, and heat damage indicator (HDI)]. As fresh (88% DM), SBM from BRA had more CF (4.9%) and NDF (10.6%) than SBM from USA (3.8 and 8.3%) or ARG (3.5 and 8.0%). Sucrose and stachyose contents were higher for USA meals (8.1 and 6.0%) than for ARG (7.2 and 5.9%) and BRA (5.5 and 4.4%) meals. TIA, KOH, PDI were higher but HDI was lower for the USA meals than for the BRA and the ARG meals. The data on AID and SID were analyzed as completely randomized design using the GLM procedure of SAS with the origin of the SBM as main effect with 3 treatments and 24 replicates per treatment. The experimental unit was a cage with 10 broilers. Birds were fed a commercial corn-soybean meal diet in mash form from 1 to 15 d of age and then, their corresponding experimental diets for 6 d. The experimental diets resulted from the combination of 42.6% SBM and 57.4% of a nitrogen free diet composed of corn starch, dextrose, soybean oil, minerals, and solka-floc. All diets were fed. At d 21, all birds were weighed and sacrificed by CO₂ asphyxiation. The contents of the ileal portion, defined as the section of the small intestine extending from the vitelline Meckel's diverticulum to 4 cm anterior to the ileocecal junction, were gently flushed with distilled water into

plastic container. The contents of the 10 birds of each replicate were pooled and frozen (-20C) for AID and SID determinations. The AID and SID of the CP and of all the indispensable AA were greater for the SBM of USA origin than for the SBM of South American origin. In this respect, the AID of the CP, Lys, and Thr of the SBM were 84.4, 87.5, and 79.6%, 83.2, 86.2 and 79.0%, and 83.8, 86.7, and 78.6% for the USA, BRA, and ARG meals, respectively. None of the differences reported, however, reached significance ($P > 0.05$).

Key Words: amino acid, crude protein, ileal digestibility, soybean origin

65 A comparison of methods to determine standardized ileal amino acid digestibility of ingredients with low and high protein quality for broiler chickens. Ana Beatriz Oliveira*, Marcos Nascimento Filho, Alvaro Burin Junior, Diana Suckeveris, Raquel Pereira, and José Fernando Machado Menten, *ESALQ - USP, Piracicaba, Brazil*.

Standardized ileal digestibility coefficient (SIDC) of amino acids (AA) is the most desirable measure to formulate diets for poultry. Whereas methodologies such as the direct method (nitrogen-free diet) and indirect method (substitution method) may be inconsistent due to applied techniques, it is important to evaluate if the values are equivalent and accurate to be associated. This study aimed to compare 2 methodology procedures to determine SIDC of ingredients with low and high protein quality fed to broilers. In study 1, 180 16-d-old Ross AP95 male chickens were placed in floor pens (10 birds/pen, $n = 6$) in a completely randomized design. Birds were assigned to 3 assay diets: semi-purified nitrogen-free diet to estimate the basal endogenous AA flow, a test diet with 37.8% of insect meal (high protein quality, HP), and a test diet with 73.8% of corn distillers bran with solubles (low protein quality, LP). Each feed ingredient was the sole source of AA. In study 2, 72 male chickens 27d-old Ross AP95 were assigned to 3 diets (4 birds/cage, $n = 6$) in a completely randomized design. A corn-soybean meal basal diet was formulated as the control diet. Test diets were formulated replacing 300g/kg of the control diet by HP and LP ingredients. Chromic oxide was used as an indigestible marker at 0.5% in both experiments. On d 22 (study 1) and d 33 (study 2), all birds were euthanized by cervical dislocation and contents of the distal third of the ileum were obtained for determination of AA digestibility coefficients. Data were analyzed by one-way ANOVA. The AA contents for HP and LP ingredients were respectively 0.80 and 0.27% Met, 3.36 and 0.81% Lys, 2.15 and 0.69% Thr, 3.32 and 0.81% Val, 2.30 and 0.56% Ile, on dry matter basis. Comparing the direct and indirect methods, calculated average values of SIDC of some indispensable AA for HP ingredient were very similar for Met, 87.5 and 87.1%; Lys, 89.0 and 90.3%; Thr, 81.6 and 82.6%; Ile, 85.6 and 87.9%, respectively, but not for Val, 85.6 and 89.6% ($P = 0.036$). In contrast, SIDC of AA for the LP ingredient were statistically different for Met, 76.2 and 88.0% ($P = 0.006$); Lys, 65.6 and 75.9% ($P = 0.039$); Val, 62.8 and 75.7% ($P = 0.004$); and Ile, 66.1 and 79.5% ($P = 0.005$), respectively, with greater values in the substitution method. For Thr, the values (62.1 and 65.6%) were not different. In conclusion, the SIDC values for ingredients with different protein quality might be influenced by the method applied. LP ingredient AA digestibility may be overestimated by the basal diet used in the indirect method, whereas HP ingredient does not follow this same pattern. Acknowledgment: FS Bioenergy for the support and FAPESP for the research grant (No 2017/05423-8).

Key Words: feed ingredients, digestibility, amino acids, broilers, methodology

66 The development of Ark NE value for soybean meal. Katie Hilton*, Antonio Beitia, Pramir Maharjan, Jordan Weil, Nawin Suesuttajit, Diego Martinez, and CN Coon, *University of Arkansas, Fayetteville, Arkansas, United States*.

The classical method of net energy (NE) assesses the value of ME minus heat increment (HI). Analyzing classic NE can be misleading as more calorie efficiency (NE/ME) is given to fat deposition than lean mass deposition. It is therefore desirable to provide an improved process of formulating energy requirements of feed ingredients that overcomes the shortcomings of the classical equation. Experiments were conducted to create the methodology of a new NE system that includes both HI and body composition. Two test diets were developed for each feeding phase, with 2 different samples of SBM evaluated for starter (0-10d, 3,008 kcals/kg, 21%CP), grower (10-22d, 3,100 kcals/kg, 19%CP) and finisher (22-42d, 3,200 kcals/kg, 18%CP) feeding periods. The highest concentration of lysine/CP/kcal for SBM was utilized as control SBM analyzed by NIR. The control SBM was formulated in a corn soybean diet to provide 80%, 100% and 120% AA requirements for starter, grower and finisher diets with both AA levels and ME set according to the breeder recommendation. The control SBM dictated the formulation inclusion levels utilized for the test SBM. Each of the SBM samples were fed equally on a percentage basis as determined for the control SBM. Birds were moved to the respiratory chambers 1d before evaluation for a period of adaptation. Heat production (HP) Kcal = $3.872 \cdot \text{VO}_2 \text{ (L/d)} + 1.195 \cdot \text{VCO}_2 \text{ (L/d)}$ (Farrell, 1974) was measured for 1d. After HP was measured, fasting heat production (FHP) was measured for 24h. HI was determined $\text{HI} = \text{HP} - \text{FHP}$ (Farrell, 1974). Body composition was measured throughout the experiments by dual energy x-ray absorptiometry (DEXA) to determine net energy gain (NEg). Near infrared spectroscopy (NIR) analysis revealed SBM A was 2.576%digLys while SBM B was 2.528%digLys. Body composition indicated birds on SBM A gained significantly more protein ($P = 0.008$), while birds consuming SBM B gained more fat ($P = 0.0062$), ultimately having more ($P = 0.0352$) NEg than SBM A. Simple linear regression indicated a high level of predictability for SBM ($R^2 = 0.97$, $P = 0.0020$) for Ark NE with total digestible lysine (%digLys) as the predictor. The model shows a linear increase for Ark NE as %digLys increases. Using the mean intercept SBM A Ark NE value 1996 kcals and SBM B Ark NE value of 1632 kcals. This study indicates utilizing the Ark NE system is a valuable means for predicting the Ark NE value for SBM, while giving more calorie value for protein containing increased %digLys than classic NE.

Key Words: net energy, heat production, body composition, digestible lysine

67 Determination of standardized amino acid digestibility and metabolizable energy in plasma protein using different methods.

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The objective of this study was to determine the metabolizable energy and amino acid digestibility of plasma protein compared with soybean meal (SBM) using 3 different assays. The 48-h precision-fed rooster assay was used to evaluate true metabolizable energy (TME_n) using conventional roosters, whereas standardized amino acid digestibility was determined using cecectomized birds. Roosters on this trial were fasted for 24 h before being crop-intubated 30 g of either plasma protein mixed with 50% corn or SBM mixed with 50% corn. All excreta were then collected for 48 h. The TME_n and amino acid digestibility values

for plasma protein and SBM were calculated by difference. Data from all 3 assays were statistically analyzed using a one-way ANOVA for a completely randomized design. Results of the rooster assay indicated that the TME_n value for plasma protein was higher ($P < 0.05$) than SBM (3.743 and 2.669 kcal/g DM, respectively). The assay also showed that the average amino acid digestibility of plasma protein was higher ($P < 0.05$) than SBM (94 and 86%, respectively). In addition, 2 chick assays were conducted using Ross 708 males to determine apparent metabolizable energy (AME_n) and standardized ileal amino acid digestibility of plasma protein and SBM. To determine the AME_n, 3 diets were fed: a corn-SBM reference diet, a 30% plasma protein diet, and a 30% SBM diet. Chicks were fed one of the 3 diets from d 7–10 and 18–21, with excreta being collected on d 10 and 21. To determine the ileal amino acid digestibility, 2 diets were fed: a semi purified diet containing 25% plasma protein and a semi purified diet containing 41% SBM. Chicks were fed one of the 2 diets from d 7–10 or 18–21; ileal digesta were collected at d 10 and 21. The AME_n values obtained from the chick assay showed that plasma protein had a substantially higher ($P < 0.05$) AME_n value than SBM at both d 10 and d 21 (4.239 and 2.849 kcal/g DM, respectively, at d 21) and an even greater difference between the 2 feedstuffs was observed at d 10. The second chick assay showed an increase in average ileal amino acid digestibility of plasma protein compared with SBM ($P < 0.05$) for chicks at both d 10 (95 and 83%, respectively) and d 21 (96 and 87%, respectively). In conclusion, the results of all 3 assays used in this study indicated that plasma protein is a highly digestible feed ingredient regardless of the age of the bird.

Key Words: plasma protein, soybean meal, amino acids, metabolizable energy, precision-fed

68 Apparent metabolizable energy value of selected modern grain sorghum varieties for feeding commercial broilers. Alissa Moritz*¹, Shanice Krombeen¹, Robert Buresh², Michael Blair³, William Bridges¹, and Tiffany Wilmoth¹, ¹Clemson University, Clemson, South Carolina, United States, ²Novus, International Inc., St. Charles, Missouri, United States, ³Devenish Nutrition, Fairmont, Minnesota, United States.

Modern varieties of US grain sorghum (milo) are nearly 99% tannin-free; however, limited data is available to support that these modern varieties can be used as an alternative feedstuff to effectively replace corn in poultry diets. Milo may be a viable alternative considering its resilience to adverse climate and soil types enabling it to be grown in locations coinciding with poultry production in the Southeastern United States. This experiment determined the apparent metabolizable energy (AME_n) of red/bronze, white/tan, and US No. 2 yellow milo varieties. Trials evaluated the AME_n response of Cobb 500 x Hubbard broilers in the grower diet phase (22 to 24 d) and finisher diet phase (43 to 45 d). Broilers ($n = 224$) were randomly distributed into 56 grower battery cages at a density of 4 birds per cage and phase-fed 1 of 4 corn-soybean meal basal diets. The diets included 80% corn-soybean meal basal and were completed with 20% milo for the treatment diets or dextrose for the control diet. The control diet included 8 replicates per treatment and each of the 3 milo treatment diets included 16 replicates per treatment. During each phase, feed intake was measured, and excreta were analyzed for nitrogen and gross energy (GE). The difference method was used to determine the AME_n response. In both trials, the temperature was set to 35°C at placement and decreased to 27°C with a 16L:8D lighting program. An ANOVA was conducted to assess the effects of a full factorial of diet, trial, and % female of grower AME_n and finisher AME_n. In the grower phase diet, the effects of diet ($P = 0.023$), trial

($P = 0.0042$), diet x trial ($P = 0.022$), and diet x trial x % female ($P = 0.035$) were significant. Overall, the AME_n of red/bronze and white/tan milo varieties in the grower phase diet were significantly greater than the control and yellow milo variety ($P = 0.023$). In the finisher diet phase, AME_n of all milo varieties were significantly greater than the control ($P \leq 0.0001$). Results from AME_n analysis indicate that modern milo varieties show potential for replacing corn in diet formulation for the growth performance of broilers from 1 to 47 d of age.

Key Words: apparent metabolizable energy, broiler, grain sorghum, milo

69 Effect of dietary inclusion level on the metabolizable energy content of distillers dried grains with solubles determined in broiler chicks at 21 d of age. Kenia Mitre*¹, Kevin Herrick², and Samuel Rochell¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²POET Nutrition, Sioux Falls, South Dakota, United States.

Assays to determine ME_n are inherently variable and can be influenced by several factors, such as inclusion level of the test ingredient. Understanding these effects is particularly important for ingredients that can vary widely in ME_n content such as distillers dried grains with solubles (DDGS). An experiment was conducted to evaluate the influence of dietary DDGS concentration on the estimation of its ME and ME_n values in broilers, and to determine if any changes in these values are associated with changes in broiler FI as dietary concentrations of DDGS increase. Five treatments consisted of a reference basal diet and diets in which 15, 30, 45, or 60% DDGS was added at the expense of all energy providing ingredients in the basal diet for ME_n determination by the difference method. A sixth treatment consisted of the 30% DDGS diet pair-fed to equalize FI with birds fed the 60% DDGS *ad libitum*. A seventh treatment included an additional reference diet in which dextrose was included at 15% for ME_n determination of DDGS using the single ingredient replacement approach. Male broilers (448 total) were randomly allocated to 56 battery cages with 8 replicates cages per treatment (8 birds/cage) and were provided a common starter diet from 0 to 14 d post-hatch. Experimental diets contained 0.5% TiO₂ and were fed from 14 to 21 d. Diets and excreta were collected from 19 to 21 d and analyzed for nitrogen, gross energy and TiO₂ for determination of ME and ME_n using the index method. Polynomial contrasts were used to assess linear and quadratic effects of DDGS inclusion level. As expected, linear decreases ($P < 0.001$) in BWG and FI were observed as dietary DDGS concentration increased at the expense of the nutritionally complete basal diet. There were decreases ($P < 0.001$) in ME and ME_n values of the complete diets as DDGS inclusion increased, indicating that these values for DDGS were lower than the ME and ME_n values determined for the basal diet (3,107 and 2,905 kcal/kg DM, respectively). Similarly, ME and ME_n values of DDGS determined by difference decreased ($P < 0.001$) as its dietary concentration increased. Pair-feeding the 30% DDGS diet to the 60% DDGS diet reduced ($P < 0.05$) ME and ME_n values of DDGS when compared with those determined in birds fed the 30% DDGS diet *ad libitum*. The ME and ME_n values determined for DDGS when fed at 15% of the diet were higher ($P < 0.001$) when using the complete basal replacement method than when using the single ingredient replacement method. These results indicate that ME_n values determined for DDGS decreased as its inclusion within the test diet increased, with these reductions in ME_n partly due to decreased feed intake.

Key Words: metabolizable energy, broiler, DDGS, inclusion level, pair-feeding

70 Effects of black soldier fly larvae meal and oil on growth performance of broiler chickens. Brittany Wall*¹, Elizabeth Koutsos², and Kimberly Livingston¹, ¹North Carolina State University, Raleigh, North Carolina, United States, ²Enviroflight, Yellow Springs, Ohio, United States.

Black Soldier Fly Larvae (BSFL), *Hermetia illucens*, is a potential alternative protein and fat source in poultry diets. However, its effects on broiler chicken performance has not been elucidated. This experiment was conducted to determine how different levels of BSFL meal (BSFLm) and BSFL oil (BSFLo) affect the growth and performance on broiler chickens. The 2 studies were conducted simultaneously. In Experiment 1, BSFLm replaced soybean meal and was included at titrated levels (0, 5, 10, 15%) in replacement of soybean meal. In experiment 2, BSFLo was included at titrated levels (0, 0.5, 1.5, 3.0%) in replacement of soybean oil. All diets were formulated to be isonitrogenous and isocaloric. Ross 708 male broilers were raised in cages, with 10 birds per cage, and 5 replicates per treatment. Bird weights, and feed intake were measured weekly and feed conversion ratios (FCR) were calculated. Five birds

from each pen were processed for breast meat yield, and color. Data were analyzed using 2 separate one-way ANOVAs using JMP Pro 14, probability < 0.05 was considered significant. In experiment 1, Chicks fed 5% BSFLm had the greatest BWG and best FCR from 0 to 37 d when compared with 10 and 15% BSFLm ($P < 0.05$). With an inclusion of 5.0% BSFLm there was a significant increase ($P < 0.001$) in live weight, hot carcass weight, and cold carcass weight. There were significant decreases ($P < 0.001$) in those parameters with the inclusion of BSFLm at 10% and 15%. There are significant increases ($P < 0.003$) in the L*, or white value, in birds being fed BSFLm in comparison to the control birds. Significant decreases ($P < 0.03$) were observed in the a* value, or the redness value, in birds being fed diets of BSFLm when compared with control birds. However, these differences were not observed in experiment 2. In conclusion, BSFLm can replace soybean meal without any adverse effects on the performance of the chickens up to 5%. Moreover, BSFLo can replace soybean oil without adversely affecting broiler performance

Key Words: black soldier fly larvae, replacement, broiler performance

Student Competition: Metabolism and Nutrition: Vitamins and Minerals II

71 Tibia quality, tissue zinc concentration and gut integrity of broiler chickens in response to different sources of zinc. T.

T. H. (Anna) Nguyen*, N. K. Morgan, J. R. Roberts, S. B. Wu, M. Toghyani, and R. A. Swick, *University of New England, Australia, Armidale, New South Wales, Australia.*

Zinc (Zn) is essential for bone development, normal intestinal barrier function and regeneration of damaged gut epithelium. Zinc hydroxychloride has covalent bonds and thus is less reactive both in the feed and on the gastrointestinal tract epithelium compared with ionic salt forms such as zinc oxide or sulfate. The objective of this study was to evaluate the effect of different sources (zinc oxide, zinc sulfate and zinc hydroxychloride) and levels of zinc hydroxychloride (IntelliBondZn - IBZ) on tibia breaking strength and ash content, zinc deposition in the liver and breast meat and gut integrity in broilers fed wheat and soybean meal based diets. A total of 784 one-day-old male Ross 308 chicks were randomly assigned to 7 dietary treatments consisting of 7 replicates of 16 chicks per pen. The 7 dietary treatments were: a positive control (PC) containing zinc from an ionic source combination (50 ppm ZnO and 50 ppm ZnSO₄), a negative control (NC) without zinc (0 ppm of Zn) and 5 treatments containing zinc from IBZ at 20, 40, 60, 80, 100 ppm, respectively. Broilers were fed starter (d1 - d14) and grower (d14 - d35) diets. On d 14, blood was collected from 3 birds/ replicate 2.5 h after they were gavaged with 1 mL fluorescein isothiocyanate-dextran (FITC-d) solution to evaluate leaky gut using a microplate reader. On d 35, the right tibia, liver and breast were excised from 3 birds/ replicate after euthanasia. Tibias were subjected to breaking strength by a Lloyd Tensile Testing Instrument. Zinc concentration in the liver and breast meat were determined using inductively coupled plasma optical emission spectrometer (ICP-OES) technique. All data were analyzed via a one-way ANOVA. Means with significant differences were separated by Tukey's HSD test at $P < 0.05$. Supplementation with 100 ppm Zn from IBZ resulted in the highest tibia breaking strength at d35 (426 N/mm², $P < 0.05$), compared with the NC (367 N/mm²) and 20 ppm IBZ groups (368 N/mm²), but tibia ash content was not affected by the treatments (average of 44.6%, $P > 0.05$). Inclusion of IBZ at 100 ppm in the diet tended to increase liver Zn content compared with the NC diet (88.9 vs. 80.82 µg/g, $P = 0.07$). The breast meat zinc content was the lowest in the NC bird group (19.50 µg/g, $P < 0.05$) compared with all the other treatments. No significant effect of treatment was observed on the measured FITC values (average of 0.1038 µg/mL, $P > 0.05$), suggesting that gut integrity was not influenced by dietary Zn source or level. These results indicate that supplementation of 100 ppm Zn from IBZ is beneficial to broiler chickens in bone development and nutrient values of breast meat.

Key Words: broiler, zinc hydroxychloride, bone strength, tissue zinc, gut integrity

72 Supplemental micro mineral amino acid complexes lead to further benefits when compared to traditional supplements.

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A study was conducted to evaluate the effect of the partial replacement of supplemental micro minerals with amino acid complexes (AAC) in broiler breeder feeds. A total of 640 Cobb 500 broiler breeder hens, 22

wks old, were placed in 32 floor pens, 20 hens and 2 roosters per pen. Treatments were provided from 26 to 66 wks after a 4 wks adaptation period. The experimental diets were composed by 4 supplemental sources and levels of Zn, Mn, Cu and Se: a Control treatment sole from sulfate (Zn: 100/0, Mn: 100/0, Cu: 10/0, Fe: 50/0, and Se: 0.30/0 ppm), partial replacement of AAC, except iron (Zn: 60/40, Mn: 60/40, Cu: 13/7, Fe: 50/0, and Se: 0.15/0.15 ppm), partial replacement of AAC (Zn: 60/40, Mn: 60/40, Cu: 13/7, Fe: 10/40, and Se: 0.15/0.15 ppm) and On Top supplementation of AAC (Zn: 100/40, Mn: 100/40, Cu: 10/7, Fe: 50/40, and Se: 0.30/0.15 ppm). Eggshell breaking strength significantly increased ($P < 0.05$) for the On Top supplementation of AAC (3.66, 3.71, 3.78 and 3.95 kg/cm², respectively) whereas eggshell thickness presented a significant interaction between dietary treatment and period ($P < 0.05$) with diets only affecting after 55 wks (396, 398, 402 and 413 µm, respectively). Eggs from 65 wks were incubated and the progeny was fed a common diet. The progeny from partial replacements as well as from the On Top supplementation had higher body weight gain in the first wk ($P < 0.05$) and a better feed conversion to 35 d ($P < 0.05$). In conclusion, supplementation of diets for broiler breeder hens with AAC improved eggshell quality, progeny body weight gain for 1 to 7 d and feed conversion ratio for 1 to 35 d.

Key Words: performance, supplementation, feed conversion, eggshell, progeny

73 Effect of cage type and dietary limestone particle size on growth performance, nutrients retention and long bones quality in Lohmann LSL-Lite pullets. Tanka Khanal*, Gregoy Bedecarrats, Tina Widowski, and Elijah Kiarie, *University of Guelph, Guelph, Ontario, Canada.*

This study reports the effect of cage type and dietary limestone particle size (LPS) on pullet growth performance, gizzard development, apparent retention (AR) of nutrients, and quality of femur and tibia at 16 wks of age (woa). The treatments were arranged in a 2x3 factorial; with cage (conventional, CC; and furnished with perches, FC) and LPS (fine, < 0.6 mm, F; medium, 0.6 to > 1.7 mm, M; and 1:1 mixture of F and M wt/wt; FM). A total of 900-d old Lohmann LSL-Lite chicks were placed in CC (20 chicks/cage; 270 cm²/chick) and FC (30 chicks/cage, 636 cm²/chick) based on BW. The 3 LPS were offered in starter (0–4 woa), grower (5–12 woa) and developer (13–16 woa) feeding program according to breeder's nutrient specifications. Within cage type, the diets were allocated in a completely randomized design to give 6 replicates. Birds had free access to feed and water. The feed intake (FI) and BW were monitored. At the end of each phase, 2 birds/cage were euthanized for sampling. At the last wk of grower and developer phases, 1 bird/cage was transferred to a digestibility cage for AR measurements. Data were analyzed for effects of cage, LPS and their interactions using a 2-way ANOVA. There was no interaction ($P > 0.05$) between cage and LPS on cumulative FI (CFI), final BW (FBW), and empty gizzard weight in any phases. There was a cage effect on CFI and FBW. At the end of starter, CFI (689 vs. 599 g/bird, ~5.02%, $P = < 0.0001$) and FBW (262 vs. 254 g/bird, ~4%, $P = 0.002$) were higher for birds in CC than in FC but the reverse was observed at the end of developer (CFI: 1,810 vs. 1,612 g/bird, ~12.3% $P = < 0.0001$; FBW: 1,235 vs. 1,160 g/bird, ~6.42; $P = < 0.0001$). The AR of nutrients was affected by cage but not by LPS in developer phase. Birds in CC had higher AR of dry matter ($P = 0.013$), organic matter ($P = 0.024$) and ash ($P = 0.002$). The cage type did not ($P > 0.05$) interact with LPS on femur and tibia breaking strength (BS).

At the end of starter phase, birds in CC had higher femur (102 vs. 95 N, ~7.4% $P = 0.067$) and tibia (93 vs. 83, ~12.1%, $P = 0.012$) BS than birds in FC. However, at the end of the developer phase, birds in FC had higher tibia BS (197 vs. 183 N, ~7.6%, $P = 0.035$) than those in CC but femur BS was similar (176 vs. 167 N, $P = 0.264$). In conclusion, the

cumulative feed intake, body weight, nutrient retention and long bone breaking strength of pullets at different phases were affected by cage type but not by the dietary limestone particle size.

Key Words: cage, particle size, limestone, bone, pullets

Extension and Instruction

74 An environmental scan of recruitment practices in poultry science departments located in the United States. Jessica Wells*, Laura Downey, Tom Tabler, Kelley Wamsley, and Chris McDaniel, *Mississippi State University, Mississippi State, Mississippi, United States.*

The commercial poultry industry in the US ranks as the world's largest poultry producer. With such a large industry that continues to grow, the demand for new jobs in this industry also rises, and they rely, in part, on poultry science graduates from various land grant universities to fill these new positions. Currently, poultry science departments do not graduate enough students to fill positions, and their recruitment efforts are not well documented. An environmental scan of current recruitment practices assessed these recruitment programs to build upon them. Environmental scans identify, study, and analyze current, as well as emerging forces that exist in the external environment of a given organization and should be conducted continuously. The current research was performed to determine the recruitment efforts utilized in the remaining 6 degree-granting poultry science departments nationwide. An environmental scan was conducted with these departments to document current recruitment plans, their perceived effectiveness, as well as 5-year student enrollment numbers and graduation rates. These data were collected from department heads through survey methods and concluded that all poultry science departments have a functioning recruitment program, with an average yearly recruiting budget of \$15,832 and an average of 0.79 employees recruiting. Overall, the poultry science department heads perceived campus visits to be the most effective recruitment method. Also, a linear increase of enrolled poultry science students across all 6 departments was observed over the last 5 years ($P = 0.0491$), with the average enrolled number of students for a department being 68.9 students. However, only 3 departments exhibited a linear increase in enrolled students over this same 5 years: Department 1 ($P = 0.0343$), Department 2 ($P = 0.0099$) and Department 6 ($P = 0.0096$). These findings can be utilized to begin building solid recruitment plans for the remaining poultry science departments.

Key Words: recruitment, undergraduate, enrollment, environmental scan

75 Current recruitment practices and their relationship with student numbers, graduation rates, and satisfaction within poultry science. Jessica Wells*, Laura Downey, Tom Tabler, Kelley Wamsley, and Chris McDaniel, *Mississippi State University, Mississippi State, Mississippi, United States.*

As early as 1972 and again in 1992, reports of stagnant numbers in enrollment of poultry science students have been observed. Even more recently in 2003, declines in poultry science student enrollment was observed. Also, in 2009, the demand for University of Georgia poultry science graduates to fill vacant positions within the industry exceeded the number of students graduating. With such small numbers of graduates and an increase in demand for graduates, it is important to understand how to recruit poultry science students to increase enrollment and work toward meeting industry demands. Therefore, this study was conducted to determine the effectiveness of recruitment efforts utilized in poultry science departments nationwide. The current study utilized data previously collected from department heads of the 6 poultry science departments and a survey conducted with poultry science students currently enrolled in these departments. Data were compared with determine if

current recruitment efforts influence student enrollment, graduation rates or student satisfaction. Five of the 6 departments indicated having a current recruitment plan. Additionally, the single department without a recruitment plan also had 242% fewer enrolled students over the course of the last 5 yr than the average of the departments with a recruitment plan. When given 5 choices ranging from "Not Effective" to "Highly Effective," the study concluded that different recruitment practices were found effective by the 6 poultry science department heads. However, a few of the activities found effective by schools with high enrollment numbers were not viewed as effective by departments with lower enrollment numbers, suggesting that these activities have importance. These activities include recruiting at FFA national convention, direct mailing, brochures/pamphlets, and hosting 4-H/FFA poultry activities. In fact, the department that, on average, considered all recruitment activities the least effective also had the lowest enrollment but the highest student satisfaction. However, it must be noted, general recruiting budgets and number of employees that recruit were not statistically correlated ($P > 0.05$) with enrolled student numbers, graduation rate, or student satisfaction. Overall, these findings suggest that there are recruitment efforts in place that may be more effective than others. Additionally, some alterations may need to be developed to strengthen recruitment efforts for poultry science departments.

Key Words: recruitment, undergraduate, enrollment, graduation rate, student satisfaction

76 Internal and external factors influencing poultry science student college choice. Jessica Wells*, Laura Downey, Tom Tabler, Kelley Wamsley, and Chris McDaniel, *Mississippi State University, Mississippi State, Mississippi, United States.*

Chapman's model of student college choice identifies internal and external factors that influence a student's choices in college selection. Many authors have used this model to explore factors that influence a student's selection of a major in agriculture and life science. However, only one published study has evaluated factors of influence on student choice to major in poultry science. Therefore, there is a need for a more current identification of the factors that influence a student's choice to major in poultry science. In the current study, a survey method was utilized to collect data from poultry science majors nationwide, detailing the most influential factors in their selection of major. These data were also compared with results obtained in a companion study pertaining to what poultry department heads perceive to be effective methods of recruitment in poultry science. Data from the current study revealed that conversations with a poultry faculty member or department representative and on-campus activities (i.e., FFA, 4-H, etc.) were the most influential in efforts to communicate. Fixed college factors that were most influential were cost, scholarships, employment opportunities, and preparation for future employment. Furthermore, the most "influential significant persons" were high school agriculture teachers and parents. With regards to "agricultural backgrounds" and "involvement in agriculture activities," 58% of students reported having an agriculture background, and of those students, 52% specify their background being in some type of poultry. Also, 65% of students reported being involved in "agricultural youth activities" with 57% being "poultry youth activities." It was also determined that some current recruitment practices that were favored by students were also viewed as "Highly Effective" by department heads when given a choice of 5 options ranging from "Not Effective" to "Highly Effective." These included on-campus student activities such

as FFA and 4-H and letter and/or information mailed from a poultry faculty member. However, department heads and students did not seem to agree on the usefulness of some recruitment methods. Students viewed participation in on-campus recruitment events, social media, brochures, and visits to high schools as not very favorable recruitment methods, but department heads viewed them as “Highly Effective.” These results will hopefully be utilized to develop effective recruitment practices based upon factors of influence on poultry science students’ choice of major.

Key Words: recruitment, undergraduate, enrollment, graduation rate, student satisfaction

77 Self-perceived barriers of women in leadership roles in science and agriculture. Christine Alvarado¹, Casey Owens², and Julie Harlin*¹, ¹*Texas A&M University, College Station, Texas, United States*, ²*University of Arkansas, Fayetteville, Arkansas, United States*.

Women are under-represented in leadership positions in science and agriculture, despite increasing numbers of women in these professions. The purpose of the study was to explore the barriers for women to move into these types of leadership positions. This study used a voluntary sample descriptive survey design that included 8 demographic, 11 importance/satisfaction, 7 negative/positive attribution, 11 barrier, and 2 open-ended questions. Responses were collected over a one-week period using women in agriculture specific social networking sites resulting in 60 respondents. Respondents were all women in leadership roles in science and agriculture with an average age of 44 years, and the majority (68.3%)

were married, with children (63.3%), making between \$60–120,000 per year. Respondents were highly educated with 63.3% having either a Masters or Doctoral degree, and 45% of respondents traveled more than 2 to 3 times per month. The largest discrepancies between rated importance and satisfaction were the availability of leadership development, mentoring opportunities, and responsibilities to raising children (discrepancy scores of 1.35, 1.33, and 0.80, respectively). Based on these respondents, it appears that women are generally able to manage responsibilities at home and feel they have support of friends, family, and significant others. Self-perceived negative attributes (guilt, fear, stress, anxiety, etc.) were relatively neutral (2.93) and positive attributes (confident, satisfied, happy, fulfilled, etc.) were relatively positive (3.50). Topping the list of identified barriers were Family Responsibilities (75.4%); Advancement Opportunities (71.9%); Time (57.9%); Home Responsibilities (57.9%); and Mentoring (47.4%). Though results of this study are limited to this group of respondents, it is an important step in understanding the barriers women face in leadership roles in science and agriculture. The perceived barrier of family responsibility from women with and without children as well as the perceived lack of mentoring and leadership development opportunities warrant further study from both a quantitative and qualitative approach to develop a better understanding of these complex issues.

Key Words: women, gender, agriculture, inequality, survey

78 Not Presented

Genetics and Genomics

79 Assessing MHC-B diversity in Silkie chickens. Katy Tarrant*¹, Rodrigo Lopez¹, Meghan Loper², and Janet Fulton³, ¹Fresno State, Fresno, California, United States, ²Pitman Farms, Fresno, California, United States, ³Hy-Line International, Dallas Center, Iowa, United States.

Known as a region which influences disease susceptibility and resistance in chickens, the major histocompatibility complex-B (MHC-B) is a highly polymorphic region on chromosome 16. Variability of MHC-B haplotypes between genetic lines has previously been identified. This study aims to understand MHC-B diversity in the Silkie breed utilizing a high-density SNP panel that encompasses the chicken MHC-B region. Blood samples from 74 females and 27 males were collected from a 5,000-head closed commercial Silkie breeder colony. The colony is maintained through minimal genetic selection practices. The only parameter utilized is selection against red-colored combs, as black combs are preferred in the consumer meat market. Further, a color other than black, or dark mulberry, is not ideal according to the breed standard. A previously described 90 SNP panel within the MHC-B region was used to evaluate the commercial population. MHC-B haplotypes were identified, and compared with haplotypes identified within the region from other breeds. Of the 27 haplotypes identified using the SNP panel, 8 have been previously described, and are commonly found in broiler and dual purpose breeds. Nineteen haplotypes are novel haplotypes discovered within this population, and include novel recombinants. Six haplotypes found have a frequency of greater than 5% in the population, of which, 2 are novel. Rare haplotypes (<5%) in the population account for 59% of the population. The novel Silkie haplotypes and recombinants were observed in 2 individuals at a frequency of 2%. Finally, Hardy Weinberg Equilibrium (HWE) was calculated for haplotypes, which were found not to be in HWE ($P < 0.001$). This study shows considerable MHC-B diversity in the Silkie breed and adds further information on diversity of the MHC-B region.

Key Words: Silkie, MHC-B, haplotypes

80 Whole-genome resequencing identifies potential genes for cold adaption in the Chantecler chicken breed. Naiyi Xu², Wei Si¹, Ming Li², Weiwei Fu², Mian Gong², Yudong Cai², Jean-Marc Larivière³, Yu Jiang², and Xin Zhao*¹, ¹McGill University, Ste. Anne de Bellevue, Quebec, Canada, ²Northwest A&F University, Yangling, China, ³ITA La Pocatière, La Pocatière, Québec, Canada.

Cold exposure is one of the most important physical environmental stressors, which significantly affects the health and welfare of poultry, especially in cold regions. The Chantecler is a unique dual-purpose breed of chicken that was developed in Québec, Canada to withstand extreme cold weather. However, the underlying genetic mechanisms of cold adaptation have not been elucidated. Thus, 10 white color Chantecler chickens were re-sequenced and the whole genome sequencing data were subjected to SNP calling, PCA analysis and selection sweep signal analysis along with 30 Middle East native chickens, 31 South Asian native chickens, 23 South East Asian native chickens and 32 Chinese native chickens. In total, 16984731 SNPs were called, using the Genome Analysis ToolKit (GATK). The PCA analysis showed that Chantecler was close to Middle East local chickens, suggesting that its ancestors might originate from Middle East. Based on top 1% population differentiation (FST) and the top 1% nucleotide diversity (π) ratio between Chantecler and the other chickens, the selection sweep signal

analysis identified 44 overlapping genes. Among these 44 genes, *CDH23* is involved in the formation of hair bundles and/or feather follicles and *ESR1* is responsible for bird's sexual development, reproductive function and skeletal tissue, while *ZDHHC8* and *TIAM2* are involved in lipid metabolism. Considering that skeleton development, lipid metabolism and feather development are vital in bird thermoregulation, we hypothesize that *CDH23*, *ESR1*, *ZDHHC8* and *TIAM2* may play important roles in Chantecler's adaptation to low temperature environment. Our findings have provided important information in identifying cold resistant-related genes in poultry and for understanding the mechanism of adaptation to climatic conditions in Chantecler chickens.

Key Words: Chantecler chicken, whole-genome resequencing, cold resistance

81 Analysis of genome wide DNA methylation in *Coturnix japonica* as a result of dietary methyl donors. Chelsea Phillips*¹, Roselina Angel², and Chris Ashwell¹, ¹North Carolina State University, Raleigh, North Carolina, United States, ²University of Maryland, College Park, Maryland, United States.

Nutrition provided during different stages of life can alter epigenetic regulation. The methylation of DNA requires enzymes and many dietary micronutrients referred to as methyl donors. Studies assessing parental methyl donor supplementation have indicated that parental nutrition plays a role in shaping the epigenetic pattern of the offspring. A diet that is high in methyl donors (HiMet) may alter DNA methylation in progeny compared with a control (CON). The methyl donors included 7030 mg/kg choline chloride, 5 mg/kg betaine, 1.5 mg/kg vitamin B12, 7.5 mg/kg folic acid, 12 mg/kg pyridoxine, and 99 mg/kg zinc sulfate in addition to nutrients endogenous to the dietary feedstuffs. In the following study, quail parents were fed either the HiMet or CON diet in the starter and layer diets, yielding the following treatments: HiMet-HiMet, HiMet-CON, CON-HiMet, and CON-CON. For the parent generation of this multi-generational trial, 300 Japanese Quail were placed in a brooder at day of hatch. 150 chicks received the CON diet while the remaining 150 received the Hi-MET diet. At 6 wk, half of each group continued on the same diet while the other half switched diets. Two generations of progeny from both treatments were evaluated, all receiving a control diet. Liver gDNA was isolated, pooled and bisulfite converted for 12 samples from the 3 generations of female quail and 4 treatments while the remaining gDNA from the 12 samples served as the non-bisulfite treated original sequence for a comparative analysis. After conversion, single stranded DNA was used to create gDNA libraries using TruSeq DNA Methylation Kit. Libraries were sequenced using Illumina NextSeq with 75bp paired end runs with 800 million paired end reads total. DNA sequence reads were mapped using CLC Genomics workbench. Percent CpG hypermethylation and hypomethylation was determined for each generation and treatment. Overall most CpG sites were unmethylated. The top differentially methylated regions were evaluated using Ingenuity Pathway Analysis to determine molecular networks associated with each treatment. Metabolic pathways surrounding methionine were found to be impacted long-term. Epigenetic changes associated with nutrient availability in the offspring leads to further questions regarding the mechanisms behind the alterations of the epigenetic landscape for the expression or suppression of specific genes. Further research is warranted on the manipulation of breeder diets to impart positive epigenetic effects on progeny. These ideas could be a useful tool to the poultry

industry by manipulating the environmental conditions of the parents to better prepare the offspring for future environmental conditions.

Key Words: epigenetics, methyl donors, DNA methylation, metabolism

82 Genomic prediction of feed efficiency, body weight, breast meat yield and walking ability in a turkey population (*Meleagris gallopavo*). Emhimad Abdallah^{*1}, Ben Wood², Flavio Schenkel¹, Ryley Vanderhout¹, Hakimeh Emamgholi Begli¹, Owen Willems², and Christine Baes¹, ¹University of Guelph, Guelph, Ontario, Canada, ²Hybrid Turkeys, Guelph, Ontario, Canada.

The use of molecular markers to enhance prediction of breeding values for quantitative traits has led to more rapid genetic improvement in animal breeding programs. The objective of this study was to compare pedigree-based best linear unbiased prediction (PBLUP) to 4 2-step genomic prediction models (GBLUP, Bayes A, Bayes B and Bayesian Lasso) in terms of prediction accuracy and bias in a turkey population. For the 2-step evaluation, 2 alternate pseudo-phenotypes were considered: estimated breeding values (EBVs) and deregressed proofs (DRPs). Records of feed conversion ratio, residual feed intake, body weight, breast meat yield and walking ability were obtained for 5,592, 5,592, 170,844, 9,634 and 170,844 animals, respectively. The data was provided by Hybrid Turkeys, Kitchener. The predictive ability of each model was assessed by placing approximately 10% of the youngest animals in a validation group, while the remaining subset was used to train the models. For all studied traits, the 2-step models outperformed the pedigree-based model with an increase in validation accuracy ranging between 8% to 36%. In general, only small gains in accuracy were observed by using a specific 2-step model for each trait compared with using GBLUP for all traits. No clear pattern for bias was observed across 2-step models, but they tended to yield slightly inflated predictions in contrast to the traditional pedigree BLUP, which produced slightly deflated predictions across traits, except for RFI. In general, the use of EBVs as pseudo phenotypes yielded to slightly increased accuracy of predictions with no clear advantage in terms of bias when compared with DRPs. This initial evaluation of genomic selection in turkey seems promising and incorporating genomic information may significantly contribute to increasing genetic progress for key traits in turkey populations.

Key Words: genomic selection, GBLUP, pedigree BLUP, two-step, Bayesian

83 Differential gene expression associated with long-term sperm storage in the turkey, *Meleagris gallopavo*. Katina Krasnec* and Julie Long, United States Department of Agriculture, Beltsville, Maryland, United States.

Sperm storage in the female reproductive tract is a biological feature common in avian species. Sperm residing in the turkey hen's sperm storage tubules (SSTs) retain fertilizing ability for 10 weeks after a single insemination; however, the molecular and cellular mechanisms of SST function remain unknown. Previous work elucidated differentially expressed genes (DEGs) of SSTs over time in the absence of sperm. To assess the effect of sperm on DEGs in the SSTs, non-inseminated (n = 10) and inseminated (twice pre-lay and once immediately after onset of egg lay; n = 22) hens were sampled at the onset (d 1), middle (d 30), and end (d 90) of reproduction. The utero-vaginal junction region containing SSTs was dissected and flash frozen. Laser capture microdissection was used to isolate SSTs from the surrounding epithelial tissue for RNA extraction. Approximately 70–80 million fragments of

150bp paired-end reads per sample were generated using an Illumina NextSeq. Sequences were aligned to the turkey reference genome (v. 5.0) and annotations were used to identify gene transcripts. At each time point, pairwise comparisons between hen groups were performed to identify DEGs that were significantly (± 1.5 -fold change, FDR p-value ≤ 0.05) up- or downregulated, relative to inseminated hens. A total of 349 unique genes were differentially expressed from onset to end of reproduction. Most DEGs occurred during the middle of reproduction compared with onset and end; however, at each time point, the pattern of expression varied. At onset of reproduction, 77.7% of DEGs were upregulated; while at the middle, 75.6% of DEGs were downregulated. By the end of reproduction, 36.3% and 63.6% were up- and downregulated, respectively. The DEGs were broadly categorized using Gene Ontology terms for molecular function including: activity (catalytic, structural, receptor, molecular transducer, and transporter), binding, and regulation. At onset of reproduction, DEGs were primarily involved in transport of ions (e.g., sodium, zinc, potassium), and catalytic activity, suggesting that these functions are important for the initial residence of sperm within the SSTs. During the middle of reproduction, DEGs were associated with functions such as receptor/signal transducer activity and transport, where potential functions could be to reduce or prevent cell death or immune response against sperm. At the end of reproduction, when most of the SSTs did not contain sperm, functions were largely related to catalytic activity and apoptosis. The overall pattern of gene regulation and ontology of SSTs with or without sperm provides novel insights regarding the function of this unique reproductive structure.

Key Words: reproduction, sperm storage tubules, turkey, insemination, RNASeq

84 Expression of genes encoding proteins associated with nutrient uptake at either the brush border (BB) or basolateral (BL) surface of the intestine, in six strains of chickens with different growth potential. Katarzyna Miska*, Lori Schreier, Stanislaw Kahl, Beverly Russell, Yang Qu, and Monika Proszkowiec-Weglarz, USDA Agricultural Research Service, Beltsville, Maryland, United States.

Over the last 60 years broiler chickens have been selected for rapid growth while maintaining a low feed conversion ratio (FCR). Today's broilers can attain FCR of approximately 1.5 while undergoing rapid weight gain, making them more efficient. This study was designed to compare expression of genes that encode proteins located at either the BB or BL surface of the gut epithelium among fast and slow growing broiler strains. Gene expression changes between fast and slow growing broilers may reflect differences in uptake of nutrients. Six strains of chicks with different growth capacities were used: Ross 708, Hubbard H1 (HH1), Cobb500, Longnecker's Heritage (LHR), Red-Bro, and the Athens Canadian Randombred Control (ACRBC). Fertile eggs were hatched, and birds were raised in floor pens and sampled at embryonic d 19, day of hatch, and days (d) 7, 14, 21, 28 and 35 post-hatch (PH). At each sampling a portion of the duodenum, jejunum, ileum, and ceca were snap frozen for RNA isolation. Performance parameters indicated that Ross 708, HH1, and Cobb500 had the greatest body weights (BW), reaching 2,172 g, 2,320 g, and 2137 g respectively at d35 PH. Red Bro and LHR birds attained BWs of 1,308 g and 1,436 g, while ACRBCs (not selected for rapid growth) weighed only 421 g at d35 PH. Quantitative RT-PCR was performed on 14 genes which encode proteins associated with nutrient processing and uptake. Analysis was carried out using SigmaPlot 14 (ANOVA) of 8 genes which encode proteins associated with the BB of the gut epithelium, including: Aminopeptidase N, 4 amino acid transporters, (ATB⁰⁺, B⁰AT, b⁰⁺AT, EAAT3) a di- and

tri-peptide transporter (PepT1), and 2 sugar transporters (GLUT5 and SGLT1). Additionally, analysis of 5 genes which encode proteins associated with the BL gut epithelium: 4 amino acid transporters (CAT1, CAT2, LAT1, and γ^+ LAT1), and a single sugar transporter (GLUT2). The results indicate that while strain specific differences in gene expression were noted they did not correlate with growth rate. In most cases, genes encoding BB associated proteins increased in expression over time ($P < 0.05$) in the duodenum, jejunum, and ileum, while in the ceca the expression decreased. On the other hand, genes encoding BL

associated proteins decreased ($P < 0.05$) in expression over time in all gut segments with exception of GLUT2 which increased in expression in the small intestine. The temporal changes in gene expression were very consistent among bird strains. In conclusion temporal and strain differences in gene expression were noted, however, differences may not be associated with growth efficiency.

Key Words: nutrient transporters, broilers, gene expression

Student Competition: Metabolism and Nutrition: Enzymes

85 Phytase efficacy during the starter phase of broiler chickens; the influence of age and feeding length. Olufemi Babatunde*¹, Aaron Cowieson², Jonathan Wilson², and Olayiwola Adeola¹, ¹Purdue University, West Lafayette, Indiana, United States, ²DSM Nutritional Products, Kaiseraugst, Switzerland.

Phytase is of importance to the poultry industry because of its ability to hydrolyze phytate and release phosphorus (P) for use by poultry. However, the effect of age on phytase efficacy is not fully understood. A total of 864 d-old broiler chicks were used to investigate the effect of age and feeding length on phytase efficacy using growth performance, mineral utilization, tibia ash and plasma myo-inositol concentration as response criteria of evaluation. The experiment was arranged as a 3 × 2 × 2 factorial in a randomized complete block design with 3 diets including; a positive control (PC) (0.4% non-phytate P (nPP)), a negative control (NC) (0.2% nPP) and a NC diet supplemented with phytase at 2,000 FYT/kg; 2 ages (i.e., d 14 and 22); and 2 feeding lengths (i.e., 2 and 5 d) with 8 replicates each. Data were analyzed using the general linear procedure of SAS with age, feeding length and diet serving as the main effects. Orthogonal contrasts were used to analyze the effect of age and feeding length on phytase efficacy and significance was set at $P \leq 0.05$. Birds fed the NC diet had decreased ($P < 0.01$) body weight, gain and feed efficiency compared with birds fed the PC diet regardless of age or feeding length. Similarly, birds fed the phytase-supplemented diet had improved ($P < 0.01$) performance as compared with birds fed the NC diet regardless of age. Birds fed the NC had reduced ($P < 0.01$) P and calcium (Ca) digestibility as compared with birds on the PC diet regardless of age or feeding length. However, supplementation with phytase improved ($P < 0.01$) the digestibility and retention of both minerals. There were no significant differences in P utilization between birds fed for 2 d to d 14 or 22 and birds fed for 5 d to both ages. However, phytase was more efficacious at d 14 than d 22 when mineral utilization was considered because the super dose of phytase elicited greater response in birds fed the phytase supplemented diet for 2 d until d 14. In contrast, percentage tibia ash improved ($P < 0.01$) in birds fed phytase supplemented diet for 5 d at both ages with an average improvement of 8.5% as compared with birds fed for 2 d with an average increase of 3% in tibia ash. Phytase supplementation increased the concentration of plasma myo-inositol in birds fed for 2 or 5 d until d 14 and 22 as compared with birds fed the NC diet for the same periods. However, the highest phytase induced improvement to myo-inositol concentration was observed in birds fed for 5 d until d 14. In conclusion, testing phytase products, even at high doses, for 2 d during the second week in the life cycle of broiler chicks, can be recommended from the results of this study.

Key Words: age, broilers, feeding length, phosphorus, phytase efficacy

86 Effect of thermo-stable phytase on growth performance and bone mineral content in broilers. Fernanda Castro*¹, Thomas Shieh², and Woo Kim¹, ¹University of Georgia, Athens, Georgia, United States, ²Vitech Ultra Bioscience Corp., Orange, California, United States.

The aim was to investigate the effect of a thermo-stable phytase (provided by Vitech Ultra Bioscience Corp.) on growth performance and bone mineral content of broilers. A total of 600-d-old male chicks (Cobb 500) were distributed randomly in 5 treatments, with 6 replicates of 20 birds. The treatments were a positive control diet (PC: 3000, 3090, 3170 kcal/kg of ME and 0.45, 0.42 and 0.38% of available phosphorus

for starter, grower and finisher diets, respectively), a negative control diet (NC: reduction in 40kcal/kg ME and 0.13% available phosphorus), and the NC diet supplemented with either 50, 100 or 200 g/T of Vitazyme Phy (T1, T2 and T3, respectively). At 14, 28 and 42 d, feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were measured. At 42 d, one bird/rep was euthanized to collect tibia for mineral composition evaluation. One-way ANOVA was performed, and means were compared by Tukey's test ($P < 0.05$). From 1 to 14 d, the BWG was higher for birds fed T3 compared with T1 and NC diet ($P < 0.001$). No other differences were found to be significant. From 14 to 28 d, BWG was higher for birds fed T3 than NC and T1, whereas the FCR was lower for birds fed T3 than NC and T1 ($P < 0.001$). From 28 to 42 d, FI was lower for birds fed NC than PC ($P = 0.036$). No other differences were found to be significant. Considering the overall period (1–42 d), BWG was the lowest and FCR the highest for birds fed NC diet ($P < 0.001$). Regarding tibia mineral content, birds fed the NC diet showed lower bone weight than birds fed T1, lower ash and Zn contents than birds supplemented with all levels of Vitazyme Phy, and the lowest Ca, Mg and P contents ($P < 0.021$). In conclusion, the use of 200g/T of Vitazyme Phy resulted in significantly better growth performance and tibia mineral content in broiler chickens compared with the negative control diet. Moreover, the use of 200g/T of Vitazyme Phy resulted in numerically superior performance results and bone mineral content than the birds fed the positive control diets.

Key Words: bone minerals, broilers, phytase

87 Viscosity and growth response of broilers fed high fiber diets supplemented with a corn-produced recombinant carbohydrase. Victoria Ayres*¹, Jon Broomhead², Xuemei Li², Michael Raab², and Joseph Moritz¹, ¹West Virginia University, Morgantown, West Virginia, United States, ²Agrivida Inc., Boston, Massachusetts, United States.

Feed ingredients containing high levels of non-starch polysaccharides (NSP), such as wheat and corn distillers dried grains with solubles (DDGS), can form viscous digesta in the gastrointestinal tract, limiting broiler performance. A study was conducted to determine efficacy of a corn-produced recombinant carbohydrase (AC1) on dietary and intestinal viscosity and broiler performance with a high NSP diet. Hubbard x Ross 708, day-old, male broiler chicks (n = 960) were fed one of 8 dietary treatments for 21 d. Diets included a corn-soybean meal based diet (PC_1) and a diet of similar essential nutrient density, but with a 10% inclusion of both wheat and DDGS (PC_2) and a negative control (NC) with similar ingredients as PC_2, but with ME reduced by 125 kcal/kg. Additional treatments had varying levels of AC1, supplying 50, 100, 200, 400, or 600 U β -Glucanase (β -Glu-U) per kg of feed, mixed into the NC diet. Dietary and digesta (d14) viscosity and weekly bird performance were measured. The inclusion of wheat and DDGS increased d1–7 feed conversion ratio (FCR) and supplementing AC1 at 50–400 β -Glu-U/kg reduced FCR equivalent to PC_1. Intestinal viscosity correlated with d1–21 FCR and inversely correlated with d1–21 live weight gain (LWG). When analyzed categorically, dietary viscosity inversely correlated with d1–21 LWG ($P < 0.05$). This study demonstrates that AC1 can reduce intestinal viscosity and improve early FCR in birds fed high viscosity diets and that in vitro viscosity can correlate to broiler performance.

Key Words: broiler, NSP, in vivo, in vitro, β -glucanase

88 Interactive effects of fibrous feed ingredients and multi-enzyme supplement: Comparative impact on gizzard attributes, plasma uric acid concentration and excreta dry matter content in broiler chickens and turkey poults fed corn or wheat-based diets. Juan Sanchez*¹, Rob Patterson², and Elijah Kiarie¹, ¹University of Guelph, Guelph, Ontario, Canada, ²Canadian Bio-Systems Inc., Calgary, Alberta, Canada.

It has been suggested that poultry requires some level of structural dietary components such as fiber to stimulate gizzard development for enhanced digestion and overall gut health. However, provision of fibrous feedstuffs is known to reduce nutrient utilization. We evaluated the impact of adding fibrous feedstuffs in corn or wheat-based diets fed without or with multi-enzyme supplement (MES) on gizzard weight and content pH, plasma uric acid (PUA) and excreta DM in broiler chickens (Exp. 1) and turkey poults (Exp. 2). High fiber diets were created by adding corn DDGS and wheat middlings at 10% in corn and wheat-based diets, respectively. The MES supplied 2,500 U xylanase, 300 U β -glucanase, 700 U invertase, 10,000 U protease, 1,200 U cellulase, 24,000 U amylase and 20 U mannanase per kg of feed. All the diets met specifications of respective species and were prepared in pellet form. A total of 960 d-old Ross x Ross 708 male chicks (Exp. 1) and 720 d-old male hybrid toms (Exp. 2) were fed 8 diets ($n = 6$). Birds had free access to feed and water for 28 d. Excreta samples were taken on d 25 to 27 for excreta DM. On d 28, 2 birds/pen were bled for PUA concentration and subsequently sacrificed for gizzard pH, full and empty gizzard weight. Data were subjected to GLM procedures of SAS with grain, fiber, MES and associated interactions as fixed factors. In Exp. 1, there was no treatment effect ($P > 0.05$) on empty gizzard weight. However, wheat diets increased ($P = 0.01$) gizzard pH than corn diets whereas fiber increased ($P = 0.05$) full gizzard weight. In Exp 2, an interaction ($P = 0.03$) between grain and MES was such that MES increased empty gizzard weight in corn diets only. Additionally, corn diets resulted in heavier ($P = 0.001$) full gizzard weight than wheat diet and high fiber diets increased ($P = 0.04$) gizzard pH in turkey poults. In Exp. 1, an interaction ($P = 0.05$) between grain, fiber and MES was such that broilers fed low fiber wheat diet had higher PUA concentration compared with low fiber corn diet. In Exp 2, there was no treatment effect ($P > 0.05$) on PUA concentration. There was no interaction ($P > 0.05$) between grain, fiber and MES on excreta DM in both species. In Exp. 1, broilers fed wheat diets had higher excreta DM than broilers fed corn diets. Additionally, broilers fed high fiber diets had higher ($P < 0.05$) excreta DM than broilers fed low fiber diets. There were no treatments effects ($P > 0.05$) on excreta DM in poults. In conclusions, fiber and MES exerted different responses on gizzard development and acidification as well as on indices of nutrients utilization (PUA) and gut health (excreta DM) in broiler and turkey.

Key Words: broiler, turkey, fiber, uric acid, gizzard

89 Effects of graded levels of hullless barley and β -glucanase on β -glucan depolymerization, and digestive tract morphology and physiology of broiler chickens vaccinated for coccidiosis. Namalika Karunaratne*¹, Nancy Ames², Andrew Van Kessel¹, Michael Bedford³, Rex Newkirk¹, and Henry Classen¹, ¹University of Saskatchewan, Saskatoon, Saskatchewan, Canada, ²Agriculture and Agri Food Canada, Winnipeg, Manitoba, Canada, ³AB Vista, Marlborough, United Kingdom.

Exogenous β -glucanase (BG) in barley-based feed has previously been shown to reduce digesta viscosity in chickens, and thereby improve nutrient digestibility and bird performance. Less well studied is the potential for BG to convert barley β -glucan into low molecular weight (MW)

fermentable carbohydrates, which in turn may influence digestive tract function and enteric disease. The objective of the study was to evaluate the effect of BG dose on ileal digesta β -glucan depolymerization, and digestive tract morphology and physiology in broilers fed variable levels of hullless barley (HB). Broilers (5346) vaccinated for coccidiosis (Coccivac-B52, Merck Animal Health) at 5 d of age were fed graded levels of high β -glucan HB (0, 30 and 60% by replacing wheat) and β -glucanase (Econase GT 200P; 0, 0.01 and 0.1%) in a 3×3 factorial arrangement. Each treatment (trt) was assigned to 1 litter floor pen (66 birds) in each of 9 independent rooms. Digestive tract tissues and contents were sampled at d 11 and 33 to study ileal β -glucan MW, and intestinal and cecal morphology, pH and gene expression. Statistical significance level was $P \leq 0.05$. At both 11 and 33 d, the peak MW of β -glucan in ileal digesta decreased with increasing BG at 30 and 60% HB levels; BG did not affect peak MW when HB was not included in the diet. The maximum MW for the 10% smallest β -glucan molecules decreased with BG at both ages for the 30 and 60% HB trts; for birds fed the 0% HB diet, 0.1% BG decreased the maximum MW. Ileal pH increased with HB and BG at both 11 and 33 d. Cecal pH decreased with 0.1% BG at 60% HB at d 11. At d 33, cecal pH was higher at 30% HB compared with the 0 and 60% HB trts. With minor exceptions, histological morphology of the ileum (villi height and width, crypt depth, goblet cell numbers) was largely unaffected by trt. Relative gene expression of IL6 and IL8 in the ileum increased with 0.1% BG at d 11 and 33, respectively, while expression of ileal MUC2 decreased with 0.1% BG at d 33. Expression of ileal AvBD2 increased with 0.1% BG at d 11 in birds fed 30% HB diets. The HB by BG interaction was significant for ileal MCT1 expression on d 11, but differences were small. Ileal MCT1 expression at d 33 was lower in birds fed 30 and 60% HB diets. In the ceca, interactions between HB and BG were significant for MCT1 and MUC5ac on d 11, but dietary trt did not affect cecal gene expression on d 33. In conclusion, BG depolymerized high MW β -glucan in HB in a dose dependent manner, resulting in reduced peak MW and a higher proportion of small MW β -glucan molecules, but had minor effects on digestive tract morphological and physiological measurements.

Key Words: prebiotic, molecular weight, pH, fermentation, viscosity

90 High levels of phytase on performance, nutrient metabolizability, and bone composition of turkey poults. Lucas Bassi*¹, Leopoldo de Almeida¹, Levy Teixeira², Rafael Sens², Jonathan Wilson³, and Alex Maiorka¹, ¹Federal University of Paraná, Curitiba, Paraná, Brazil, ²DSM Nutritional Products, São Paulo, São Paulo, Brazil, ³DSM Nutritional Products, Kaiseraugst, Switzerland.

The aim of this study was to evaluate the performance, nutrient metabolizability and bone mineral composition of turkey poults fed diets with high levels of phytase inclusion. A total of 320 one-d-old male turkeys were distributed in a completely randomized design with 4 treatments with 8 replicates of 10 birds each. The birds received feed and water *ad libitum*. Feed was offered in mash form, based on corn-soybean meal and maize gluten meal. The targeted treatments were: positive control (PC), negative control (NC, -0.15% Av P and -0.18% Ca), NC + 2000 FYT/kg, NC + 4000 FYT/kg. Performance were measured by feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) from 1 to 28 d. Partial excreta samples were collected daily from 26 to 28 d-old, freeze-dried and grounded for analysis and calculation of the apparent metabolizability coefficient of dry matter (AMC_{DM}), mineral residue (AMC_{MR}), crude protein (AMC_{CP}), calcium (AMC_{Ca}), phosphorus (AMC_P) and apparent metabolizable energy (AME). At 28 d-old, 2 birds of each repetition were euthanized to remove tibiae to analyze Ca and P content. Data was submitted to ANOVA and means

compared with Tukey test at 5% probability. The regression analysis was conducted among NC diet and 2 inclusions levels of phytase for all parameters evaluated. The inclusion of 2000 and 4000 FYT/kg resulted in higher FI (1231.2 g and 1219.7 g respectively) and higher WG (810.9 g and 806.5 g respectively) when compared with NC diet (FI = 1167.9 g; WG = 738.6 g) ($P < 0.05$), both were similar to PC diet (FI = 1226 g; WG = 815.7 g). FCR was not affected by treatments ($P > 0.05$). Phytase showed a linear response on P content in the tibiae with maximum of 8.96% ($P < 0.05$), but it did not affect ($P > 0.05$) Ca content. Phytase presented a linear improvement ($P < 0.05$) for all AMC parameters evaluated with maximums of 71.74%, 57.93%, 69.88%, 75.33, 73.21% and 3486 kcal/kg for DM, MR, CP, Ca, P and AME, respectively. In conclusion, the supplementation of turkey diets with high levels of phytase improves metabolizability of nutrients and minerals, therefore increasing phosphorus content in the bone.

Key Words: enzymes, metabolizability, bone composition, phosphorus, turkey

91 Evaluation of the precision-fed rooster assay for detecting effects of supplemental carbohydrase enzymes on true metabolizable energy. Annalise Anderson*, Pamela Utterback, and Carl Parsons, University of Illinois at Urbana-Champaign, Urbana, Illinois, United States.

The precision-fed rooster assay has been used extensively to determine nitrogen-corrected true metabolizable energy (TME_n) of feed ingredients for poultry. However, this assay has not generally been used to evaluate effects of supplemental carbohydrase enzymes for this purpose. Therefore, 2 precision-fed rooster assays were conducted to evaluate several different carbohydrase enzymes on TME_n for a corn/soybean meal diet, a pearled barley diet, and diets containing different inclusion levels of rye/corn. In both rooster assays, Single Comb White Leghorn roosters were fasted for 26 h and then crop-intubated with either 25 or 30 g of the test diets, depending on the diet. Excreta were then collected quantitatively for 48 h after feeding. Data from all assays were analyzed by one-way ANOVA for completely randomized designs. In the first rooster assay, 6 carbohydrase combinations and/or levels were evaluated using a corn/soybean meal diet. All carbohydrases either numerically or significantly ($P < 0.05$) increased TME_n and the mean increase for the enzyme treatments was 0.066 kcal/g DM compared with the corn/soybean meal control diet. A single degree of freedom contrast for all diets containing carbohydrase versus the control was significant ($P < 0.05$). The second assay consisted of 20 dietary treatments; roosters were crop-intubated with 25 g of diets that were composed of 100% barley, 100% rye, 50% rye: 50% corn, or 25% rye: 75% corn. The 100% barley diet was fed with and without inclusion of 2 different levels of either glucanase only or 2 levels of a multi-carbohydrase blend. The rye-containing diets were fed with and without inclusion of 2 different levels of either xylanase only or 2 levels of the same multi-carbohydrase blend. As expected, TME_n of the 100% barley diet was significantly ($P < 0.05$) higher than the 100% rye diet, and TME_n of the diets containing rye increased ($P < 0.05$) as level of corn increased and rye decreased. Both glucanase alone and the multi-carbohydrase blend significantly ($P < 0.05$) increased TME_n of the 100% barley diet, with the blend increasing it from 3.722 to 4.086 kcal/g DM at the highest inclusion rate. The xylanase alone and multi-carbohydrase blend either numerically or significantly ($P < 0.05$) increased TME_n of the 100% rye diet, with the blend increasing it from 3.581 to 3.909 kcal/g DM at the highest inclusion rate. The magnitude of enzyme response decreased as the level of rye in the diets decreased. Overall, results of this study indicated that the precision-fed rooster assay

can detect effects of carbohydrase enzymes on TME_n of diets containing corn/soybean meal, pearled barley, and/or rye.

Key Words: carbohydrase, glucanase, xylanase, barley, rye

92 Effect of fine and coarse limestone particle size and addition of phytase on broiler live performance. Shuja Majeed*, Rasha Qudsieh, and Frank Edens, North Carolina State University, Raleigh, North Carolina, United States.

Limestone particle size can influence broiler performance by altering rate of Ca release in GI tract due to varying solubility. The objective of this research was to determine, using a 2 × 2 × 2 factorial arrangement in a completely randomized experimental design, the influence of limestone particle size (190 μm and 900 μm) and phytase supplementation (0 FYT/kg and 1000 FYT/kg) to diets formulated with 2 Ca and P levels (positive control [PC] and negative control [NC]) on broiler live performance. In the starter (0–14 d), PC had 0.90 Ca and 0.45 P, while NC had 0.72 Ca and 0.30 P. In the grower (15–35 d), PC had 0.76 and 0.38 while NC had 0.52 and 0.23 for Ca and P, respectively. Phytase supplemented was of bacterial origin (*Citrobacter braakii*) and limestone added (fine and coarse) in the diets was of Hubercarb® Q series which was obtained from mines in Quincy, Illinois, USA. Phytate P was determined to be 0.22% in the starter and 0.20% in the grower diets. The 8 experimental diets were randomly assigned to a total of 1512 birds, with 21 birds per pen and 9 pens per dietary treatment. Body weight (BW) and feed intake (FI) were recorded at 15, 28 and 35 d. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated for each period and the overall period (0–35 d). Pen was considered an experimental unit and Data were analyzed using SAS GLM procedure. The main effects of limestone particle size and Ca and P level had no influence on either BW, BWG, FI, or FCR among all periods of the study. Adding phytase improved BW among all periods ($P \leq 0.05$), BWG and FI at 0–14 d ($P \leq 0.05$) and 0–35 d ($P \leq 0.05$), but the improvement was not significant for FCR ($P \geq 0.05$). Phytase improved BWG from 0 to 35 d by 50 g. PC and addition of phytase to NC improved BW, BWG, and FI for 0–14 d ($P \leq 0.05$) and for BWG during the 14–28 d period ($P \leq 0.05$) when compared with NC without phytase. Birds fed PC without phytase, and PC or NC with phytase were about 96 g heavier than NC without phytase. The NC coarse and PC fine performed better ($P \leq 0.05$) than PC coarse and NC fine for BW at 14 and 28 d ($P \leq 0.05$), BWG for 0–14 d and 15–28 d ($P \leq 0.05$). The results indicate that phytase enzyme improved broiler performance without being affected by limestone particle size. The NC control supplemented with phytase performed similarly to PC and PC + phytase with respect of BW, BWG, and FI. With coarse limestone inclusion, a lower Ca and P level in the diet can be utilized without negatively affecting performance for the first 28 d.

Key Words: broilers, limestone particle size, phytase, calcium, phosphorus

93 Effects of inositol and elevated phytase supplementation on phytate degradation in gizzard content and ileal digesta of broilers from 1 to 43 days of age. Ruben Kriseldi*¹, Carrie Walk², Michael Bedford², and William Dozier, III¹, ¹Auburn University, Auburn, Alabama, United States, ²AB Vista, Marlborough, United Kingdom.

An experiment was conducted to evaluate effects of elevated phytase supplementation on phytate degradation in gizzard contents and ileal digesta of broilers from 1 to 43 d of age. A total of 1,920 Yield Plus × Ross 708 male chicks was placed in 64 floor pens (30 birds/pen; 0.07

m²/bird). Each pen received 1 of 8 dietary treatments from 1 to 43 d of age. Treatment 1, the negative control, was formulated to contain 0.16 and 0.15% lower Ca and P, respectively, compared with Treatment 7, the positive control. Treatments 2 to 6 were formulated similarly to Treatment 1 with phytase additions of 500, 1,500, 4,500, 13,500, and 40,500 phytase units (FTU)/kg, respectively. Treatment 8 was formulated similarly to Treatment 7 with the addition of inositol, which was calculated based on the possible maximum inositol liberation in Treatment 6. At 15, 29, and 43 d of age, gizzard contents and ileal digesta were collected for analysis of inositol and inositol phosphate (IP) 6, 5, 4, 3, and 2 concentrations. Increasing phytase activity from 0 to 40,500 FTU/kg resulted in log-quadratic reductions ($P < 0.01$) of IP6 and IP5 concentrations in the gizzard contents and ileal digesta of broilers at 15, 29, and 43 d of age. Similarly, the increase in phytase activity in Treatments 1 to 6 reduced IP4 and IP3 concentrations in a log-quadratic manner ($P < 0.05$) at each collection period in gizzard contents, but only at 43 d of age in the ileal digesta of broilers ($P < 0.01$). Broilers fed diets with increasing phytase activity did not alter ($P > 0.05$) IP2 concentrations in the ileal digesta at any age period. Total IP6 to IP2 concentrations in the ileal digesta of broilers had approximately 78 and 94% reductions when broilers were fed diets with 4,500 and 13,500 FTU/kg phytase, respectively, compared with diets having no phytase addition. Log-quadratic increases ($P < 0.05$) of inositol concentrations were observed in both gizzard contents and ileal digesta when increasing phytase activity from 0 to 40,500 FTU/kg at each collection period. The increase of inositol concentration due to elevated phytase supplementation also translated to a log-quadratic increase ($P < 0.01$) in plasma inositol concentration of broilers at 15, 29, and 43 d of age. Phytase supplementation of 4,500 FTU/kg or greater may benefit broilers by reducing inositol phosphate concentrations in the gizzard contents and ileal digesta, which may increase the availability of other nutrients. Moreover, further phytate degradation may increase inositol liberation in the ileal digesta, which subsequently increase plasma inositol concentration of broilers.

Key Words: broiler, phytase, phytate, inositol

94 In vitro phytase activity post steam conditioning and pelleting may not consistently correlate with broiler performance, tibia mineralization, and blood myo-inositol concentration among commercial phytases. Angela Lamp^{*1}, Nelson Ward², Jonathan Wilson², and Joseph Moritz¹, ¹West Virginia University, Morgantown, West Virginia, United States, ²DSM Nutritional Products Inc., Parsippany, New Jersey, United States.

The current study hypothesized that decreased phytase activity in feed due to increased steam conditioning temperature during pelleting is correlated to a decrease in broiler performance upon feeding. The objective of the study was to feed broilers diets containing different phytase enzymes conditioned at different temperatures and correlate phytase activity post pelleting with broiler performance, tibia mineralization, and blood myo-inositol concentration. Dietary treatments included a positive (PC) (0.9% calcium (Ca) and 0.4% non-phytate phosphorus (nPP)), negative control (NC) (0.7% Ca and 0.2% nPP), and 4 additional diets containing commercially available phytase products (A, B, C, and D) added to the NC based on analyzed activity and manufacturer's recommendations. Each diet was conditioned at 70, 80, and 90°C and fed as crumbles to 10 replications of 10 male Hubbard x Ross 708 broiler chicks for 21d. Treatments were arranged in a 6 (diet formulation) x 3 (conditioning temperature) factorial in a randomized complete block design. Correlations were determined with categorical and replicated data. Broiler chicks provided PC and NC produced expected performance,

tibia ash, and blood myo-inositol differences ($P < 0.05$). In general, phytase activity decreased with increased conditioning temperature. A conditioning temperature x diet formulation interaction occurred for d14 and 21 FCR and d21 tibia ash ($P < 0.05$). Day 14 and 21 FCR increased and d21 tibia ash decreased as conditioning temperature was increased from 70 to 90°C for all phytase treatments except phytase B that did not demonstrate performance or tibia ash detriment. Correlations were most apparent with replicated data, demonstrating the importance of analyzing multiple samples for phytase activity; however, correlations between phytase activity and performance metrics were not consistent among phytase treatments. All phytase activity values demonstrated a negative correlation with increased conditioning temperature ($P < 0.05$, r values ranged from -0.52 to -0.86). However, phytase A activity correlated with 21d FCR ($P = 0.04$, r = -0.38), phytase B did not demonstrate correlation ($P > 0.05$), phytase C correlated with tibia ash mg/chick ($P = 0.01$, r = 0.46), and phytase D showed a moderate correlation with tibia ash mg/chick ($P = 0.16$, r = 0.26) and blood myo-inositol concentration ($P = 0.15$, r = 0.27). This study did not demonstrate a common performance metric that was correlated with phytase activity post manufacture among commercial phytase products that decreased in activity due to increased conditioning temperature. The absence of a common correlation may have been due to the NC diet not being deficient enough in nPP.

Key Words: phytase activity, correlation, myo-inositol, feed manufacture, tibia mineralization

95 Addition of multi-carbohydrase into a nutrients deficient diet containing wheat and wheat by-products sustained growth performance of broiler chickens. Samiru Wickramasuriya^{*1}, Shemil Macelline¹, Hyun Min Cho¹, Jun Seon Hong¹, Yu Bin Kim¹, Rob Patterson², and Jung Min Heo¹, ¹Chungnam National University, Daejeon, Daejeon, Korea (the Republic of), ²Canadian Bio-Systems Inc., Calgary, Alberta, Canada.

This study was performed to investigate the effect of dietary Multi-Carbohydrase (MC) supplementation on growth performance, jejunum morphology, and nutrient digestibility in broiler chickens fed low nutrient density corn soybean-meal based diets containing wheat and wheat by-products. A total of 378 one-day-old Ross 308 broiler chickens were randomly assigned to one of 7 dietary treatments to give 6 replicates pens per treatment (9 birds per pen). Diets were corn-soybean meal based and formulated based on Ross 308 (Aviagen, 2014) specifications. Dietary treatments were as follows: (1) positive control (PC; energy and amino acids sufficient diet); (2) negative control 1 (NC-1; 120 kcal/kg deficient energy); (3) negative control 2 (NC-2; 3% lower digestible amino acids). The remaining 4 dietary treatments were formulated with the addition of MC (MC; Superzyme-CS, Canadian Bio-Systems Inc., Canada) into the 2 NC treatments with dosages of 0.025% and 0.05%, respectively. Birds were fed their respective experimental diets *ad-libitum* for the entire 35 d study period and management practices were performed according to the Ross 308 broiler management guide (Aviagen, 2014). Improved body weight, weight gain and feed conversion ratio ($P < 0.05$) were observed in broiler chickens fed the low energy diet supplemented with MC from hatch to 35 d. Moreover, birds fed lower energy diet with 0.05% MC showed similar ($P > 0.05$) performance as the PC diet fed broiler chickens throughout the 35 d experimental period. The addition of MC into low digestible amino acid (AA) diets also improved ($P < 0.05$) growth performance during the starter period (d 1–21), however, it did not overcome the hindrance of growth potential from the lower AA profile compared with PC diet during the starter period. Broiler chickens fed MC supplemented diets showed higher ($P < 0.05$) villus height to

crypt depth ratio compared with birds fed lower nutrient diets without MC on d 21 and 35. Similarly, improved ($P < 0.05$) dry matter, crude protein and energy digestibility were observed when birds were fed MC supplemented low energy diets on d 21 and crude protein and energy digestibility on d 35. Our results suggest that supplementation of MC at the rate of 0.05% into energy deficient diets would significantly improve

growth performance and nutrient digestibility of broiler chickens, and would result in similar performance as compared with broiler chickens fed energy sufficient diets.

Key Words: broiler, growth performance, gut morphology, multi-carbohydrase, nutrient digestibility

Student Competition: Microbiology and Food Safety

96 Effects of *in ovo* probiotic administration on the incidence of avian pathogenic *Escherichia coli* (APEC) in broilers and an evaluation on the virulence and antimicrobial resistance properties of APEC. Tianmin Li*, Claudia Castañeda, Julio Miotto, Chris McDaniel, Aaron Kiess, and Li Zhang, *Mississippi State University, Mississippi State, Mississippi, United States.*

Avian pathogenic *Escherichia coli* (APEC) cause colibacillosis, which can be controlled by antibiotics. However, antibiotics are being removed from poultry diets due to the emergence of multidrug-resistant bacteria. Therefore, alternatives to control APEC are required. This study aimed to evaluate the effects of *in ovo* injection of probiotics on the incidence of APEC in broilers. On embryonic D18, 4 *in ovo* treatments (T) were applied: T1 = Marek's vaccine (MV), T2 = MV+*Lactobacillus animalis*, T3 = MV+*L. reuteri*, T4 = MV+*L. rhamnosus*. A total of 180 male broilers of each T were randomly placed in 10 pens. Heart, liver, and spleen samples were collected on D1, 14 and 28. Yolk sac samples were collected on D1 and 14. *E. coli* colonies were isolated on MacConkey agar and confirmed by *E. coli* specific real-time PCR. The *E. coli* isolates were further screened for the presence of APEC by detecting 5 APEC associated genes (*iroN*, *ompT*, *hlyF*, *iss*, and *iutA*). A randomized complete block with a split plot in time was utilized to evaluate the incidence of APEC. Then, the APEC isolates were examined for 5 virulence factors and 22 antimicrobial resistance genes. A total of 165 *E. coli* were isolated and 115 isolates harbored at least 3 of the 5 APEC genes. No differences ($P > 0.05$) among T were observed for the APEC incidence in all organs when averaged over sampling days. When averaged over T, however, the incidence was different ($P < 0.05$) among sampling days for each organ. In heart, APEC incidence was not different ($P > 0.05$) between D14 (52.5%) and D28 (35.0%), which were both greater ($P < 0.05$) than D1 (5.0%). In spleen, APEC was detected in 35.0% of birds on both D14 and D28, but none was detected on D1. In liver, APEC incidence was greater ($P < 0.05$) on D14 (57.5%) than D28 (20.0%) and D1 (5.0%). In the yolk sac, 39.3% of birds were APEC positive on D14, which was greater ($P < 0.05$) than D1 (15.0%). No APEC isolates harbored *stx1/2* (Shiga toxin 1/2), *eaeA* (adhesin intimin) or LT (heat-labile enterotoxin) genes. However, 44.3% APEC were positive for *astA*, a gene encoding the enteroaggregative *E. coli* heat-stable enterotoxin, indicating that these strains were at a higher zoonotic risk to cause human intestinal disease. Antimicrobial resistance gene testing results revealed that 93.2% APEC carried at least one resistance gene; most isolates carried genes that respond to 3 (24.3%) or 4 (46.1%) antimicrobials. High frequencies of genes encoding resistance to arsenic (83.5%), trimethoprim (48.7%), quaternary ammonium compounds (47.0%), tetracycline (43.5%) and gentamicin (25.2%) were detected. Further research toward developing efficient antibiotic alternatives is essential to control APEC incidence in broilers.

Key Words: avian pathogenic *Escherichia coli*, probiotics, *in ovo*, antimicrobial resistance, broiler

97 Persistence of *Salmonella* Enteritidis and Typhimurium on hatching eggshells after 1, 6, or 24 hours. Caitlin Harris*^{1,3}, Nicole Bartenfeld Josselson¹, Dianna Bourassa², and Richard Jeff Buhr¹, ¹USDA-ARS, US National Poultry Research Center, Athens, Georgia, United States, ²Auburn University, Auburn, Alabama, United States, ³University of Georgia, Athens, Georgia, United States.

Salmonellae are associated with eggshells, which predominantly become contaminated when contacted with infected feces from the cloaca or after lay. Our previous research has shown that holding inoculated eggs for 24 h depressed *Salmonella* recovery. The first objective of this study was to determine the impact of 3 holding times (1, 6, and 24 h) on *Salmonella* recovery from eggshells. The second objective was to determine if the presence of intact cuticle or egg contents impacted *Salmonella* recovery. Two experiments were performed with the following 4 treatments: control (no eggshell modification), chemically or mechanically altered cuticle, and eggshell without contents. On d 1, eggs were assigned to one of the 4 treatments ($n = 36$ exp 1, $n = 48$ exp 2). For the chemically altered cuticle treatment, eggs were submerged in 100% alcohol for 10 min (exp 1) or 30 min (exp 2). For the mechanically altered cuticle treatment, sandpaper was used to buff the cuticle off a 4.5² cm area on each egg. For the empty egg treatment, a hole was ground through the shell and the contents were removed. All eggs were then placed into an incubator (42°C) for 16 h incubation time before inoculation. For exp 1, eggs were inoculated with 10 μ L of 10⁷ *S. Enteritidis* and for exp 2, half of the eggs were inoculated with 10⁵ *S. Enteritidis* and the remaining half inoculated with 10⁵ *S. Typhimurium*. Each treatment was then divided between the 3 holding times after which eggs were cracked, contents were discarded, and eggshells crushed into a centrifuge tube containing 30 mL of 1% buffered peptone water. For direct samples, 2 loops (20 μ L) of eggshell rinsates were streaked onto Brilliant Green Sulfa agar containing 100 μ g/mL nalidixic acid. Plates and eggshell rinsates were incubated for 24 h at 37°C, plates recorded as positive or negative, and enriched plating was performed. Prevalence data was analyzed using Chi Square. Total direct counts had low prevalence (17% exp 1; 27% exp 2) while enriched samples had higher prevalence (84% exp 1; 82% exp 2). For both experiments, as holding time increased, recovery of *Salmonella* decreased for direct plates (1 h: 43 and 47%; 6 h: 6 and 25%; 24 h: 0 and 9%). The control and both cuticle treatments were statistically similar while shells with no contents had higher recovery of *Salmonella* compared with the controls for both experiments at 1 h ($P = 0.0002$ and < 0.0001) and exp 2 at 6 h ($P = 0.001$). For exp 2, recovery of *S. Enteritidis* was lower than *S. Typhimurium* for direct plating (1 h: 31 vs 63%; 6 h: 6 vs 44%; 24 h: 0 vs 19%). In conclusion, increase in holding times of inoculated eggs before sampling reduced shell *Salmonella* recovery and removal of egg contents improved *Salmonella* recovery.

Key Words: *Salmonella*, eggshells, holding time, cuticle stripping, chicken

98 Genomic epidemiology of *Pasteurella multocida* and global trends in antibiotic resistance and virulence. Emily Smith*¹, Elizabeth Miller¹, and Timothy Johnson^{1,2}, ¹University of Minnesota, St. Paul, Minnesota, United States, ²Mid-Central Research and Outreach Center, Willmar, Minnesota, United States.

Pasteurella multocida is a Gram-negative bacterium that infects multiple species with a variety of clinical presentations, including fowl cholera in avian hosts. This pathogen is distributed globally and is often categorized according to Multi-locus sequence type or capsular serotype, however, the association between sequence types, serotypes, and genetic indicators of virulence or antibiotic resistance have yet to be determined. The purpose of this study is to gain a better understanding of the ecologic and evolutionary relationships between *P. multocida* strains of different sequence types and serotypes, as well as describe the global distribution of antibiotic resistance and virulence genes as

they pertain to domestic poultry and wild birds. All publically available *P. multocida* whole-genome sequences from the NCBI Assembly and Short Read Archive databases were downloaded and combined with 156 *P. multocida* isolates from commercial turkeys sequenced at the University of Minnesota Mid-Central Research and Outreach Center. Genomes were assembled de novo using SPAdes and annotated with Prokka, and blast searches were performed to identify capsular serotypes *in silico*, as well as known *P. multocida* virulence genes, antibiotic resistance genes, and plasmid sequences. Sequence typing was also done on each genome, which categorizes bacterial genomes into types based on the allelic profiles of 7 housekeeping genes. A SNP alignment was also performed to construct a maximum-likelihood phylogenetic tree. Four serotypes and 60 sequence types were identified from 471 whole genome sequences of *P. multocida*. Among the 264 genomes of avian origin, serotype A was predominant, but serotypes D and F were also found. Genomes of serotype A had significantly increased odds of containing acquired antibiotic resistance genes such as *erm(42)*, *sul2*, and *tetH*, which confer resistance to erythromycin, sulfonamide, and tetracycline antibiotics, respectively. An Inc.Q1 plasmid encoding resistance to aminoglycosides and tetracyclines was found in multiple sequences of serotype A from California, Minnesota, and North Carolina. Some sequence types were host and continent-specific, such as the dominant ST235, consisting of 54 genomes from avian hosts in the United States from 2012 to 2018, as well as ST129 in chickens and ducks from Asia with collection dates going back to 1953. Characterizing these genetic determinants of resistance and virulence in the context of traditional typing methods can provide researchers, clinicians, and public health professionals important information regarding potential clinical outcomes for different *P. multocida* strains when access to next-generation sequencing technologies is limited.

Key Words: *Pasteurella multocida*, genomics, virulence, antibiotic resistance

99 Effect of *Lactococcus lactis*-derived cell-free extract on sensitizing multidrug-resistant *Salmonella* Heidelberg to ampicillin and tetracycline. Brigitta Yaputri^{*1}, Divek V. T. Nair², and Anup Kollanoor Johnny², ¹University of Minnesota Twin Cities, Minneapolis, Minnesota, United States, ²University of Minnesota, Saint Paul, Minnesota, United States.

Salmonella Heidelberg (SH) is a major *Salmonella* serovar implicated in several foodborne outbreaks in the United States and frequently isolated from poultry. Most of the SH strains implicated in poultry-associated outbreaks have reported resistance to several important antibiotics, including ampicillin and tetracycline. The objective of the study was to determine the efficacy of *Lactococcus lactis* (a beneficial bacteria of dairy origin) derived cell-free extracts (CFEs; bacteria free) in sensitizing multidrug-resistant (MDR) SH to 2 antibiotics, ampicillin, and tetracycline. Ampicillin (16, 32, and 64 µg/ml), tetracycline (32, 64, and 128 µg/ml) and *L. lactis* CFEs [5, 7.5, 10, 12.5 or 15% (vol/vol)] were tested either alone or in combination for their efficacy against SH using 12-well tissue culture plate assays. Each well, containing 2 mL of tryptic soy broth, was added with CFEs and antibiotics, either alone or in combination, with the MDR SH inoculated at 5 log₁₀cfu/ml. Since the addition of CFEs reduced the pH of the test medium, pH controls were set for each concentration in addition to the negative and positive controls. The wells were incubated at 37°C for 24h and absorbance at OD₆₀₀ was determined at 0 and 24h. The experiment was repeated 6 times with 6 technical replicates per treatment and data were analyzed using ANOVA. Results revealed that CFE concentrations > 5% resulted in a significant reduction of MDR SH compared with the controls (*P* <

0.05). The magnitude of MDR SH reduction obtained with ≥ 7.5% CFE was higher than that of 64 µg/ml ampicillin alone (*P* < 0.05). Similarly, CFE at ≥ 10% resulted in higher MDR SH reduction than 128 µg/ml tetracycline. The synergistic effect of CFEs and tetracycline was observed when ≥ 7.5% CFE was tested in combination with 32, 64, and 128 µg/ml of tetracycline against MDR SH. The combinations resulted in a higher reduction of MDR SH compared with the individual treatments (*P* < 0.05). However, 7.5% CFE showed synergistic activity with 16, 32, and 64 µg/ml of ampicillin resulting in significant reduction of MDR SH (*P* < 0.05). All other combinations of CFE (>7.5%) in combination with ampicillin had similar MDR SH reduction as that of the reduction observed with CFE alone (*P* > 0.05). Results of this study indicate the potential of *L. lactis* CFEs in enhancing the sensitivity of SH to ampicillin and tetracycline. Studies are underway to explore the mechanisms behind the CFE-mediated antibiotic sensitivity in SH (University of Minnesota Undergraduate Research Opportunities Scholarship and Minnesota Agricultural Experimentation Station grant# MIN-16-120).

Key Words: probiotics, antibiotics, sensitizing, multidrug-resistant, *Salmonella* Heidelberg

100 Inhibiting horizontal transfer of antibiotic resistance gene between multi-drug resistant *Salmonella* Heidelberg and *E. coli* by select lactic acid bacteria. Poonam Vinayamohan^{*1}, Abraham Joseph Pellissery¹, Annie Donoghue², and Kumar Venkitanarayanan¹, ¹University of Connecticut, Storrs, Connecticut, United States, ²Poultry Production and Product Safety Research Unit, ARS, USDA, Fayetteville, Arkansas, United States.

Antibiotic resistance (AR) is a global public health threat. Bacteria have the ability to share genes, resulting in AR spread in a variety of environmental niches, including animal gut, manure and water. Horizontal gene transfer (HGT) represents the major route for AR spread in bacteria. Since food animals, including poultry constitute a potential source of AR bacteria, strategies that inhibit HGT between AR pathogens and commensal bacteria could potentially reduce the dissemination of AR genes in the environment and poultry-derived foods. *Salmonella* Heidelberg (SH) is a major AR foodborne pathogen associated with poultry. Lactic acid bacteria (LAB) produce several antimicrobial compounds including bacteriocins which have proven inhibitory action against pathogenic bacteria. This study investigated the efficacy of supernatant from 5 LAB (Two species of *Lactobacillus plantarum*, *L. johnsoni*, *L. casei*, *L. gasseri*) in reducing β-lactamase gene (*bla*_{TEM}, ampicillin resistance) transfer between *S. Heidelberg* and commensal *Escherichia coli* in Luria-Bertani broth (LBB), water and chicken manure. *S. Heidelberg* (*bla*_{TEM}⁺) and nalidixic acid (NA)-resistance induced *E. coli* were inoculated in 1:1 ratio in LBB, fresh poultry manure and water with and without sub-inhibitory concentration (8% vol/vol, highest concentration not inhibiting growth) of LAB supernatants (LABS) and incubated at 37°C for 24 h. Appropriate pH controls were included. Transconjugant colonies were recovered by plating on tryptic soy agar (TSA) containing ampicillin and NA, donor cells (SH) were recovered on TSA+ ampicillin, and recipients (*E. coli*) were enumerated on TSA+NA plates. The HGT of AR was calculated as the ratio of the number of trans-conjugants to the number of recipient cells. Additionally, the effect of LABS on genes critical for conjugation in SH was determined by RT-qPCR. The study was replicated thrice with duplicate samples. The data were analyzed using Proc GLM of SAS. All 5 LABS reduced HGT frequency of *bla*_{TEM} gene between MDR *Salmonella* and *E. coli* (*P* < 0.05) by 69 to 98% compared with the control. RT-qPCR results revealed that LABS upregulated (1.5 to 2 fold) the *finO* (repressor of conjugation), and decreased (1 to 7 fold)

the transcription of *tra* genes (responsible for direct cell to cell contact) compared with control ($P < 0.05$). Results suggest the potential use of LABS for controlling AR spread in poultry gut, poultry manure, and water, however, the functional component(s) in LABS that inhibit HGT need to be characterized. Additionally, follow up studies in the chicken gut in vivo are warranted.

Key Words: Antibiotic resistance, poultry, lactic acid bacterial supernatants, horizontal gene transfer, *Salmonella*

101 Prevalence of plasmid carried virulence genes in *Escherichia coli* isolates from litter, feces, and cloacal and tracheal swabs collected from 'no antibiotics ever' commercial broiler farms. Mercedes Smith*, Alyssa Easterling, Nikhil Nuthalapati, Li Zhang, Aaron Kiess, and Anuraj Theradiyil Sukumaran, *Mississippi State University, Mississippi State, Mississippi, United States.*

The objective of the current study was to identify the prevalence of plasmid-carried virulence genes *iroN*, *ompT*, *hlyF*, *iss*, and *iutA* in *Escherichia coli* isolated from litter, feces, cloacal and tracheal swabs of commercial broiler chicken raised under 'no antibiotics ever (NAE)' policy in Mississippi. Chicken litter ($n = 8$) samples were collected before chick placement on d0 from 4 NAE broiler farms. On d 28 of the grow-out, 8 litter, fecal, cloacal swab and tracheal swab samples were collected. The litter and fecal samples (10 g) were homogenized in 90 mL of BPW and the cloacal and tracheal swabs in 10 mL BPW. From each suspension, a loop full was streaked onto duplicate MacConkey agar plates and incubated at 37°C for 24 h. Two presumptive pink colored colonies were selected from each plate and DNA was extracted by heating the cells to 95°C for 5 min in nuclease-free water. *E. coli* isolates were confirmed by PCR amplification of the *ybbW* gene. The positive *E. coli* isolates ($n = 363$; 157 from litter, 73 from feces, 78 from cloacal swabs, and 55 from tracheal swabs) were screened further for the presence of 5 virulence genes, *iroN*, *ompT*, *hlyF*, *iss*, and *iutA*, using the following PCR reaction mixture (10 μ L); 1 μ L of cell free DNA, 5 μ L PowerUp SYBR Green master mix (2X), 0.25 μ L of forward and reverse primers (10 μ M), and 3.5 μ L nuclease-free water. The mixture was amplified in a QuantStudio 3 real-time PCR system (Applied Biosystems, CA, USA) for 40 cycles (denaturation at 95°C for 1 s, annealing and extension at 60°C for 40 to 60 s). The effect of grow-out day on the prevalence of virulence genes in *E. coli* isolates from litter was analyzed using the PROC MIXED procedure of SAS 9.4 with grow-out day as the fixed effect and farm being the random block effect. Overall, 49.1, 56.2, 35.9, and 47.3% of the isolates from litter, feces, cloacal, and tracheal swabs, respectively had all virulence genes; whereas, 5.7, 12.3, 6.4, and 7.3% of the isolates, respectively had none of the tested virulence genes. The genes, *iroN*, *ompT*, *hlyF*, *iss*, and *iutA* were present in 65.5, 73.0, 85.9, 70.5, and 62.1% of the isolates, respectively from litter, 68.4, 69.6, 79.0, 68.4, and 62.5% of the isolates, respectively from feces, 45.7, 70.5, 80.6, 48.8, and 82.2% of the isolates, respectively from cloacal swabs, and 63.5, 76.4, 85.2, 68.7, and 70.0% of the isolates, respectively from tracheal swabs. However, there was no difference in the prevalence of any of the virulence genes in litter isolates between d0 and d28 of the grow-out ($P \geq 0.746$). The results from this study show very high prevalence of virulent *E. coli* in broilers raised under NAE system suggesting the potential for avian pathogenic *E. coli* outbreaks

Key Words: *E. coli*, broiler, virulence, prevalence, antibiotic free

102 Prevalence of *Salmonella enterica* on processing equipment after sanitization procedures. Tomi Obe*, Rama

Nannapaneni, Wes Schilling, Chris McDaniel, and Aaron Kiess, *Mississippi State University, Mississippi State, Mississippi, United States.*

Salmonella is a major poultry-borne pathogen that causes illness throughout the world. Consequently, it is critical to control *Salmonella* during the processing of poultry meat. Sanitization of a poultry processing facility and equipment is a crucial control measure. However, prevalence of *Salmonella* on equipment after sanitization and its potential risk to food safety has not been well evaluated. Hence, the objective of this study was to evaluate the persistence of *Salmonella* on poultry processing equipment before and following sanitization procedures. A total of 15 locations within 6 commercial processing plants were sampled at 3 time points defined as post processing (A), cleaning (B) and sanitization (C) on 3 separate visits for a total of 135 samples per plant. *Salmonella*-positive isolates were recovered from samples using the USDA MLG 4.09 protocol. Presumptive *Salmonella* colonies were subjected to biochemical tests for confirmation. *Salmonella* isolates recovered after sanitization were serotyped and tested for virulence genes associated with *Salmonella*. A randomized complete block design with a 3×15 factorial arrangement was utilized to analyze results for *Salmonella* prevalence between processing plant where plants are the block. Means were separated using Fisher's protected LSD when $P \leq 0.05$. For *Salmonella* prevalence between processing plants, differences ($P < 0.0001$) were observed in the 6 plants tested where the maximum and minimum prevalence was 29.6% and 7.4% respectively. As expected, there was a difference ($P < 0.0001$) in the recovery of *Salmonella* due to sampling time. *Salmonella* prevalence at time A (35%) was significantly higher, while there was no difference between time B (12%) and C (9%). There was a location effect ($P < 0.0001$) for the prevalence of *Salmonella* with the head puller, picker, cropper, and scalding having a significantly higher prevalence when compared with several other locations. At sampling time C, a trend toward a difference ($P = 0.0899$) was observed for *Salmonella* prevalence between the 6 plants while differences were observed due to location ($P = 0.0031$). Five prominent *Salmonella enterica* serovars were identified, including Kentucky, Schwarzengrund, Enteritidis, Liverpool and Typhimurium with *S. Kentucky* being the most prevalent. PCR analysis of 8 *Salmonella* virulence genes showed that the *invA*, *sipB*, *spiA*, and *sseC* were detected in all isolates. Whereas the genes carried on plasmids and/or fimbriae varied remarkably among all isolates. This study established *Salmonella* prevalence and persistence in different poultry processing facilities after antimicrobial application which could result in contamination of poultry carcasses and food safety risks in poultry meat.

Key Words: *Salmonella*, prevalence, sanitization, poultry plants, virulence

103 Effect of caprylic acid in scalding water on broiler drumsticks contaminated with multidrug-resistant *Salmonella* Heidelberg. Shijinaraj Manjankattil*¹, Divek V. T. Nair¹, Claire Peichel¹, Annie Donoghue², and Anup Kollanoor Johny¹, ¹University of Minnesota, Saint Paul, Minnesota, United States, ²USDA ARS, Fayetteville, Arkansas, United States.

Salmonellosis continues to be a major foodborne illness attributed to the consumption of poultry meat and demands interventions at processing steps for improved food safety. In this study, we determined the antimicrobial efficacy of caprylic acid (CA; generally recognized as safe-status medium chain fatty acid) against multidrug-resistant *Salmonella* Heidelberg (SH) on broiler chicken drumsticks during scalding conditions intended for organic poultry processing. Chicken drumsticks were spot inoculated with SH [lower (~3.0 log₁₀ cfu/g) or higher (5.0 log₁₀ cfu/g) inoculum] and immersed in scalding water containing treatments for

2 min at 54°C (USDA-recommended time-temperature combination for scalding). The treatments included in the study were 0.5% CA, 1% CA, 0.05% peracetic acid (PAA), 0.5% CA + 0.05% PAA and 1.0% CA + 0.05% PAA. Samples inoculated with or without SH and immersed in scalding water containing neither of the antimicrobial treatments served as the positive control (PC) and negative control, respectively. Immediately after scalding, the drumsticks (n = 6) were homogenized in phosphate buffered saline (PBS) and surviving SH populations were recovered. Similarly, SH populations that survived in scalding water was also determined. Additionally, the efficacy of the scalding treatments against SH survival on drumsticks during storage for 48 h at 4°C was determined. The effect of these treatments on the surface color of the drumsticks was also evaluated. Data were analyzed using the PROC-MIXED procedure of SAS. The antimicrobial treatments resulted in a significant reduction of SH on drumsticks. For the lower inoculum study, 0.5% CA, 1% CA, 0.05% PAA, 0.5% CA + 0.05% PAA and 1.0% CA + 0.05% PAA resulted in 0.7-, 1.0-, 2.5-, 1.3- and 1.5- log₁₀ cfu/g reduction of SH on drumsticks ($P < 0.05$). The same treatments resulted in 0.9-, 1.3-, 2.4-, 2.2- and 2.6- log₁₀ cfu/g reduction of SH when the drumsticks were contaminated with the higher inoculum level ($P < 0.05$). The antimicrobial treatments inactivated SH to non-detectable levels in scalding water whereas 2.0- to 4.0- log₁₀ cfu/ml SH survived in PC ($P < 0.05$). Also, the scalding treatments were effective in inhibiting SH on the drumsticks compared with the respective controls during storage at 4°C for 48 h; the magnitude of reduction remained the same as observed during the scalding treatment ($P < 0.05$). Additionally, none of the treatments affected the surface color of the drumsticks. Caprylic acid could be used as a natural strategy against SH on chicken drumsticks at scalding step of organic poultry processing to improve microbiological safety of meat products (MIN-16–120).

Key Words: *Salmonella* Heidelberg, caprylic acid, scalding, organic, antimicrobial

104 Competitive exclusion of intragenus *Salmonella* serovars.

Megan Pineda*¹, Michael Kogut², Kenneth Genovese², Xi Wang¹, Dan Zhao¹, Annah Lee¹, Kristin Moncada¹, Denise Caldwell², and Morgan Farnell¹, ¹Texas A&M University, College Station, Texas, United States, ²United States Department of Agriculture, College Station, Texas, United States.

Average poultry consumption is 109.6 pounds per capita in the United States, and an estimated 400,000 *Salmonella* cases come from consuming contaminated poultry annually. We hypothesized that inhibition would occur between *Salmonella* serovars and colonization will vary depending on the virulence of the strain. *Salmonella* spp. were selected for resistance to rifampicin or gentamicin. Chicks were confirmed negative for wild-type *Salmonella* by enriching tray papers. Neonatal broilers were randomly distributed between treatments. Each treatment consisted of 30 birds, of which 20 were removed for sampling. Birds were orally challenged with 0.5 mL of phosphate buffered saline (PBS) only, 10³ cfu/ml *Salmonella* Kentucky (SK) on D1 only, 10³ cfu/ml *Salmonella* Typhimurium (ST) on D1 only, 10³ cfu/ml SK on D1 then 10⁴ cfu/ml ST on D2, 10³ cfu/ml ST on D1 then 10⁴ cfu/ml SK on D2 or 10³ cfu/ml SK and 10³ cfu/ml ST concurrently. Chicks were humanely euthanized on D3 and samples were aseptically removed for culture. Livers and spleens were combined and macerated to determine organ invasion. Organs were pre-enriched in buffered peptone water then sub-cultured in Rappaport-Vassiliadis broth. Cecal contents were serially diluted in PBS and plated onto xylose-lysine-tergitol 4 agar. Colonies with black centers were confirmed as *Salmonella* and were randomly selected to be confirmed using antiserum, lysine iron agar and triple sugar iron agar slants. Significant differences were determined via ANOVA at $P < 0.05$ in both trials. Negative controls did not exhibit any growth of either serovar in both trials. Groups only administered SK or ST recovered only the respective serotypes in both trials. Administration of SK before ST significantly decreased ST in both trials by 5.36 log cfu/g and 2.55 log cfu/g, respectively ($P < 0.001$). Chicks challenged with ST before SK significantly reduced SK in both trials by 0.97 log cfu/g and 0.64 log cfu/g, respectively ($P < 0.001$). Concurrently challenged chicks had a significant decrease in ST by 1.62 log cfu/g and 1.03 log cfu/g, respectively ($P < 0.001$). There were no significant differences in SK in either trial when concurrently infected. All enriched cecal contents were 100% positive for the respective challenge strains with the exception of the SK D1 and ST D2 treatment, where no ST was recovered. Incidence of organ invasion was too low to observe a treatment effect. *Salmonella* Kentucky is infrequently associated with disease in humans, whereas *Salmonella* Typhimurium is the most common serovar detected. These results suggest *Salmonella* Kentucky could be used to displace *Salmonella* Typhimurium in neonatal broilers.

Key Words: broiler, competitive exclusion, *Salmonella* Kentucky, *Salmonella* Typhimurium

5-Minute Abstract Presentations

105 Effect of *Histomonas meleagridis* on broiler breeder egg production and quality. Elle Chadwick*, Ramon Malheiros, Edgar Oviedo, Christina Sigmon, Maria Alfaro Wisaquillo, Gustavo Quintana-Ospina, Viviana San Martin, Hernan Cordova-Noboa, and Robert Beckstead, *North Carolina State University, Raleigh, North Carolina, United States.*

The objective of this study was to determine if inoculation with *Histomonas meleagridis* would alter broiler breeder hens' egg production and egg quality due to field cases suggesting negative effects of this parasite. Previously, we demonstrated that inoculating a flock of broiler breeder hens with *H. meleagridis* caused limited morbidity and the inoculation did not cause mortality. The onset of lay was also not altered due to infection compared with the control flock. Because of these results, we hypothesized that this parasite would not alter broiler breeder egg production or quality. 520 breeder hens were transferred from a rearing to a production house at 21 weeks of age. Four pens represented the control flock and 4 pens had previously been inoculated with *H. meleagridis* and were still positive for the parasite. Both flocks started laying at 24 weeks where egg collection occurred twice daily. Feed intake and egg production were analyzed per period (28 d) with 10 periods in this trial. Six eggs per pen were measured for: shell strength, shell thickness, egg weight, Haugh Units and vitelline membrane on wk 30, 34, 39, 42 and 56. One hundred and 80 eggs per pen were analyzed for hatchability on wk 27, 37 and 60 while fertility was analyzed on wk 27 and 37. At 64 weeks, individual bird weights were recorded and cecal samples collected to determine presence of *H. meleagridis*. Differences between treatments were analyzed in JMP Pro 14 using a *t*-test ($P \leq 0.05$). The number of eggs produced per hen differed significantly during one period ($P = 0.021$), where the infected birds produced more eggs per hen (17 vs 13 eggs) and had a lower FCR (3.12 vs 3.47) ($P = 0.0347$). On wk 30, the infected birds produced heavier eggs (59 vs 57 g) ($P = 0.04$) and on wk 42 they produced eggs with a thicker shell (0.35 mm vs 0.34 mm) ($P = 0.0305$). Hatchability and fertility did not differ between treatments. At 64 weeks, the inoculated birds weighed significantly less than the control (4.16 vs 4.29 kg) ($P = 0.0016$) and *H. meleagridis* was observed in the cecal samples analyzed via light microscopy. This study indicates that *H. meleagridis* has limited effects on broiler breeder egg production and quality. Recovery of the parasite a year after infection infers the broiler breeder as an ideal carrier of *H. meleagridis*.

Key Words: *Histomonas meleagridis*, broiler breeder, egg production, egg quality, hatchability

106 Breast physical measurements of the standard and breast yield bird lines associated with wooden breast. Barbara de Almeida Mallmann*¹, Ashleigh Muller¹, Ashunti Jackson², Andy Mauro-moustakos¹, and Casey Owens¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²Cobb-Vantress, Siloam Springs, Arkansas, United States.

Woody breast (WB) is a current topic in meat quality characterized by the hardness of the meat, but it is typically only reliably detected after deboning. Considering this, the aim of this study is to relate the WB in live birds by the size of the breast. A total of 480 birds from standard (n = 240) and high breast yield (n = 240) strains were measured for breast characteristics and body weight weekly from wk 1 to 7. The measurements were made with a fabric tape on the cranial region (Tcranial; wing to wing) and a caliper on the caudal region (Ccaudal).

WB palpation scoring of live birds was based on established scoring methodology for deboned breast fillets, which is based on the degree and extent of hardness of the muscle. The same person scored both the live birds and deboned fillets in this study. At 52 d of age, birds were processed, deboned, and fillets were scored for WB. Measurement responses were compared for the 3 categories of WB (0 = normal, N; 1 = mild, M; and 2 = moderate/severe, S) using Fit Model with JMP® 14. The means for the significant responses were separated using Tukey test at 5% significance level, and trend contrasts were performed. Three parameters of Gompertz growth curves were fit for both measurements by bird group and comparisons of the parameters were performed among the curves of WB categories. Differences on Gompertz for body weight of the standard and high breast yield birds were that S birds had an inflection point 2 d after the N suggesting they begin a slower growth rate 2 d later. Both measurements, Ccaudal and Tcranial, were significantly different ($P > 0.005$) for the WB and the bird categories (standard vs high breast yield), but there was no interaction. There was a significant Ccaudal difference ($P < 0.05$) of 4.2 and 7.5 mm between N and S scores for the high breast yield and standard birds, respectively. Tcranial differences were not significant ($P > 0.05$). Gompertz curves for the different bird groups and WB scores had R^2 all above 95%. The parameters of the measurements were not significantly different ($P > 0.05$) for the Gompertz curves. Finally, bird measurements could help to differentiate the WB scores between the strains, but other factors should be considered in future research to determine models that are predictive of WB during growth.

Key Words: wooden breast, measurements, relationship, breast fillet size, growth curve

107 Does a mathematical method beat the comparative slaughter technique in determining net energy values for feed? Sasha van der Klein* and Martin Zuidhof, *University of Alberta, Edmonton, Alberta, Canada.*

Optimal feed formulation requires accurate estimation of feed energy values, however the often-used comparative slaughter technique (CST) is a resource intensive method. The objective of this study was to compare a mathematical methodology with the CST to estimate energy partitioning to heat production (HP) and energy retention (RE) and determine the NE for gain (NE_g). At hatch, 48 broilers were randomly allocated in one of 4 pens equipped with a precision feeding station. From d 14 to d 45 they were either fed a Low ME (3,111 kcal/kg ME) or a High ME (3,383 kcal/kg ME) diet. At d 19, birds were assigned to pair-feeding with master birds eating *ad libitum* (100%) and followers eating at either 50%, 60%, 70%, 80%, or 90% of the paired master's cumulative feed intake. HP and RE were estimated by the CST and with a nonlinear mixed model explaining daily ME intake (MEI) as a function of metabolic BW and daily gain (ADG). The energy partitioning model predicted $MEI = (145.10 + u) BW^{0.83} + 1.09 \times BW^{-0.18} \times ADG^{1.19} + \epsilon$ ($P < 0.001$ for all parameters), where $(145.10 + u) BW^{0.83}$ was total HP and $1.09 \times BW^{-0.18} \times ADG^{1.19}$ was RE. The random variable u was the animal specific (random) term for energetic requirement for maintenance ($u \sim N(0, \sigma^2)$). Estimated HP and RE per 4-d period were summed to reflect total HP and total RE over the experimental period (d 14 to d 45). NE_g of the diets for both methods was calculated as RE divided by the cumulative feed intake over the same measurement period and expressed as kcal/kg. Pairwise differences between means were considered significant at $P \leq 0.05$. The model estimated HP 15.4% and

11.4% lower and RE 29.2% and 16.9% higher, compared with the CST for the Low ME and High ME diet, respectively. The model estimated NE_g for the High ME diet at 1,498 kcal/kg compared with 1,456 kcal/kg for the Low ME diet. The CST estimated NE_g for the High ME diet at 1,261 kcal/kg compared with 1,115 kcal/kg for the Low ME diet. The model overestimated the NE_g value for both diets, but the difference in NE_g between the methods was lower for the High ME diet compared with the Low ME diet (237 kcal/kg vs. 340 kcal/kg, $P = 0.001$). The model provided a non-invasive real-time method to estimate HP and RE, but could not estimate the NE_g values of the 2 diets accurately; the mathematical method underestimated the NE_g values derived using the CST. Therefore, the mathematical method was inferior to the CST in determining NE_g values for feed.

Key Words: precision feeding, modeling, energy, production

108 Use of orange corn to reduce prevalence of footpad dermatitis in broiler chickens. Meagan Abraham^{*1}, Shawna Weimer¹, Kailynn VanDeWater¹, Evan Rocheford², Torbert Rocheford^{1,2}, Lisa Hoverman², and Darrin Karcher¹, ¹Purdue University, West Lafayette, Indiana, United States, ²Nutramaize, West Lafayette, Indiana, United States.

Footpad dermatitis (FPD), which is necrosis of the plantar surface of the foot, is not only a welfare concern but also causes chicken paw downgrades and condemnations. Orange corn, unlike yellow and white corn, has larger quantities of carotenoids, which are antioxidant pigments that include Vitamin A precursors. Carotenoids are thought to play an important role in skin and feather health. This study's objective was to evaluate the effect of orange corn on FPD prevalence in broiler chickens. This project was a 3x2 factorial design with 4 replicates of yellow, orange, and white corn diets fed to broilers raised on wet or dry litter. The wet litter moisture reached 60% or greater through application of water to the litter. Twenty-four pens of 40 birds each were reared to 42 d of age. Footpads were scored with a 0–2 visual scale of increasing severity on 20% of birds in each pen ($n = 192$) at 20, 27, 35, and 42 d of age. Body weights were recorded at placement and at wk 2, 4, and 6. Liver and fat color were assessed with a colorimeter at d 42. Data was analyzed using SAS Proc-Mixed analysis and a chi-squared option for footpad scores. At 42 d of age, litter treatments differed with the wet group having higher prevalence of FPD (scores of 1 or 2) compared with the control group (87.5% vs 13% respectively; $P < 0.0001$). There was a diet effect at 42 d of age; prevalence of birds with footpad scores of 1 or 2 were least on the orange corn diet (33%), followed by white corn (56.25%), and yellow corn (62.5%). Birds fed the orange corn diet had greater body weights throughout the study ($P = 0.004$). The fat and liver color were more yellow from birds on the orange diet compared with those on the white and yellow diets ($P < 0.005$). Overall, the orange corn diet did not completely protect against footpad dermatitis but did decrease prevalence of footpad scores of 1 and 2. The main determinant of footpad dermatitis prevalence was exposure to wet litter. Therefore, orange corn may have some utility in the reduction of footpad scores.

Key Words: welfare, footpad, dermatitis, orange, corn

109 Evaluation of black soldier fly larvae substituting soybean meal at two inclusions on broiler performance. Kimberly Gardner^{*}, Momin Khan, John Padgett, Jeff Tomberlin, Christopher Bailey, and Delbert M. Gatlin, III, Texas A&M University, College Station, Texas, United States.

Black soldier fly larvae (BSFL) have been a proposed alternative protein source in poultry feeds. The aim of the study was to evaluate the effect of substituting dehulled soybean meal (SBM) with BSFL. A corn-soybean meal diet was formulated to contain 22% CP and 3050 kcal ME/kg and 2 additional diets were made to include either 5% or 10% BSFL in place of SBM. The experiment design consisted of 3 dietary treatments with 6 replicates per treatment and 5 birds per replicate for a total of 90 Cobb 700 males. Proximate analysis was performed on the dried BSFL and AA content were determined by HPLC after acid hydrolysis. Average body weight (BW), mortality adjusted feed conversion ratio (FCR), and feed consumption were determined on d 7, 14, and 21 of the trial. All data were subjected to an ANOVA using the GLM Procedure and multiple comparisons were determined using Tukey's HSD Test. The BSFL averaged 95.2% DM, 42.5% CP, 28.1% EE and 9.4% ash. The lysine, methionine, and threonine content averaged $2.99\% \pm 0.38$, $0.68\% \pm 0.05$, and $1.85\% \pm 0.16$ respectively on an as fed basis. Inclusion of 5% BSFL increased ($P = 0.001$) 21-d body weight (896 ± 9 g) and decreased ($P = 0.003$) FCR (1.26 ± 0.01) when compared with the control (818 ± 13 g and 1.33 ± 0.01 respectively). Body weight for birds fed 10% BSFL averaged 859 ± 12 g compared with the control ($P = 0.07$). FCR for birds fed 10% BSFL averaged 1.30 ± 0.02 which was not different from the control treatment. In conclusion, BSFL included in the diets at 5% showed improved performance when compared with the control corn-soybean meal diet.

Key Words: black soldier fly, larvae, broilers, insects, performance

110 Effect of sub-zero saline chilling on chilling efficiency and meat quality of broiler carcasses. Morgan Wonderly^{*}, Samira Viliani, Hong Chul Lee, Darin Bennett, Sean Hurley, and Ike Kang, California Polytechnic State University, San Luis Obispo, California, United States.

The purpose of this research was to investigate the effects of sub-zero saline chilling on carcass chilling, breast fillet tenderness and product safety. A total of 12 eviscerated broiler carcasses were randomly picked from the processing line in a local process plant. The birds were then subjected to one of 3 chilling solutions: 1) 0% NaCl/0.5°C (water control), 2) 3% NaCl/-1.8°C, and 3) 4% NaCl/-2.41°C. Data in 3 replications were analyzed using one-way ANOVA and a completely randomized design. A post-hoc analysis was performed using Duncan's multiple range test to evaluate difference among treatments at $P < 0.05$. Broiler carcasses in subzero saline solutions were chilled efficiently and reduced the chilling time by 11% in 3% NaCl/-1.8°C and 37% in 4% NaCl/-2.41°C over the ice slurry control. The breast fillets of broiler carcasses in 4% NaCl/-2.41°C were significantly tenderized than those in water control ($P < 0.05$), with an intermediate value observed in 3% NaCl/-1.8°C. Before chilling, broiler carcasses possessed mesophilic aerobic bacteria (MAB), *Escherichia coli* (*E. coli*), and total coliforms for 3.81, 0.78, and 1.86 log cfu/g, respectively. Both *E. coli* and coliforms were significantly reduced when the carcasses were chilled in 3% NaCl/-1.8°C or 4% NaCl/-2.41°C than the water control ($P < 0.05$), except MAB. Based on these results, chilling of boiler carcass in 4% NaCl/-1.8°C solution appears to improve carcass chilling efficiency, and meat tenderness over the traditional water immersion chilling.

Key Words: sub-zero saline chilling, chilling efficiency, broiler carcass quality, bacterial reduction.

111 Effect of the combination of 25-hydroxyvitamin D₃ and modified levels of calcium and phosphorus in the diets on bone 3D structural development in pullets. Dima White^{*}, Chongxiao

Chen, and Woo Kim, *University of Georgia, Athens, Georgia, United States*.

Previously, our lab found additional 25-hydroxyvitamin D₃ (25OHD) in the pullet diet stimulated bone growth, increased the bone size, and created more pores in the cortical bone during a pullet period, which allowed more minerals deposit in the bones during the laying period. However, due to the larger structure size, the bone mineral density (BMD) was decreased in 25OHD treatment during the pullet period. It may be attributed to the Ca & available P level and ratio in the diets which were not ideal to provide sufficient Ca & P to build the larger mineralized bones. We hypothesized that the changes in Ca&P levels and ratios on the top of 25OHD supplementation could reduce porosity and improve BMD and bone quality during the pullet period. A study was conducted to explore the effect of 25OHD combined with modified Ca/P level and ratio in the diets on bone 3D structural development in pullets. A total of 560 d-old Hyline W36 pullets were randomly assigned to 5 treatments (8 rep; 14 birds/rep) until 17wks. Dietary treatments were; 1) vitamin D₃ at 2,760 IU/kg(D), 2) vitamin D₃ at 2,760 IU/kg+31.25mg 25OHD/ton (L25D), 3) vitamin D₃+62.5mg 25OHD/ton (H25D), 4) vitamin D₃+62.5mg 25OHD/ton+ high Ca&P (H25D+Ca&P), and 5) vitamin D₃ (2,760 IU/kg) + high Ca&P (D+Ca&P). The high Ca&P diet was made by increasing 30% of both Ca&P (2:1) for the first 12wks, and then only increase P for 12–17wks to reduce the Ca:P ratio. At 17wk, femur bones were scanned using a Micro-CT for bone 3D structure analysis. The data were subjected to a one-way ANOVA using the GLM procedure, with means deemed significant at $P < 0.05$. Results indicated that H25D+ Ca&P treatment had lower open pore volume space, open porosity (% cortical tissue volume), total volume of pore space, and total porosity (% cortical tissue volume) in the cortical bone compared with D+ Ca&P. It also showed that a higher cortical BV/TV (bone volume/tissue volume) in H25D+Ca&P than D+Ca&P. Furthermore, 25D+Ca&P treatment had lowest trabecular pattern factor and structure model index compare with the other treatments, which indicates its beneficial effects on trabecular structural development. Moreover, 25D+Ca&P had a higher trabecular percentage (trabecular bone/cavity volume%) compare with D and 25D, which suggested the additional Ca&P supplementation on the top of 25D increased trabecular content in the cavity. In conclusion, the combination of 25D with higher level of Ca/P could improve cortical bone quality in pullets and showed a beneficial effect on trabecular bone 3D structural development. The combination of higher bio-active form of vitamin D₃ and higher levels of Ca&P could become a potential feeding strategy to improve the pullets bone quality.

Key Words: vitamin D₃, calcium, phosphorus, pullets, bone 3D structure

112 Not Presented

114 CpG injection on day 15 post-hatch leads to metabolic changes on day 16 post-hatch in modern broilers and adaptive immune responses in a heritage breed. Bridget Aylward¹, Casey Johnson¹, Famatta Perry¹, Iyana Admasu¹, Rose Whelan², and Ryan Arsenaault¹, ¹*University of Delaware, Newark, Delaware, United States*, ²*Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany*.

Modern broiler breeds selected for rapid growth are more susceptible to pathogen challenge than older genetic lines, such as heritage chickens, which have not been selectively bred for a specific trait since 1957. We hypothesize this is due to an inappropriate immune response in modern strains when stimulated. To test this, we injected modern broiler and heritage chickens intra-abdominally with a known immunostimulatory

agent to observe immune response differences between the 2 bird breeds. Birds were injected at multiple time points throughout the grow-out period with 25 µg of either CpG or a GpC control, and tissue samples were collected from each group before and 24 h post-injections. Kinase activity in the tissues was assessed using a species-specific kinome peptide array to measure the degree of phosphorylation of target peptides in key immunometabolic signaling pathways, which was then visualized by staining the array with phosphoprotein-specific fluorescent dye so that fluorescence could be quantified. The raw fluorescence data were collected and normalized using variance stabilizing normalization in the vsn package in R, and normalized fluorescence in the treatment birds' tissues was compared with control birds' tissues using a paired *t*-test to generate a fold-change value and p-value. Normalized fluorescence fold changes with p-values < 0.05 are considered either significantly more or significantly less phosphorylated in the treatment birds compared with the control birds' tissues. Our previous work has shown that modern birds but not heritage birds injected just once on the day of hatch with CpG have decreased immune activation and signaling on d 15 post-hatch, and we wanted to observe how this pattern may change when birds are treated multiple times, including at this time point. In the heritage birds, the significantly differentially phosphorylated peptides unique to post-injection (d 16) compared with pre-injection (d 15) indicate increased T cell receptor signaling and activation. Specifically, key proteins such as ZAP70, NFATC1, PAK2 were unique to this time point and indicated T cell activation. In the modern birds, there was inhibition of fatty acid synthesis as well as destabilization of PGC-1 α indicating a lack of fatty oxidation unique to the modern birds' cecal tonsils post-injection. 4E-BP1 was uninhibited in the modern birds post-injection, which leads to suppression of protein synthesis; this suppression was also seen in inhibited PI3KCD signaling. Due to CpG treatment, the heritage birds exhibited adaptive immune activation, whereas we observed more metabolic changes in the modern bird.

Key Words: immune response, broilers, gastrointestinal health, kinome, signaling

115 Soiling and ammonia reductant application: How they impact the perceived value of wood shavings for turkeys. Valerie Monckton*, Nienke van Staaveren, Agnese Balzani, Peter McBride, Isabelle Kwon, and Alexandra Harlander, *University of Guelph, Guelph, Ontario, Canada*.

Turkeys' natural environments differ greatly from modern turkey farms. While farms attempt to provide outlets for turkeys' natural behaviors by providing litter (typically wood shavings), the lack of information on how to manage this litter often leads to the build-up of excreta. Proper litter management is crucial to control the build-up of excreta, litter moisture and atmospheric concentrations of ammonia, which can compromise turkey welfare. Replacement of litter or application of ammonia reductants are both methods to manage litter conditions; however, it is unknown how these practices alter the perceived value of litter to turkeys. The objective of this study was therefore to assess how soiling and ammonia reductant application affect turkeys' relative preference for wood shaving litter using a consumer-demand approach. We housed 24 11-week-old female turkeys in 6 pens (4 birds/pen) and raised them on litter consistent with commercial standards (soiled wood shavings). Each pen comprised a 'home' (H) and 'treatment' (T) compartment partitioned by a barrier with 2 one-way push-doors. We compared the hens' relative preference of H to T when feed (F) was present only in T and when one of 4 litters was present in T: unsoiled wood shavings (US), soiled wood shavings (SS), soiled wood shavings treated with an ammonia reductant (AS), and no litter (concrete floors covered in

rubber mats, NL). To assess motivation to access the treatments, the doors to T randomly weighed 0, 20 or 40% of the hens' average weight, while T and H traded compartments with each treatment/door weight combination. Over 22 d of testing, birds' presence in each compartment was determined via video-recordings using instantaneous scan sampling every 30 min for a mean of 14 h/combination. A generalized linear mixed model (PROC GLIMMIX, SAS V9.4) was used to analyze the effect of treatment, door weight, and their interaction on the percentage of time birds spent in T. Turkeys successfully pushed the doors. Door weight did not significantly affect where turkeys spent their time ($P > 0.05$). More time was spent in T with F present ($64\% \pm 9.0\%$, $P < 0.001$) than with US ($21\% \pm 13.3\%$), AS ($24\% \pm 12.9\%$), or NL ($20\% \pm 13.1\%$), while they spent the same amount of time in T with SS ($45\% \pm 10.8\%$) present. To the authors' knowledge, this is the first study to investigate turkeys' motivation to access soiled and ammonia reductant treated litter. We conclude that turkeys spent an equal amount of time on SS and F, while they had a lower relative preference for US, AS, and NL. SS may not have been soiled enough compared with H litter to influence turkeys' preference, or SS may possess attractive properties to turkeys (e.g., better foraging).

Key Words: motivation, preference, turkeys, litter, excreta

116 Not Presented

117 Effect of free choice large-particle calcium on performance and shell quality in laying hens. Diana Suckeveris*¹ and Doug Korver², ¹University of Sao Paulo, Piracicaba, Brazil, ²University of Alberta, Edmonton, Canada.

Laying hens are fed a portion of dietary Ca as large particles (LP) to ensure Ca supply during peak shell formation during the night. We investigated the effect of free-choice LP Ca in older laying hens on feed and total Ca intake, egg production, shell quality and internal quality. Individually-caged Lohmann LSL laying hens ($n = 72$; 70 wk of age) were distributed in a randomized complete block design with 6 dietary treatments. The Control group was fed a balanced wheat-soy diet according to the primary breeder management guide, with 1/3 small particle (<0.5 mm limestone), and 2/3 large particle Ca (>2.0 mm oystershell), without access to free-choice LP Ca (Control diet group). Birds were fed the Control diet, but with LP provided free-choice (T100 group). The other 4 dietary treatment groups, T75, T50, T25, and T0, were the Control diet with LP supplementation reduced to 75, 50, 25, or 0% of the Control group, respectively. These birds in T0 through T100 were given free-choice LP Ca as oystershell. Feed intake, LP and total Ca intake, egg production, and egg weight were recorded at 10:00 a.m. and 3:00 p.m. throughout the experiment. Total Ca consumption was calculated as intake of Ca from the mixed feed (small and LP) plus free-choice oystershell. Feed conversion ratio (FCR) was calculated as total feed (mixed feed plus free-choice oystershell) intake per kg of eggs produced. At d 0, 20, and 47 of the experiment, specific gravity, shell thickness and breaking strength, and Haugh units were measured. The Glimmix procedure was used for statistical analysis. The model assumed treatment (diet) as a fixed effect, and the blocks (cage) as a random effect. Feed intake, egg production, egg weight, FCR, eggshell traits and Haugh units were not influenced ($P > 0.05$) by dietary treatments. However, as dietary LP Ca level in the mixed diet was decreased, there was an increase in free choice Ca intake ($P < 0.001$), suggesting that hens are able to compensate for reduced mixed feed Ca content by increasing voluntary consumption of LP Ca. Total daily Ca intake was lower for T0 than for T100 (3.932 vs. 5.004 g; $P = 0.005$). However,

all groups consumed approximately the amount of Ca recommended by the primary breeder management guide (4.50 g), indicating that older laying hens regulate Ca intake. Therefore, use of free-choice LP Ca could allow for a reduction in LP in the mixed feed without reducing egg production and shell quality. Furthermore, free-choice LP Ca could reduce the abrasiveness of mixed layer feed in feed mill equipment.

Key Words: calcium appetite, feed intake, large-particle calcium, shell quality, laying hen

118 SNP-based breeding for broiler resistance to ascites and correlated production traits. Katie Lee*, Douglas Rhoads, and Nick Anthony, University of Arkansas, Fayetteville, Arkansas, United States.

The goal of this study is to identify the genes that influence ascites syndrome, a manifestation of pulmonary hypertension syndrome (PHS), in broiler chickens. In doing so, we intend to create a catalog of genetic regions for evaluation by poultry breeders for reducing ascites in the industry. Although controlled by better management, ascites has remained an issue in some markets world-wide. Genetic selection for ascites resistance could lead to a reduction in other positive production traits such as growth rate or muscle quality. Our previous studies have revealed 31 genetic regions with possible association with ascites resistance or susceptibility. Using assays of 2 single nucleotide polymorphisms (SNPs) per genetic region on our experimental ascites broiler lines and commercial broiler breeders, we will analyze genotypic data to see what effect these regions have, and which are the most influential on development of ascites. Our genotype association data has shown that the CPQ and LRRTM4 genes are QTLs for ascites phenotype and exhibit a strong epistatic interaction. This interaction also extends to some commercial broilers. We will present these genotype association data. Based on our data we are currently using these two regions to selectively breed 2 generations of our broiler research line. From the second generation of birds, 300 will be reared in a hypobaric chamber compared with 300 unselected birds. Another 300 s-generation birds will be reared as broilers compared with 300 unselected birds and then processed at 6 weeks of age. These data will be used to analyze the effects selection has on production traits and ascites resistance. The correlations we find between ascites resistance and production traits will help broiler geneticists in the industry make informed decisions about the relative costs for reducing ascites syndrome. Additionally, we are examining the underlying molecular mechanisms for some of the genes we have already identified, for their contribution to ascites phenotype.

Key Words: ascites, broiler, marker-assisted-selection, single-nucleotide-polymorphism, genetics

119 Effect of dietary brown seaweed (*Ascophyllum nodosum*) on yolk colour in laying hens. Cassie Stupart*, Bruce Rathgeber, and Janice MacIsaac, Dalhousie University, Truro, Nova Scotia, Canada.

Seaweed is considered a prebiotic and contains beneficial micronutrients that have shown to positively influence gut function and production performance in laying hens. Brown seaweeds contain the pigment xanthophyll, which has the potential to influence yolk color. The purpose of this study was to measure yolk color of laying hens eggs fed the commercialized brown seaweed *Ascophyllum nodosum* (AD), trademark name TascO®. The experiment was a $3 \times 2 \times 9$ factorial in a completely randomized design with inclusion level (0, 0.25, and 0.5%), strain of hen (Lohmann Lite-LSL White and Lohmann Brown Lite) and

age, denoted “period” (1, 2, 3, 4, 5, 6, 7, 8, 9) as the main effects. Each period was 28 d in length, starting from the first day of the trial. A total of 80 birds at 34 weeks of age were used for the 9-period study, with 8 replicate cages per treatment combination (5 birds per cage). A total of 3 eggs per cage (48 eggs total) were collected at the end of each period and yolk color was determined using a Hunter Lab MiniScan EZ. Eggs were broken open and the yolks separated from the albumen. Each individual yolk was placed in a quartz glass cuvette where the yolk membrane was punctured and the loose yolk was stirred. Color readings were measured in replicate, with stirring in between each reading. The colorimeter measured yolk color based off L* (black to white), a* (green to red) and b* (blue to yellow) color scales. The results were analyzed as a factorial arrangement using the Proc Mixed Procedure of ANOVA. An interaction effect ($P < 0.05$) between strain and level was observed for all color measures (L*, a* and b*). For a*, Lohmann LSL White Lite (LL) hens fed 0.25 and 0.5% AD had significantly lower values (10.69 and 10.50, respectively) compared with Lohmann Brown Lite (LB) hens fed 0.25% and 0.5% AD (11.59 and 11.22) and hens fed the control diets (11.62 for LB and 11.62 for LB). For b* values, LL birds fed 0.25% AD had significantly lower b* values (81.62) compared with LL hens fed the control and 0.5% inclusion levels (84.75 and 83.21) and LB birds fed the 0, 0.25 and 0.5% inclusion levels (84.95, 84.93 and 84.94). For L* values, LL birds fed the 0.25 and 0.5% AD had significantly higher values (62.01 and 62.26) than LB birds fed 0, 0.25 and 0.5% AD (61.09, 60.08, 61.08). LL hens fed the control diet (61.66) were significantly different only from LB hens fed the 0.25% inclusion level. It is clear that AD had a significant effect on yolk color, particularly within the LL birds. However, these differences were undetectable by the human eye, making them suitable for the marketplace.

Key Words: seaweed, laying hen, yolk colour

120 The identification of feeding regulatory neuropeptide, agouti-related peptide (AgRP), of two United States and two European strains of turkeys in the arcuate nucleus of the hypothalamus. Brooke Bartz*, Jesse Grimes, and Casey Nestor, *North Carolina State University, Raleigh, North Carolina, United States.*

Poultry feeding behaviors are influenced by various regulators which include environment factors, health status, and different strains. As a central hub in the regulation of several homeostatic processes, the arcuate nucleus (ARC) of the hypothalamus is an important brain region for appetite regulation. The co-expression of 2 opposing populations of energy homeostatic neurons: the orexigenic agouti-related peptide (AgRP) and anorexigenic pro-opiomelanocortin (POMC) neurons are localized in the ARC. It is well known in multiple species that AgRP acts as an endogenous antagonist to α -melanocyte stimulating hormone (α -MSH), a product of POMC neurons, ultimately promoting a food seeking behavior. While administration of AgRP has been shown to stimulate food intake and increase body weight both mammals and avian species, relatively little is known about differences in the endogenous AgRP expression that exist between different strains of turkeys. Therefore, the objective of this study was to examine the protein expression of AgRP in the arcuate nucleus of 4 different strains of commercially available turkeys using immunohistochemistry (IHC). Fertilized eggs of 2 United States (Strains A and B) and 2 European turkey strains (Strains C and D) were obtained and incubated under similar conditions. At hatch, birds were placed in pens based on strain with bird numbers per treatment based on successful hatching (Strain A = 20 birds total End of Study (EOS); Strain B = 19 birds total EOS; Strain C = 31 birds total EOS, Strain D = 13 birds total EOS) and performance data was calculated by pen. At 5 weeks of age, birds were weighed and euthanized individually

just before tissue collection. Birds were euthanized by cervical dislocation, followed by rapid decapitation and gender identification was obtained post-mortem. Whole brain tissue was excised and placed in 4% paraformaldehyde at 4°C for 48h, followed by 20% sucrose at 4°C until the time of sectioning. Tissue was cut from the caudal to rostral plane, on a freezing-stage microtome at 30 μ m and select sections containing the ARC were used for IHC with a primary antibody targeting AgRP. AgRP cell bodies and neuronal fibers were successfully observed in all 4 strains of birds. Feed consumption per pen positively correlated with the AgRP pixel % calculated (R-Squared = 0.9033; Strain A: 2.45kg/bd, 0.55%; B: 2.58kg/bd, 0.82%; C: 2.55kg/bd, 0.58%; and D: 2.74kg/bd, 1.29%, respectfully). Based on these pilot study results, orexigenic feeding behaviors are likely increased by the AgRP neurons located in the ARC of the hypothalamus. Further research must be completed to determine the extent of these physiological implications.

Key Words: feeding neuropeptides, agouti-related peptide, turkey, arcuate nucleus, hypothalamus

121 Data-driven, student-centered curriculum redesign in poultry science. Emily Berger*, Christine Alvarado, and Audrey McElroy, *Texas A&M University, College Station, Texas, United States.*

Poultry Science academic departments strive to update curriculum to better align with current agriculture practices, employer needs, and student readiness for future careers. The poultry industry continues to embrace new technologies, better management practices, and changes within the agriculture sector. To fulfill these responsibilities, the Department of Poultry Science at Texas A&M University chose to engage in the Program (Re) Design (PRD) process for its undergraduate curriculum. Existing curriculum strengths and weaknesses were evaluated using feedback on program evaluation surveys (153 responses) from stakeholders, including current students, former students, current faculty, and employers within the poultry industry. Additionally, job postings for careers in the poultry industry and the curricula offered at peer institutions were reviewed. The data supported discrepancies in knowledge areas of food safety, regulatory compliance, and quality assurance, as well as deficient transferrable skills in general business, understanding implications of consumer and public perceptions, and professional communication. The curriculum committee (4 faculty members, 2 academic advisors, 2 undergraduate students, and 3 curriculum consultants) developed 3 disciplinary learning outcomes and 5 outcomes focused on transferrable skills. Performance indicators were reviewed and grouped into existing courses and potential new courses. Each course included indicators for assessment of both disciplinary knowledge and transferrable skills, to ensure that students continue developing these much-needed competencies throughout their time in the program. Based on this review process, the undergraduate curriculum was updated by the addition of 7 courses and revision of existing courses to include progressive learning integrating both disciplinary knowledge and transferrable skills. While the PRD process was implemented in the context of a Poultry Science program at a large research-extensive US university, it can be adapted to a variety of programs across disciplines and institutions.

Key Words: curriculum redesign, undergraduate students, instruction, stakeholder needs, program evaluation

122 Thyroid hormone and estradiol differentially modulate pituitary gonadotropin responses in turkey hens with differential egg production. Kristen Brady*¹, Julie Long², Hsiao-Ching Liu³,

and Tom Porter¹, ¹University of Maryland, College Park, Maryland, United States, ²ARS, USDA, Beltsville, Maryland, United States, ³North Carolina State University, Raleigh, North Carolina, United States.

Low egg producing hens (LEPH) and high egg producing hens (HEPH) show differences in both gene expression and in vitro hormone responsiveness in the hypothalamo-pituitary-gonadal (HPG) axis. Hypothalamic, pituitary, and ovarian transcriptome analysis in LEPH and HEPH inferred upregulation of thyroid hormone production and metabolism in LEPH and upregulation of estrogenic genes in HEPH. The pituitary gonadotropic hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH), are glycoprotein hormones that are encoded by unique β subunits (*LHB* and *FSHB*, respectively) and a common α subunit (*CGA*). The effect of thyroid hormone (T_3) and estradiol (E_2) on *LHB*, *FSHB*, and *CGA* mRNA levels was examined in pituitary cells isolated from LEPH and HEPH ($n = 3$ per group). Isolated pituitary cells were pretreated with no pretreatment (NPT), 1.5 ng/mL T_3 , or 1.5 ng/mL E_2 for 12 h, followed by treatment with gonadotropin releasing hormone (GnRH) at 0, 10^{-9} , and 10^{-8} M for 6 h. After incubation, mRNA levels were assessed through RT-qPCR. Data were analyzed by a 3-way ANOVA using the mixed models procedure of SAS, with significance denoted at $P < 0.05$. T_3 pretreatment decreased *LHB*, *FSHB*, and *CGA* mRNA levels compared with NPT in HEPH cells, regardless of GnRH treatment concentration. T_3 pretreatment also decreased *LHB*, *FSHB*, and *CGA* mRNA levels in LEPH cells, but only at 10^{-9} M GnRH for *LHB*, 0 M and 10^{-9} M GnRH for *FSHB*, and 0 M and 10^{-8} M GnRH for *CGA*. E_2 pretreatment decreased *LHB* mRNA levels in HEPH cells compared with NPT at 10^{-8} M GnRH. E_2 pretreatment also decreased *FSHB* mRNA levels in HEPH cells relative to NPT at 0 M GnRH and increased *FSHB* mRNA levels in LEPH cells at 10^{-9} M GnRH. T_3 pretreatment negatively impacted *LHB*, *FSHB*, and *CGA* mRNA levels in cells from LEPH and HEPH, however the effect was more prominent in HEPH cells. E_2 pretreatment in HEPH cells decreased *FSHB* mRNA levels at lower GnRH treatment concentrations but decreased *LHB* mRNA levels at higher GnRH treatment concentrations. In contrast, E_2 pretreatment upregulated *FSHB* in cells from LEPH at 10^{-9} M GnRH. Overall, T_3 pretreatment negatively regulated gonadotropin production, independent of GnRH treatment concentration; whereas, E_2 pretreatment had varied impacts on gonadotropin production, depending on the rate of egg production of the hens. In vitro, T_3 and E_2 impacted pituitary gonadotropin subunit mRNA levels, both with and without GnRH treatment. Furthermore, pituitary cells from LEPH and HEPH responded differently to each pretreatment in term of gonadotropin subunit mRNA levels, indicating functional differences in the HPG axis regulation that may be related to egg production level.

Key Words: gonadotropin, thyroid hormone, estradiol, HPG axis, turkey

123 Improving biosecurity through the use of a low cost, open source vehicle cleaning and disinfection system. Eric Benson¹, Robert Alphin¹, Daniel Hougentogler¹, Adrianna Szostek¹, and Jonathan Moyle², ¹University of Delaware, Newark, Delaware, United States, ²University of Maryland, Salisbury, Maryland, United States.

Avian influenza (AIV) and Newcastle disease (NDV) are examples of highly infectious diseases causing significant outbreaks impacting the poultry industry. Biosecurity is one of the few preventative techniques available for most major avian diseases. Biosecurity can be used both to prevent diseases from entering a facility (“isolation”) and to keep diseases already present on a farm from leaving the farm (“containment”). A current virulent Newcastle disease virus (vND) outbreak in California has resulted in 409 confirmed cases as of April 1, 2019. Poor biosecurity

and quarantine led to expansion of the outbreak. An outbreak of highly pathogenic avian influenza (HPAI) in 2014 – 2015 resulted in over 220 confirmed cases and 50 million poultry dying due to disease or control measures at a cost of \$3.5 billion. A team developed a low-cost, open source and portable vehicle undercarriage decontamination system. The system was improved, and a 2-step system was used to evaluate the process. First, the coverage of the system was evaluated using litmus paper strips adhered to various locations on the truck exterior and undercarriage and sprayed with a diluted citric acid solution. In the second step, galvanized steel coupons inoculated with NDV were affixed to the truck using magnets and the truck driven through the decontamination system. Three coupon orientations (horizontal, vertical, and complex) were chosen to test the system’s ability to target various locations on the truck. One peroxide disinfectant and one detergent, were used to determine the differences in decontamination effectiveness between a disinfectant and detergent. The viral material from the steel coupons was pooled by orientation type and inoculated into specific pathogen free eggs. Chorioallantoic fluid was collected from each egg and used in hemagglutination (HA) and hemagglutination-inhibition assays (HI). The horizontal coupon orientation passed both trials with no positive HA results and a neutralizing index above 2.8. The vertical and complex coupon orientations did not pass the disinfectant guidelines, but a decrease in virus titer was observed in both groups. The system was able to achieve approximately a 2 log drop in virus titer.

Key Words: biosecurity, disinfection, vehicle, avian influenza, Newcastle disease virus

124 Strain differences and effects of different stocking densities during rearing on the musculoskeletal development of pullets.

Danielle Fawcett* and Tina Widowski, University of Guelph, London, Ontario, Canada.

There are few published studies on the effects of stocking density (SD) on the physical development of pullets, particularly between different genetic lines. The objectives of this study were to determine if strain or SD affects musculoskeletal development of pullets reared in furnished pullet cages and to determine if these rearing conditions have an impact on keel bone health of adult hens. Three strains were used: Lohmann Selected Leghorn Lite (LSL), Dekalb White (DW) and Lohmann Brown (LB). Floor space and number of birds per cage were 247cm², 91 birds; 270cm², 83 birds; 299cm², 75 birds; and 335cm², 67 birds. At 16 wks of age, the keel bone, muscles of the breast, wings and legs and long bones of the wings and legs were collected from a sample of 8 birds per treatment-strain combination to compare keel development, muscle growth and bone breaking strength (BBS). DW and LB pullets that were reared at a SD of 247 cm²/bird or 299cm²/bird were transferred to large furnished cages at a SD of 748cm²/bird to assess keel damage at 35 and 50 wks of age. A generalized linear mixed model was used to assess the effects of SD, strain and their interaction on muscle and keel bone characteristics and a generalized linear model was used to analyze BBS data. Muscle weights and keel measures were corrected for BW to account for body size difference between strain. SD did not affect the majority of keel bone characteristics, muscle weights or BBS. However, strain differences were found for all keel bone characteristics, all muscle weights and the majority of BBS measures. The keel metasternum, height and overall area of the keel bone were found to be smaller in LB pullets compared with LSL and DW pullets ($P < 0.0001$); however, cartilage length and overall percentage of the cartilage present on the keel bone was greatest in LB pullets ($P < 0.0001$). Leg muscles were heaviest in LB pullets ($P < 0.05$); however, breast muscles were heaviest in LSL and DW pullets ($P < 0.0001$). LB pullets had greater

BBS of the humerus ($P < 0.0001$) and radius ($P < 0.0001$), whereas BBS of the tibia was greatest in LSL pullets ($P = 0.001$). Additionally, there was a higher prevalence of keel fractures at 50 wks of age in LB hens compared with DW ($P = 0.0144$). Overall, rearing SD had little impact on the musculoskeletal growth of pullets; however, significant differences were found between strains which may reflect strain-specific behavior. Additionally, differences in keel development between strains may lead to differences in keel damage in adult hens.

Key Words: pullet, keel bone, musculoskeletal growth, rearing stocking density, strain

125 What are the needs of the U.S. table egg industry? Darrin Karcher*¹, Alva Muhammad¹, Deana Jones², Oscar Garrison³, and Lesa Vold⁴, ¹Purdue University, West Lafayette, Indiana, United States, ²USDA ARS, Athens, Georgia, United States, ³United Egg Producers, Johns Creek, Georgia, United States, ⁴Egg Industry Center, Ames, Iowa, United States.

Land-grant universities must continue to meet the needs of the table egg industry even as companies are faced with new and unique challenges in today's business environment. Providing Extension and outreach programming, undergraduate education and research are all needed to help the industry overcome these challenges. To these ends, a needs assessment survey was created with input from stakeholder groups including the United Egg Producers, United Egg Association and the Egg Industry Center. The survey consisted of 30 questions across extension, teaching and research. The instrument was disseminated via email from the stakeholder groups as well as state poultry associations and individuals, mostly allied industry, who sent it to their customers. The survey was open November and December 2018. There were 447 responses with 192 completing the entire survey. The respondents' jobs were classified as: 53% production, 17% processing, 17% feed mill, 7% breeders/hatchery and 6% allied industry/other and most respondents (92%) were associated with complexes housing more than 50,000 hens. The top 3 egg producing states were best represented: 14% IA, 13% OH, 13% IN. When asked where the company seeks information/education/training, responses were 30% university, 24% consultants and 23% commodity associations. Local and state extension services are used by the table egg industry 51 – 68% of the time. Need for additional employee training across different areas was assessed by ranking on a 1–10 scale (none to extremely beneficial). The mean scores for different areas were animal welfare (7.72), management (7.72), biosecurity regulations (8.23), manager training (7.57), food safety (7.49) and product quality (7.42). When asked about using research information to drive day-to-day decisions, 63% indicated they use it monthly or more frequently while 29% use it a few times a year. Participants were asked open-ended questions throughout the survey to understand more specific programming or research needs. Example responses included: "Smaller companies are the ones most in need of training and most will not likely send their non-supervisory employees far off for training. Training that can be developed, shared and implemented locally may be more utilized and effective." "Cage free production assistance" and "More on site education and guidance." Overall, the needs assessment indicates there are numerous programmatic areas that should be addressed over the next 3–5 years. The impact could be magnified if we rethink collaborations among extension personnel, commodity associations and private consultants.

Key Words: laying hen, extension, teaching, research, assessment

126 Eggshell cuticle reduces bacterial adherence in table eggs. Garima Kulshreshtha*¹, Nicholas D. Calvert¹, Ty Diep², and Maxwell Hincke¹, ¹University of Ottawa, Ottawa, Ontario, Canada, ²Burnbrae Farms Limited, Upton, Ontario, Canada.

Food safety of table eggs is critical, as consumption of contaminated eggs and egg products leads to outbreaks of food poisoning. The eggshell cuticle is the first line of defense to restrict the entry of egg-associated food safety pathogens such as *Salmonella*. The completeness of cuticle coverage and its chemical composition play an important role in limiting bacterial entry; however, bacterial load on eggshell surface is another key factor. The active components that block bacterial adhesion to the eggshell surface are largely unknown. The present study describes the effect of differences in cuticle quality related to hen strain and egg washing on bacterial adhesion to eggshell surface. Graded (washed) and ungraded (unwashed) eggs collected from both brown and white egg laying Lohmann flocks (mid lay) were used in the study. A novel enumeration method was developed using green fluorescent protein (GFP)-expressing *Salmonella* Typhimurium to monitor bacterial adhesion to the eggshell surface, with independent measurement of autofluorescence to quantify cuticle deposition. Eggshell cuticle and adhered bacterial cells were quantified / visualized on eggshell fragments ($n = 3/\text{egg}$) from blunt, equatorial and sharp regions of each egg ($n = 3$). Images ($n = 4/\text{fragment}$) of ES samples with or without fixed bacteria were acquired using a Zeiss LSM 880 with AiryScan confocal microscope through a 20x objective. Both tile-scanning and Z-stacking were acquired using excitation and emission spectra profiles for both enhanced green fluorescent protein (eGFP) and mCherry (green and red, respectively). Images were displayed using Imaris software, and ImageJ was used for data processing. Integrated density for all channels was measured to quantify autofluorescence (cuticle deposition), and the green channel was used to quantify GFP-bacteria. Staining of eggshell fragments with Cuticle blue showed that washing of eggs reduced the cuticle coverage. Bacterial cell density was quantified on graded eggs with less cuticle, bleach treated eggs with no cuticle and ungraded eggs with best cuticle coverage. Higher bacterial density was observed on graded and bleach treated eggs compared with ungraded eggs ($P \leq 0.05$, $n = 3$; one-way ANOVA), which permitted an inverse relationship between *Salmonella* Typhimurium cell adhesion and cuticle quality to be established ($R^2 = -0.79$). These results indicate that the cuticle constituents significantly reduced bacterial load on eggshell surface, independent of bacterial penetration. The outcome of our research will provide new tools to breeders and egg producers to assess cuticle quality to improve the safety of table eggs. (Supported by Egg Farmers of Canada)

Key Words: eggshell, cuticle, confocal microscopy, pathogens, antimicrobial

127 A meta-analysis of the effects of dietary supplementation of a novel microbial muramidase on broiler's performance growing in Latin American conditions. Leticia Bittencourt*¹, Vitor Fascina¹, Maria Elena Rubio², Javier Ameri³, Marcelo Hidalgo³, Rual Lopez-Ulibarri⁴, Rafael Hermes¹, and Estefania Calvo⁵, ¹DSM Nutritional Products, Sao Paulo, Brazil, ²DSM Nutritional Products, Guadalajara, Mexico, ³DSM Nutritional Products, Tortuguitas, Argentina, ⁴DSM Nutritional Products, Kaiseraugst, Switzerland, ⁵DSM Nutritional Products, Village Neuf, France.

Muramidases are enzymes that hydrolyze peptidoglycans (PGNs) that are structural polymers conforming, and exclusively found in nature, in bacterial cell walls. It is hypothesized that excessive PGNs in the gastrointestinal track of chickens, as result of natural bacterial dead and turnover in the gut microbiota, could negatively impact gastrointestinal

functionality by interfering with its capability for nutrient absorption and, consequently, on performance. To demonstrate the consistency of the beneficial effects on broiler's performance by the dietary inclusion of a novel microbial muramidase, a meta-analysis was carried out using the results of 7 independent *in vivo* performance trials conducted in different geographical locations of Latin America (LATAM): Brazil, Argentina, Chile and Mexico. A total of 5,312 one-day male broilers (Cobb 500 or Ross 308) were dietary supplemented with a microbial muramidase doses between 15,000 to 45,000 muramidase units (LSU (F)) per kg diet from 1 to 42 d. The simultaneous analysis of all trials was based on a linear mixed effects model with feed conversion ratio (FCR) as continuous endpoint parameter and muramidase levels as a continuous exploratory variable, including a random trial effect. Meta-analysis was performed in the SAS PROC MIXED procedure at 5% probability. The results indicated a linearly improved FCR response to increasing muramidase levels on broiler diet ($P < 0.0001$; $Y = 1.7525 - 0.00142x$). On average, an improvement by 0.0142 FCR per 10,000 microbial muramidase units added to broilers diet was determined. It can be concluded that the dietary supplementation of novel microbial muramidase in broiler diets growing under LATAM conditions significantly improves feed efficiency of broilers and the beneficial effects are dose-dependent.

Key Words: muramidase, peptidoglycans, gastrointestinal functionality, performance

128 Algal polysaccharides to improve gut health. Maria Garcia*^{1,2}, ¹*Olmix Group, Velp, Netherlands*, ²*INRA, Nouzilly, France*, ³*Brest University, Brest, France*.

The cell wall of marine algae is mainly composed of water soluble sulfated polysaccharides with several biological activities such as modulation of the immune response and reinforcement of gut barrier function. The present study aimed at assessing the ability of different seaweed extracts on improving gut health. The first part of the work tested a specific extract from green algal *Ulva* sp to upregulate the expression of immune mediators and the molecular mechanisms underlying this immunomodulatory activity by identifying the cell receptor and the signaling pathways involved. The second part of the work focused on the potential enhancement properties of a red algal extract from *Solieria Chordalis* on intestinal integrity using *in vitro* cell models HT-29 MTX and Caco-2 cells, under physiological and inflammatory conditions. Results showed that *Ulva* sp extract has the capacity to upregulate the expression of immune mediators: TNF α , CCL20, IL-1 α , TGF- β that are involved in cell differentiation and proliferation, recruitment of immune cells and anti-inflammatory activities. *Ulva* sp extract interacts with TLR4 and TLR2 and leads to rapid activation of transcription factors PI3K and NF- κ B. The red algal extract was shown to upregulate the expression of different target genes related to claudin-2, ZO-1 and ZO-2. The red algal extract upregulates the expression of mucin targeted genes: MUC4, MUC2, MUC5B and MUC5AC. The combination of both algal extracts can reinforce gut health targeting barrier function which is the first line of mucosal defense and via modulation of local innate and adaptive immune responses and induction of anti-inflammatory activities. The use of macroalgal sulfated polysaccharides can play an important role within the reduction of antibiotics in farms.

Key Words: macroalgae, immunomodulating agent, gut barrier, sulfated polysaccharides, mucin

129 Broiler enrichment: Welfare and performance benefits. Elizabeth Bobeck*, Meaghan Meyer, and Anna Johnson, *Iowa State University, Ames, Iowa, United States*.

Genetic selection for increased growth rate has contributed poor locomotion or leg lameness in up to 30% of modern commercial broilers, necessitating up to 2% culls within the flock. The 2017 National Chicken Council animal welfare guidelines have recognized this challenge and require gait scoring of 100 birds per flock to evaluate leg health within one week of slaughter. Past broiler research reports that motivating physical activity increased tibia strength and decreased lameness. Enrichment options that motivate birds to move have been sparingly explored; however, none have effectively captured broiler attention beyond object novelty in conjunction with improving growth and performance. We designed a novel enrichment device for poultry based on innate visual and predatory feeding behavior, wherein a red laser dot moved randomly on the pen floor several times per day. Twelve-hundred Ross 308 broilers were housed 30/pen for a 6-week grow-out where half of the pens received laser enrichment treatment daily (4 "laser periods"/day at 05:30, 11:30, 17:30, and 23:30; LASER) and the other half served as control with no laser enrichment exposure. Seven pens each of control and laser enrichment treatment (total $n = 14$) were video recorded daily d0–8 and once weekly 2–6. Five randomly selected focal birds/camera pen ($n = 70$) were marked with animal-safe food coloring on d0 and observed for active and feeding behavior (video) and lameness weekly (individual live evaluation). Performance outcomes were calculated by pen and averaged per bird for each 2-wk performance period and overall (d0–42). Data were tested for normality and analyzed using a one-way ANOVA (Proc Mixed [performance] or Proc Glimmix [behavior], SAS Version 9.4). LASER birds were more active on d0, 1, 3, 4, 5, 7, and 8, and on wks 2–5, moving up to 17.4% more than control birds in the first 9d and up to 12.2% more weekly ($P \leq 0.05$). LASER birds spent more time at the feeder on d0, 1, 2, 5, and 8, and on wk 1 and 5 ($P \leq 0.05$), with no lameness differences (LASER vs. control). LASER birds had greater feed intake and weight gain in all periods, with an overall increased gain of 0.24 ± 0.03 kg/bird ($P \leq 0.05$). FCR was improved in LASER birds in the grower and finisher periods, as well as overall ($P \leq 0.05$), with an overall improvement of 0.07 ± 0.01 points. ADG was improved in laser enriched birds in the starter and finisher periods, and overall ($P \leq 0.05$), with an overall increase of 5.7 ± 0.0006 g/bird/d. In conclusion, the laser enrichment was able to motivate broilers to move from wk 1–5 until the last trial wk 6, while improving weight gain and FCR. Future work will determine optimum exposure length and frequency to maintain performance and welfare outcomes.

Key Words: broiler, enrichment, welfare, behavior, performance

130 Broiler euthanasia by cervical dislocation or Koechner Euthanizing Device: Severing both carotid arteries immediately after application does not hasten the time to death. Richard Jeff Buhr*¹, Caitlin Harris^{1,2}, Nicole Bartenfeld Josselson¹, Leonie Jacobs³, Ranjit Boyal⁴, and Dianna Bourassa⁴, ¹*USDA ARS USNPRC, Athens, Georgia, United States*, ²*University of Georgia, Athens, Georgia, United States*, ³*Virginia Tech, Blacksburg, Virginia, United States*, ⁴*Auburn University, Auburn, Alabama, United States*.

The time to brain death during euthanasia of broilers using cervical dislocation (CD) has been partially attributed to the stretching and/or severing of the carotid arteries that would contribute to ischemia and accelerate brain hypoxia. The current study was undertaken to determine if immediately severing both carotid arteries in broilers euthanized by CD or using the Koechner Euthanizing Device-C (KED) impacted the time to cessation of musculoskeletal movements (indication of brain stem and spinal cord death). Male broilers (22/treatment) at 6 wk-of-age were assigned to either CD, CD+bled, KED, or KED+bled in sequential-rotating order. Each broiler was placed into a metal bleeding cone, either

CD or KED was performed, and the bled treatments immediately had the carotid arteries severed with a hand-held knife mid neck (to avoid cutting the damaged spinal cord at the base of the skull). CD was performed by 1 individual, KED and post-application observations by 2 individuals, and the necropsy and identification of the carotid arteries by 1 individual. The persistence of musculoskeletal movements was observed every 15 s for 4 min, or until there was cessation of movement for 1 min. The cessation of movement following CD occurred at 114 ± 33 s and was significantly ($P = 0.0387$) shorter compared with KED at 142 ± 42 s. Compared with the not bled treatments, immediately severing both carotid arteries did not alter the cessation of movement for either CD+bled (125 ± 38 s $P = 0.7281$) nor KED+bled (134 ± 40 s $P = 0.8339$). During necropsy dissection, the severing of both carotid arteries during CD or KED was not observed and the frequency of 1 carotid artery being severed was 4/22 (18%) for CD and 2/22 (9%) for KED. Five of the 6 (CD and KED) broiler carcasses with 1 severed carotid artery had shorter than average time to cessation of movement. However, 15/38 (39%) broilers with both carotid arteries intact had the same or shorter time to cessation of movement, compared the carcasses with 1 severed carotid artery. Although the carotid arteries are frequently stretched and displaced laterally by the disarticulated neck vertebra, they predominately (86%) remain intact during both CD and KED euthanasia. These results indicate that reducing blood flow to the brain by severing the carotid arteries during euthanasia did not accelerate the time to death, and were unable to aid in explaining the physiology underlining the earlier attainment of death following CD when compared with KED. Brain ischemia most likely occurs by the severing of the bilateral vertebral arteries that occurs during both CD and KED. The ruptured vertebral arteries are likely the source of the pooled blood at the skull-vertebra gap during CD and KED euthanasia.

Key Words: euthanasia, cervical dislocation, Koechner euthanizing device, carotid arteries, brain stem death

131 Effects of strain and stocking density on broiler yield and meat quality. Shawna Weimer^{*2}, Stacy Zuely¹, Kailynn VanDeWater¹, Melissa Davis¹, Darrin Karcher¹, and Marisa Erasmus¹, ¹Purdue University, West Lafayette, Indiana, United States, ²University of Maryland, College Park, Maryland, United States.

Consumer concern for the welfare of broiler chickens has increased interest in broilers from slower growing strains. However, raising slower growing broilers will lower chicken production. Furthermore, there may be differences in meat quality between faster and slower growing chicken strains that could be affected by stocking density. Our objective was to evaluate the effects of strain and stocking density on the yield and meat quality of broilers. Our study was a 2x2 factorial with main effects of strain and stocking density. The broiler strains used reached market weight (2.8 kg) at 42 d (conventional strain, CONV) and at 63 d (slow growing strain, SG). Birds were reared in 16 pens at either 29 kg/m² or 37 kg/m². Birds were provided the same starter, grower and finisher diets with SG phase changes occurring when CONV birds' body weights were matched. At the beginning of the trial, 8 focal birds per pen were identified and these birds were processed at market weight ($n = 118$ birds). At processing, live weight, carcass weight and part weights of the wings, quarters, breasts, tenders and rack were recorded to calculate carcass and part yield. The right breast and thigh from each bird was measured for color, 24 h pH, cook loss and Warner-Bratzler shear force. Data were analyzed using a 2-way ANOVA with the main effects of strain and stocking density and the random effect of pen. There was no effect of strain or stocking density on live weight or carcass weight ($P > 0.05$). There was an effect of strain on carcass and part yields as

well as breast and thigh color, 24 h pH, cook loss and shear force and an effect of stocking density on breast shear force. CONV birds had 3.3%, 13.0%, and 2.8% higher carcass, breast and tender yield ($P \leq 0.002$), respectively. SG birds had 2.7%, 5.0%, and 7.5% higher wing, quarter and rack yield ($P < 0.0001$), respectively. SG thigh meat had higher L* (lightness) and a* (redness) values than CONV ($P \leq 0.002$). SG breast meat had higher L* values and CONV had higher b* (yellowness) values ($P < 0.001$). CONV breast meat had a higher 24 h pH than SG (6.12 vs. 5.97, $P < 0.0001$). SG thigh meat had higher cook loss than CONV (9.19% vs. 7.47%, $P = 0.007$). CONV breast meat had a higher shear force than SG (29.13 N vs. 18.29 N, $P = 0.002$) and birds raised at 37 kg/m² had a higher shear force than birds raised at 29 kg/m² (26.64 N vs. 20.66, $P = 0.04$). CONV thigh meat had a lower shear force than SG (10.37 N vs. 15.47 N, $P = 0.0008$). These results indicate that the strain differences in yield and meat quality may warrant the suitability of chicken products from different broiler strains for distinct product markets.

Key Words: broiler, strain, stocking density, yield, meat quality

132 External parasites (bed bugs, *Cimex lectularius*) in cage free housing systems: Hen welfare and productivity. Marisa Erasmus^{*}, Kailynn VanDeWater, Yiru Dong, Aaron Ashbrook, Sudip Gaire, and Ameya Gondhalekar, Purdue University, West Lafayette, Indiana, United States.

External parasites such as bed bugs (*Cimex lectularius*) are a threat to laying hen welfare and farm worker health, and are becoming increasingly problematic as the US egg industry transitions to cage free production systems. A field trial was conducted to examine the effects of bed bug infestations on hen welfare and productivity. Data were collected on a total of 4 flocks housed in commercial egg barns (2 barns with bed bugs (BUG) and 2 control (CON) barns) with the same type of housing system (Big Dutchman, Inc. MI) and genetic line of hens. Data were collected at 39 (1 BUG and 1 CON flock), 48 (1 BUG and 1 CON flock), 57 (2 BUG and 2 CON flocks), 66 (2 BUG and 2 CON flocks) and 75 (1 BUG and 1 CON flock) weeks. At each age, hen welfare (feather condition of the head, neck, back, wings and tail; keel bone fractures and deviations; and footpad condition) was scored based on the Welfare Quality® Assessment Protocol using 100–120 hens/barn. Bed bugs were also counted at each age when welfare assessments were conducted. Average hen body weight, hen day production and mortality for each age were obtained from flock records. Age-related differences in welfare measures were analyzed with the Friedman test, differences in welfare measures between BUG and CON flocks were analyzed with the Mann-Whitney U test, and hen mortality, productivity and body weight data were analyzed using the MIXED procedure with time as a repeated measure (SAS 9.4). No bed bugs were found in CON barns. For both CON and BUG flocks, feather condition, footpad condition and keel damage worsened with age ($P < 0.0001$). Feather damage on the head ($P = 0.03$), neck ($P < 0.0001$), back ($P = 0.0006$), rump ($P < 0.0001$), right wing ($P < 0.0001$), left wing ($P < 0.0001$) and tail ($P < 0.0001$), and footpad condition ($P < 0.0001$) were worse for hens in CON barns. However, keel bone deviations and fractures ($P < 0.0001$) were worse for hens in BUG barns. Mortality increased with age (39 weeks: $0.09 \pm 0.06\%$; 48 weeks: $0.2 \pm 0.06\%$; 57 weeks: $0.02 \pm 0.04\%$, 66 weeks: $0.49 \pm 0.04\%$, 75 weeks: $0.52 \pm 0.06\%$, $P = 0.02$) but did not differ between CON and BUG flocks. Hen-day production declined with age (39 weeks: $93 \pm 1.4\%$; 48 weeks: $91 \pm 1.4\%$; 57 weeks: $90 \pm 1.0\%$, 66 weeks: $87 \pm 1.0\%$, 75 weeks: $82 \pm 0.4\%$, $P = 0.02$) but did not differ between CON and BUG flocks. While bed bugs did not negatively influence hen feather condition and productivity in this

small field trial, keel bone fractures and deviations were more severe, indicating a possible relationship between bed bugs and hen skeletal health. Research is ongoing to understand the influence of bed bugs on hen activity level, behavior and skeletal health.

Key Words: laying hen, cage free, parasites, welfare, productivity

133 Development and establishment of organoid biobanking: long-term *ex vivo* cultures for three-dimensional organoids from chicken intestinal crypts. Yuhua Farnell*¹, Dan Zhao¹, Michael Kogut², Kenneth Genovese², Laurie Davidson³, and Robert Chapkin³, ¹*Department of Poultry Science, Texas A&M AgriLife Research, Texas A&M University, College Station, Texas, United States*, ²*Southern Plains Agricultural Research Center, Agricultural Research Service, United States Department of Agriculture, College Station, Texas, United States*, ³*Program in Integrative Nutrition & Complex Diseases, Texas A&M AgriLife Research, Texas A&M University, College Station, Texas, United States*.

Intestinal organoids, known as “mini-guts,” are derived from intestinal stem cells, which are located at the base of the villi crypts. Recently, organoids derived from different organs including intestine, brain, liver have been used as a novel pathophysiological model to study the mechanism of host-pathogen interactions. However, there is still a need for long-term cultures of chicken intestinal epithelial cells as well as the development of an *ex vivo* cell culture model. Therefore, the objectives of this study were to generate crypt-derived organoids from chicken intestines and to optimize conditions for cell cryopreservation and passages. Crypt samples from duodenum, jejunum, ileum, and cecum were collected from layer and broiler chickens with ages ranging from day one to 20 weeks. Five-hundred isolated crypts were embedded in a basement membrane matrix, and cultured with media prepared in house. For cryopreservation, dissociated organoids were resuspended in a freezing medium. The characteristics of intestinal organoids upon extended passages and freeze-thaw cycles were analyzed using reverse transcription-PCR, immunoblotting, and live cell imaging. We then established a long-term 3-dimensional *ex vivo* culture system of the chicken organoids from the duodenum, jejunum, ileum, and cecum. The crypt-derived organoids were successfully grown and propagated to form 3-dimensional epithelial structures that can be cultured for up to 3 weeks. Organoids were formed on day one, budding appeared on d 3, and robust budding was observed on d 7 and beyond. Immunoblotting using E-cadherin (the marker for epithelial cells) and leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5, the marker for stem cells), confirmed that crypt-derived organoids are epithelial cells that contained LGR5-positive stem cells. To the best of our knowledge, this work was the first comprehensive report for establishing long-term, organoid cultures from duodenum, jejunum, ileum, and cecum of layer and broiler chickens, which will help us better understand host-pathogen interactions, eventually leading to the discovery of pathogen intervention strategies in poultry.

Key Words: organoid, crypt, stem cells, chicken, intestines

134 Not Presented

135 Synbiotic supplementation to decrease cecal *Clostridium perfringens* load in broiler chickens induced with necrotic enteritis. Revathi Shanmugasundaram*¹, Ashley Markazi², Mohammad Mortada¹, Lisa Bielke², Basharat Syed⁴, Chasity Pender³, Shelby Curry³, Raj Murugesan³, and Ramesh Selvaraj¹, ¹*University*

of Georgia, Athens, Georgia, ²*The Ohio State University, Wooster, United States*, ³*BIOMIN America Inc, Overland Park, United States*, ⁴*BIOMIN Holding GmbH, 3131 Getzersdorf, Austria*.

A series of studies were conducted to determine the efficacy of synbiotic applications to combat the negative effects of necrotic enteritis (NE). An *in vitro* study was conducted to test the effect of probiotics species supernatants to decrease *Clostridium perfringens* (CP) proliferation. *Lactobacillus reuteri*, *Enterococcus faecium*, *Bifidobacterium animalis*, and *Pediococcus acidilactici* culture supernatants decreased the proliferation of CP at 1:1 supernatant: pathogen dilution. Subsequently, 2 *in vivo* studies were conducted to determine the *in vivo* response of synbiotic supplementation containing the same probiotic strains as above on production performance and cecal CP load in broilers challenged with *Eimeria maxima* and CP to induce mixed NE infection. In Experiment I, 75 broiler chicks were randomly allotted to 3 treatment groups, control (basal diet), ionophore (Salinomycin), and synbiotic (PoultryStar® me) from day of hatch and induced with NE in 5 replications (5 birds/replicate). There were no significant treatment effects on BW, feed consumption, and feed conversion ratio. However, ionophore or synbiotic supplementation increased ($P < 0.05$) villi height and decreased IL-1 mRNA abundance, while synbiotic supplementation increased ($P < 0.05$) IL-10 mRNA abundance compared with the control group, respectively. In Experiment II, 360 broiler chicks were randomly allotted to 3 treatment groups (8 replicates/group with 15 birds/replicate), negative control (control) or positive control (NE; basal diet) or NE + synbiotic group (synbiotic). At both 21 and 42 d of age, NE birds had decreased ($P < 0.05$) BW, feed conversion, and jejunal villi height compared with control while synbiotic birds were intermediate. At 42 d of age, NE birds had 2.2 log/g increased CP in the ceca contents compared with control, while synbiotic birds had comparable CP load compared with the control group. Synbiotic birds had significantly greater amounts of bile anti-CP IgA compared with the control and NE group. It can be concluded that synbiotic supplementation decreased CP proliferation *in vitro* and cecal CP load *in vivo* while improving production parameters during a mixed NE infection in broilers.

Key Words: *Clostridium perfringens*, *Eimeria maxima*, coccidiosis, performance, salinomycin

136 In ovo feeding dose response of Clostridiales strains on hatch and early growth performance. Shaymaa Abousaad*¹, Ramon Malheiros¹, Shawn Jones², Bryan Tracy², and Peter Ferket¹, ¹*NC State University, Raleigh, North Carolina, United States*, ²*White Dog Laboratories Inc., New Castle, Delaware, United States*.

Due to current public concern about antimicrobial resistance, the use of live microorganisms to manage enteric health is becoming a feasible practice, especially when administered early in a broiler's productive life. Strains within the Clostridiales order, including many butyric acid-producing anaerobes, are found in the intestines of healthy animals have been shown to promote growth performance and improve gut health. The objective of this study was to examine the dose response of BioTyton consisting of unique Clostridiales strains, delivered by *in ovo* feeding (IOF) on hatch performance, hatch window and early growth performance. Viable eggs of equal weight (± 0.5 std.) were segregated for 6 experimental treatments. At ~17 d of incubation, 100 μ L of either anaerobic control solution (Cont) or 10^0 , 10^2 , 10^4 , 10^6 , 10^8 cfu BioTyton/egg was administered into the embryonic amnion. Hatch yield were determined at 4 h intervals starting at ~480 h until 504 h of incubation, which is a typical hatch pulling time at commercial hatcheries. Embryos for all unhatched eggs were examined to determine proportion of external and internal pipped, late dead and early dead. At the day of hatch,

10 chicks from each treatment group were placed randomly in battery cages (5–8 Cages/treatment). Data were subjected to one way ANOVA and the significant differences ($P \leq 0.05$) among treatment means were determined by Duncan's multiple range test. A regression analysis for the hatchability rate revealed the optimum dose for improved the hatchability rate was between 10^4 and 10^6 cfu/egg. In comparison to control, doses of 10^4 and 10^6 cfu/egg showed significantly ($P \leq 0.05$) higher relative yolk-free embryo weight (g), lower embryonic death rate after external pipping, higher daily weight gain (g/d) from 0 to 14 d post-hatch, higher 2-week-body weight (g), and lower mortality rate during the first week. In addition, 10^6 cfu/egg reduced hatch window by 5 h and had the lowest mortality rate from 0 to 21 d, and 10^4 cfu/egg resulted in the lowest FCR (g feed/g gain) from 7 to 14 d. There is no significant difference among treatment groups in the hatch weight, chick quality score, and tonic immobility of the birds. In conclusion, in ovo administration of 10^4 and 10^6 cfu BioTyton/egg into the amnion of the chicken embryos improved hatch performance and early growth performance.

Key Words: in ovo feeding, *Clostridium*, hatch, growth, broilers

137 Evaluation on control of mite infestations of flocks under tropical climatic conditions through the use of Fluralaner 1% solution. Gustavo Perdoncini*, Jorge Werlich, Lucas Colvero, and Harvey Souza, *Merck Animal Health, São Paulo, Brazil.*

Mite control on commercial farms is one of the challenges faced by farmers. The main negative impacts related to infestations are reductions in egg productivity and quality, as well as the susceptibility of transmission of *Salmonella* spp. or other pathogenic microorganisms to birds. The choice of tool that guarantees zero-day withdrawal period for eggs, high efficacy and convenient application features characteristics of an ideal product. The objective of this work was to evaluate the efficiency of the Fluralaner (Exzolt®) molecule against infestations of *Ornithonyssus sylvii* and *Dermanyssus gallinae* on farms. The mite infestation was characterized in 18 commercial egg farms in Brazil and the elimination of mites were carried out using Fluralaner 1% aqueous solution (Exzolt®). In total, 463 thousand hens were treated. Feathers and facilities of infested birds were analyzed through the taxonomic identifications of the isolated individuals to characterize the mite involved. The hens received the oral treatment by drinking water on d 0 and 7, at a dose of 0.5 mg fluralaner per kg of Body Weight (BW) (equivalent to 0.05 mL Exzolt/kg BW). The treatment was given using a tank of medication for an average period of 5 h. The determination of primary efficacy was based upon the of *Ornithonyssus sylvii* and *Dermanyssus gallinae* inside the houses or on the hens pre- and post-treatment. In the evaluated farms *Ornithonyssus sylvii* was identified in 78.22% of the egg farms and *Dermanyssus gallinae* in 27.78%. This evaluation made it possible to characterize *Ornithonyssus sylvii* as a prevalent species. It was not identified the presence of both species in the same farm. Infestations have always been characterized as the only prevalent species. The use of Fluralaner orally by drinking water allowed the efficient elimination of both mites on 100% of the farms. The verification of the viable presence of the mites was performed 7 d after the second application. The oral application via drinking water offered an innovative new approach for comprehensive management of both species identified. Currently, conventional insecticides are used to control mite infestation through sprayed directly over the birds, which show low effectiveness due to ability to reach mite refuges in crevices and cracks close to the birds. The great results were easily identified in the farms, due to the rapid mite elimination. Furthermore, it brought zero egg withdrawal time for layers. The use of Fluralaner was efficient in the

elimination of the mites present in the farms, characterizing an effective product for this purpose. Exzolt is not approved for sale in Canada.

Key Words: eggs, Exzolt, Fluralaner, hen, mite

138 Prevalence of *Salmonella enterica* in chicken meat retail in Mexico: A longitudinal study. Gerardo Nava*, Ivan Regalado-Pineda, Carolina Resendiz-Nava, and Elizabeth Saenz-Garcia, *Universidad Autonoma de Queretaro, Queretaro, Qro, Mexico.*

Worldwide, *Salmonella* is one of the most important zoonotic pathogens; however, in many countries its prevalence remains unknown. The objective of the present study was to perform the first long-term longitudinal study of *Salmonella* prevalence in chicken meat retail in Mexico. A total of 1,500 samples were collected monthly from 2016 to 2018 in wet markets ($n = 590$) and supermarket chains ($n = 910$). Each month, chicken legs and thighs were collected from 5 markets ($n = 10$ or 32 pieces) and 5 supermarkets ($n = 10$ or 64) from Queretaro, Mexico; and transported on ice to the laboratory for processing within 4 h. Culture dependent (pre-enrichment, enrichment and selective media) and molecular methods were used to isolate and identify *S. enterica* isolates. Results were compared using the *t*-test. Overall, *S. enterica* was recovered from 19% of the meat samples. Remarkably, a higher prevalence ($P < 0.05$) was observed in supermarkets (25%) than in wet markets (9%); it was 2.6 times more likely (odds ratio = 2.6, $P < 0.0001$) to recover *S. enterica* from supermarkets than wet markets. Within the 3 years analyzed, the pathogen was always more prevalent in supermarkets and in spring and winter seasons ($P < 0.05$). Interestingly, the recovery rate of *S. enterica* from supermarkets has increased gradually from 20% to 42% during the study period. The higher occurrence of *Salmonella* in supermarkets uncovers an important health threat that needs to be urgently addressed by poultry meat producers and retailers.

Key Words: *Salmonella*, prevalence, chicken, retail, longitudinal study

139 Up-regulated hypoxia-inducible factor 1 alpha in *pectoralis major* of commercial broilers affected with white striping myopathy. Yuwares Malila*, Krittaporn Thanatsang, Sopacha Arayamethakorn, Tanaporn Uengwetwanit, Yane Srimarut, Wanalada Rungrassamee, and Wonnop Visessanguan, *National Center for Genetic Engineering and Biotechnology, Pathum Thani, Thailand.*

Numbers of studies have pointed out an association between development of white striping (WS) abnormality in commercial broilers and myodegeneration initiated through low oxygenation within broiler breast muscle. However, an actually etiology of this myopathy has not been fully understood. The objective of this study was to determine hypoxia-inducible factor 1 α (HIF1A) in *pectoralis major* of 42-d-old Ross 308 broilers exhibiting WS myopathy. It was hypothesized that this transcription factor would be increased in the WS relative to non-defective samples as the increased abundance of this protein was previously demonstrated within several tissues exposed to an experimentally reduced oxygen availability. The chicken breast samples were collected within 20-min postmortem. One side of the breasts was dissected, snap frozen in liquid nitrogen, and stored at -80°C until total RNA isolation and protein extraction. The other side was used for defect classification at 24-h postmortem. The breast was classified as "WS" or "non-defective" based on its appearance. Those with white lines on the surface were categorized into WS group ($n = 7$) whereas non-defective samples ($n = 4$) were ones without any lesion. Expressions of HIF1A at gene and protein levels in *pectoralis major* were examined using droplet digital polymerase chain reaction (ddPCR) and immunoblot,

respectively. Statistical differences in HIF1A expression between WS and non-defective breast muscles were identified using Student's *t*-test ($\alpha = 0.05$). Based on ddPCR, absolute HIF1A transcript abundance in the WS samples (8.0 ± 0.7 copies/ng template) was significantly greater than that of non-defective samples (5.4 ± 0.6 copies/ng template) ($P = 0.01$). At protein level, although there was no significant difference in average values of protein band intensity (MW = 120 kDa) between WS and non-defective groups ($P = 0.14$), the average band intensity

of the abnormal samples was approximately 2-fold greater than that of non-defective ones. The results indicated an upregulation of HIF1A at transcriptional level in WS breast muscles, suggesting the molecular activities encountering oxidative stress within the WS muscle.

Key Words: droplet digital polymerase chain reaction, immunoblot, oxidative stress, white striping

Student Competition: Immunology, Health, and Disease I

141 Withdrawn

142 Not Presented

143 A machine learning approach to characterizing microbial profiles in tibial joints of Pekin ducks. Shelly Nolin^{*1}, Zachary Lowman^{1,2}, Jocelyn Romano³, and Chris Ashwell¹, ¹*NC State University, Raleigh, North Carolina, United States*, ²*Joe Jurgielewicz & Son, Hamburg, Pennsylvania, United States*, ³*Romano Veterinary, PLLC, Jacksonville, North Carolina, United States*.

To more efficiently satisfy world-wide demands for poultry, birds have been selected for faster growth rates, with the unfortunate side effects of leg problems such as tibial dyschondroplasia (TD) which can arise when bones cannot grow and mineralize adequately to support the growing body. Leg problems are leading causes of late mortality in commercial production as well as being an issue of welfare as the birds may suffer painful lameness. Resident microbe populations often correlate with matters of health and disease, and it was the goal of this trial to better understand TD and lameness by characterizing the microbes inhabiting the proximal tibial joint of Pekin ducks. To induce lameness, ducklings were raised on a slanted floor. At 18 and 39 d of age 8 ducks exhibiting lameness and 8 non-lame controls were euthanized, and had their left tibia scored 0–4 for TD, based on appearance and extent of lesions. Lesions were observed in 28/32 samples, but there was no significant statistical correlation between score and lameness. The right tibias were flash frozen for microbial DNA isolation, library construction, 16s sequencing, and bioinformatic analysis for microbial composition and abundance. A total of 390 microbial taxa were identified. The Waikato Environment for Knowledge Analysis, “WEKA” (Waikato, New Zealand) machine learning program suite was utilized to run support vector machine (SVM) and artificial neural network (ANN) algorithms, using a stratified hold out and 66–34% split for model cross-validation. Taxa were ranked by WEKA based on its’ entropy based ranking feature. Algorithms run sequentially as taxa are reduced to maximize prediction performance for age and lameness, and to determine optimal microbial profiles for each comparison. Microbial abundance values could predict age with 100% accuracy using the top 2–25 taxa in non-lame samples and top 4–50 taxa in lame samples. Lameness at d 18 could be predicted with 100% accuracy using the top 73–87 taxa, however the algorithms could only predict d 39 lameness with 68–87% accuracy using the top 6–7 taxa. The results of machine learning indicate abundance of only a few taxa are needed to accurately differentiate samples by age, whereas more taxa are necessary to differentiate between young lame and not lame birds. At 39 d there is too little distinction between the joint microbiomes of lame and not lame birds to predict with high accuracy. Better understanding of microbial populations and their effects on changes within the joints may aid in future targeted approaches to control leg problems in poultry production.

Key Words: machine learning, tibial dyschondroplasia, joint microbes, lameness, 16S sequencing

144 Early cytokine responses in tracheas from congenic MHC haplotype chickens with distinct susceptibility to infectious bronchitis virus. Ana da Silva^{*} and Rodrigo Gallardo, *University of California, Davis, Davis, California, United States*.

Infectious bronchitis virus (IBV) is an upper respiratory pathogen of chickens that undergoes mutation and recombination events during replication. Due to its genetic variability, some IBV genotypes are able to evade immune responses elicited by commonly used vaccine strains. Therefore, local innate and non-specific immune responses play an important role in protection against IBV. To better understand innate immunity to IBV, we have been using MHC congenic chicken lines that present distinct susceptibility to IBV. The relatively resistant 331/B2 and susceptible 335/B19 chicken lines share the same genetic background, with differences only in their MHC haplotype. In previous experiments, we used tracheal organ cultures to assess cytokine levels after an in vitro challenge with IBV. However, we were not able to distinguish cytokine responses to IBV because of the inflammation induced by the tracheal processing. It was noticeable that line 335/B19 presented an exaggerated inflammatory response compared with 331/B2, which might be related with its relative susceptibility. The goal of the present study was to assess cytokine gene expression and production in tracheas from these 2 chicken lines after a challenge with IBV in vivo. A total of 120 birds, 60 from each chicken line (331/B2 and 335/B19), were raised in isolators for one week. Half of the birds of each line were challenged with IBV M41 at 7 d of age, totalizing 4 groups (331/B2 and 335/B19, challenged and unchallenged). Five birds/group were euthanized by cervical dislocation at 18, 24, 36, 48 and 60 h post-infection. Tracheas were carefully removed and immediately placed in liquid nitrogen to stop cellular processes such as cytokine production and mRNA synthesis. Tracheas were flushed with PBS and the tracheal wash used for IFN- β , IL-1 β , IL-6 and IL-10 quantification by ELISA. Total RNA was extracted from tracheas and gene expression of the same cytokines was evaluated by RT-qPCR. Preliminary results corroborate previous findings that 335/B19 birds presented higher inflammatory response than 331/B2. Unlike the in vitro experiment, this experiment was able to provide valuable information about the local innate responses elicited after the challenge with IBV. This knowledge can assist the development of targeted prevention approaches that focus on unspecific innate immune responses against multiple IBV genotypes.

Key Words: infectious bronchitis, innate immunology, cytokines

145 Chitosan oligosaccharide supplementation alleviates stress stimulated by in-feed dexamethasone in broiler chickens. Saheed Osho^{*} and Olayiwola Adeola, *Purdue University, West Lafayette, Indiana, United States*.

This experiment was conducted to investigate the effect of dietary chitosan oligosaccharide (COS) on growth performance, nutrient digestibility, jejunal morphology, gene expression, and plasma antioxidant enzymes in male broiler chickens under experimentally induced stress via in-feed dexamethasone (DEX). On d 3 post hatching, male broiler chicks (initial BW = 61 \pm 1.8 g) were assigned to 2 diets supplemented with COS at 0 or 1 g/kg in a randomized complete block design with BW as a blocking factor and fed to d 27 post hatching. Birds were pooled within each diet (0 or 1 g/kg COS) to equalize the average BW and fed 2 diets supplemented with 0 or 1 g/kg DEX, within each dietary COS, from d 20 to 27 post hatching. This resulted in a 2 \times 2 factorial arrangement of treatments with 2 levels each of COS and DEX, 8 replicate cages and 7 birds per cage. On d 27 post hatching, birds were weighed and euthanized, and samples were collected. In the group of birds fed diet supplemented with 0 g/kg COS, dietary DEX at 1 mg/kg decreased BW gain from 378 to 91 g, whereas in birds fed diet supplemented with 1

g/kg COS, inclusion of DEX at 1 mg/kg decreased BW gain only from 477 to 243 g thus, resulting in a COS × DEX interaction ($P < 0.05$). Dietary COS also decreased ($P < 0.05$) DEX-induced effects (interaction; $P < 0.05$) on gain:feed. Dietary COS supplementation attenuated the DEX effects (interaction; $P < 0.05$) on villus height, crypt depth, villus height to crypt depth ratio, and ileal digestibility of DM and energy. The DEX-induced effect of relative mRNA expression of intestinal IL-6, IL-10, and claudin-1 was reduced by dietary COS supplementation (interaction; $P < 0.05$). Responses (interaction; $P < 0.05$) in the activity of plasma superoxide dismutase, catalase, and glutathione peroxidase to COS and DEX were similar to that observed with the relative mRNA expression. In conclusion, dietary COS improved growth performance, nutrient digestibility, jejunal morphology, gene expression, and plasma antioxidant enzymes in broiler chickens under DEX-induced stress. These implies that dietary COS may be useful for ameliorating adverse influences of inflammation on gut health of broiler chickens.

Key Words: broiler chickens, chitosan oligosaccharide, dexamethasone, gene expression, stress

146 Study of toll-like receptor (TLR) 3 mediated immune responses in avian knockout cell lines. K. C. Mahesh*¹, John Ngunjiri¹, Joonbum Lee², Amir Ghorbani¹, Mohamed Elaish¹, Michael Abundo¹, Kichoon Lee², and Chang-Won Lee¹, ¹The Ohio State University, Wooster, Ohio, United States, ²The Ohio State University, Columbus, Ohio, United States.

TLR3 is a key receptor for sensing dsRNA and induction of immune responses. Although previous studies of avian TLR3 have reported cytokine induction by a synthetic analog of dsRNA, poly(I:C), the role of TLR3-mediated immune responses in viral pathogenesis is not well understood. Our recent study shows that quail express unique TLR3 isoforms compared with chicken. This led us to hypothesize that species differences in avian TLR3 isoforms reflect differences in stimulation of immune responses upon viral infection. To test our hypothesis, we generated TLR3 knockout (KO) chicken (DF-1) and quail (QT-35) cell lines using CRISPR/Cas9 system optimized for avian species. The wild-type (WT) and KO cell lines were compared in terms of innate immune responses to poly(I:C) treatment and infection with different viruses. Interestingly, poly(I:C) treatment induced rapid cell death in WT DF-1 cells, which was not observed in KO DF-1 cells, and prevented a side-by-side comparison of immune responses to be made between DF-1 and QT-35 cells. In QT-35 cells, poly(I:C) treatment significantly increased IFN- β and IL-8 gene expression along with the cytoplasmic dsRNA receptor melanoma differentiation-associated gene 5 (MDA5) in WT cells while almost no change in gene expression level was observed in KO cells, suggesting that the TLR3 could be the only functional endosomal dsRNA sensing receptor in quail cells. The TLR3-dependent upregulation of MDA5 gene expression is likely through a loop involving secreted cytokines. The potential antiviral effects of poly(I:C)-induced cytokines were assessed using influenza A virus (IAV) and vesicular stomatitis virus (VSV). Replication of both viruses was significantly inhibited in poly(I:C)-treated WT cells compared with untreated WT cells, with VSV being more sensitive to antiviral effects than IAV. Poly(I:C) pre-treatment did not block virus replication in KO cells which further confirms the antiviral activity of TLR3-mediated IFN in WT cells. Students' *t*-test was used for analyzing gene expression data and one-way ANOVA followed by Tukey post hoc test for comparing virus titer, respectively. Currently, we are determining immune responses upon direct virus infections (IAV, VSV, and Reovirus) in relation to TLR3. Overall, our results show that the avian TLR3 is directly or indirectly involved in the expression of dif-

ferent cytokine and cytosolic viral receptor genes. Our ongoing studies on effect of TLR3 on expression of other related cytokines along with comparative study with chicken cells will further delineate the species-specific roles of avian TLR3.

Key Words: avian TLR3, CRISPR/Cas9, quail, chicken, immune responses

147 Effect of essential oils (EO) on mRNA expression of antiviral and pro-inflammatory innate immunity genes on chicken macrophages infected with IBDV. Santiago Uribe-Diaz*^{1,2}, Joaquin F. Pirateque², Beatrice Despres¹, Julian Reyes³, and Juan Rodriguez-Lecompte¹, ¹Atlantic Veterinary College, Charlottetown, Prince Edward Island, Canada, ²Promitec, Bucaramanga, Santander, Colombia, ³Biogenesis, Medellin, Antioquia, Colombia.

A study was conducted to assess the effects of a single (*Lippia origanoides*)(Lo) or blend Lo and *Rosmarinus officinalis*)(Ro) of essential oils supplementation on immune innate response in chicken macrophages infected with Infectious Bursal Disease Virus (IBDV). Methods: Chicken macrophages (HD-11) cell line were cultured by triplicate at a concentration of 5×10^5 cells / well in plates of 24 wells. Macrophages were divided into 6 groups: 1. Negative control (No virus, no EOs); 2. No virus + EO (Lo); 3. No virus + EO (Lo + Ro); 4. Positive control (Virus, no Eos); 5. Virus + EO (lo); 6. Virus + EO (Lo + Ro). After 12h of culture, cell were inoculated as a modified intermediate live vaccine of IBDV (UNIVAX-BD®) with a MOI = 1 (Groups 4, 5 and 6). Followed of 1 h of virus absorption, the macrophages received media supplemented with 50 ppm of *Lippia origanoides*(groups 2 and 5) or with 10-ppm *Lippia origanoides* and 100 *Rosmarinus officinalis* (groups 3 and 6). This study evaluates periods of time 0, 6, 12 and 24 h post-infection (p.i). The research tested the effect of EOs single or blended supplementation on chicken macrophages gene expression of toll-like receptors 3 and 21, MDA5, cytokines IL-1 β , IL-10, IRF-7, IFN- β , OAS and PKR as indicators of immunological cell activation. IBDV virus was quantified and detect in chicken macrophages and supernatant using quantitative RT-PCR with SYBR green dye. Relative quantification and standardization was achieved using a log 10 dilution series of RNA extracted from IBDV intermediated strain. An ANOVA model was calculated to determine the effect of Treatment group, gene and Time on the natural logarithm of CT values; a pairwise comparison was determined on the marginal predictions for the 6 treatment groups using Tukey adjustment. Results showed that there were not statistically significant differences for the contrasts: 2 vs 1, 6 Vs 1, 6 Vs 2 and 5 Vs 4 ($P > 0.05$), however the rest of the contrasts showed significant differences ($P < 0.001$). There was a significant difference ($P < 0.001$) between EO blend (group 5) and single (group 6) affecting virus replication, and gene expression of TLR3, IL-1 β , IFN- β , OAS and PKR. Conclusion: The supplementation with a blend of EOs was shown to reduce virus attachment and replication, and reduction of IFN inducible antiviral effectors pathway in chicken's macrophages

Key Words: innate immunity, macrophages, IBDV, essential oils, health

148 The effect of oral tea saponins to relieve oxidative stress and immune suppression in chickens. Xiaoqing Chi*, Shicheng Bi, Yong Zhang, Xiaodan Ma, and Songhua Hu, Zhejiang University, Hangzhou, Zhejiang, China.

The present study was designed to evaluate the effects of tea saponins on oxidative stress induced by cyclophosphamide in chickens. One hundred and 20 one-day-old specific-pathogen-free (SPF) chickens (male) were

randomly divided into 5 groups. Groups 3 to 4 received intramuscular injection of cyclophosphamide to induce oxidative stress and immunosuppression. After that, groups 2 and 4 were orally administered tea saponins in drinking water for 7 d. Groups 5 were not medicated and served as a control. Then, groups 1 to 4 were intranasally immunized twice with a live bivalent vaccine of Newcastle disease virus and Infectious bronchitis virus at 2 weeks intervals. Blood samples were collected before Cy treatment and 7, 14 and 21 d after immunization for analysis of oxidative parameters and specific antibody titers, meanwhile, splenocytes were prepared from 6 birds of each group before Cy treatment and 7, 14, 21 d after immunization for lymphocyte proliferative assay. The dose of tea saponins used in this study was based on our previous investigation. Serum oxidative parameters (total antioxidant capacity, total superoxide dismutase, catalase, glutathione peroxidase, glutathione, ascorbic acid, α -tocopherol, malondialdehyde, and protein carbonyl) and Infectious bronchitis virus -specific antibody titers were determined using detection kits according to the manufactures' protocols, Newcastle disease virus -specific HI titers were determined as described using Hemagglutination inhibition test, lymphocyte proliferation test were induced by either Concanavalin A or lipopolysaccharides for the thiazolyl blue tetrazolium bromide (MTT) assay. Analysis of data was performed using SPSS software (version 20.0, SPSS Inc., Chicago, IL). One-Way ANOVA with Duncan post hoc test was used for multiple comparisons between groups. Values were expressed as the mean \pm standard deviation (SD). P values of less than 0.05 were considered statistically significant. The results showed that administration of tea saponins significantly increased total antioxidant capacity, total superoxide dismutase, catalase, glutathione peroxidase, glutathione, ascorbic acid and α -tocopherol, and decreased malondialdehyde, protein carbonyl. Enhanced immune responses such as lymphocyte proliferation induced by Concanavalin A and lipopolysaccharides, and serum Newcastle disease virus - and Infectious bronchitis virus -specific antibodies were also observed in chickens injected with or without cyclophosphamide. In addition, no side effects were found in chickens throughout the study. Therefore, tea saponins may be a potential agent to improve immunosuppression induced by oxidative stress in chickens.

Key Words: tea saponin, oxidative stress, cyclophosphamide, immunosuppression

149 Combined adjuvant effect of ginseng stem-leaf saponins and selenium on immune responses to a live bivalent vaccine of Newcastle disease virus and infectious bronchitis virus in chickens. Xiaodan Ma*, Shicheng Bi, Yueming Wang, Xiaoqing Chi, and Songhua Hu, *Zhejiang University, Hangzhou, Zhejiang, China.*

Vaccination with a live bivalent vaccine of Newcastle disease virus (NDV) and infectious bronchitis virus (IBV) is a routine practice in poultry industry in China. This study was designed to evaluate ginseng stem-leaf saponins (GSLs) in combination with selenium (Se) for their adjuvant effect on the immune response to vaccination against NDV and IBV in chickens. Experiment 1. To evaluate the effect of GSLs and/or Se on the vaccine antibody response, 70 chickens at 12 d old were randomly divided into 5 groups with 14 birds in each and received intraocular-and-intranasal immunization with a live bivalent vaccine of NDV and IBV in saline solution (50 μ l) with or without GSLs (5 μ g), Se (25 μ g) or GSLs (5 μ g) + Se (25 μ g). Blood samples were collected at 10, 14, 18 d post immunization for tests of NDV-specific HI titers and IBV-specific antibody titers. Experiment 2. To observe the changes of maternal or vaccine-induced serum antibody responses, 45 chickens at 12 d old were randomly divided into 3 groups with 15 birds in each and immunized with the same method as Experiment 1. Every 3 d

post immunization, blood samples were collected for NDV-specific HI titers and IBV-specific antibody titers tests. Experiment 3. To evaluate serum cytokines, neutralizing antibodies and lymphocyte proliferative responses, 45 chickens at 12 d old were randomly divided into 3 groups and immunized with the same method as Experiment 1. At 48 h post immunization, blood samples were collected for measurement of serum IFN- γ and IL-4. At 2 weeks post immunization, blood was sampled for isolation of lymphocytes to test lymphocyte proliferation and neutralizing antibodies. NDV specific HI titers were determined by Hemagglutination inhibition test. IBV specific antibody titers, serum IFN- γ and IL-4 were determined by ELISA according to manufactures' instructions. Lymphocyte proliferation test were induced by concanavalin A, lipopolysaccharides or NDV antigen for MTT assay. NDV-specific neutralizing antibodies were estimated by inoculating CEF while IBV-specific neutralizing antibodies were estimated by inoculating TOC. Results showed that GSLs promoted significantly higher NDV- and IBV-specific antibody responses with the highest antibody response detected in GSLs-Se group. The increased antibody was capable of neutralizing NDV and IBV. In addition, GSLs-Se enhanced lymphocyte proliferation and production of IFN- γ and IL-4. More importantly GSLs-Se was found to promote early production and prolong the duration of the antibody responses. To improve the efficacy of vaccination in chicken flocks, the diluent containing GSLs-Se deserves further studies to evaluate its effect on other chicken vaccines.

Key Words: ginseng stem-leaf saponins, selenium, Newcastle disease, infectious bronchitis

150 Evaluation of green yerba mate extract as a phytogetic additive to control *Salmonella enterica* Heidelberg in broiler chickens. Aline Tujimoto Silva*¹, Ana Heemann¹, Bruna Belote¹, Adrien Sanches¹, Paulo Hümmlgen¹, Igor Soares¹, Breno Beirão², Celso Fávaro Filho², Max Ingberman², Rodrigo Heemann⁵, Anderson Cunha³, Izabela Alvim⁴, and Elizabeth Santin¹, ¹Federal University of Paraná, Curitiba, Brazil, ²Imunova, Curitiba, Brazil, ³Federal University of São Carlos, São Carlos, Brazil, ⁴Institute of Food Technology - ITAL, Campinas, Brazil, ⁵Heide Natural Ingredients, Pinhais, Brazil.

Phytogetic additives have been studied as candidates to reduce pathogen colonization in the gut due to its antimicrobial properties. In this study, we evaluated the green yerba mate (*Ilex paraguariensis*) (YM) as phytogetic additive to control *Salmonella enterica* Heidelberg (SH) in broilers and its effects over performance and gut histology. A total of 216 one-day-old Cobb 500 were housed in isolated rooms, randomly divided into 6 groups: non-challenged (NC); non-challenged + free YM 0.1% (NF); non-challenged + microencapsulated YM 0.1% (NM); challenged (CH); challenged + free YM 0.1% (CF) and challenged + microencapsulated YM 0.1% (CM). The birds from challenged groups received, at 3 d of age, 10⁸ cfu/mL of SH by gavage. The birds and feed were weighted at 7, 14 and 21 d of age for performance evaluation and the birds were euthanized for sampling of cecum and liver for bacteriological analysis and histological evaluation by I See Inside (ISI) method. For SH evaluation each bird was considered one replicate. The data was submitted to Shapiro-Wilk normality test, one-way ANOVA and Tukey tests ($P < 0.05$). Differences in performance were not observed among groups at any age and the non-challenge birds were negative in all periods evaluated. At 7 d, birds fed with CM presented a higher counting of SH in cecum compared with CH and CF. At 21 d, CF showed a reduced counting of SH compared with CH. There was no difference between challenged groups for SH in liver at all periods. Regarding ISI results, there was a significant difference between challenged and non-

challenged groups. In cecum for non-challenged groups, at 7 and 14 d, NF and NM had a higher ISI score compared with NC due to increased inflammatory cell infiltration (ICI) on lamina propria at 7 d and lamina propria and epithelial ICI at 14 d. At 21 d, NM had a higher ISI score compared with NC due to an increase of lamina propria and epithelial ICI. For challenged groups, CM had a higher ISI score at all periods due to an increase in goblet cells and lamina propria and epithelial ICI compared with CH. In liver for non-challenged groups, at 7 d NF and NM had a higher ISI score compared with NC due to congestion and pericholangitis. For challenged groups, at 21 d, CH had a higher liver ISI score compared with CF and CM due to congestion and ICI. The YM demonstrated having impacts over the histology of cecum and liver of broilers, especially when microencapsulated. However, the ISI results obtained did not seem to affect animal performance, since no statistical difference was observed in this aspect. Therefore, the reduction of counting in cecum with the use of free YM indicates this product as a good candidate on controlling *Salmonella* Heidelberg.

Key Words: cecum histology, I See Inside, *Ilex paraguariensis*, liver histology, microencapsulation

151 Effect of select Gram-negative enteric strains on inflammation in chicks. Kaylin Chasser*¹, Kate McGovern¹, Audrey Duff¹, B. Graham², Whitney Briggs¹, Denise Russi Rodrigues¹, Emily Winson¹, Michael Trombetta¹, and Lisa Bielke¹, ¹The Ohio State University, Wooster, Ohio, United States, ²University of Arkansas, Fayetteville, Arkansas, United States.

Gastrointestinal (GIT) inflammation, caused by inappropriate microbial colonization, can predispose poultry to opportunistic infections and reduce growth performance. Two independent studies were completed to test wild-type Gram-negative poultry GIT isolates for potential to induce inflammation and decrease body weight gain (BWG). In exp 1, non-inoculated control (NC) was tested against *E. coli* Huff and E2, chicks were orally dosed with 10³cfu/chick on day of hatch. Feed and BW were measured on d0, 7 and 15, and d15 serum was collected for chicken α -1-acid glycoprotein (AGP). In exp 2, NC, E2, LG, Huff, and *Salmonella* Typhimurium (ST) were tested, where EC groups received 10³cfu/chick and ST received 10⁴cfu/chick orally on d0. Feed and BW were measured on d0, 2, 8, and 15, with blood and yolk sacs (YS) collected on d2, 8, and 15 for serum AGP analysis and bacterial enumeration, respectively. In both experiments, all data were subject to Analysis of Variance as a completely randomized design, with significant differences among means determined using Student's *t*-test in Exp1 and Tukey's Honestly Significant Difference test in Exp2. Exp 1 resulted in decreased ($P < 0.05$) BW on d7 and BWG d0–7 for Huff, as well as BW d15 and BWG d7–15 Huff and E2. Feed conversion ratio was increased ($P < 0.05$) d0–7 and d7–15 for E2, and AGP on d15 was reduced ($P < 0.05$) in Huff and E2 compared with NC, though NC was high, possibly due to pen effects. In exp 2, only LG reduced ($P < 0.05$) BW and BWG on d2 and 8, as well as d0–2 and d2–8, respectively. No differences were observed in feed conversion ratio or number of retained YS observed at each time point compared with NS, but YS enumeration increased ($P < 0.05$) in E2 on d8. Additionally, E2 was the only treatment that increased ($P < 0.05$) AGP concentration on d15. Changes observed in BW and BWG for both Huff and E2 in exp 1 and LG in exp 2 indicate an influence of bacteria on growth parameters during wk 1. In exp 2, trends of elevated cfu in YS for all inoculated groups compared with NC on d8 suggested a potential for bacterial entry into the GIT to cause inflammation. Additionally, AGP levels were elevated at d2 and d8 for all groups except ST, which may indicate AGP is not the best method to measure inflammation for ST. Elevated AGP in E2 on d15 indicated

inflammation on d15. Both E2 and LG resulted in changes or trends indicating treatment effect on GIT inflammation. These isolates may serve as predisposing factors for opportunistic disease and ideal candidates for studying the role of pioneer colonization in health of poultry.

Key Words: Gram-negative, *E. coli*, inflammation, opportunistic disease

152 Development of an *Escherichia coli* spray challenge model for neonatal broiler chickens. Callie Selby*, B. Graham, Lucas Graham, Kyle Teague, Christine Vuong, Guillermo Tellez, and Billy Hargis, University of Arkansas, Fayetteville, Arkansas, United States.

Escherichia coli (*E. coli*) is an opportunistic pathogen often introduced to neonatal chicks during the hatching process. This commensal bacterium, particularly as a pioneer colonizer of the gastrointestinal tract, can have substantial implications in the rearing of poultry due to reduced flock performance. To mimic the effects of the natural bacterial bloom present during hatch, a seeder challenge model was developed to expose neonatal chicks to virulent *E. coli*. On d 20 of embryogenesis, selected chicks, or seeders (n = 18/hatcher), were briefly removed from hatchers and spray challenged with saline (vehicle) or *E. coli* at 1x10⁷ cfu/chick (Exp 1) and 2.5x10⁷ cfu/chick (Exp 2) and placed back into the hatchery to transmit the pathogen to the indirect challenged, or contact chicks (n = 195/hatcher). For 2 7-d experiments, the efficacy of transmission was evaluated via enteric bacterial recovery, body weight gain (BWG), and mortality. For Exp 1 and Exp 2, significantly ($P < 0.0001$) more Gram-negative bacteria were recovered from the seeder and contact gastrointestinal samples vs. the negative control samples on day-of-hatch. Additionally, there was a significant ($P < 0.05$) reduction in 7-d BWG and significantly ($P < 0.0001$) higher mortality in the contact-challenged chicks compared with the negative control chicks in both Exp 1 and Exp 2. These data suggest that this model could be utilized to evaluate different methods of controlling the bacterial bloom that occurs in the hatching environment.

Key Words: *Escherichia coli*, challenge, broiler, hatchery, model

153 Evaluation of dietary administration of sodium chlorate, sodium nitrate, arginine, or a combination of dietary lactose with lactic acid bacterial gavage, for *Histomonas meleagridis* prophylaxis in poults. Thaina L. Barros*, Lesleigh Beer, Christine Vuong, Samuel Rochell, Guillermo Tellez, and Billy Hargis, University of Arkansas, Huntsville, Arkansas, United States.

Histomoniasis is currently a re-emerging disease of major significance for many commercial turkey production companies and some broiler breeders due to the unavailability of chemicals in the US. *Histomonas meleagridis* (HM) requires the presence of the cecal microflora to promote the disease, with some evidence for preference of *Enterobacteriaceae*. Inclusion of sodium chlorate (SC; 500 ppm) or sodium nitrate (SN; 100 or 500 ppm) in HM xenoculture markedly reduced HM survival over a 20h culture period. Selected lactic acid bacterial (LAB) administration with inclusion of 0.1% lactose (LAC) has been shown to reduce recoverable cecal *Enterobacteriaceae*, and addition of arginine (ARG) has been shown to enhance enteric innate immune responses with elevated nitric oxide release from macrophage lineage cells. Presently, 32 poults were randomly assigned to each of 6 treatment groups: dietary inclusion of SC (3,200 ppm), SN (500 ppm), selected mixed LAB culture (10⁹ cfu by oral gavage on d of hatch and d 12–14) combined with dietary inclusion of 0.1% LAC, or 2.44% dietary ARG during 12d to 28d, with all groups challenged with 2x10⁵ histomonads intracloacally at 14d. Controls consisted of basal diet (challenged, PC;

and unchallenged control, NC). BWG was determined weekly beginning on the day of challenge (14d), and mortality was recorded. Mortalities and all surviving poult (d28) were lesion scored for hepatic and cecal lesions (0–3 scale), and ceca were collected for bacterial recovery in MacConkey, MRS, and TSA agar. BWG and cecal bacterial recovery were analyzed by ANOVA. Lesion scores were analyzed using PROC MIXED procedure (SAS 9.4). Mortality and morbidity were compared using the chi-squared test of independence ($P < 0.05$). No mortalities or lesions were observed in the NC group and mortality ranged from 18.8 to 31.3% ($P > 0.05$) in the other groups. Lesion scores were not different between treatments for either cecal or hepatic lesions. BWG was suppressed by HM challenge in all groups but was not significantly different between treatments at 28d. Interestingly, presumptive cecal LAB recovery was significantly ($P = 0.007$) suppressed in PC vs. NC, while presumptive Gram-negative recovery was elevated ($P = 0.1$) in challenged birds at 28d but neither were affected by treatments. These data are not encouraging for these candidate approaches for controlling HM as no beneficial effects of these treatments and time frames were observed. Higher dietary concentrations of SC, SN, ARG, nor LAC are not likely candidates for evaluation due to known negative effects (SC and SN), cost (ARG), or dysbiosis (lactose) at higher concentrations in turkeys or data extrapolated from other monogastrics.

Key Words: blackhead, antihistomonal, turkeys, alternative compounds, protozoa

154 Evaluation of a highly-passaged *Histomonas meleagridis* as a vaccine candidate against blackhead. Lesleigh Beer*, Juan Latorre, Guillermo Tellez, Billy Hargis, and Christine Vuong, *University of Arkansas, Fayetteville, Arkansas, United States.*

Histomoniasis, commonly known as blackhead, is caused by *Histomonas meleagridis* and is an important protozoal disease primarily associated with turkeys. No vaccines are currently available to alleviate this disease. Three experiments were conducted to evaluate a highly in vitro passaged *H. meleagridis* as a potential live-attenuated vaccine candidate

(VC). At selected dosages and time points, turkeys ($n = \sim 40/\text{group}$) were vaccinated via different routes (oral vs. cloacal) with the VC and subsequently challenged with a wild-type (WT) *H. meleagridis*. Experiment 1 included negative control, VC, or the WT administered intracloacally on d14 (2×10^5 *Histomonas* cells/poult). On d29, a subset of negative control and all VC poult were similarly WT-challenged. On d11 post-challenge, liver and cecal lesions were tabulated. Histomoniasis-related mortalities, liver and cecal lesions were significantly lower ($P < 0.05$) in the VC as compared with the WT control. Experiment 2 included negative control, oral VC (doses: 2×10^3 or 2×10^4 cells/poult), cloacal VC (doses: 2×10^3 , 2×10^4 , or 2×10^5 cells/poult), or cloacal WT control (2×10^5 cells/poult). On day-of-hatch, vaccinations were administered pre-feeding via their respective routes and doses. On d21, VC turkeys were subsequently challenged with 2×10^5 WT *Histomonas* cells/poult intracloacally. Two challenged-only control groups were introduced on d21, receiving the 2×10^5 WT cells/poult either cloacally or orally. Lesions were evaluated d14 post-challenge. Mortalities, liver and cecal lesions were higher in all groups as compared with the orally-administered WT. No differences were observed between the VC groups and the cloacal WT control for liver or cecal lesions. Experiment 3 included negative control, cloacal VC (d0 or d14), oral VC (d0), or cloacal WT control (d0, d14, or d28). A dose of 2×10^5 cells/poult was used for both the VC and WT challenge. On d28, all treatment groups were intracloacally challenged with WT, and lesions evaluated d14 post-challenge. Mortality and liver lesions were lower in the d14 cloacal VC as compared with WT control. Cecal lesions were not different in any VC treatment group as compared with the WT control. Although Histomoniasis was not completely prevented, d14 intracloacal administration of the VC yielded lower lesions and mortalities in experiments 1 and 3. Although the practicality of this administration method may be limited; taken together, these data suggest that further research with an attenuated strain could potentially be efficacious for lessening blackhead.

Key Words: blackhead, histomoniasis, *Histomonas meleagridis*, turkey, vaccination

Student Competition: Management and Production

155 Dietary supplementation of yeast fermentation metabolites affects gene expression and intestinal morphology in stressed broilers. Jill Nelson*, Mohamed Ibrahim, Giri Athrey, and Greg Archer, *Texas A&M University, College Station, Texas, United States.*

The yeast fermentation products XPC and AviCare (Diamond V Mills, Cedar Rapids, IA) have been shown to reduce stress susceptibility in poultry, but their mode of action remains to be investigated. Two experiments were conducted to assess supplementation of XPC or AviCare and changes in gene expression related to stress and immune function, and on indicators of intestinal dysfunction during heat stress. Day-of-hatch male Cobb 500 broilers were placed in floor pens (0.91 × 1.83 m) lined with pine shavings and grown to 42 d of age. Where noted, AviCare was administered in the drinking water (160mL/100L) d0–42 and XPC was added to the feed (1.25kg/metric ton) d0–42. There were 3 treatments (T) in Experiment 1: stressed control (CS), stressed + XPC (XS), stressed + AviCare (AS). Birds were vaccinated for coccidiosis (d0) and Newcastle/Bronchitis (d18), and heat stressed with feed/water withdrawal for 12h on d18. On d42 spleen and adrenal glands were obtained from 20 birds/T: expression of DEF1β and IL-10 was measured in the spleen, and expression of CYP1A2 and MC2R was measured in the adrenal glands. Experiment 2 consisted of 6 treatments: CS, XS, AS, non-stressed control (CN), non-stressed + XPC (XN), non-stressed + AviCare (AN). Stressed treatments were exposed to cyclic heat stress d28–42 for 16h/d. On d42 blood samples were collected from 12 birds/T to assess plasma chemistry, hematocrit, IL-1α, IL-8, and α₁-AGP. Ileum sections were collected from 12 birds/T to assess goblet cell density, villus height, crypt depth, and villus/crypt ratio. Data was analyzed using one-way ANOVA and means were separated using Fisher's LSD; ileal data was analyzed using Kruskal-Wallis followed by Dwass-Steel-Critchlow-Fligner where significant differences existed. A significant difference was defined as $P < 0.05$. In Experiment 1 both supplements reduced CYP1A2 ($P < 0.05$) and MC2R ($P < 0.05$) compared with CS birds. Both supplements reduced IL-10 ($P < 0.05$) compared with CS, and IL-10 was lower in XS than AS, but DEF1β expression was not affected ($P > 0.05$). In Experiment 2 differences were observed in plasma chemistry, IL-1α and IL-8. Goblet cell density was higher in XS (28.01 ± 1.76) and AS (26.3 ± 1.18) compared with CS (19.5 ± 1.02), but was lower in AN (15.87 ± 0.63) than CN (20.75 ± 1.23). Villus/crypt ratio was higher in XN (5.83 ± 0.3) and AN (5.48 ± 0.31) than CN (4.95 ± 0.2), and higher in XS (6.13 ± 0.24) and AS (6.69 ± 0.23) than CS (5.87 ± 0.31). Dietary inclusion of XPC or AviCare reduces broilers' susceptibility to rearing stressors by reducing expression of MC2R and CYP1A2, and improves intestinal integrity by supporting mucus secretion and epithelial cell survival during cyclic heat stress.

Key Words: stress, intestinal health, gene expression, broiler, yeast fermentation product

156 Evaluation of phosphatidic acid supplementation in conjunction with vitamins, minerals, and betaine on the production efficiency of broilers subjected to cyclic heat stress. Eric Sobotik*, Gabrielle House, Austin Stiewert, Austin Brown, Jill Nelson, and Greg Archer, *Texas A&M University, College Station, Texas, United States.*

Heat stress is one of the most important environmental stressors challenging poultry production. Detrimental effects of heat stress on broilers include lower body weight, reduced feed intake, higher feed conversion,

reduced meat quality, and increased stress. A study was conducted to evaluate the effects phosphatidic acid (PA) supplementation and various dietary feed additives to broilers reared under heat stress temperatures. Day-old Ross 708 male broiler chicks were randomly allocated to one of 10 dietary treatments: T1, Control (CON); T2, Vitamin C (250mg/kg); T3, Chromium (0.50kg/MT); T4, Zinc (0.33kg/MT); T5, Betaine (2.0kg/MT); T6, PA (5mg/bird/day); T7, PA + Vitamin C; T8, PA + Chromium; T9, PA + Zinc; T10, PA + Betaine. All birds were weighed on d12, d26, d41, and d49 to obtain performance parameters: body weight (BW), feed consumption (FC), feed conversion ratio (FCR), and mortality percentage (MORT). From d35 to d49, birds were exposed to elevated temperatures (32°C-35°C) for 16hrs/day. On d49, blood was collected from 2 birds per pen. Blood was analyzed for plasma corticosterone (CORT). On d49, 5 birds per pen were processed to determine processing yields and breast meat quality. All data was analyzed via One-Way ANOVA using GLM with treatment means deemed significantly different at $P < 0.05$. From d1 to d41, T5, T4, T8 and T1 had a lower FCR (1.614 ± 0.003, $P < 0.05$) than T7 and T9 (1.644 ± 0.006, $P < 0.05$). On d49, T1 had higher CORT levels (4225.59 ± 963.43, $P < 0.05$) than T6, T8, T2, T3, T9, T7, and T5 (2275.56 ± 220.04, $P < 0.05$). Leg quarter weight was greater in T1 (0.889 ± 0.126 kg, $P < 0.05$) than all treatments except T2 (0.849 ± 0.004 kg, $P < 0.05$). Breast meat yield was greater in T5 and T10 (30.48 ± 0.22, $P < 0.05$) than all treatments except T8 (29.48 ± 0.11, $P < 0.05$). Tender yield was greater in T9, T10, and T8 (5.67 ± 0.07, $P < 0.05$) compared with T2, T1, and T3 (5.37 ± 0.04, $P < 0.05$). Leg quarter yield was greater in T1 (31.41 ± 0.19, $P < 0.05$) compared with T4, T6, T5, T8, T3, and T10 (30.51 ± 0.08, $P < 0.05$). Results from this study indicate supplementation of PA in conjunction with vitamins, minerals, and betaine may alleviate the negative attributes associated with heat stress, ultimately improving production efficiency and welfare in broilers.

Key Words: broiler, heat stress, phosphatidic acid, performance, yield

157 Effect of air movement on chick body temperature under different brooding temperatures during the first week of age. Tsunglin Lu*, Brian Fairchild, and Michael Czarick, *University of Georgia, Athens, Georgia, United States.*

The use of circulation fans has been introduced during brooding of the chicks to generate a more uniform heat distribution inside the broiler house. However, the effect of air movement over the chicks on their body temperature is less known. The objectives of this study were to (1) utilize subcutaneous flank temperature (T_{SF}) as an alternative method in measuring chick temperature and a prediction of rectal temperature (T_R). (2) investigate the effect of air movement under 35°C, 32°C, and 29.4°C brooding temperature on chick temperature and on the relationship between T_R and T_{SF} during the first week of age. Sixteen newly hatched broiler chicks were randomly selected and RFID temperature sensors were surgically inserted into the subcutaneous layer of the flank. All the chicks were grown in a single floor pen and raised to an age of 7 d. Twice a day for a period of 1.5 h, chicks in treatment group were placed in individual cells (10 cm X 18 cm) with 1 m per second (m/s) air movement while those in control group were placed in cells with no supplemental air movement. T_R and T_{SF} were measured 3 times during each test period as sets to perform regression analyses. All statistical analyses were performed in JMP software. T_{SF} was 0.1°C significantly lower than T_R ($P < 0.01$). Air movement had no significant effect on the difference between T_R and T_{SF} ($P > 0.05$). The difference between T_R

and T_{SF} in 35°C, 32°C, and 29.4°C were 0.105°C, 0.118°C, and 0.107°C respectively and was reported significantly different ($P < 0.01$). However, as the accuracy of the sensor was 0.1°C, these differences between the 3 brooding temperatures were too small to be accurately measured by the RFID sensor. Regression analysis showed that the overall relationship between T_R and T_{SF} was $T_R = 0.975 * T_{SF} + 1.139$ ($R^2 = 0.95$) with a root mean square error of 0.1°C. One meter per second moving air on the drop of chick temperature was brooding temperature dependent ($P < 0.01$), where the T_{SF} decreased 0°C, 0.4°C, and 0.4°C under 35°C, 32°C and 29.4°C brooding temperature respectively. The effect of air movement on chick temperature was also significantly affected by chick age ($P < 0.01$). T_{SF} had a 0.5°C drop on d 1 of age while only a 0.1°C drop on d 7 of age. In conclusion, our data suggest that T_{SF} measured by surgically implanted sensors is a promising method in measuring chick temperature and can be used to predict T_R . Air movement over chicks during the first week of age can cause a drop of body temperature and sufficient heat should be provided.

Key Words: management, circulation fan, brooding, chick temperature, air movement

158 Comparison of the effects of low versus high air movement at floor level on bird health and welfare in a commercial broiler house. Connie Mou*, Michael Czarick, and Brian Fairchild, *University of Georgia, Athens, Georgia, United States.*

A field study was conducted on 2 commercial broiler farms to compare the effect of 2 different circulation fan systems on litter conditions and paw quality over a total of 4 flocks. The 12 m X 152 m houses were managed to maintain a house relative humidity (Rh) of approximately 60%. One house on each farm was equipped with 8, commonly used 45 cm 125 W low volume circulation fans (CTL) while the adjacent house was equipped with 8–60 cm 350 W high volume circulation fans (TRT). Average air velocity at floor level was 0.28 ± 0.14 m/s in the control house and 0.76 ± 0.03 m/s in the treatment house. Weekly litter moisture, water activity (A_w) and footpad scores were collected and analyzed from both houses. On both farms, litter moisture was higher in CTL versus TRT house. By Day 14, litter moisture for the 3 flocks on Farm 1 was on average 25%, 19%, and 19% in CTL houses versus 17%, 16%, and 17% in TRT houses. By the end of each flock on Farm 1, litter moisture was 32%, 32%, and 29% in CTL houses versus 24%, 24%, and 26% in TRT houses. On Farm 2 by Day 14, litter moisture in the CTL house was 19% versus 15% in TRT house. At the end of the flock on Farm 2, litter moisture was 26% in CTL versus 24% in TRT house. On both farms, litter moisture had a greater degree of variation between sampling locations in the CTL house while TRT house litter moisture profiles were more uniform. Sidewall litter moisture tended to be 10% higher in CTL vs. TRT (20% vs. 30%). Water activity levels were kept below 0.80 on average in the TRT house versus 0.90 in the house with lower air movement. By Day 14, 21, and 28, the house with higher air movement had on average 76%, 54%, and 40%, less occurrence of minor and severe lesions versus lower air movement, respectively. By the end of each flock on both farms, there was double the occurrence of severe lesions in CTL versus TRT house.

Key Words: litter management, air movement, water activity, footpad health, bird welfare

159 Reduced feed intake rate suggests reduced hunger in broiler breeders with higher body weight. Chantele Gouliquer*¹, Nicole Zukiwsky¹, Mohammad Afrouziyeh¹, Jihao You¹, and Martin

Zuidhof², ¹University of Alberta, Edmonton, Alberta, Canada, ²Martin Zuidhof, Edmonton, Alberta, Canada.

This study was designed to analyze the effect of BW on feed intake rate and feed seeking behaviors of precision-fed broiler breeders. Assuming that birds on a higher BW treatment would be less hungry and less motivated to eat, it is hypothesized that they would have a lower feed intake rate and display fewer aggressive behaviors. The current study consisted of 10 BW treatments ranging from the breeder-recommended target BW (CON) to 22.5% higher (CON+22.5%) in 2.5% increments. Ross 708 broiler breeder pullets ($n = 30$) were randomly assigned to these treatments and fed individually by 2 precision feeding (PF) stations. An additional 6 ad libitum fed birds were also observed as an unrestricted reference treatment. Feed intake rate data was collected from PF system records from 16 to 23 wk of age. Live behavior observations were conducted by continuous observation 1 h per day from 23 to 25 wk of age. Behaviors that were observed included standing, preening, feather pecks, pecks at head, dislodging other birds to gain station access, and threats. All data were subjected to 2-way ANOVA using the MIXED procedure of SAS, with age and treatment as sources of variation. Means were compared using the LSD test and were reported as different where $P \leq 0.05$. Behaviors in all of the treatments showed few differences. During 6-second feeding bouts, no difference in feed intake rate was observed between the control and other treatments. However, feed intake rate differed between CON+12.5% (10.3 g/min) and CON+15% (10.2 g/min) vs. CON+22.5% (8.3 g/min). Meal size following each observed behavior was determined from PF system data. Independent of BW, birds that displayed threat behaviors had a greater feed intake rate (13.7 g/min) compared with birds that did not display threat behaviors (8.4 g/min). No other behaviors correlated with meal size. From 23 to 25 wk of age, unrestricted birds had the lowest feed intake rate (7.3 g/min), which did not differ from the CON+17.5% birds (9.5 g/min). The current data supports the hypothesis that relaxing feed restriction in pullets with higher BW trajectories would reduce feed intake rate.

Key Words: broiler breeders, precision feeding, feed intake rate, feed seeking behaviors, hunger motivation

160 A strategic approach to study reproductive performance in broiler breeders. Mohammad Afrouziyeh* and Martin Zuidhof, *University of Alberta, Edmonton, Alberta, Canada.*

The amount and timing of growth are important factors affecting age at first egg, body conformation, and reproductive performance in broiler breeders. To optimize growth pattern 10 growth trajectories were designed using a 3-phase Gompertz model with the form: $BW = \sum_i W_{mi} * \exp(-\exp(-b_i * (t - t_{INF_i})))$ where W_m was the mature BW (kg); b was the rate of maturing; t was age (wk); t_{INF} was the inflection point (wk); i was the growth phase ($i = 1$ to 3). The growth curves were designed with 2 levels of BW gain in phase 1 (discrete variable). W_{m1} was equivalent to breeder recommended BW target or 10% higher. The inflection point of the second (pubertal) growth phase (t_{INF2}) was advanced by 5, 10, 15, or 20% (continuous variable). We hypothesized that earlier growth (advancing the inflection point of the pubertal growth phase) and higher BW could optimize body frame size, early egg production and weight, and also advance age at onset of lay by providing a sufficient metabolic status and fat threshold. Forty females and 11 males were randomly subjected to the growth trajectories with a precision feeding system. Age at first egg, early hen-day egg production and egg weight from 23 to 26 wk of age, and abdominal skinfold thickness at 24 wk of age were measured. Analysis of covariance was conducted on all dependent variables using the MIXED procedure of SAS, with t_{INF2} and W_{m1} as sources of variation. Egg weight was significantly increased

($P = 0.028$) during the early production phase by advancing t_{INF2} . For every day that the pubertal growth phase was delayed (relative to the 20% t_{INF2} advancement), egg weight decreased by 0.103 and 0.093 g for high and standard BW, respectively. Advancing inflection point of the second growth phase significantly increased BW at photostimulation ($P < 0.0001$) and shank length ($P = 0.013$) as an indicator of the body frame size. For every day that the pubertal growth phase was delayed, BW at 22 wk of age decreased by 17.0 and 16.8 g, and shank length decreased by 0.21 and 0.05 mm for high and standard BW, respectively. Age at first egg, hen-day egg production, and skinfold thickness were not affected by BW or advancing the time of second growth. The current data support our hypotheses that advancing time of the second growth phase can advance sexual maturity by increasing BW, body frame size and early egg weight.

Key Words: broiler breeder, multi-phasic growth, sexual maturity, skinfold thickness

161 The effect of skip a day and everyday rearing feeding programs in protein synthesis and degradation, heat production, and body composition of broiler breeder pullets. Antonio Beitia^{*1}, Pramir Maharjan¹, Jordan Weil¹, Katie Hilton¹, Justina Caldas-Cuevas², Nawin Suesuttajit¹, Diego Martinez¹, and Chris Coon¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²Cobb-Vantress, Siloam Springs, Arkansas, United States.

Skip a day (SK) and every day (ED) feeding programs have an effect on hen uniformity ultimately affecting hen performance. The objective of the study was to evaluate the effects of 2 different feeding programs on protein turnover (PT), heat production (HP), and body composition (BC) of broiler breeder pullets. Two-hundred and 40 d-old pullets were reared in 6 pens on 2 feeding schedules from 0 to 16 wk of age. Pullets were reared following the Cobb 500FF parent stock recommended growth curve. All treatments were fed the same Breeder 1 diet from 0 to 16 wk. Pullets were restricted-fed with feed amount and weighed weekly with the feed allotment adjusted to achieve desired BW. The SK birds were fed feed 2 times the ED amount every other day. At 16 wk, 6 birds from each growth curve were sampled every 4 h for PT study. This was done for a 24-h period for the ED birds and for 48-hour period for SK birds with a total of 7 and 13 sampling points respectively. Sampled birds were given an intravenous flooding-dose of ¹⁵N-Phe (150 mM, 40% atom percent excess) at 10 mL/kg for PT determination. After 10 min, birds were sacrificed by CO₂ inhalation, and *Pectoralis major* (breast muscle) and excreta samples were taken. Fractional synthesis and degradation rates (FSR and FDR) were determined (in %/D) using data collected from mass spectrometer where selected ion monitoring was done for m/z ratio of 302 and 303 for enrichment measurement. HP was measured every 2-wk from 3 to 16 wk rearing period by indirect calorimetry (metabolic chambers) using the expression, HP (kcal) = 3.872*VO₂ (L/d) + 1.195 VCO₂ (L/d). BC was measured every 2 wk from 0 to 16 wk by dual-energy x-ray absorptiometry. Significant means were separated at $P < 0.05$ using one-way ANOVA (JMP Pro 16). The FSR values measured for SK and ED after 4 h post feeding were 12.41% and 12.25%. FSR decreased afterward and remained steady for ED (11.02%) and SK (10.91%) until post 24 h feeding. FSR then increased for SK at 32 h post feeding (15.94%) followed by its tendency to decrease and remain steady until post 48 h feeding (11.71%). The average FBR value observed was higher for SK (17.17%) than ED (6.73%) ($P < 0.05$). HP from 3 to 16 wk, showed SK pullets had higher HP than ED ($P < 0.01$). BC data showed that SK pullets had lower lean mass, protein mass, and higher fat mass g/kg BW at 6 wk than ED pullets (P

< 0.05). Overall results indicated that SK pullets were expending more protein mass than ED birds for their growth during the rearing period.

Key Words: protein synthesis, protein degradation, body composition, heat production, broiler breeders

162 Assessment of current egg density methods. Laura Oxford^{*1}, Carla Aranibar¹, Deana Jones², and Jeanna Wilson¹, ¹University of Georgia, Athens, Georgia, United States, ²US National Poultry Research Center, USDA-ARS, Athens, Georgia, United States.

Specific gravity has been used in the poultry industry as a measure of egg shell quality and density for many years. It is performed by sinking intact eggs into water with varying levels of salinity. Eggs that float at each known salinity are removed and recorded. This method is widely accepted, however, it is subject to human error and inaccurate observations. A novel method of determining egg shell density measurements is the use of a VolScan Profiler. The device was developed to aid in determining the volume of bakery products and has been proven effective in determining various measurements of eggs. The VolScan is advantageous over conventional specific gravity measures because the egg is not destroyed, there is no risk of human error, and the device is very accurate. The objective of this observational study was to determine the accuracy of conventional specific gravity measures compared with the VolScan predicted specific gravity derived from density. For this study, eggs from the same breeder flock were taken weekly from 28 to 40 weeks of age. A minimum of 443 eggs were sampled weekly using conventional specific gravity methods and a minimum of 290 additional eggs were tested weekly using the VolScan. There was an average difference between the VolScan and conventional specific gravity measures of 0.0078 over the 12 weeks. Statistical analysis was performed using JMP Pro 14 statistical software. One-way ANOVA analysis results showed that the VolScan measure of specific gravity was significantly higher ($P < 0.0001$) than conventional specific gravity methods. The VolScan method has improved accuracy over traditional floatation methods, however, VolScan Profiling requires equipment that may not be readily available across the industry.

Key Words: specific gravity, shell quality, density, VolScan Profiler, salinity

163 Impact of northern fowl mites on cage-free layer performance from 17 to 48 weeks of age. Rachel Jarrett^{*1}, Marisa Erasmus¹, Deana Jones², Kailynn VanDeWater¹, Amy Murillo³, and Darrin Karcher¹, ¹Purdue University, West Lafayette, Indiana, United States, ²USDA ARS, Athens, Georgia, United States, ³University of California - Riverside, Riverside, California, United States.

The northern fowl mite (*Ornithonyssus sylvarium*) is an obligate blood-feeding ectoparasite of poultry that can cause decreased egg production, profit loss, anemia, irritation to flocks and personnel, and death to hens in extreme cases. This study aimed to investigate the effects of the northern fowl mite (NFM) on laying hen performance and welfare quality between 17 and 48 weeks of age. 800 Tetra brown hens (n = 200 per room) were housed in 4 cage-free rooms at the Purdue University Poultry Unit. Two rooms were infested with NFM and 2 rooms served as controls. Initial NFM infestation occurred at 24 weeks on 2% (4 hens) in each of the NFM rooms. Since no NFM were present, a second infestation on 2% of hens occurred at 35 weeks on 2% of hens. A final attempt to infest with NFM occurred at 41 weeks with all hens in NFM rooms being infested. Egg production and mortality were recorded daily and case weights were recorded weekly. Monthly Welfare Quality[®] assessments were taken, as

well as monthly mite counts on all birds beginning at 28 weeks. Mite checks were conducted periodically on 25% (50 hens) in all rooms between wk 25 and 38. Data were analyzed in SAS® using the Glimmix procedure and all significant statistical differences were reported at $P < 0.05$. Regardless of treatment, mortality increased dramatically after 21 weeks, leading to a loss of 473 hens by wk 48. Case weights and egg production increased over time. NFM hen day peaked in-line with the Tetra management guide at 28 weeks but was approximately 1% lower and control hen day peaked 3 weeks later but was 3% higher. At 38 weeks of age, egg case weights in NFM rooms were approximately 4.63 kg higher than in control rooms ($P < 0.05$). The NFM population spiked shortly after infestation at 28 weeks and again at 36 weeks (1–10 mites/bird). From 28 weeks on, 60% of birds received an overall feather score of 2 during Welfare Quality® assessments, meaning that feathers were missing in at least one area of 5 cm or greater. This was due to pecking behaviors in all 4 rooms. There was no correlation between feathering and NFM presence ($P > 0.05$). Cannibalism and pecking in both treatments led to extreme feather loss, high mortality, and negatively impacted production parameters. As a result, the feather loss and high mortality contributed to the low NFM populations.

Key Words: *Ornithonyssus sylvarium*, mite, poultry, cage-free, layer

164 Chick length and navel score of four bird strains provided *in ovo* photo stimulation. Janessa Henry*, Bruce Rathgeber, and Xujie Li, *Dalhousie University, Truro, Nova Scotia, Canada.*

Measures such as chick length and navel score are practical non-lethal methods for determining chick quality in a hatchery. Providing producers, a robust healthy chick is key for hatcheries. In the broiler industry, this is even more important as chicks spend a shorter time in the rearing facility to achieve the desired market weight. Hatchery practices are constantly changing, an example that has been under investigation is to provide light during incubation. The objective of this study was to determine if chick quality is improved in very different types of chickens when light during incubation is provided. Two replicate trials were performed using the following strains: Ross 308, Cobb 500, Lohmann LSL Lite and a 1978 random bred broiler line from the University of Alberta. All strains were represented in each of the 8 incubators for each trial. Incubation lighting consisted of 4 treatments: dark (no light), white LED, dim to red LED, and dim to blue LED. Lighting treatments had a 12L:12D photoperiod and were replicated for each trial. At 512 h of incubation, chicks were removed from the incubators, batched weighed and navel scored. A sample of 10 birds per strain per incubator were measured for chick length in trial 1 and 12 in trial 2. A 3-point scoring system was adapted from Tona et al., 2003 chick quality assessment. The experiment was a split plot completely randomized design, incubation treatment representing the main plot, and strain as the subplot. Data were analyzed in SAS 9.4 using the GLIMMIX procedure. There was a significant difference in chick length among lighting treatments ($P = 0.0013$). Birds regardless of strain were longer in the dim to blue LED treatment (16.90cm) and in the dark control (16.89cm) compared with those incubated in white (16.54cm) or red (16.49cm). Chick length differed among strains, with Cobb (17.54cm) being longer than the other strains. Although Ross (16.93cm) were shorter than Cobb they were longer than the 1978 birds (16.20cm) and layers (16.15cm). There were no differences among lighting treatments on navel score or percent of birds exhibiting the best score. However, there were strain differences were reported ($P = 0.0146$), Ross birds (1.80) had a superior average navel scores compared with the layers (1.93), with Cobb (1.87) and 1978 birds (1.85) intermediate. Blue light did not negatively affect chick length and could be considered for use in incubators, while white

and red produced shorter chicks. Considering the provision of light during incubation is a relatively new concept, more research should be conducted to determine if there is an optimum light color for hatching and chick performance.

Key Words: incubation lighting, chick length, navel score, broiler, layer

165 The effects of simulated transport conditions on pullet blood physiology, body temperature, and behaviour. Samantha Lalonde*, Kailyn Beaulac, Trever Crowe, and Karen Schwean-Lardner, *University of Saskatchewan, Saskatoon, Saskatchewan, Canada.*

The objective of this study was to determine the effects of various temperature/RH combinations and exposure durations on the physiology and behavior of white-feathered layer pullets (sourced from commercial farms) during simulated transport. The experiment, a 5x2 factorial arrangement of temperature (T) and RH, and exposure duration (D), consisted of 3 replications. Pullets ($n = 240$, 8 birds per T/RH and D) were randomly exposed to 1 of 5 T/RH combinations (21°C30%RH (21/30), 21°C80%RH (21/80), 30°C30%RH (30/30), 30°C80%RH (30/80), and -15°C (-15)), for either a 4 or 8 h D, in 1 of 2 environmental chambers at a crated density of 45.5 kg/m². Before exposure birds were orally administered a miniature data logger to record core body temperature (CBT). Blood samples were collected before and after exposure from 5 birds/T/RH and D and analyzed for stress levels via heterophil to lymphocyte (H/L) ratio. Blood samples from 3 of 5 birds were analyzed for partial pressure of CO₂ (pCO₂), bicarbonate (HCO₃⁻), and glucose (G) using an iSTAT machine. Surface foot and comb temperatures were recorded for all pullets before and after exposure. Behavior during exposure was video recorded, then analyzed using scan sampling at 5-min intervals. After exposure, all birds were slaughtered using a small-scale facility, and all data loggers were retrieved. In a randomized complete block design, data were analyzed by a one-way ANOVA (Proc Mixed (SAS 9.4)), with farm of origin as the block. Significant differences were determined at $P \leq 0.05$. Final G was higher in birds exposed to 30/80 compared with 21/80 and 21/30, however delta (Δ) values did not differ. Compared with 21/30, 30/30 and 30/80 pullets had higher Δ comb T, whereas pullets exposed to -15 had lower Δ comb T. Final foot T were highest after exposure to 30/80, and lowest after exposure to -15, however Δ T values did not differ. Panting and surveying occurred more in the 30/30 and 30/80 birds, while birds from 30/80 also spent more time performing active behaviors. Pullets exposed to 30/30 pecked other birds more often. Preening occurred more often in 30/30 and 21/80 pullets. Pullets spent more time motionless in 21/30 and -15. Compared with the 4 h D, Δ CBT was higher in the 8 h D. With increasing D, Δ pCO₂ and Δ HCO₃⁻ decreased. Pullets were more active in the 4 h, compared with the 8 h, D. In conclusion, pullets used behavioral (performing thermoregulatory behaviors), and physiological mechanisms (mobilization of energy stores, and increased respiration) to cope with hot T and high RH, and increased exposure D. Differences were noted in time spent performing various behaviors, mobilization of G stores, surface foot and comb T, Δ CBT, and blood Δ pCO₂ and Δ HCO₃⁻.

Key Words: cold stress, heat stress, thermoregulation, hematology, welfare

166 Interior environment of three laying hen systems during winter conditions. Yijie Xiong*, Richard Gates, and Kenneth Koelkebeck, *University of Illinois, Urbana, Illinois, United States.*

Proper design and management of the environment in conventional cage and cage-free laying hen facilities are reasonably well understood. Maintaining acceptable air quality during winter can be a challenge. Thus, there is a critical need to better understand the housing environment in laying hen systems for balancing comfortable environment and energy efficiency. This field study aimed to assess interior environmental parameters, including temperature, ammonia (NH₃), and carbon dioxide (CO₂) concentrations, in 3 types of commercial laying hen houses during winter conditions. Six portable monitoring units (PMUs) designed to continuously measure NH₃ and CO₂ concentrations were installed in 2 types of aviaries and an enrichable cage manure-belted house on a commercial layer farm. The 2 aviaries measured 15 × 159 m and consisted of 2 different types of aviary systems (AV1 and AV2), and the enrichable cage house (EC) measured 25 × 168 m (9 rows, 12 tiers high). At the time of deployment, there were 46,400 hens (19 wk), 36,300 hens (77 wk) and 497,000 hens (25 wk) in AV1, AV2, and EC. Two PMUs and 2 temperature/RH dataloggers were placed in each house. Two 30.5 m long air sampling hoses were run from the PMUs along the building centerline to measure the gas concentrations at 2 elevations (approximately 1.5 and 2.5 m for AV1 and AV2; 1.5 and 5.3 m for EC). The 2 temperature/RH dataloggers were placed at the same locations where air was sampled. House air was sampled every 10 s for 10 min followed by a 20 min fresh air purge, and the process was repeated continuously. A 2-sample *t*-test was used to determine variation in 2 elevations for each measurement within each house. Results for February showed that, among 125 h of continuous monitoring, mean values for temperature and gas concentrations were 22.6, 26.9, and 25.4°C, 31, 20, and 36 ppm (NH₃), and 2375, 2823, and 2376 ppm (CO₂), for AV1, AV2, and EC, respectively. Mean daily outside temperature ranged from -3.9 to 6.1°C. Substantial temperature stratification (3 to 6°C) was observed in the 2 aviaries, but not EC. A difference in vertical variation was observed for each measurement in all houses ($P < 0.01$). The proportion of hours below the temperature setpoint in each house was 45.6, 62.4, and 53.7% for AV1, AV2, and EC, respectively. For NH₃, the occurrence of hours exceeding the 25 ppm OSHA 8h time-weighted average was 71.8, 23.9, and 98.3% in AV1, AV2, and EC, respectively. The 25 ppm value is proposed for humans but is often used for management guidelines within poultry houses. These measurements suggest further improvement to minimum ventilation management would be helpful. Monitoring will continue for all 4 seasons at the site.

Key Words: Indoor air quality, ammonia, carbon dioxide, aviary, cage-free house

167 Housing environment and hen strain alter egg production, egg quality, and tibia strength as well as eggshell and cloacal microbiology in post-peak laying hens. Milan Sharma*, Chris McDaniel, Aaron Kiess, and Pratima Adhikari, *Mississippi State University, Mississippi State, Mississippi, United States.*

An experiment was conducted to determine the effects of housing environment and laying hen strain on hen-day egg production (HDEP), egg quality, and tibia breaking strength (TBS) as well as eggshell and cloacal microbiology. A total of 1152 laying hens (Hy-Line brown (HB) and Hy-Line W36 (HW)) were randomly allocated into conventional cages (CC), enriched colony cages (EC) or cage-free free-range housing (CF) creating a 2 × 3 factorial arrangement with a total of 6 treatments. The experiment was conducted when hens were 38 to 65 wk of age. Feed was formulated according to Hy-Line management guidelines, and both feed and water were provided *ad libitum*. Egg production was recorded daily, while egg weight (EW), Haugh unit (HU), and eggshell breaking strength (EBS), as well as eggshell and cloacal microbiology, were

measured once every 6 wk. Data were analyzed as a split plot over time with 6 replicates for each treatment. A 3-way interaction was observed among house, strain, and age for HDEP ($P = 0.0571$). Overall, HDEP was higher in CF and CC than EC. However, HDEP for HW EC hens was consistently lower than all other treatments every week, yet HDEP for HB EC hens did not become lower than the other treatments until wk 46. An interaction between strain and age ($P = 0.007$) was observed for EW where at wk 59 HW had lower EW than HB. There was an interaction of housing and age for HU ($P = 0.0303$). Additionally, CC and CF had higher EBS than EC ($P = 0.0009$). Main effects of housing environment and strain were observed ($P < 0.0001$) for TBS where CF and HW hens had higher TBS than other treatments. A 3-way interaction was observed among house, strain, and age for feed intake ($P = 0.0074$). Feed intake (FI) was lower in EC than CC, with the highest FI observed in CF in wk 50–51 and wk 62–63. A higher FI was observed in HB than HW throughout the study for CC and EC. Feed conversion ratio per dozen eggs (FCR) was higher in EC than CF and CC in wk 54–55 and wk 58–59 ($P < 0.0001$). A significantly higher count of total aerobic, coliform and anaerobic bacteria was observed in the cloaca ($P < 0.0001$) of CF when compared with CC and EC. Similarly, bacterial eggshell contamination was higher in the CF when compared with the CC and EC for total aerobes ($P < 0.0001$), anaerobes ($P < 0.0001$) and coliforms ($P = 0.0003$). To conclude, both the housing environment and laying hen strain had effects on HDEP, egg quality, FI, FCR, and bacterial population. Although CF hens have high egg production, egg quality, and TBS, the rate of eggshell contamination, FI and FCR were also higher. Our findings challenge us to further investigate food safety and economic benefits, which need to be considered when deciding on a housing environment to use.

Key Words: housing environment, laying hen strain, egg production, egg production, egg and cloacal microbiology

168 Effects of housing types on egg production and cecal microbiota of two different strains of laying hens during the late production stage. Bishnu Adhikari¹, Se-Ran Jun², Young Min Kwon¹, Aaron Kiess³, and Pratima Adhikari³, ¹*University of Arkansas, Fayetteville, Arkansas, United States,* ²*University of Arkansas for Medical Sciences, Little Rock, Arkansas, United States,* ³*Mississippi State University, Starkville, Mississippi, United States.*

Due to animal welfare issues, EU banned use of conventional cages (CC) for laying hens, and non-EU countries including the US are also under constant public pressure to restrict their use in egg production. Enriched colony cages (EC) were developed to provide hens more comfort movement and allow natural behaviors. Although previous studies have investigated the performance parameters and welfare of laying hens housed in CC and EC, there is very limited information regarding the changes in gut microbiota and their possible roles in egg production. Thus, this study was conducted to explore the effects of CC and EC housing on egg production and cecal microbiota of 2 commercial laying hen strains, Hyline Brown (HB) and White Leghorns (WL). Hens were assigned in a 2x2 factorial arrangement in a completely randomized design: HB in CC (120) and EC (311), and WL in CC (120) and EC (355). Hen-day egg production (HDEP) was recorded weekly, and cecal samples (n = 6/group) were collected at 53, 58, 67, and 72 weeks of age for microbiota analysis by MiSeq sequencing of 16S rRNA gene. Statistical analysis of HDEP data was carried out in split-plot in time design with significance level set at $P < 0.05$, whereas sequence reads were analyzed using QIIME2 ver. 2018.8. Differentially abundant taxa were identified by LefSe ($P < 0.05$, log₁₀LDA score > 2.0) analysis. WL had significantly higher HDEP compared with HB on wk 58 (90 vs.

78.13%) and 72 (64.86 vs. 56.34%). Hens housed in CC had significantly higher HDEP compared with EC on wk 67 (82.22 vs. 68.11%). Moreover, the composition and diversities of cecal microbiota were affected by breed, housing, and age in descending order. LEfSe analysis at genus level revealed 51, 48, 58, and 15 differentially abundant taxa between HB and WL at 53, 58, 67, and 72 weeks, respectively. Interestingly, *Bifidobacterium* was significantly enriched in WL at all time periods, and in addition, butyrate producing genera such as *Butyricoccus* and *Subdoligranulum* were significantly higher in WL as compared with HB at 58 and 72 weeks. Moreover, 13, 8, 23, and 8 differentially abundant taxa between CC and EC housing were observed at 53, 58, 67, and 72 weeks, respectively. At 67 weeks, *Campylobacter* and the unknown genus of family Campylobacteriaceae were significantly enriched in EC which might be associated with reduced egg production in EC. Likewise, there were significant differences in both α and β diversity between HB and WL at all time periods, while a significant difference was observed between CC and EC only at 67 week ($P < 0.05$). The overall results suggest that the difference in egg production between HB and WL, and CC and EC might be achieved through alterations of cecal microbiota.

Key Words: laying hens, egg production, enriched colony cage, convention cage, cecal microbiota

169 Poultry in the classroom: Effectiveness of an online poultry-science-based education program for high school STEM instruction. MaryGrace Erickson*, Darrin Karcher, Neil Knobloch, Marisa Erasmus, and Elizabeth Karcher, *Purdue University, West Lafayette, Indiana, United States.*

Public knowledge of the poultry industry is limited, yet attitudes toward the industry shape consumer behavior and influence the attractiveness of poultry careers and future study. This study assessed an online learning program contextualizing STEM learning within poultry science which was designed to increase poultry knowledge and interest. High school student participants ($n = 169$) across 16 classes ($n = 12$ teachers) completed 730-min online modules during the fall 2018 semester. This case study used a mixed-methods, sequential explanatory design. Student knowledge and interest in poultry and teacher perceptions of the program were examined using quantitative measures. The study's qualitative portion assessed perceptions of the learning experience and comprised open-ended student and teacher survey questions and a teacher focus group. Qualitative data suggested that the program increased students' poultry knowledge, including awareness of the industry and related careers. Paired *t*-tests showed moderate to large effect sizes regarding increases in content quiz scores following each module ($P < 0.001$, Cohen's $d = 0.45$ to 0.80). Students' mean interest in poultry was initially low and remained statistically similar upon completion of the program ($M = 1.84, 1.87; P = 0.67$). Student and teacher statements indicated that design features of the program enhanced interest for some students. Other students did not perceive the program and its poultry science topics to be interesting or relevant to their lives. Results from this study can be used to inform the creation of effective poultry learning resources to contribute to workforce development and enhance the industry's public image.

Key Words: online, public awareness, interest, education, learning

Student Competition: Metabolism and Nutrition: Amino Acids

170 Determining reactive lysine and amino acid availability in soybean meal using near-infrared reflectance and reference analysis. Kara Dunmire*¹, Janak Dhakal¹, Kendre Stringfellow², Charles Stark¹, and Chad Paulk¹, ¹Kansas State University, Manhattan, Kansas, United States, ²AB Vista, Plantation, Florida, United States.

Soybean meal, the most widely used protein source in animal feed, undergoes oil extraction and heat processing which can lead to variation in amino acid (AA) availability. Diet formulation and animal performance can be affected when AAs are not accurately predicted. Therefore, the objective of this study was to evaluate the correlations between chemical assay and near-infrared reflectance spectroscopy (NIR) when measuring protein quality. Crushed soybean white flakes (Hershey, PA) exposed to mechanical oil extraction but not heat processing were used in this experiment. Soybean white flakes were autoclaved to simulate heat processing to create under processed, adequately processed, and over processed soybean meal. Flakes were ground using a blender and 500 g samples were put into calico cotton bags to be autoclaved at 128°C for 0, 5, 10, 15, 30, 45, and 60 min at 200 kPa. A total of 2 samples per treatment were autoclaved at 3 separate time points to provide 3 replications per treatment. The duplicate samples were divided and analyzed using NIR or laboratory analysis. Total AA, available Lys, all other AA, and urease activity were analyzed to determine the correlation coefficient for reference method for AA and NIR. Increasing soybean exposure time to the autoclave decreased (linear, $P < 0.05$) total AA, Arg, and tended to decrease CP (linear, $P < 0.061$) when measured using NIR. Total Lys, Lys/CP, Reactive Lys, Asp, Cys, Glu, Gly, His, Iso, Leu, Met, Phe, Pro, Ser, Thr, Tyr, and Val decreased (quadratic, $P < 0.05$) when measured using NIR with increasing soybean exposure time to the autoclave. Total AA, urease, Ala, Asp, Glu, Gly, Iso, Leu, and Val decreased (linear, $P < 0.05$) when measured with official analytical methods with increasing autoclave exposure time. Amino acid measurements using analytical methods decreased (quadratic, $P < 0.05$) total Lys, Lys/CP, available Lys and Cys with increasing autoclave exposure time. There was a positive correlation ($r = 0.82$) between NIR Lys and analytical Lys. The NIR reactive Lys was positively correlated ($r = 0.98$) to analytical available Lys. The NIR Lys/CP was positively correlated ($r = 0.99$) to analytical Lys/CP. In conclusion, increasing soybean autoclave exposure decreased total AAs, CP, available Lys, Lys/CP, and urease. Analytical methods and NIR were highly correlated for total Lys, available Lys, and Lys/CP.

Key Words: soybeans, soybean meal, heat processing, lysine, amino acid availability

171 The effect of varying steam conditioning temperature and time on pellet manufacture variables, digestible amino acid concentration, and feed enzyme recovery. Timothy Boltz*¹, Nelson Ward², Victoria Ayres¹, Angela Lamp¹, and Joseph Moritz¹, ¹West Virginia University, Morgantown, West Virginia, United States, ²DSM Nutritional Products Inc., Parsippany, New Jersey, United States.

This study hypothesized that increased steam conditioning temperature and conditioning time would improve feed manufacture metrics while decreasing the digestible amino acid concentration and recovery of a lysozyme feed additive. The objectives of the study were to assess how varying steam conditioning temperatures and conditioning times interacted to affect pellet mill motor load, pellet quality, digestible amino

acid concentration, and enzyme recovery. Corn and soybean meal based diets that included DDGS and meat and bone meal were conditioned at either 76°C, 82°C, or 88°C with conditioning times of either 30 or 60 s. The soybean meal used in the formulations had an analyzed trypsin inhibitor content of 3.64 TIU/mg. Treatments were arranged in a 3 (steam conditioning temperature) x 2 (conditioning time) factorial in a randomized complete block design. Digestible amino acid concentration was estimated using cececotomized roosters in a Latin Square with crossover design. Conditioning temperature and time interacted to affect pellet mill motor load ($P < 0.05$). Motor load decreased for 30 s conditioning compared with 60 s conditioning when diets were subjected to 76 and 82°C, but motor load was similar among conditioning times at 88°C. This interaction was likely the result of lower feed volume in the conditioner as per 30 s conditioning obtaining greater moisture condensation and lubrication at lower conditioning temperatures. Pellet quality increased with increased conditioning temperature ($P < 0.05$), likely associated with greater starch gelatinization and protein gelation. Lysozyme activity decreased when conditioned at 88°C ($P < 0.05$), but was not affected by retention time. Conditioning temperature and time interacted to affect digestible amino acid concentrations of methionine, lysine, threonine, alanine, aspartic acid, glutamic acid, isoleucine, leucine, proline, and valine ($P < 0.05$). Increased conditioning temperatures at 30 s increased digestible amino acid concentrations. Similarly, diets conditioned at 60 s increased digestible amino acid concentration between 76 and 82°C. However, the digestible amino acid concentration decreased when diets were conditioned at 60 s and 88°C. Perhaps negative effects of trypsin inhibitor complexes and ingredient aleurone layers on digestibility were decreased with increasing temperatures; however, the 60 s 88°C treatment may have been too thermally aggressive and rendered the proteins indigestible.

Key Words: conditioning, pelleting, amino acid, digestibility, enzyme

172 Nutritional value of *Tenebrio molitor* meal for broiler chickens: standardized ileal amino acid digestibility. Marcos Nascimento Filho*, Raquel Pereira, Alvaro Burin Junior, Ana Beatriz Oliveira, Diana Suckeveris, Caio Alexandre Paes Soares, and José Fernando Machado Menten, University of São Paulo, Piracicaba, São Paulo, Brazil.

There has been a worldwide interest and investment in the production of insects for human and animal nutrition. These products are seen as novel sources of animal origin protein, which are sustainable in production and desirable as nutrient-rich feed ingredients. To be used in feed formulation for poultry, the composition and nutritional specifications of the insect products, such as larvae meal, must be known. This study aimed to determine the standardized ileal digestibility coefficients (SIDC) of amino acids of tenebrio (*Tenebrio molitor*) meal (TM) for broiler chickens. A total of 120 one-day-old male broilers chickens of commercial strain were weighted, placed in floor pens (10 birds/pen, $n = 6$) in a completely randomized design and fed a basal corn-soybean meal starter diet to 15 d of age. On d 16, birds were assigned to 2 experimental diets: semi-purified nitrogen-free diet to estimate the basal endogenous amino acids flow, and a test diet with TM as the sole source of amino acids, formulated to reach approximately 20% crude protein. Chromic oxide was used as an indigestible marker at 0.5%. The experimental period lasted 6 d. Feed and water were available *ad libitum*. On d 22, all birds were euthanized by cervical dislocation and contents from distal third

of the ileum until approximately 2 cm above the ileocecal junction were collected gently by flushing with distilled water. The samples were kept refrigerated during collection and frozen at -20°C . Total fresh digesta from all birds in each pen were pooled for lyophilization. Samples of the diets, TM, and ileal digesta were analyzed for dry matter, nitrogen, amino acids and chromium to estimate the digestibility coefficients. Analyzed values for TM were 97.0% dry matter, 50.0% crude protein, 29.7% fat, 3.8% minerals, 0.07% calcium, and 0.54% phosphorus. The amino acid contents were 0.78% Met, 3.26% Lys, 2.09% Thr, 3.22% Val, 2.23% Ile, 2.92% Arg, 2.36% Phe, 3.74% Leu, and 1.82% His, as fed basis. The calculated average values of SIDC of the indispensable amino acids were Met, 87.5%; Lys, 89.0%; Thr, 81.6%; Val, 85.6%; Ile, 85.6%; Arg, 91.9%; Phe, 90.0%; Leu, 88.4%; His, 81.2%. Overall, the present study shows values of SIDC for TM similar to those found in the literature for other types of insect meal as feed ingredient for chickens. These are the first measurements on standardized ileal digestibility of amino acids of TM, which allows adequate formulation using this ingredient in diets for poultry. Acknowledgment: FAPESP for the research grant (No 2017/05423–8) and MS scholarship (No 2017/19751–7).

Key Words: insect meal, *Tenebrio molitor*, digestibility, amino acids, broilers

173 Effects of high methionine supplementation on tissue redox, inflammation, and health status of broiler chicks under heat stress. Guanchen Liu^{*1}, Andrew Magnuson¹, Tao Sun¹, Samar Tolba¹, Rose Whelan², and Xingen Lei¹, ¹Cornell University, Ithaca, New York, United States, ²Evonik Nutrition & Care GmbH, Hanau, Germany.

Methionine as a sulfur-containing amino acid is known for its antioxidant capacity. We determined if adding 30% extra supplemental DL-methionine (MET) or crystalline DL-methionine hydroxyl analog (MHA) to grower and finisher diets improved tissue redox, inflammation, and health status of chicks under heat stress. A total of 360 (day-old) male Cornish cockerel chicks were divided into 4 treatments (10 pens/treatment, 9 chicks/pen), and were fed the same corn-soy starter diet and then respective grower diets (5.8 g SAA/kg) supplemented with MET (3.09 or 4.02 g/kg) or MHA (7.19 or 9.34 g/kg) and finisher diets (5.4 g SAA/kg) supplemented with MET (2.77 or 3.60 g/kg) or MHA (6.44 or 8.37 g/kg). Grower and finisher chicks were raised at a high room temperature of 31°C with 25% humidity. Blood and tissues were sampled at the end of each period, and data were analyzed by 2-way ANOVA. The higher concentration of MET or MHA produced greater ($P < 0.05$) hepatic, but lower ($P < 0.05$) plasma GSH concentrations than their normal concentration controls during the grower and finisher periods, respectively. Breast and thigh GSH concentrations in the grower chicks fed MET were higher ($P < 0.05$) than those fed MHA. Supplementing extra MET improved ($P < 0.05$) plasma ferric reducing ability of the finisher chicks, but tissue malondialdehyde levels, plasma corticosterone concentrations, or hepatic protein carbonyl concentrations were not affected by the dietary treatments. Neither the form nor the concentration of methionine displayed consistent effects on activities of 4 antioxidant enzymes (GPx, GR, GST, and SOD) in 4 tissues across the 2 periods. However, MHA seemed to induce higher ($P < 0.05$) enzyme activities than MET in the grower chicks. There were interactions ($P < 0.05$) between the form and concentration of methionine on activities of GR in the thigh and adipose tissues, GST in the liver, or GPx in the breast. Likewise, such interactions ($P < 0.05$) were also shown on the expression of most of the assayed inflammation-related genes in tissues of the finisher chicks. Chicks fed MET had higher ($P < 0.05$) expression of *HSP90* and *AKT* in the liver and all tested genes except for *AKT* in

the breast than those fed MHA. Supplementing extra MET or MHA suppressed ($P < 0.05$) expressions of *IL-10*, *HSP70*, and *HSP90* in the thigh. The higher concentration of MET or MHA decreased ($P < 0.05$) plasma inorganic phosphorous concentrations at both time points, and elevated plasma alkaline phosphatase activity of finisher chicks. In conclusion, both forms and concentrations of dietary supplemental methionine exerted major effects on redox, inflammation, and health status of grower and finisher chicks in response to heat stress.

Key Words: antioxidant, heat stress, methionine, oxidative stress, poultry

174 Effects of high methionine supplementation on growth performance, meat quality, and bone strength of broiler chicks under heat stress. Tao Sun^{*1}, Guanchen Liu¹, Andrew Magnuson¹, Samar Tolba¹, Charles Starkey², Rose Whelan³, and Xingen Lei¹, ¹Cornell University, Ithaca, New York, United States, ²Auburn University, Auburn, Alabama, United States, ³Evonik Nutrition & Care GmbH, Hanau, Germany.

It remains unclear how methionine concentration or source affects the production and health of broilers at heat stress. This study determined effects of 30% extra supplemental DL-methionine (MET) or crystalline DL-methionine hydroxyl analog (MHA) to grower and finisher diets on growth performance, health status, meat quality, bone strength, and feather coverage of chicks under heat stress. A total of 360 (day-old) male Cornish cockerel chicks were divided into 4 treatments (10 pens/treatment, 9 chicks/pen), and were fed the same corn-soy starter diet and then respective grower diets (5.8 g SAA/kg) supplemented with Met (3.09 or 4.02 g/kg) or MHA (7.19 or 9.34 g/kg) and finisher diets (5.4 g SAA/kg) supplemented with either MET (2.77 or 3.60 g/kg) or MHA (6.44 or 8.37 g/kg). Grower and finisher chicks were raised at a high room temperature of 31°C with an average humidity of 25%. Growth performance was recorded at the end of each period and tissue samples were collected at the end of the study. Data were analyzed using 2-way ANOVA and Duncan multiple mean comparisons. Neither the form nor the concentration of methionine affected the growth performance except for a lower ($P < 0.05$) feed intake in the finisher chicks fed MHA than those fed MET. Dressing percentage and the meat to bone ratio of the thigh were not affected by the diets, chicks fed MET had higher ($P < 0.05$) hardness of the breast muscle than those fed MHA and the higher MET or MHA concentration improved ($P < 0.05$) chewiness of the breast muscle (tested by TA.XT texture analyzer). Incidences of woody breast and white striping were affected by treatments. Plasma and tissue lipid profiles were unaffected by the diets except triglyceride concentration in the thigh was higher ($P < 0.05$) in the chicks fed MHA than those fed MET. Hepatic fatty acid content was lower ($P < 0.05$) in the grower chicks fed MHA than those fed MET, with similar decreases in saturated (SFA), monounsaturated (MUFA), and poly-unsaturated fatty acids (PUFA). In contrast, concentrations of the 3 types of fatty acids in the breast were higher ($P < 0.05$) in the finisher chicks fed MHA than those fed MET. Higher MET or MHA concentration enhanced ($P < 0.05$) SFA and MUFA contents in the thigh and all types of fatty acids in the adipose tissue of finisher chicks. Higher MET and/or MHA concentration elevated ($P < 0.05$) energy at maximum load of tibias of grower chicks but decreased that of finisher chicks. Feather coverage was not affected by the diets. In conclusion, the form and concentration of supplemental dietary methionine showed effects on eating quality and fatty acid composition of meat and tibia strength of grower and finisher chicks under heat stress.

Key Words: antioxidant, heat stress, methionine, meat, poultry

175 Responses of varying levels of DL-methionine and TSAA on [¹³C]Met and [¹³C]Cys oxidation in broilers. Jordan Weil*¹, Pramir Maharjan¹, Antonio Beitia¹, Katie Hilton¹, Nawin Suesuttajit¹, Victor Naranjo², and CN Coon¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²Evonik Nutrition and Care, Hanau, Germany.

For feed formulations and protein synthesis, the metabolism of sulfur amino acids (SAA) is important because methionine is the first limiting amino acid for poultry. Additionally, considering the metabolite, glutathione, plays a key role in tissue building, muscle repair, and the reduction of oxidative stress, understanding the metabolite is increasingly important as the poultry industry moves toward antibiotic-free (ABF) production settings. In 2 experiments, 2400 Cobb 500 chicks were reared to determine the rate of CO₂ produced (VCO₂) through stable isotopes and indirect calorimetry as well as the oxidation of [1-¹³C]Met and [1-¹³C]Cys. Birds were fed grower diets containing varying levels of TSAA in both experiments (Evonik AMINOChick®2.0). At 21d old, birds were selected for either control, intravenous (IV) or intragastric (IG) infusion of [1-¹³C]Met (Exp 1), or [1-¹³C]Cys (Exp 2), followed by sampling at intervals ranging from 5 to 420 min post-infusion. Additionally, birds were selected for metabolic chambers. Blood was collected and ¹³CO₂ enrichment was determined by isotope ratio mass spectrometry (IRMS). Small intestine, pancreas, kidney, and liver tissues were harvested at each of the time periods to determine the amino acid enrichment over time. Samples were analyzed using a gas chromatography-mass spectrometer (GC-MS) and high pressure liquid chromatography (HPLC), depending on the metabolite of interest. Results were analyzed using JMP Pro 13.1. Deficient levels of TSAA resulted in birds that were unable to overcome such deficiency, resulting in poorer feed conversions and greater loss of methionine label as ¹³CO₂. Comparison of ¹³CO₂ revealed differences in IG and IV infusions ($P < 0.05$). Loss of [1-¹³C]Met through ¹³CO₂ in birds fed adequate TSAA increased (18 vs 11%; $P < 0.05$), as determined through IRMS. Eventual loss of [1-¹³C]Met determined through whole blood and plasma enrichment showed an increase in oxidation for IG infusions when compared with IV infusions (20.4 vs 10.2; $P < 0.05$). Differences in IG and IV infusions also existed when looking at diets deficient in cysteine (9 vs 3%). These deficiencies in cysteine also resulted in a decrease in glutathione production in the liver as observed through a ratio of reduced: oxidized glutathione (11 vs 327; $P < 0.05$). As the poultry industry moves toward more ABF production settings, understanding methionine, TSAA, and glutathione pathways becomes increasingly important. Ultimately, the loss of the Met and Cys labels to ¹³CO₂ proves worthy for understanding TSAA utilization and metabolism, considering greater ¹³CO₂ production from [¹³C]Met may signify increased cysteine synthesis.

Key Words: methionine, sulfur amino acids, glutathione, stable isotopes, broilers

176 Digestible sulfur amino acid requirements of broiler chicks over short time periods in the starter phase of production. Albaraa Sarsour* and Michael Persia, Virginia Tech, Blacksburg, Virginia, United States.

Sulfur amino acids are the first limiting amino acids in common poultry diets and are crucial for growth and muscle development. Generally, requirement experiments have focused on defined periods of time called starter, grower, finisher phases resulting in requirements over a 10 to 20 d feeding period. It is impractical economically in commercial production to mix diets and transport them to the farm every week. However, Pope et al., 2002 put forward the idea to formulate high and low nutrient dense diets placed in 2 different bins on the farm. These diets would be fed at

different proportions within a phase to better meet the requirements of the birds that were hypothesized to linearly decrease as the chicks' age. This idea was put forward on assumption of a linear response without validating data, therefore, experiments were conducted to determine the requirement of Digestible sulfur amino acids (DSAA) from 3 to 6, 6–9 and 9 to 12 d of age. A deficient diet containing 0.63% DSAA was formulated using corn, soybean meal, and corn gluten meal. Experimental diets were formulated from this basal diet by adding DL-methionine in 0.07% increments to generate 0.70, 0.77, 0.82, 0.89, 0.96, and 1.03% DSAA. In each experiment, 350 male Hubbard x Ross 708 chicks were classified by body weight and randomly assigned to the 7 diets with 10 replicates of 5 birds in battery cages. Chicks and feeders were weighed at the start and end of each 3-d period. All chicks were sacrificed and breast weight was determined. Relative breast weight, BWG and FCR were calculated. Data were analyzed using a one-way ANOVA in SAS ($P \leq 0.05$). Requirements were estimated using broken line regression in JMP 14. No differences were observed in feed intake in any of the experiments ($P > 0.05$). The DSAA requirement for BWG was estimated to be 0.717, 0.787, and 0.838% or 136, 179, and 287 mg/d DSAA from 3 to 6, 6–9 and 9–12 d, respectively. The requirement for relative breast weight was estimated to be 0.841, 0.812, and 0.871% or 160, 235, and 296 mg/d DSAA from 3 to 6, 6–9 and 9–12 d, respectively. Lastly, the requirement for FCR was estimated to be 0.693, 0.730, and 0.675% or 132, 155, and 230 mg/d DSAA from 3 to 6, 6–9 and 9–12 d, respectively. These data may suggest that DSAA requirements for BWG do respond in a linear manner, but both relative breast weight and FCR may not respond in a linear manner over the starter phase of production.

Key Words: digestible sulfur amino acids, requirement, broiler

177 Evaluation of a valine fermentation product on performance of male broilers fed valine deficient corn-soy diets. Bryce Leopold*¹, Austin Jasek¹, Jason Lee², Keith Haydon², and Craig Coufal¹, ¹Texas A&M University, College Station, Texas, United States, ²CJ America, Downers Grove, Illinois, United States.

Two experiments were conducted to evaluate a new valine fermentation product (70% L-valine) on male broiler performance. Experiment 1 was designed to determine the level of digestible valine reduction necessary to impact performance. Experiment 2 evaluated the inclusion of the valine fermentation product compared with L-valine (98.5%) when included in a valine deficient diet through 28 d of age. Diets in both experiments were corn and soybean meal based. Performance data in both experiments were analyzed via a one-way ANOVA with differences determined at $P < 0.05$ and means separated using Duncan's Multiple Range test. Experiment 1 consisted of 3 dietary treatments varying in levels of digestible valine (60, 68, and 76% dVal:dLys) with 16 replicates per treatment and 7 birds per replicate for a total of 336 Cobb 500 male broilers. Growth performance was decreased ($P < 0.05$) with reducing dVal level. The 60% dVal diet resulted in lower BW and higher FCR as compared with the 76% dVal diet throughout the trial. The intermediate level dVal (68%) was intermediate ($P > 0.05$). Experiment 2 was designed using the lowest level of dVal:dLys as the negative control (NC). Experiment 2 evaluated 3 dietary treatments, each with 15 pens with 35 Cobb 500 male broilers per pen. Experimental treatments included a positive control (PC) with L-valine (76% dVal:dLys), a NC with valine deficiency (60% dVal:dLys), and NC + the valine fermentation product equal to the level of PC. The NC broilers had the lowest BW and higher FCR ($P < 0.05$) compared with all other treatments throughout the experiment. Following the starter phase, broilers fed the fermentation product had elevated BW and reduced FCR compared with the L-valine fed broilers, presumably due to the additional nutritive value

present in the fermentation product. At the conclusion of the study (28 d), broilers fed different valine sources had similar BW and FCR. These data confirm the importance of adequate dietary valine and demonstrate a new valine fermentation product provides equivalent performance to L-valine when included at equivalent valine concentrations.

Key Words: broiler, valine, amino acid, performance

178 Influence of branched chain amino acid inclusion in diets varying in ingredient composition on broiler performance, processing yields, and paw quality and litter characteristics. Trevor Lee*¹, Jason Lee², and Samuel Rochell¹, ¹University of Arkansas, Farmington, Arkansas, United States, ²CJ America, Downers Grove, Illinois, United States.

The current experiment evaluated the influence of supplementing the branched chain amino acids (BCAA) Val and Ile in corn-soybean meal-based (CSBM) diets with or without either animal protein meal (APM) or peanut meal (PM) on broiler growth performance, processing characteristics, and paw quality and litter characteristics. Ross 708 male chicks (1,512 total) were allocated 6 treatments (21 birds/pen) in a factorial arrangement of 3 diet types (CSBM, PM, and APM) with or without BCAA inclusion from 0 to 48 d: 1) CSBM, 2) CSBM+BCAA, 3) CSBM+PM, 4) CSBM+PM+BCAA, 5) CSBM+APM, 6) CSBM+APM+BCAA. Starter diets were formulated to contain 1.28% digestible Lys with minimum ratios of digestible amino acids relative to Lys set to 0.74, 0.67, 0.75, 0.67, and 1.07 for TSAA, Thr, Val, Ile, and Arg, respectively. Supplementation with BCAA reduced analyzed CP content by 1.87, 1.43, and 1.24 percentage units in the CSBM, PM, and APM starter diets, respectively, with analyzed total Gly+Ser concentrations of the BCAA-supplemented diets of 1.28, 1.24, and 1.39%. Treatments were fed in starter (0 to 14 d), grower (14 to 28 d), finisher (28 to 39 d), and withdrawal (39 to 48 d) phases with 12 replicate pens per treatment. Body weight gain, FI, and FCR were evaluated for each phase and cumulatively. At 48 d, footpad lesions were scored and litter analyzed for nitrogen, pH, and moisture. At 49 d, 6 birds per pen were randomly selected for processing. There were no interactive effects ($P > 0.05$) between diet type and BCAA inclusion on any performance or processing measurement. From 0 to 28 d, birds fed PM diets had higher ($P = 0.014$) BWG and lower ($P = 0.001$) FCR than birds fed APM or CSBM diets. However, effects of diet type on performance diminished by d 39. Supplementation of BCAA increased ($P = 0.014$) FI of broilers, but did not influence ($P > 0.05$) BWG or FCR. At processing, carcass weights were heaviest for PM-fed birds, lightest for APM-fed birds, and intermediate for CSBM-fed birds ($P < 0.05$). Inclusion of BCAA increased total white meat weight ($P = 0.042$) and yield ($P = 0.016$), but also increased relative ($P = 0.001$) and absolute ($P = 0.002$) fat pad weights. Footpad lesions were lowest for APM-fed birds, highest for CSBM-fed birds, and intermediate for PM-fed birds ($P < 0.05$), with no effects ($P > 0.05$) of BCAA supplementation. There were no treatment effects ($P > 0.05$) on litter moisture or pH, but BCAA supplementation reduced ($P < 0.05$) litter nitrogen content. In conclusion, these data indicate that BCAA inclusion can support CP reductions for high amino acid density diets that vary in ingredient composition while maintaining broiler growth performance, improving breast meat yield, and reducing nitrogen excretion.

Key Words: valine, isoleucine, peanut meal, animal protein meal, broiler

179 Determination of the 4th limiting amino acid for male and female Cobb MV × 500 broilers from 15 to 35 d in low crude protein vegetable based diets. Craig Maynard*¹, Sonia Liu², Jason

Lee³, Justina Caldas-Cuevas⁴, Edward Diehl⁴, Samuel Rochell¹, and Michael Kidd¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²The University of Sydney, The Oaks, New South Wales, Australia, ³CJ America, Downers Grove, Illinois, United States, ⁴Cobb-Vantress, Siloam Springs, Arkansas, United States.

With more interest in reducing environmental nitrogen, determination of the 4th limiting amino acid (AA) is necessary to ensure good broiler performance when fed low CP corn-soybean meal diets. Therefore, 2 concurrent experiments (Exp) were conducted to determine the 4th limiting AA for male and female Cobb MV × 500 broilers for the 15 to 35 d period using the diet deletion technique. Least cost formulation constraints for AA minimums were allowed to fall in the test diet from 20.29% to 18.65% CP (positive control: PC). Test diets were adequate in TSAA, Lys, and Thr. Nine subsequent diets were created by removing all (negative control: NC) or a single AA and included: NC, -Val, -Ile, -Leu, -Trp, -Arg, -His, -Phe, and -Gly+Pro deletions. Experiment 1 utilized male broilers and the former diets, whereas Exp 2 utilized female broilers fed the NC and all AA deletion diets. One thousand-9 hundred and 20 sexed broiler chicks were distributed across 96 pens (20 birds/pen) and randomly assigned experimental diets. Body weight gain, feed intake, and feed conversion ratio (FCR) were determined for the 15 to 35 d experimental period. At 36 d, 6 birds per pen were processed for the evaluation of carcass traits. All data were analyzed using a one-way ANOVA and means were separated using a repeated *t*-test where appropriate. No differences ($P > 0.05$) were detected for any live performance parameter in Exp 2 for females, whereas BW gain ($P = 0.051$) and FCR ($P < 0.05$) were impacted in Exp 2. Removal of supplemental Val resulted in reduced ($P = 0.051$) BW gain compared with birds fed the PC diet, similar to birds fed the NC diet ($P > 0.05$). The removal of supplemental Ile decreased ($P < 0.05$) 15 to 35 d FCR in males, while all other AA deletions resulted in FCR similar to the PC ($P > 0.05$). Carcass yields were unaffected ($P > 0.05$) by supplemental AA removal in either Exp. However, in Exp 1, removal of supplemental Val, Phe, and Gly+Pro resulted in reduced ($P < 0.05$) breast and wing weights compared with birds fed the PC diet, similar to birds fed the NC diet ($P > 0.05$). Removing Val and Gly+Pro from the diet reduced ($P < 0.05$) carcass weight, whereas removing Val and Phe reduced ($P < 0.05$) leg quarter weight to values similar ($P > 0.05$) to birds fed the NC diet. No carcass trait weights were effected ($P > 0.05$) by supplemental AA deletion in Exp 2 except wing weights which increased ($P < 0.05$) for birds fed diets devoid in Phe. In conclusion, the 4th limiting AA for corn-soybean meal based low CP vegetable diets appears to be either Val or Gly+Pro, but further research is needed to identify the effects of non-essential AA, specifically Gly and Pro, and their individual impact on performance and carcass traits.

Key Words: limiting amino acid, broiler, performance, carcass traits

180 Effects of feeding reduced crude protein diets to broilers while maintaining amino acid concentrations on growth performance, ammonia production, and meat yield. Tanner Wise*², Jeremiah Davis², Victor Naranjo¹, Vincent Hess¹, and William Dozier, III², ¹Evonik Nutrition & Care GmbH, Hanau, Germany, ²Auburn University, Auburn, United States.

An experiment was conducted to determine the effects of feeding reduced crude protein (CP) diets to broilers while providing adequate essential amino acid (AA) concentrations on growth performance, ammonia concentrations, and carcass characteristics from 1 to 33 d of age. Fifteen hundred Ross × Ross 708 male chicks were distributed into 60 floor pens (25 birds/pen; 0.09 m²/bird). Broilers received 1 of 6 dietary treatments (10 replicate pens per treatment) varying in CP con-

tent. Diet 1 (control) was formulated with DL-Met, L-Lys, and L-Thr (23.2, 20.7, and 19.1% CP) in the starter (1 to 14 d of age), grower (15 to 25 d of age), and finisher (26 to 33 d of age) periods, respectively, while additional L-Val, Gly, L-Ile, L-Arg, and L-Trp were sequentially supplemented in the order of limitation respective of diets 2 through 6. Dietary CP was reduced in diets 1 to 6 by 3.4, 3.4, and 2.3% points in the starter, grower and finisher periods, respectively. Standardized ileal digestible (SID) Lys concentrations in the starter, grower, and finisher diets were formulated to 1.24, 1.08, and 1.00%, respectively, with minimum ratios of SID SAA, Thr, Val, Ile, Arg, Trp, and total Gly + Ser at 0.76, 0.68, 0.80, 0.69, 1.05, 0.17, and 1.70 (starter period), respectively. Broilers fed reduced-CP diets supplemented with DL-Met, L-Lys, L-Thr, L-Val, Gly, L-Ile, L-Arg, and L-Trp had similar ($P > 0.05$) body weight gain and feed intake compared with those provided the control diet from 1 to 25 and 1 to 33 d of age. Feeding reduced-CP diets supplemented with DL-Met to L-Arg did not affect ($P > 0.05$) feed conversion ratio compared with those receiving the control diet. Feed conversion ratio differences ($P \leq 0.05$) were observed when L-Trp was supplemented to the diets compared with broilers fed Diets 2 to 5 from 1 to 25 and 1 to 33 d of age. Equilibrium ammonia concentrations of the litter at the end of experimentation was 52% lower (48.85 vs. 93.68 mg/kg; $P \leq 0.05$) for broilers fed diets supplemented with DL-Met to L-Trp compared with the control-fed birds. Dietary treatments did not alter ($P > 0.05$) weights and yields of carcass, drums, thighs, wings, tenders, or fillets. Results from this study indicated that sequential addition of supplemental AA in the order of limitation from DL-Met to L-Trp allowed reduction of dietary CP content beyond 2.0% points without depressing growth rate and meat yield of broilers from 1 to 33 d of age while reducing ammonia emissions.

Key Words: amino acid, broiler, crude protein, glycine

181 The effect of amino acid density on performance and processing yield in Cobb 700 × Cobb MV mixed-sex broilers. Corey Johnson^{*1}, Rocky Latham², Jason Lee³, Christine Alvarado¹, Craig Coufal¹, and Tri Duong¹, ¹Texas A & M University, College Station,

Texas, United States, ²Tyson Foods, Springdale, Arkansas, United States, ³CJ America, Downers Grove, Illinois, United States.

The objective of this study was to investigate the phase effects of feeding increased digestible amino acid (dAA) density on the performance and processing yield of Cobb 700 × Cobb MV mixed-sexed broilers. Experimental animals were allocated to 7 treatment groups with 10 replicate pens of 34 birds arranged as a randomized complete block design and were fed diets with varying dAA densities using a 4-phase feeding program (starter: d 0–12, grower: d 12–26, finisher: d 26–36, and withdrawal: d 36–49). Broilers were fed one of 3 dAA levels (Low, Medium and High) during the starter phase and one of 4 dAA levels (Low, Medium, High and High+) during the grower, finisher, and withdrawal phases. Treatment combinations consisted of LLLL, MLLL, MMMM, MHHH HMMM, HHHH and HH⁺H⁺H⁺. Diets were formulated to maintain equivalent dEAA: dLys ratios across all treatments within a given phase. The dLys content of the low diets was 1.22, 1.10, 0.95, and 0.90% for the starter, grower, finisher, and withdrawal phases, respectively. Body weight (BW), feed intake (FI), and mortality-adjusted feed conversion ratio (FCR) were evaluated on d 12, 26, 36 and 49. On d 50, 3 males and 3 females from each pen were processed to determine boneless-skinless breast and fat pad yield. Data were analyzed via ANOVA, and means were separated using Duncan's Multiple Range test ($P < 0.05$). Increasing the dAA density reduced FCR and increased BW during the starter ($P < 0.01$) and grower ($P < 0.01$) phases. On a cumulative basis, FCR was reduced ($P < 0.05$) by 5.3 points in broilers that were fed the HH⁺H⁺H⁺ compared with those fed the LLLL treatment. Breast meat yield (% of live weight) was increased in broilers fed MMMM and HHHH (+4.6%) and in those fed HH⁺H⁺H⁺ (+8.2%) compared with broilers fed LLLL ($P < 0.01$). Fat pad yield followed a classical decrease as dAA increased, ranging from 1.70% in the LLLL to 1.27% in broilers fed HH⁺H⁺H⁺ ($P < 0.01$). The results of this study can be utilized to determine the viability and impact of feeding diets with increased dAA supplementation to the Cobb 700 x MV.

Key Words: heavy broiler, digestible amino acids, breast yield, Cobb 700, amino acid density

Student Competition: Metabolism and Nutrition: Nutrition III

182 Cricket meal (*Gryllus sigillatus*) for use in broiler chicken (*Gallus gallus domesticus*) diets. Holly Fisher^{*1}, Stephanie Collins¹, Bruce Rathgeber¹, Beth Mason², and Stefanie Colombo¹, ¹Dalhousie University, Debert, Nova Scotia, Canada, ²Cape Breton University, Sydney, Nova Scotia, Canada.

Access to a variety of protein sources for use in animal feeds is vital to ensure food security and efficient food production. Insect-based proteins, such as crickets, are an emerging sector, but there is a gap in knowledge regarding the use of crickets in broiler chicken diets. This study investigated dietary inclusion of cricket meal (CM) (Midgard Insect Farm, Inc.) at 0% (non-medicated control), 0% (medicated control, *Virginiamycin and Coban*), 5%, 10%, 15%, and 20% CM, on growth parameters, organ weights, and carcass quality of broiler chickens. The objective was to determine the effect of 5 dietary inclusion levels of CM on growth, feed intake, health, and meat quality of broiler chickens. This experiment was run as a completely randomized design. Six hundred and 24 Ross 508 broilers were randomly housed in 24 floor pens (26 birds/pen) in an environmentally controlled room. Bird weights and feed intake were recorded weekly and growth parameters were calculated. On d 13, 20, and 35, 3 birds from each pen were euthanized and individual organs were weighed. On d 35, meat quality was analyzed and liver color (L* a* b*; lightness, red/green, yellow/blue) was measured using a Hunterlab MiniScan EZ 4500L spectrophotometer. To assess meat quality, the right breasts from 3 birds per pen were weighed and cooked in one batch to determine % cook loss. The texture of both raw and cooked meat was measured using a TA.XTPlus texture analyzer. Data was analyzed as an ANOVA using IBM SPSS 25 and statistical significance was determined using the REGWF-test ($\alpha = 0.05$). Data was tested for normality and non-parametric statistics were used when required. The final average weight of broilers fed 5% CM (1933.4 g) was lower than that of broilers fed the non-medicated control (2095.6g) and 10% CM (2063.5g) ($P < 0.05$). The total weight gain of chickens fed 5% CM was lower than that of chickens fed CM at the higher inclusion levels and the controls ($P < 0.05$). Bursa size was highest in birds fed 15% CM (0.23% of body weight) and lowest in birds fed the medicated control (0.16% of body weight; $P < 0.05$), with no significant differences from other treatments. Breast weight and meat texture were not influenced by feed treatments. Breast meat from broilers fed 15% CM had higher % cook loss ($P < 0.05$). The livers from broilers fed 5% CM had lower a* color values than birds fed the medicated control diet ($P < 0.05$). The results suggest it is best to include cricket meal at a dietary inclusion level above 5% when incorporating this ingredient in a non-medicated broiler chicken diet.

Key Words: cricket, broilers, nutrition, meat quality, growth performance

183 Nutrient intake in a free-choice feeding trial using insect meal as a novel ingredient. Raquel Pereira*, Marcos Nascimento Filho, Ana Beatriz Oliveira, and José Fernando Machado Menten, University of Sao Paulo, ESALQ, Piracicaba, Brazil.

Chickens have the natural behavior of feeding themselves from a variety of insects during their entire lifecycle. A free-choice feeding trial using insect meal as a feed ingredient for chickens was conducted to study the preference and to determine the nutrient intake compared with a complete diet. A total of 60 14-d-old male broiler chickens of commercial strain were divided into 2 dietary treatments: a control group,

and *Tenebrio molitor* (larvae) meal (TM) group. Control group was fed a standard corn-extruded semi-whole soybean diet and the TM group had the choice to feed among TM, ground corn, extruded semi-whole soybean, or a supplement mixture (vitamin-mineral premix) offered separately in 4 identical feeders. Each group consisted of 6 replicate floor pens (5 birds/pen) assigned in a completely randomized design. Feed and water were available *ad libitum*. The control group was used as a reference for feed and nutrient consumption by the chickens. All feeders were weighed and refilled daily to assess each ingredient intake and feed conversion ratio. The intake of metabolizable energy digestible protein, total lipids, crude fiber, and minerals was calculated in kcal or g per day and adjusted to the body weight (kcal or g.kg BW⁻¹.d⁻¹) for 3 weeks. After Shapiro-Wilk analysis, Student's *t*-test was performed at $P < 0.05$. The TM was the most consumed ingredient accounting up to 70% of the daily feed intake. The TM-fed group had improved feed conversion ratio on wk 2 (1.22 vs 1.58) and on wk 3 (1.36 vs 1.63) compared with the control group. The nutritional specifications of TM are 93.0% dry matter, 5,004 kcal AMEn.kg⁻¹, 49.3% CP, 85.9% protein digestibility, 29.3% ether extract, 4.5% crude fiber and 3.8% ash. Energy intake per kg BW was higher for the TM group by 22% in wk 2 and 15% in wk 3 ($P < 0.01$). Digestible protein intake per kg BW was also higher by 33% and 17% ($P < 0.01$) on wk 2 and 3, respectively, compared with control. The greatest differences were found for lipid intake per kg BW, which was 139% higher, and mineral intake per kg BW, which was 58% lower for the TM group for the entire period ($P < 0.01$) compared with control. Similarly, crude fiber intake was 19% higher for the TM group on wk 2 ($P < 0.05$). In conclusion, the free-choice trial indicated a clear preference of the chickens for TM. Also, the daily intake of metabolizable energy, protein, and lipids was higher for the TM group compared with the standard diet resulting in improved feed conversion. We thank the research grants from FAPESP to Dr. José F. M. Menten (No 2017/05423-8) and Vida Proteína for the *Tenebrio molitor* meal supply

Key Words: protein, energy, lipids, feed conversion, broiler

184 True metabolizable energy and amino acid digestibility for seven insect meals. Nahal Matin*, Pamela Utterback, and Carl Parsons, University of Illinois at Urbana-Champaign, Champaign, Illinois, United States.

Seven precision-fed rooster assays were conducted to determine nutrient composition, nitrogen-corrected true metabolizable energy (TME_n) and standardized amino acid digestibility for 3 black soldier fly larvae meals (BSFL), one partially defatted BSFL, one cricket meal and 2 mealworm meals (regular and honest). The TME_n values were determined in 3 rooster assays using conventional roosters and the standardized amino acid digestibility values were determined in 3 rooster assays using cecectomized roosters. In the rooster assays, the birds were fasted for 24 h and then crop incubated with 25 to 30 g of the BSFL or mealworms. The cricket meal was fed at 15 g and mixed with 15 g of corn and TME_n and amino acid digestibility values were calculated by difference. Data were analyzed within rooster assay using a one-way ANOVA for a completely randomized design. Nutrient analysis of the meals indicated that the crude protein varied from 44 to 54% among the 4 BSFL, was 64% for the cricket meal and varied from 49 to 53% for the 2 mealworms. Crude fat was 18 to 19% for the BSFL, 12% for the partially defatted BSFL, 19% for the cricket meal and 29% for the 2 mealworms. Total phosphorus content of the 7 meals ranged from 0.7 to 1% and calcium

varied from 0.04 to 3.5%. Neutral detergent fiber varied greatly from 9 to 35% among the meals with the 2 mealworms being much lower in fiber than the other insect meals. The TME_n values for the 3 BSFL were generally consistent and averaged 4079 kcal/kg DM. As expected, the partially defatted BSFL contained a lower level of TME_n. Due to their low fiber content and high fat content, the TME_n values for the 2 mealworms were high and in excess of 5000 kcal/kg DM. Amino acid concentrations of the various insect meals ranged from 0.67 to 1% for methionine, 0.38 to 0.52% for cysteine, 2.7 to 4% for lysine, and 1.6 to 2.1% for threonine. Standardized amino acid digestibility values were generally high (85–95%) for the 4 BSFL and 2 mealworms. Amino acid digestibility values were slightly lower for the cricket meal. Digestibility of cysteine was generally lower and more variable than other amino acids in the 7 meals. The results of this study indicated that the nutrient composition can vary substantially among different insect meals but all insect meals contained high levels of TME_n and digestible amino acids compared with feed ingredients commonly used in poultry diets.

Key Words: insect meals, metabolizable energy, protein, amino acid digestibility, black soldier fly

185 The effects of feeding high-oleic peanuts on the growth performance of broilers. Elliot Sanders*¹, Matthew Livingston¹, Brittany Wall¹, Thien Vu¹, Ramon Malheiros¹, Kimberly Livingston¹, Luiz Carvalho¹, Peter Ferket¹, and Ondulla Toomer^{1,2}, ¹North Carolina State University, Raleigh, North Carolina, United States, ²USDA-ARS, Raleigh, North Carolina, United States.

Locally grown feed ingredients of high energy and protein content, such as peanuts, maybe economically feasible alternatives to corn and soybean meal in broiler diets. Even though normal-oleic peanuts have been demonstrated to be an economically viable feed ingredient for poultry, their dietary inclusion increases the risk of fat oxidation and aflatoxin contamination which may adversely affect growth performance and enteric health. In contrast, new high-oleic peanut cultivars (80% oleic fatty acids and 2% linoleic fatty acids) have greater oxidative stability during storage and manufacturing, and they may be more resistant to aflatoxin contamination. We hypothesized course ground high-oleic peanuts as a feasible feed ingredient for broilers when formulated into amino acid-balanced corn-soy based diets. The objective of this study was to determine the effect of dietary full-fat high-oleic peanuts on the growth performance and jejunum villi morphology of broilers. Age-appropriate feed phases were manufactured into 3 experimental diets of similar caloric and digestible amino acid content to meet breeder nutrient recommendations: 1) dietary inclusion of ~10% coarse-ground full-fat high-oleic acid peanuts (HOP); 2) a corn-soybean meal positive control diet with 5.5% added poultry fat (PC); and 3) a negative control diet containing 6% defatted peanut meal and 5.5% peanut oil (NC). Three-hundred Ross 708 broilers were randomly placed in 10 replicate pens of 10 chicks/treatment and raised until 42 d. Body weights (BW) and feed intake (FI) were determined weekly, and feed conversion ratio (FCR) was calculated after adjustment for mortality rate (averaging 1.3% over 42 d). Intestinal samples were collected from 5 birds per pen at 42 d for jejunum villi histomorphometric analysis. ANOVA was performed on all variables using a general linear mixed model in SAS (9.4). Broilers in the HO PN group had significantly lower body weights than the PC and NC groups, respectively, at 14d (419 g versus 469g and 471 g/bird, $P < 0.05$) and weekly through to 42 d (2,430g versus 2,546 and 2,588, $P < 0.06$). Similarly, the broilers in the HO PN group had significantly higher 0–42d feed conversion ratio (FCR) than broilers in the PC and NC groups, respectively, (1.68 versus 1.56 and 1.55, $P < 0.05$). There were no significant differences in jejunal villi surface area between the

treatment groups, indicating no significant dietary effects on enteric mucosa inflammation. Evidently, dietary inclusion of coarse ground high-oleic peanuts may have lower than expected nutrient availability but without allergenic effects. Further studies are justified to define the nutritional value relative to corn and soybean meal.

Key Words: high-oleic peanuts, peanut oil, broilers, growth performance, villi morphology

186 Effect of almond hull as an alternative ingredient on broiler performance and nutrient digestibility. Jinquan Wang*, Woo Kim, and Fanbin Kong, University of Georgia, Athens, Georgia, United States.

The objective of this study was to evaluate 2 varieties of almond hulls (Non-pareil: NP; Carmel: CA), by-products derived from the almond industry, as alternative feed ingredients for broilers. In experiment 1, NP and CA nutritional values were determined by proximate analysis and rooster model. The dry matter (DM), nitrogen (N), fat, crude fiber, and ash for NP and CA was 85.50, 0.77, 1.62, 13.11, 8.54, 57.43% and 88.11, 0.80, 1.82, 26.35, 5.98, 48.90% respectively. The N corrected true metabolizable energy (TME_n) values from NP and CA were 1,624 and 1,514 kcal/kg respectively from 20 roosters. The essential amino acids in NP and CA were determined non-digestible by 20 cecaminized roosters. In experiment 2, a total of 560 one-day old Cobb 500 male chicks were randomly placed to 7 experimental treatments with 8 replicates as 10 birds each for 19 d with a completely randomized design. Treatments consisted of a control diet based on corn and soybean meal; T2 to T4 were formulated to contain 3, 6 and 9% of NP, respectively; and T5 to T7 were formulated to contain 3, 6 and 9% of CA, respectively. Data of T2 to T4 (NP) with control and T5 to T7 (CA) with control were subjected to SAS using one-way ANOVA following GLM procedure separately. The significance level was set at $P < 0.05$. Means were separated using Duncan's Multiple Range Test. For T2 to T4 (NP) with control, inclusion of NP at 3% decreased ($P < 0.05$) feed intake compared with control during d 0–7 of age. No difference was found on body weight gain and feed conversion ratio within treatments. Inclusion of NP at 3% decreased ($P < 0.05$) DM digestibility and N corrected apparent metabolizable energy (AMEn) compared with control. 9% NP inclusion further decreased ($P < 0.05$) DM digestibility compared with 3% NP inclusion group. However, inclusion of NP at 6 and 9% group increased ($P < 0.05$) AMEn compared with inclusion of 3% NP and reached to a similar level to control. Only 9% NP inclusion group decreased ($P < 0.05$) ileal N digestibility compared with control, however, the N retention was not influenced by treatments. For T5 to T7 (CA) with control, inclusion at 9% increased feed intake and feed conversion ratio compared with control group during d 8–19 and d 0–19 of age. No difference was found on body weight gain within treatments. Inclusion at 3 and 9% of CA decreased ($P < 0.05$) DM digestibility compared with control group, however, 6% inclusion of CA showed a similar DM digestibility to control. No difference was found on AMEn, ileal N digestibility and N retention within treatments. In conclusion, despite the moderate negative effect on nutrient digestibility from NP and CA, both almond hulls can be used in broiler diet up to 9% without compromising body weight gain.

Key Words: almond hull, alternative ingredient, broiler, growth performance, nutrient digestibility

187 Nutrient profile and digestibility of Brassica carinata meal in chicken and in vitro anticoccidial activity of glucosinolate-sinigrin of Brassica carinata meal. Sudhir Yadav*, Po-Yun Teng,

Lorraine Fuller, and Woo Kim, *University of Georgia, Athens, GA, Georgia.*

Coccidiosis is one of the main risks to production and economic losses in the poultry industry. Furthermore, anticoccidial drugs resistance could add up to cause more devastating condition, which mandates exploration of novel and effective therapies. *Brassica carinata* also known as Ethiopian mustard, is oilseed crop grown for jet bio-fuel and residue after extraction of oil is of interest to animal nutritionists. This study was conducted to determine the nutrient profile, total metabolizable energy (TME), and amino acid (AA) digestibility of 2 groups: *B. carinata* meal containing low glucosinolate (CMLG) and *B. carinata* meal containing high glucosinolate (CMHG) for chickens. The CMLG was analyzed to contain 28µmol/g glucosinolate, 44.3% CP, 9% CF, 1.25% EE, and 8.37% total ash and CMHG contains 100µmol/g glucosinolate, 43.9% CP, 8.9% CF, 1.47% EE, and 7.74% total ash on DM basis. The precision-fed rooster assays with conventional (randomly allocated 10 birds/group) and cecectomized roosters (randomly allocated 10 birds/group) were conducted to determine TME and AA digestibility respectively. After 24h of feed withdrawal, each rooster was then precision-fed 35 g of *B. carinata* meal, and excreta samples were collected for a period of 48h. The TME of CMLG and CMHG was determined to be 2,046 Kcal/kg and 1,878 Kcal/kg DM, respectively. Standardized digestibility for Lys, Met, Cys, Thr, and Val were 72.4%, 87.8%, 69.3%, 74.9%, and 78.6% for CMLG and 79.5%, 89.3%, 70.7%, 75.5%, and 80% for HGCM, respectively. This study also evaluates the anticoccidial activity of sinigrin, a glucosinolate found in plants of Brassicaceae family. Sinigrin at doses of 0.01, 0.04, 0.07, and 0.1 mg/mL along with positive control (monensin at 0.5 µg/mL) and negative control (RPMI media) were challenged with *Eimeria tenella* sporozoites in vitro in Madin-Darby bovine kidney (MDBK) cells. Data from in vitro were analyzed using MIXED procedure of SAS. One-way ANOVA was run, and Tukey's was used to compare means between different doses effect on the sporozoites count. Results showed that higher doses 0.07 and 0.1 mg/mL significantly reduced the sporozoites count compared with negative control. In conclusion, *B. carinata* meal nutrient profile and digestibility are comparable to soybean meal, canola meal and rapeseed meal. Sinigrin present in *B. carinata* exhibited a marked reduction effect in vitro on *E. tenella*. Thus, *B. carinata* meal can be a potential feedstuff for chicken to replace conventional protein sources in the diet along with benefit against coccidiosis.

Key Words: *Brassica carinata*, chicken, digestibility, glucosinolate, in vitro

188 Zinc source and microalgae supplementation in broiler breeder diet affects the growth plate morphology of offspring leg bones at hatch. Marquisha Paul*, Szamare Crowder, Lizza Macalintal, Anthony Pescatore, Tuoying Ao, Mike Ford, and Karl Dawson, *Alltech-University of Kentucky Nutrition Research Alliance, Lexington, Kentucky, United States.*

Previous research has demonstrated that broiler breeder nutrition directly affects offspring skeletal development. Long bone elongation is driven by chondrocyte proliferation and differentiation in the growth plates, which are located at the bone epiphyses. This study was conducted to evaluate the effect of supplementing microalgae (FORPLUS, Alltech, Inc.; source of docosahexaenoic acid) and 2 sources of Zn, ZnO or Zn-proteinate (Bioplex® Zn, Alltech, Inc.), in broiler breeder diets on offspring growth plate morphology of femurs and tibias at hatch. An RCB design with a 2x2 factorial trt structure was used for this study. A total of 240 Cobb500 broiler breeder hens and 20 Cobb500 broiler breeder roosters were randomly assigned to a corn-soybean meal diet

containing either 0 or 2% microalgae and an additional 40 mg Zn/kg diet as ZnO or Zn-proteinate for the entire rearing period. Breeders were raised in floor pens with *ad libitum* access to water and nest boxes, 15L: 9D lighting, and controlled access to feed. Each dietary trt consisted of 5 rep floor pens (12 hens + 1 rooster/pen). Fertile eggs were collected and set in 10-week intervals at 3 time points: 19–20 wk of lay, 30–31 wk of lay, and 41–43 wk of lay. At hatch, the femurs and tibias of breeder offspring were collected and processed for morphological evaluation of the proliferative zone (PZ), hypertrophic zone (HZ), and proliferative + hypertrophic zones (PZ+HZ) of the proximal growth plates. Data were subjected to 2-way ANOVA (GLM procedure of SAS v9.4) with means separated using Fisher's LSD test. A main effect of breeder dietary Zn source when breeders were 19–20 wk of lay was observed for offspring femur growth plate morphology. Offspring from breeders fed Bioplex® Zn had greater ($P < 0.05$) femur PZ, HZ, and PZ+HZ heights than offspring from breeders fed ZnO. At 30–31 wk of lay, an interaction effect of breeder dietary microalgae supplementation level and Zn source was observed for offspring tibia growth plate morphology. Offspring from breeders fed 0% FORPLUS + Bioplex® Zn had the greatest ($P < 0.05$) tibia HZ and PZ+HZ heights compared with offspring from breeders fed other diets. Main effects of breeder dietary microalgae supplementation level and Zn source when breeders were 41–43 wk of lay were observed for offspring tibia growth plate morphology. Offspring from breeders fed 2% FORPLUS or Bioplex® Zn had greater ($P < 0.05$) tibia HZ and PZ+HZ heights compared with offspring from breeders fed ZnO and 0% FORPLUS, respectively. In conclusion, dietary supplementation of Bioplex® Zn or FORPLUS in broiler breeder diets increased the growth plate zone heights in the leg bones of offspring at hatch.

Key Words: broiler breeder, zinc, microalgae, offspring bone, growth plate morphology

189 Effects of encapsulated cinnamaldehyde and citral on growth performance, gut health, and cecal microbiota in broiler chickens. Chongwu Yang*^{1,2}, Yan Martel Kennes³, Dion Lepp², Xianhua Yin², Qi Wang², Hai Yu², Chengbo Yang¹, Joshua Gong², and Moussa Diarra², ¹University of Manitoba, Winnipeg, Manitoba, Canada, ²Guelph Research and Development Centre, Agriculture Agri-Food Canada, Winnipeg, Manitoba, Canada, ³Centre de Recherche en Sciences Animales de Deschambault, Deschambault, Quebec, Canada.

This study investigated the effects of encapsulated cinnamaldehyde (CIN) and citral (CIT) alone or in combination (CIN + CIT) on the performance, gut health, and cecal microbiota of broilers vaccinated or not against coccidiosis. Two groups of 3,200 d-old male Cobb 500 broilers (vaccinated = 1,600; non-vaccinated = 1,600) were randomly allocated to 5 treatments: Non-medicated feed (control basal diet) and basal diet supplemented with bacitracin (BAC, 55 ppm), CIN (100 ppm), CIT (100 ppm) and a combination of CIN (100 ppm) + CIT (100 ppm). Statistical analyses on growth performance and severity of intestinal lesions were conducted according to a complete randomized design using the GLM procedure (SAS, 9.4. SAS) with treatment groups as sources of variation (4 pens/treatment) and the individual pen as experimental units. Differences in relative abundances between treatment groups were analyzed by Dunn's test. In general, average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) were significantly improved in both BAC- and CIN-treated birds ($P < 0.05$). The incidence of minor coccidiosis (sub-clinical) was significantly higher ($P < 0.05$) in vaccinated birds than in non-vaccinated birds in accordance with high oocyte counts in the vaccinated birds. Diet supplementation with BAC or tested encapsulated essential oils showed similar effects

on the coccidiosis incidences. CIN appeared to be more effective than CIT in controlling necrotic enteritis (NE), although an additive effect was observed in CIN+CIT treated birds. Cecal microbiota analysis by the 16S rRNA sequencing showed a clustering by vaccination group. No treatment effects were observed on the abundance of total 4 detected phyla including *Actinobacteria*, *Firmicutes*, *Proteobacteria*, *Tenericutes*. At the genus level, abundances of 3 of 40 detected genera including *Leuconostoc* and 2 unassigned genera were found to be affected by treatments ($P \leq 0.05$). Other beneficial genera including *Lactobacillus*, present at a relative abundance $\geq 1\%$ appeared to be prevalent but not statistically significant in essential oils fed birds. The results suggested that CIN alone or in combination with CIT in feed could influence cecal microbiota composition and improve the intestinal health and performance of broiler chickens.

Key Words: broiler, encapsulated essential oils, growth performance, gut health, cecal microbiota

190 Evaluation of the effects of putrescine, as a component in “in ovo” nutrition of broilers. Emanuele Goes*, Gabriela Cardoso Dal Pont, Priscila Raijche, Rosiane de Araújo, Chayane da Rocha, and Alex Maiorka, *Universidade Federal do Paraná, Curitiba, Paraná, Brazil.*

In ovo feeding technique consists on inoculating solutions containing nutrients and/or compounds within the egg with specific functions, resulting in a greater energy reserve for the embryo and accelerating enteric development before hatching. Thus, this study aimed to develop nutrient solutions with putrescine and, after inoculation *in ovo*, evaluate its effects on hatching rates, intestinal morphophysiology and growth performance of broilers at pre-initial phase. The eggs used came from broiler breeders with 32 weeks of age and average weight of 61.5 ± 1.5 g. After selection, eggs were distributed in a completely randomized design in 5 treatments divided into incubation trays. Each tray was considered a repetition, totaling 8 replicates with 5 treatments of 18 eggs each, in a total of 144 eggs per treatment equally represented in each tray within hatchery machine. Experimental groups consisted of 4 nutrient solutions containing different concentrations of putrescine (0.05, 0.1, 0.15 and 0.2%) diluted in 0.9% physiological solution and a control group (without inoculation). Inoculation of the sterile solutions occurred at 17 d of incubation and later eggs were transferred to the hatcher. After hatch, 330 birds were housed according to their treatments, totalizing 5 treatments of 6 replicates with an average of 11 birds each. Two d after hatching, ileum of 4 animals were collected per treatment for preparation and subsequent reading of histomorphological slides. Broiler's performance was evaluated only in the pre-initial phase, and all birds were fed *ad libitum* with the same corn and soybean meal based diet. The polynomial regression model was used to evaluate putrescine levels. Hatchability (%) fell while the putrescine levels were increased in the treatments ($P < 0.01$). For intestinal histomorphometry analyzes, there was a linear increase ($P < 0.01$) in response to putrescine levels for villi height (μm) and number of goblet cells. On the other hand, crypt depth (μm) was lower on treatments where higher doses of putrescine were inoculated. About performance, there was no significant difference ($P > 0.05$) for feed intake and average weight gain, while feed conversion ratio was better for the control group ($P = 0.01$). The increase of mucus production to protect the intestinal epithelium of animals that received putrescine *in ovo* resulted in worse feed conversion ratio at pre-initial phase. Putrescine *in ovo*, showed effects on cell proliferation and behaved as a trophic agent in the development of intestinal villi, but the doses used caused some toxicity to broilers.

Key Words: 1,4-amino-butane, biogenic amines, embryo nutrition, intestinal development, intestinal health

191 Influence of coccidiosis vaccination and starter diet lipid concentration on nutrient and energy digestibility, and broiler growth performance and processing characteristics. Alyson Gautier* and Samuel Rochell, *University of Arkansas, Fayetteville, Arkansas, United States.*

Subclinical infections elicited by coccidiosis vaccination impair lipid digestibility and energy utilization in broilers. An experiment was conducted to determine if live performance, processing characteristics, and apparent ileal digestibility (AID) of nutrients and energy (IDE) in coccidiosis vaccinated broilers can be improved by feeding diets that account for potential vaccine-induced reductions in lipid digestibility. Six experimental treatments based on a factorial arrangement of 2 coccidiosis control methods [control group with in-feed diclazuril (CTL) or vaccinated (VAC)] and 3 starter diets with varying supplemental soybean oil (SBO) concentrations were administered to male Cobb 500 broilers in floor pens with 12 birds per pen ($0.09 \text{ m}^2/\text{bird}$). A 1x dose of a live coccidiosis vaccine was given to VAC birds via oral gavage on day of hatch. Experimental starter diets were fed from 0 to 18 d and contained 2.10% (standard), 2.67% (moderate), and 3.24% (high) SBO and calculated AME_n contents of 3,008, 3,058, and 3,108 kcal/kg, respectively. All birds were fed common grower (18 to 31 d) and finisher (31 to 43 d) diets. Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were determined at 18, 31, and 43 d. On d 11, blood and ileal digesta were collected from all birds in 7 replicate pens to measure plasma carotenoids and determine AID of nitrogen, ether extract (EE), and IDE. For performance, 12 replicate pens were used to 43 d, and all birds from 8 replicate pens were processed at d 44. At d 11, VAC increased ($P < 0.05$) excreta oocyst counts and decreased ($P < 0.05$) plasma carotenoids and AID of nitrogen and EE in all diets when compared with CTL birds. Interactive effects ($P < 0.05$) were observed for IDE, whereby the vaccine-induced reductions in IDE were greatest for the standard and moderate SBO starter diets. From 0 to 18 and 0 to 31 d, VAC decreased ($P < 0.05$) BWG, although FI was only reduced ($P < 0.05$) from 0 to 18 d. Interactive effects ($P < 0.05$) were observed for FCR from 0 to 18 and 0 to 31 d, whereby VAC increased ($P < 0.05$) FCR of birds fed moderate and high SBO levels but not those fed the standard SBO. From 0 to 43 d, no treatment effects ($P > 0.05$) on BWG and FI were observed, whereas VAC increased FCR of birds fed the moderate SBO starter but not those fed the other SBO diets (VAC x Diet, $P < 0.05$). Carcass yields were lower ($P < 0.05$) for VAC birds than for CTL birds, but there were no treatment effects ($P > 0.05$) on breast or abdominal fat pad yield. In conclusion, these data demonstrated that starter diet lipid concentration and coccidiosis vaccination had interactive effects on broiler FCR that extended beyond the starter period.

Key Words: coccidiosis, digestibility, vaccination, lipid, broiler

192 The role of dietary calcium in necrotic enteritis development and pathogenesis. Christopher Eagleson*¹, Ariel Bergeron¹, Chance Williams², Craig Wyatt⁴, Dan Klamfoth³, and Audrey McElroy¹, ¹Texas A&M University, College Station, Texas, United States, ²Wayne Farms, Oakwood, Georgia, United States, ³Lhoist North America, Fort Worth, Texas, United States, ⁴AB Vista, Plantation, Florida, United States.

Previously, we have published the influence of dietary calcium (Ca) inclusion levels on naturally occurring necrotic enteritis (NE). This trial was conducted to further investigate dietary Ca involvement in NE

pathogenesis and evaluate the performance alterations associated with natural NE occurrence. A randomized, complete block design trial was conducted to determine the impact of 2 limestone sources of 2 particle sizes with 2 dietary inclusion rates on the pathogenesis of NE. A total of 3600 d-old Ross male broilers were assigned to 8 dietary treatments (trt) fed in 3 phases (0–14 d, 14–28 d, and 28–35d). Each trt was replicated by 9 pens and all birds received a coccidia vaccine at day of age. NE was allowed to occur naturally following mild intestinal response to day of age coccidia vaccination. All diets included 1000 FTU/kg of phytase and the Ca:P ratio remained at approximately 2:1. Dietary treatments were: 1.05% Ca 1 at 120 sieve (trt 1), 1.05% Ca 1 at 200 sieve (trt 2), 1.05% Ca 2 at 120 sieve (trt 3), 1.05% Ca 2 at 200 sieve (trt 4), 0.75% Ca 1 at 120 sieve (trt 5), 0.75% Ca 1 at 200 sieve (trt 6), 0.75% Ca 2 at 120 sieve (trt 7), and 0.75% Ca 2 at 200 sieve (trt 8). All data were subjected to ANOVA using the GLM procedure. Means were deemed significantly different at $P \leq 0.05$ and separated using Tukey's HSD. A 2x2x2 factorial analysis was used to determine the impact of dietary Ca inclusion rate, Ca particle size, and Ca source. Beginning at d16 numerous birds were clinically morbid, and mild mortality resulting from NE increased and persisted through d23. At d14, interaction of particle size and limestone source indicated birds fed the 120 sieve Ca 1 had reduced BW compared with other sizes and source combinations. At d28 and d35, interaction of limestone source and inclusion rate indicated the higher inclusion rates of Ca 1 reduced BW. During d14–28 and d0–28 there was an inclusion rate and source interaction with birds fed higher Ca 1 having increased FCR. During the finisher phase and d0–35 a 3 way interaction indicated birds fed trt 1 had increased FCR. During d0–35 birds fed trt 1 or 2 (1.774 and 1.679 respectively) had approximately 1 point decrease in FCR compared with trt 3, 5, and 8 (1.593, 1.601, 1.589 respectively). Trt 4, 6, and 7 had FCR similar to trt 2. NE caused increased mortality, predominantly during d14–21, with Ca rate and particle size interaction. Birds fed 1.05% total Ca at 200 sieve (2.9%) had increased mortality compared with birds fed 0.75% total Ca at 200 sieve (0.0%). Collectively, this data indicates that not only inclusion rate of dietary Ca, but also limestone source and solubility characteristics effect broiler performance and mortality attributable to NE.

Key Words: calcium, necrotic enteritis, performance

193 Impact of feeding dietary sources of docosahexaenoic and α -linolenic acids to ISA brown and Shaver white pullet breeders and/or progeny on tibia attributes of 18-wk pullets. Reza Akbari Moghaddam Kakhki¹, Kayla Price², Janna Moats³, Niel Karrow¹, Tina Widowski¹, and Elijah Kiarie¹, ¹University of Guelph, Guelph, Ontario, Canada, ²Alltech Canada, Guelph, Ontario, Canada, ³O & T Farms, Saskatoon, Saskatchewan, Canada.

Structural bone development ceases at sexual maturity suggesting modification of nutrition before puberty may be a better strategy for optimizing bone health in hens than post-puberty. We evaluated the effects of maternal and rearing dietary n-3 fatty acids (FA) and genetics on tibia attributes of progeny at 18 wks of age (woa). ISA brown (ISA) and Shaver white (SHAV) female and male breeders (26 woa; 3 group pens of 27♀ and 4♂ per strain) were fed 3 different diets: Control (CON); CON + 1% of dried micro-algae (*Aurantiochytrium limacinum*) fermentation product, as a source of docosahexaenoic acid (DHA); and CON + 2.48% of LinPRO, a dry extruded product consisting of full-fat flaxseed, as a source of α -linolenic acid (ALA). Test diets (DHA and ALA) had equal amounts of n-3 FA and ratio of n-6: n-3 FA. Hatched female offspring were divided into post-hatch treatments (5 replications with 9 ISA or 10 SHAV pullets) as follows: A) breeder CON: CON (CON-CON), DHA (CON-DHA) and ALA (CON-ALA),

B) breeder DHA: CON (DHA-CON) and DHA (DHA-DHA) and C) breeder ALA: CON (ALA-CON) and ALA (ALA-ALA). Pullets were kept in conventional cages and sacrificed for tibia sampling at 18 woa. The left tibia was surgically separated into 3 parts: epiphysis, medullary and cortical. Dry (DW), ash weight (AW) and ash percentage (AP) were measured in the 3 parts and normalized by body weight (BW). The right tibia breaking strength (TBS) was measured by the Instron machine as the amount of applied Newton (N/m²) pressure at 2 mm/sec. Data were analyzed as a complete randomized design with 2 (strains) × 7 (diets) factorial arrangement. Except for AW in cortical, there was no interaction between strain × diets or the main effect of diet ($P > 0.05$) on wk-18 BW and whole or other parts of tibia attributes. Strain effects ($P < 0.05$) was such that ISA pullets had heavier 18-wk BW (1,785 vs. 1,427 g), tibia (4.9 vs. 4.3 g/kg BW) and medullary (0.64 vs. 0.44 g/kg BW) than SHAV pullets. Moreover, although ISA pullets exhibited heavier ($P < 0.05$) tibia epiphysis and cortical than SHAV pullets, the later had higher AP in the whole tibia (39 vs. 35%) and medullary (1.21 vs. 0.69%). The interactive effect of strain × diet improved TBS of DHA-DHA and ALA-ALA ($P = 0.024$) by 47 and 36%, respectively relative to CON-CON in SHAV pullets. The cortical AW was improved in SHAV ($P = 0.007$) by 13.1 and 23.8% in DHA-CON and DHA-DHA, respectively compared with CON-CON. The strain effects were strong on tibia attributes on 18-wk old pullets. Maternal feeding of n-3 FA was more effective when concomitant with rearing feeding of n-3 FA compared with the CON in supporting structural bones (cortical) in SHAV pullets.

Key Words: maternal feeding, rearing feeding, pullets, bone quality, n-3 fatty acids

194 Effect of dietary supplementation with EconomasE™ and two sodium sources on production parameters, egg quality, and blood electrolyte responses of laying hens exposed to elevated temperatures. Opeyemi Olojede^{*1}, Mike Ford¹, Anthony Pescatore¹, Tuoying Ao², and Sunday Adedokun¹, ¹University of Kentucky, Lexington, Kentucky, United States, ²Alltech Inc, Nicholasville, Kentucky, United States.

Acute heat stress is often associated with reduced feed intake which can influence the intake of micronutrients. Furthermore, temperature above the thermoneutral zone can result in the loss of CO₂ through panting affecting acid-base homeostasis in the blood. In this study, we investigated the effect of an antioxidant EconomasE (0 or 0.2 g/kg) and 2 sodium sources (NaCl or NaCl+NaHCO₃) on laying hens exposed to elevated temperature in terms of egg quality, egg production, and blood metabolites. Hy-line Brown hens at 26 wk of age were randomly assigned to one of 4 dietary treatments with 9 replicates per diet with 12 hens per rep. The hens were housed 2 birds per cage (25 × 41 cm) and photo-stimulated with 16L: 8D. Feed and water were provided ad libitum. The birds were maintained at a thermoneutral (TN) temperature of 23.8°C until 31 wk of age. Then, all birds were maintained under cycling elevated temperature conditions (HS) at 32.2°C for 8 h per day (from 9:00 a.m. to 5:00 p.m.) for 5 wk and allowed to recover at TN for another 5 wk (REC). This was repeated following the same sequence. Throughout the experiment, egg production was recorded daily. Twice a week for 3 weeks within each temperature period, 6 eggs per replicate per treatment were collected, weighed and evaluated for eggshell breaking strength and albumen height. One bird from each 6 replicates (6 bird per treatment) was sampled, before heat treatment, at the end of HS, and 5 weeks after heat treatment. Serum glucose, mineral concentrations, and acid-base equilibrium related parameter (HCO₃⁻, Na⁺, K⁺, Cl⁻, Ca) levels were determined. Dietary supplementation with EconomasE or different sodium sources did not affect egg production or egg quality

parameters. Before HS, the glucose level was elevated ($P < 0.05$) in birds fed a diet with NaCl+NaHCO₃ compared with those fed other diets. During the HS period, dietary NaCl+NaHCO₃ significantly increased ($P < 0.01$) HCO₃⁻ and decreased ($P < 0.01$) Cl⁻. At the end of the REC period, K⁺ decreased ($P < 0.05$) and Cl remained lower ($P < 0.05$) with the inclusion of NaCl+NaHCO₃. An interaction was observed with Economase and sodium source, where hens fed the diet containing no Economase and only NaCl as the sodium source, had the lowest body weight during the REC period. These results indicate that during HS, NaCl+NaHCO₃ supplementation elevated serum HCO₃⁻ and reduced Cl levels circumventing the effects of respiratory alkalosis.

Key Words: layers, electrolyte balance, egg quality, heat stress, blood metabolites

195 Effects of phosphorus source and level of insoluble fiber in the diet on growth performance, gizzard development, and bone mineralization of broilers from 1 to 14 days of age.

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The effects of source of phosphorus and level of inclusion of oat hulls (OH) in the diet on growth performance, bone mineralization, and gizzard development were studied in Ross 308 straight-run chicks from 1 to 14 d of age. All birds were fed *ad libitum* a common corn-soybean meal diet in crumble form. The experiment was conducted as a completely randomized design with 6 diets arranged as a 2x3 factorial with source of phosphorus [monosodium (MSP), monocalcium (MCP), and calcined bone (CBP) phosphates] and level of inclusion of OH (0 and 3%) as main effects. Each treatment was replicated 10 times and the experimental unit was a cage with 12 birds. The estimated phosphorus digestibility

coefficients were 91.0, 88.5, and 86.9% for the MSP, MCP, and CBP, respectively. All the diets contained 0.25% digestible P (dP). The Na⁺ and Cl⁻ contents of the diets were 0.19 and 0.17%, 0.17 and 0.32%, and 0.17 and 0.32% for the MSP, MCP, and CBP diets, respectively. At 7 and 14 d of age, 2 birds per cage were euthanized by CO₂ asphyxiation and weighed individually. In addition, representative samples of the bones were obtained by severing the foot at the tibio-metatarsal joint. To study the development of the proximal part of the GIT the gizzard was excised and clamped to avoid digesta contamination and the full and empty weight and the pH of the contents were recorded. Data were analyzed as a completely randomized design with the source of phosphorus and level of OH in the diet as main effects. The source of phosphorus did not affect any of the growth performance or gizzard traits studied of the birds from 1 to 14 d of age. An interaction ($P < 0.05$) between phosphorus source and age was observed for FCR. Broilers fed the diets containing 3% OH consumed more feed ($P < 0.05$), and tended to have higher ADG ($P = 0.062$) but had similar FCR than broilers fed the control diets. Furthermore, energy efficiency, defined as Kcal of energy intake per gram of BWG, improved ($P < 0.001$) with OH inclusion. In absolute (g) and relative (% BW) terms the full and the empty gizzards were heavier ($P < 0.001$) in broilers fed 3% OH than in broilers fed the control diets, but no differences were observed for gizzard pH. Foot ash content was neither affected by phosphorus source, nor by OH inclusion level. In summary, based on the P digestibility used for the different phosphorus sources, phosphorus source, in diets formulated with equal dP did not affect neither broiler performance, nor bone mineralization, nor gizzard development of young broilers. The inclusion of 3% OH improved the size of the gizzard and improved BW gain and energy efficiency in young broilers.

Key Words: bone mineralization, broilers, fiber, gizzard, phosphorus

Student Competition: Immunology, Health,, and Disease II

207 Effects of riboflavin and *Bacillus subtilis* on growth performance of Ross 708 male broilers with or without coccidial challenge. Sabin Poudel* and Wei Zhai, *Mississippi State University, Mississippi State, Mississippi, United States.*

The study was aimed to improve broiler growth performance by reducing bile salt hydrolase enzyme (BSH) activity in the intestine. Both intestinal microflora and probiotics produce BSH, which hydrolyze conjugated bile salt. Reduced conjugated bile salt concentration reduces fat digestion and absorption. The objective was to determine the effects of riboflavin (as a BSH inhibitor) and probiotics (*Bacillus subtilis* PB6) on broiler performance, carcass yield, and woody breast condition (WB) under coccidial challenge. A total of 1,248 d-old Ross 708 male broilers were randomly divided into 12 treatments in 8 blocks in a 3 × 2 × 2 (riboflavin × *Bacillus subtilis* × coccidial challenge) factorial arrangement. The 3 levels of riboflavin used were 0.75, 6.6, and 20 ppm. On d 14, birds were gavaged with 20 × doses of commercial cocci vaccine. Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were determined on d 14, 28, 35, and 41. On d 41, 5 birds/ pen were processed and deboned. Data were analyzed using 3-way ANOVA, with significance level set at $P \leq 0.05$. None of the riboflavin, *Bacillus subtilis*, or coccidial challenge treatments affected overall mortality from d 0–41. However, the supplementation of *Bacillus subtilis* lowered mortality from d 35–41 ($P = 0.050$). *Bacillus subtilis* also lowered overall FI from d 0–41 ($P = 0.028$), but did not affect BWG or FCR. *Bacillus subtilis* lowered carcass, breast, and tender weights of birds only when they were supplemented with 0.75 ppm riboflavin ($P = 0.053$, 0.005, and 0.008). The birds were successfully challenged as coccidial lesions were increased in duodenum and jejunum ($P < 0.0001$) caused by *Eimeria acervulina* and *Eimeria maxima* on d 27. The coccidial challenge exhibited different effects on growth performance during different growth phases. The challenge reduced FI, BWG, and increased FCR between d 14–28 ($P < 0.0001$); did not alter the FI, but increased BWG ($P = 0.0011$) and lowered FCR ($P = 0.003$) between d 28–35; did not influence FI between d 35–41. From d 35–41, the challenge lowered FCR only in birds fed 0.75 ppm riboflavin ($P = 0.013$), but increased BWG only in birds fed 6.6 ppm riboflavin ($P = 0.009$). The coccidial challenge also increased abdominal fat pad weight and slight WB incidence ($P = 0.020$ and 0.040). In conclusion, birds' response to riboflavin, *Bacillus subtilis*, and challenge varied with age. Riboflavin inclusion level affected birds' response to *Bacillus subtilis* and coccidial challenge. More research should be conducted to determine the effects of these 3 factors on intestinal microbiota, gut health, and internal organ development to reveal how to use probiotics more effectively in antibiotic-free broiler production.

Key Words: riboflavin, *Bacillus subtilis*, coccidiosis, growth performance, broiler

208 How do dietary protein source and graded level of pea interact in coccidiosis-challenged, cage-housed broiler chickens? Rachel Savary*, Andrew Van Kessel, and Henry Classen, *University of Saskatchewan, Saskatoon, Saskatchewan, Canada.*

Dietary protein of animal origin is often considered a risk factor for the development of enteric disease in broiler chickens. Evidence suggests that carbohydrate fermentation reduces these negative effects by increasing the protein utilized for microbe growth and decreasing protein fermentation. Undigested starch (e.g., pea) is a potential fermentation

source to accomplish this goal. This experiment studied the impact of diets containing animal- (ABP) or plant-based protein (PBP) and graded levels of pea on the response of cage-housed broilers to 30 times (x) label dose of coccidial vaccine. A RCBD in a 2x3x2 factorial arrangement with 10 replicate cages of 5 birds was used. The main factors were protein source (ABP or PBP), level of pea (0, 15 or 30%) and coccidiosis challenge (0x or 30x). Un-medicated diets, formulated to meet broiler grower recommendations, were fed for the duration of the trial. Wheat and soybean meal were used in PBP diets while ABP diets replaced a portion of the soybean meal with fish meal (7%) and porcine meal (3%). At 21d birds were gavaged with 1 mL of Coccivac-B52 (Merck Animal Health, approx. 2.83×10^4 sporulated oocysts) or distilled water. Response criteria were evaluated at 28d. Analysis of variance was completed in SAS 9.4 with significance set at $P \leq 0.05$. Means are listed as 0x versus 30x or in the following order: protein x challenge, ABP-0x, ABP-30x, PBP-0x, PBP-30x; pea x challenge, 0%-0x, 0%-30x, 15%-0x, 15%-30x, 30%-0x, 30%-30x. Coccidial challenge induced disease as indicated by the presence of oocysts in challenged birds, reduced body weight gain (BWG; 635, 611 g bird⁻¹), feed intake (915, 892 g bird⁻¹) and excreta moisture (74.8, 72.5% moisture), and increased small intestine (SI) digesta content and SI, ceca and colon size. Results were inconsistent on whether ABP or PBP caused more severe disease. Challenge reduced excreta moisture of ABP-fed birds more than PBP-fed birds (73.2^c, 70.1^d, 76.4^a, 74.8^b) but *Eimeria maxima* shedding was less for ABP than for PBP (4.23 and 4.97 log oocyst per g). Level of pea did not reduce the negative effect of either protein source as shown by the lack of 3-way interactions among main effects. Including pea increased challenge severity. Increased pea reduced BWG and the reduction due to challenge was greater at 15 or 30% pea inclusion (656^a, 655^a, 653^a, 600^b, 596^b, 579^b g bird⁻¹). Similar effects were noted for excreta drying and SI weight. Total oocyst shedding increased with 15 and 30% pea (5.07^b, 5.47^a, 5.50^a log oocyst per g). In conclusion, dietary protein source did not consistently affect the response to coccidiosis challenge, and increasing dietary pea level increased the severity of the infection regardless of protein source.

Key Words: *Eimeria*, fish meal, porcine meal, soybean meal, wheat

209 Evaluation of four different doses of *Eimeria*-challenge on growth performance and gut health parameters of broilers. Po-Yun Teng*, Sudhir Yadav, Fernanda Castro, Alberta Fuller, and Woo Kim, *University of Georgia, Athens, Georgia, United States.*

Coccidiosis is an intestinal tract disease caused by *Eimeria* spp. infection. These parasites are able to penetrate and reproduce in the lining cells of gut which causes severe damage in the intestine. This study was conducted to evaluate different *Eimeria* challenge doses on growth performance and parameters of intestinal health in broilers. The non-challenge control and 4 different doses were used in the study. A mixed *Eimeria* spp. solution with 50,000 *E. maxima*, 50,000 *E. tenella*, and 250,000 *E. acervulina* per milliliter was prepared for the highest dose challenge treatment. The mixed solution was diluted by 2-fold serial dilution to make the medium-high (25,000 *E. maxima*; 25,000 *E. tenella*; 125,000 *E. acervulina*), the medium-low (12,500 *E. maxima*; 12,500 *E. tenella*; 62,500 *E. acervulina*), and the lowest dose (6,250 *E. maxima*; 6,250 *E. tenella*; 31,250 *E. acervulina*). A total of 360 13-d-old male Cobb 500 (males from the female line) broilers were randomly allocated into 5 treatments with 6 replicated cages. Growth performance was calculated from 0 to 6-d post infection (DPI), and intestinal lesion

was scored on 6 DPI. Gut permeability was measured on 3, 5, 6, 7, and 9 DPI to test dynamic change of gut leakage in *Eimeria*-challenged broilers. Data were analyzed as a PROC GLM Contrast in SAS version 9.4 with significance at $P \leq 0.05$. The results indicated significant linear reduction in body weight gain (370, 270, 243, 205, and 189), and feed intake (547, 481, 440, 438, and 427) with the increase of *Eimeria* dose, whereas significant linear increase was found for feed conversion rate (1.48, 1.79, 1.83, 2.15, and 2.32) ($P < 0.01$). Similarly, the percentage of higher lesion scores was increased by dose of *Eimeria* challenge in the ceca (Score 3 and 4, 0%, 13%, 27%, 33%, and 67%). There was no significant difference in the gut permeability on 3 DPI, but a significant linear trend was found on 6 DPI (14, 136, 207, 216, and 234 ng/ml) and 7 DPI (4, 47, 69, 48, and 230 ng/ml) ($P < 0.01$). On 9 DPI, the gut permeability of birds in each challenged treatment was recovered to normal levels. In conclusion, the higher *Eimeria* dose that birds were challenged, the more damage was caused in the gut of broilers. Additionally, gut permeability is a well-operated parameter to measure dynamic change of gut health in the *Eimeria*-challenge model.

Key Words: coccidiosis, *Eimeria*, broiler, gut permeability

210 Comparing of gut health by I See Inside methodology in broilers raised in controlled environment and common backyard structure. Bárbara Schreiner Spiassi^{*1}, Adrien Sanches¹, Bruna Belote¹, Igor Soares¹, Amanda Tirado¹, Ana Heemann¹, Aline Tujimoto Silva¹, Paulo Hümmelgen¹, and Elizabeth Santin¹, ¹Universidade Federal do Paraná, Curitiba, Paraná, Brazil, ²ISI Institute, Curitiba, Paraná, Brazil.

The Free Range systems are gaining more popularity mostly because consumer pressure and market opportunities. There are few studies comparing the effect of the Free Range systems in gut health. A trial was conducted to compare the gut health by ISI methodology, in broilers raised in controlled environment (NC) and raised in a common backyard structure with (WS) and without (60g/T) salinomycin (WTS), in diet. A total of 96 birds were used, divided in 3 groups (each bird is a replicate), all were male broilers, Cobb 500. Weakly, feed and birds were weight and 8 birds by treatment were euthanized and ileum samples were collected for ISI evaluation. In the ISI methodology, an impact factor (IF) that ranges from 1 to 3 is defined for each tissue alteration according to the reduction on organ functionality and a score (S) that ranges from 0 to 3 is given according to the intensity or frequency of each alteration. To ISI final value, the IF of each alteration is multiplied by the respective score and summed according to the formula $ISI = \sum(IF * S)$. The data were submitted to one-way ANOVA and Tukey's test ($P < 0.05$). At 14 and 28d the birds from WTS (14D 8.46; 28D 11.56) and WS (14D 4.32; 28D 10.4) groups displayed higher ($P < 0.05$) ileum ISI total scores when compared with NC (14D 3.47; 28D 8.83). It was observed lower ($P < 0.05$) body weight gain (BWG) for WS (438g) and WTS (448g) at 14 d compared with NC (604g). The feed intake was lower for NC at 7d, but higher in 21 and 28d (7D 163g; 21D 1808g; 28D 3005g) than WS (7D 202g; 21D 1479g; 28D 2598g) and WTS (7D 229g; 21D 1508g; 28D 2356g). A relation between ISI scores for ileum and BWG was observed during 14 and 28d - When the higher was the ileum score, the lower was the BWG. Also, the feed conversion ratio was better ($P < 0.05$) for NC in 7 and 14d (7D 0.85; 14D 1.05) than WS (7D 1.05; 14D 1.56) and WTS (7D 1.47; 14D 1.74). This trial can show that birds raised in Free Range system presented lower gut health and performance when compared with birds for the same lineage raised in controlled system, since they have much more contact with environment challenges. The Free Range system does not mean that the animal have better health and welfare. Despite the salinomycin used,

there is no improve in intestinal parameters for Free Range, suggesting that new strategies or dosages should be explored to control coccidiosis and improve gut health in this system.

Key Words: free range, salinomycin, coccidiostatic, gut health, ISI

211 A field evaluation on the effect of feeding spray-dried plasma in the starter period on the overall performance and gut health of broilers. Bruna Luiza Belote^{*1,2}, Bruno Carvalho³, Camila Martins⁵, Ricardo Gonzalez Esquerre⁴, Amanda Tirado², Aline Tujimoto Silva^{1,2}, Igor Soares², Luis Rangel⁴, and Elizabeth Santin¹, ¹ISI Institute, Curitiba, Brazil, ²Federal University of Paraná, Curitiba, Brazil, ³Federal University of Viçosa, Viçosa, Brazil, ⁴APC, Ankeny, United States, ⁵AAC&T Assessoria em Pesquisa Científica, São Paulo, Brazil.

A field evaluation was undertaken to test the effect of feeding spray-dried plasma (SDP) on the performance, gut health of commercial broilers, value of the I See Inside methodology (ISI®) in assessing gut health under field conditions. A total of 105 farms were used at a broiler integrator in Brazil, 51 fed a control starter diet and 54 fed a starter diet with 1% SDP formulated into the diet. Both diets were fed from 0 to 10 d of age after which both groups were fed common diets. Diets contained zinc bacitracin as growth promoter and 200 ppm of CuSO₄ since d 1. Data were analyzed altogether or by type of farm: Positive pressured (PP), negative pressured (NP) or environmentally controlled (EN); with 79.0, 16.2 and 4.8% of the total farms evaluated, respectively. Birds were sent to market as they reached the desired weight therefore age at slaughter (AS) was evaluated as a dependent variable along with other performance measures. At age of 12 to 16 d, 6 birds per farm were randomly selected, euthanized, and their ileum sampled for ISI analysis in 35 farms (16 and 19 from Control and SDP farms, respectively). The Shapiro-Wilk test revealed that the distribution of the data was not normal and therefore the Mann-Whitney U test was used using SPSS® 20.0 software. AS was 1 d lower ($P < 0.05$) for SDP birds irrespective of type of barn (54 d vs 53 d on PP and 53 d vs 52 d on NP for Control vs SDP, respectively). Feeding SDP also improved FCR Adj to 2.8 kg vs Control by 4.4 and 5.0% ($P < 0.05$) and FCR in PP farms by 5.6 ($P < 0.05$) while numerical FCR improvements were observed in NP barns (3.0%; $P > 0.5$). Mortality was reduced by feeding SDP by 0.51% and 0.8% points ($P < 0.05$) on PP and NP farms, respectively. Out of the 35 farms sampled for ileum histology evaluations, 16 and 19 were fed the SDP vs the Control diets, respectively. Significantly lower ($P < 0.05$) ileal histological alterations (ISI scores) were observed in chickens from SDP vs Control farms for lamina propria thickness (2.52 vs 2.61), inflammatory cell infiltration in epithelium (0.25 vs 0.29), inflammatory cell infiltration in lamina propria (1.40 vs. 1.58), congestion (0.02 vs 0.05) and Total ISI score (8.28 vs 8.57), which is an indicator of overall intestinal health and its inflammatory status. In conclusion, feeding 1% SDP in the starter period to broilers resulted in improved performance under field conditions irrespective of type of barn. Gut health was also improved by feeding SDP and the intestinal measurements taken with the use of the ISI® methodology correlated well with the performance improvements observed

Key Words: spray-dried plasma, field evaluation, broilers, gut health, ISI histology

212 Microscopic enteritis in broilers—A chronological evaluation by the I See Inside histologic analysis. Igor Soares^{*1}, Adrien Sanches¹, Bruna Belote¹, Paulo Hümmelgen¹, Ana Heemann¹, Amanda Tirado¹, Aline Tujimoto Silva¹, Anderson Cunha²,

and Elizabeth Santin³, ¹Universidade Federal do Paraná, Curitiba, Brazil, ²Universidade Federal de São Carlos, São Carlos, Brazil, ³ISI Institute, Curitiba, Brazil.

The inflammation of the intestinal mucosa has been related to disease in humans and animals. The concept of Microscopic Enteritis (ME) used in human pathology through the Marsh Classification system has no counter-part in veterinary medicine. In poultry science, the I See Inside (ISI) methodology, unlike the current linear measures of villi and crypts, displays possibilities to describe and understand the avian ME. Herein, 2 trials were conducted to describe the development of ME through the ISI methodology in broilers under enteric challenge or not. In each trial, a total of 64 birds were divided into 2 groups of 4 replicates with 8 birds each: non-challenged (NCH) and challenged (CH) by gavage with *Eimeria* vaccine in a 10X higher dose at d 1 and 10⁸ cfu/mL of *Clostridium perfringens* at 10, 11, and 12 d. Weakly, feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were evaluated and birds were euthanized for the sampling of ileum for ISI histologic evaluation, analysis of IL-8 and IL-10 mRNA expression and quantification of macrophages, CD4+ and CD8+ cells. In the ISI methodology, an impact factor (IF) that ranges from 1 to 3 is defined for each tissue alteration according to the reduction of the organ functionality and a score (S) that ranges from 0 to 3 is given according to the intensity or frequency of each alteration. To obtain an ISI final value, the IF of each alteration is multiplied by the respective score and the results are summed according to the formula $ISI = \Sigma(IF \cdot S)$. The data were submitted to one-way ANOVA and Tukey's test ($P < 0.05$). The CH group presented lower BWG at 1–14 d, 1–21 d and 1–28 d and worst FCR at 1–7 d and 1–21 d and displayed higher ISI final value at 7 d (NCH 5.24 - CH 8.66), 14 d (NCH 5.50 - CH 9.19) and 28 d (NCH 11.29 - CH 13.31) due to increased inflammation and thickness of lamina propria and epithelium, enterocytes proliferation and congestion. Comparing the histologic parameters, both groups presented the same chronological increase in intestinal inflammatory alterations since the 7 d, but the intensity was higher in the CH group. The CH group showed a higher expression of IL-8 mRNA at 7 d and IL-10 mRNA at 21 d compared with the NCH group. At all ages, higher counting of macrophages was observed in the CH group. This group displayed lower counting of CD4+ cells at 7 d and higher counting at 14 d and 21 d. The number of CD8+ cells was higher in the CH group at 21 d. The histopathologic data allowed the description of the ME as a basal enteritis in unchallenged broilers and the infection, associated to the expression of cytokines, increases the intensity of the inflammatory alterations which are linked to losses in zootechnical performance.

Key Words: microscopic enteritis, basal enteritis, ISI, regenerative inflammation, histopathology

213 Evaluation of a novel probiotic *Bacillus subtilis* in the control of necrotic enteritis in broilers. Amanda Tirado^{*1}, Bruna Belote¹, Patrino Thiele¹, Bárbara Schreiner Spiassi¹, Rafael Vieira¹, Wanderley Quinteiro-Filho², and Elizabeth Santin¹, ¹Universidade Federal do Paraná, Curitiba, Paraná, Brazil, ²Adisseo, São Paulo, Brazil.

The aim of this experiment was to evaluate the efficiency of a *Bacillus subtilis* strain (DSM 29784) on gut health in non-challenged birds and challenged with *Eimeria* and *Clostridium perfringens*. 160 male broilers (from 1 to 28 d of age) were distributed in a completely randomized design, 2X2 in factorial arrangement model, divided into 4 treatments with 4 repetitions each, starting with 10 birds in each repetition. The treatment groups were: NC (negative control) – non-challenged with no additive in the diet; NCBS – non-challenged with *B. subtilis* (BS)

in the diet; CH - challenged with no additive in the diet; CHBS - challenged with BS in the diet. At d 1 of the trial, the groups CH and CHBS received *Eimeria* vaccine 15 times the manufactured recommendation dose. At d 10, 11 and 12, they were inoculated with 10⁸ cfu/bird of *C. perfringens* (CP) by oral gavage. The non-challenged groups received the same quantity of sterilized water by oral gavage as well. The bird's performance was weekly evaluated for feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) and it was sampled ileum and cecum for histopathological analysis using the I See Inside (ISI) method. Data were analyzed using ANOVA and means were compared by Tukey's test at 5% probability. All the challenged groups, at 7, 14 and 21 d, had significantly higher total ISI score than the non-challenged ones, meaning they had more microscopical intestinal lesions. When BS was added to the diet, the birds showed less lesions at 7 and 14 d. At 7 d, CH had significantly higher total ISI score (7.62) than CHBS (6.51), NC (6.35) and NCBS (5.22) due to higher lamina propria thickness found in the histology. This could explain why the non-challenged groups had better BWG than all the other groups. At 14 d, due to *Eimeria* cycle is expected higher presence of oocysts, proliferation of enterocytes, lamina propria thickness, epithelial thickness, inflammatory cell of the lamina propria and goblet cells proliferation in challenge groups what is related to better BWG at 14 d in the non-challenged groups (601.64g) than in the challenged ones (472.51g). At this critical age, the CH had higher ISI total score (11.36) compared with CHBS (5.74) indicating a positive effect of BS in this groups. At 28 d, the non-challenged birds had better BWG and FCR. All other groups did not show any significant difference. The results demonstrated that using BS as a feed additive for broilers improve the gut health in birds challenged with *Eimeria* and *C. perfringens*.

Key Words: coccidiosis, clostridiosis, histopathology, probiotic, gut health

214 Increased ileal deoxycholic acid levels were associated with reduced necrotic enteritis in broiler chickens. Mohit Bansal*, Rohana Liyanage, Mussie Abraha, Ayidh Almansour, Hong Wang, Anamika Gupta, Billy Hargis, and Xiaolun Sun, University of Arkansas, Fayetteville, Arkansas, United States.

Chicken necrotic enteritis (NE), caused by *Clostridium perfringens* and coccidiosis, is responsible for billions of dollars in loss annually. The withdrawal of antibiotic growth promoters is the main factor for the reemergence of NE in chickens. Dietary supplementation with secondary bile acid deoxycholic acid (DCA) prevents NE in broiler chickens. The objective of this study was to examine the impact of dietary DCA on the dynamics of ileal bile acids and NE in chickens. Day old broiler chicks were randomly assigned to 6 groups of diets supplemented with 0 (basal diet), 0.8, 1.0 and 1.5 g/kg (on top of basal diet) DCA. The birds were challenged with *Eimeria maxima* (20,000 oocyst/bird) at d 18 and *C. perfringens* (10 cfu/bird/day) at d 23 and d 24 to induce NE. Birds were sacrificed at d 26 and ileal tissue and content samples were collected for histopathology evaluation and bile acid analysis. Gas chromatography and mass spectrometry (GC-MS) were used to measure primary bile acids of chenodeoxycholic acid (CDCA), cholic acid (CA), and secondary bile acids of deoxycholic acid (DCA), ursodeoxycholic acid (UDCA) and lithocholic acid (LCA) in ileal content. The significant differences between groups were calculated by Student's unpaired *t*-test with statistical significance when $P \leq 0.05$. Interestingly, the composition in the ileal content of non-infected healthy broiler chickens was mainly primary bile acids, composed of 3.47 mM CDCA, 1.20 mM CA, 0.04 mM DCA, and 6.89×10^{-3} mM UDCA and 5.48×10^{-3} mM of LCA. After infection, UDCA levels in NE infected birds were reduced

compared with non-infected birds (6.89×10^{-3} mM vs 3×10^{-6} mM, $P \leq 0.05$). Dietary DCA supplementation increased the concentration of the secondary bile acid in ileal content. Feed supplemented with 0.8, 1.0 and 1.5 g/kg DCA increased ileal content concentration by 123 (2.73 mM), 107 (2.38 mM), and 136 (3.00 mM) folds compared with control NE birds ($P \leq 0.05$). At the cellular level, *E. maxima* and *C. perfringens* infection-induced NE showed inflammation and shortening of villi, crypt hyperplasia, and massive infiltration of immune cells in lamina propria. Importantly, increased ileal DCA concentration was coupled with reduction of NE induced intestinal histopathology compared with NE birds (ileitis score 6.6, 5.6, 2.8 vs 12.4 respectively). In conclusion, levels of secondary bile acid DCA in ileal content may influence the outcome of NE in broiler chickens.

Key Words: necrotic enteritis, bile acids, GC-MS, ileal content, histopathology

215 Experimental induction of necrotic enteritis in broiler chickens based on performance, intestinal morphology, gut microflora changes, and gut lesion scores indices. Ali Daneshmand¹, Hassan Kermanshahi¹, M. H. Sekhavati¹, Ali Javadmanesh¹, Monire Ahmadian¹, Marzie Alizadeh¹, and Raveendra Kulkarni², ¹Ferdowsi University of Mashhad, Mashhad, Khorasan Razavi, Iran (the Islamic Republic of), ²North Carolina State University, Raleigh, North Carolina, United States.

The present study was conducted to compare different methods for experimental induction of necrotic enteritis (NE) in broiler chickens. 360 one-day-old male Cobb chickens were randomly assigned to 4 experimental groups with 6 replicates of 15 birds each. The experimental groups were: 1. Control diet (corn-soybean meal) group (Ctrl), 2. Ctrl + *Eimeria* + *Clostridium perfringens* co-inoculation group (ECp), 3. Ctrl + 25% corn replaced by wheat (W) + *Clostridium perfringens* inoculation group (WCp), and 4. W + *Eimeria* and *Clostridium perfringens* co-inoculation group (WECp). Birds were inoculated with *Eimeria* on d 11 and orally gavaged with *C. perfringens* on d 15. On d 17 and 24, 2 birds from each replicate of treatments were randomly selected, euthanized and the contents and segments of small intestine were carefully separated for microflora, histomorphology and lesion score indices evaluations. All data were analyzed by ANOVA in a completely randomized design using the GLM procedure of SAS software. The results of the present study showed that chickens receiving ECp and WECp reduced ($P < 0.05$) body weight gain and negatively affected FCR at d 24. Chickens showed a severe ($P < 0.05$) morphological damage of the jejunal epithelium compared with those of other groups at d 17 and 24. Chickens fed with ECp and WECp showed higher ($P < 0.05$) lesion scores in the jejunum and ileum compared with those of birds received Ctrl feed at 17 and 24 d of age, while WCp had no significant effects on lesion scores. The inclusion of ECp and WECp increased ($P < 0.05$) the population of *Clostridia* and *E. col* and decreased ($P < 0.05$) the *Lactobacillus* population in the ileum of broilers compared with other groups at d 17 and 24. In conclusion, the co-inclusion of *Eimeria*

and *C. perfringens* with or without inclusion of wheat can effectively reproduce NE in broiler chickens and thus, can be proposed as reliable methods for future NE-related investigations.

Key Words: necrotic enteritis, NE inducers, gut morphology, microbial population, broiler chickens

216 Abundance of peptide transport 1 and mucin 2 mRNA in response to delayed access to feed in the small intestine of early posthatch broilers. Kuan-Ling Liu* and Eric Wong, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, United States.

Newly hatched broilers are often subjected to delayed access to feed (DAF) and water up to 48 h posthatch, which impacts small intestinal development. The objective of this study was to determine the effect of DAF on the abundance of peptide transporter 1 (PepT1) and mucin 2 (Muc2) mRNA in small intestines of early posthatch broilers. The peptide transporter PepT1 plays an important role in transporting short peptides, while Muc2 secreted from goblet cells forms a layer of mucus that lines the intestinal epithelial tract as a protective coating. Cobb-500 broiler chicks hatching within a 12-h window were randomly distributed into 3 groups: control with no feed delay (ND), 24-h feed delay (D24), and 36-h feed delay (D36). The quantification of cells expressing Muc2 and PepT1 mRNA was investigated by quantitative PCR on all 3 segments of the small intestine at hour (h) 0, h24, h36, h72, h120, and h168 posthatch. The cell localization was determined by in situ hybridization at the same time points until h120. Statistical analysis was performed using JMP Pro 14 and significant differences were determined by *t*-test and one-way ANOVA. In the duodenum at h72, PepT1 mRNA abundance was elevated in both D24 ($P = 0.015$) and D36 ($P = 0.0012$), while Muc2 mRNA was lowered in D24 ($P = 0.012$) and D36 ($P = 0.0154$) compared with ND. In the jejunum, PepT1 mRNA abundance was elevated in D24 and D36 at h24 ($P = 0.063$) and h36 ($P = 0.0008$), respectively. A similar pattern was found in the ileum, where PepT1 mRNA abundance was greater in D24 at h24 ($P = 0.062$) and in D36 at h36 ($P = 0.056$). In contrast, D36 birds at h36 had lower Muc2 mRNA abundance in the jejunum ($P = 0.0009$). An analysis of the number and distribution of goblet cells in both the upper half and the lower half of the villi was determined and expressed as a ratio (VU/VL). The VU/VL was greater in D24 at h24 in the jejunum ($P = 0.026$) and ileum ($P = 0.012$), and in D36 at h36 ($P = 0.0008$) in the jejunum. This result showed that DAF leads to a reduction in the number of goblet cells in the lower half compared with the upper half of the villus. In summary, broilers with DAF increased the abundance of PepT1 mRNA but decreased Muc2 mRNA and the number and distribution of goblet cells. This may result in enhanced uptake of amino acids as short peptides in the small intestines, but a decrease in the mucus layer, which can increase the risk of infection from pathogens.

Key Words: PepT1, Muc2, goblet cell, delayed access to feed, small intestine

Student Competition: Metabolism and Nutrition: Nutrition IV

217 Blood pH and blood gasses of laying hens as affected by limestone particle size, phytase inclusion, and time after oviposition. Micaela Sinclair-Black*^{1,2}, Roselina Angel³, Wenting Li⁴, and Peter Plumstead⁵, ¹Chemuniqu (Pty) Ltd., Johannesburg, Gauteng, South Africa, ²University of Pretoria, Johannesburg, Gauteng, South Africa, ³University of Maryland, College Park, Maryland, United States, ⁴DuPont Animal Nutrition, Wilmington, Delaware, United States, ⁵Chemuniqu (Pty) Ltd., Johannesburg, South Africa.

The objectives of this study were to determine if blood pH, blood gasses [partial pressure of CO₂ (PCO₂), partial pressure of O₂ (PO₂), total CO₂ (TCO₂), O₂ saturation percentage (sO₂), bicarbonate (HCO₃⁻), blood sodium (Na) and blood base excess (BE_{ecf})] in laying hens were affected by limestone (LM) particle size, phytase inclusion and time post oviposition (OP). The experimental design was a 2x2 factorial of 2 particle sizes of LM, grit (1.5mm geometric mean diameter (GMD)) or fine (0.2mm GMD) and 2 levels of added phytase from *Buttiauxella* sp. (0 or 600 FTU/kg). Amberlink hens at 31 wk of age were individually housed. A single hen was a replicate (n = 1 hen) and there were 16 replicates per treatment. A corn basal diet was mixed to contain no LM or inorganic P (1.14 g/kg Ca and 3.6 g/kg P). The basal diet was then used to mix 4 dietary treatments. LM containing 38.05% Ca was added to the basal diet to achieve 3.70% Ca. Each hen received a meal of 130 g of feed each day and the exact time of OP recorded. For the same hen, blood collection commenced within 5 min of OP and then every 3h for 24h following OP. All blood parameters were measured using an i-STAT point-of-care laboratory system (Abbott Point of Care, East Windsor, NJ). The effects of LM particle size, phytase, blood collection time point, and their interactions on the various blood parameters were tested by SAS MIXED model using repeated measures (SAS, 9.4). Individual hens were treated as random effect and pre-trial average daily feed intake and body weight were included in the model as covariates. Time post OP affected all blood parameters ($P < 0.01$). Blood pH increased from 6.35 at OP to 6.44 by 6h post OP and decreasing after 18h post OP. Hens fed LM grit compared with hens fed LM fine respectively, had higher PCO₂ (38.4%, 37.1%), lower PO₂ (35.43%, 37.95%) and lower SO₂ (66.20%, 70.75%). Significant 2-way interactions between LM particle size and time post OP were observed for BE_{ecf}, HCO₃⁻, TCO₂ ($P < 0.05$), all of which increased more rapidly from 3 to 12h post OP, when hens received fine LM. Phytase had no significant effect on blood gasses, with the exception of Na ($P < 0.05$). The results of this study demonstrate that blood gasses changed significantly throughout the egg formation cycle in the laying hen and were altered by LM particle size. The addition of different LM particle sizes used in the diet, may have effects on the physiological status of the hen.

Key Words: blood parameters, limestone, phytase, laying hen, oviposition

218 Quantitative relationships among intestinal histology, nutrient absorption and performance of broilers: A meta-regression approach. Diego Martinez*¹, Carol Ponce-de-Leon², and Carlos Vilchez¹, ¹La Molina National Agrarian University, Lima, Peru, ²LIAN Development & Service, Lima, Peru.

Villus height (VH), crypt depth (CD) and their relationship (V/C) are used as indicators of nutrient absorption potential, nutrient expenditure and feed efficiency, respectively. However, the accuracy and consistency of these assumptions are unknown. The objective was to determine the

quantitative relationships among the effects on histology variables (D, duodenum; J, jejunum; I, ileum; A, average), on measured apparent metabolizable energy (AME) -as indicator of nutrient absorption- and on performance (ADG, daily weight gain; FCR, feed conversion ratio). Scientific papers including all these variables in the same experiment, regardless of the factor being evaluated, were collected. Data from 2001 to 2017 were considered including 66 registries, 884 independent groups of birds, 1254 intestinal samples, 20,952 intestinal readings and 956 AME measurements. Criteria to determine data suitability were residuals of random-effects model normally distributed (Shapiro-Wilk; $P > 0.05$), significant heterogeneity of effect-sizes (HG) (Q test; $P < 0.01$) and no bias (Egger's test; $P > 0.10$). Effects were expressed as effect sizes (Σ) (kcal/kg, g/d and g/g for AME, ADG and FCR, respectively) or as percent changes (Δ) for response or moderator variables, respectively. For each model, HG was quantified using mixed-effects models, τ^2 , τ , I^2 and H^2 were calculated, and 95% confidence intervals (IC95) of slopes were determined. Relationships (slopes) were considered consistent if the bounds of the IC95 had the same sign, and if so a "●" symbol appears after the slope value. Multiple linear models were constructed with moderator variables significantly related ($P < 0.10$) when tested one at a time. Model evaluation criteria were pseudo-R² and AIC and BIC information criteria. The analysis was performed with the Metafor package in R version 3.5.2. All variables were normally distributed ($P > 0.05$), presented significant HG ($P < 0.0001$) and were unbiased ($P > 0.10$). Results showed that per each percent increase (+1 Δ) in VH_D, VH_I, VH_A, V/C_I, V/C_A or V/C_D, Σ in AME is +3.8●, +3.2●, +4.3●, +3.2●, +2.8● ($P < 0.01$) and +1.5● ($P < 0.5$), respectively; per +1 Δ in AME, CD_J, V/C_I or V/C_A, Σ in ADG is +0.71● ($P < 0.01$), -0.13● ($P < 0.05$), +0.07 and +0.06 ($P < 0.10$), respectively; and per +1 Δ in ADG, AME or VH_D, Σ in FCR is -0.005●, -0.012● ($P < 0.01$) and -0.002● ($P < 0.10$), respectively. In addition, multiple linear models were obtained for Σ in AME based on Δ in VH_D and V/C_I with slopes of +2.88● and +2.52●, respectively ($P < 0.01$) and for Σ in FCR based on Δ in AME and ADG with slopes of -0.006 ($P < 0.10$) and -0.004● ($P < 0.01$), respectively. In conclusion, the assumed relationships referred at the beginning were demonstrated and, most of them, are consistent.

Key Words: meta-analysis, meta-regression, AME, intestinal histology, nutrient absorption

219 Growth performance and tight junction gene expressions in broilers fed a lower protein diet for 14 days post-hatching. Shemil Macelline*¹, Samiru Wickramasuriya¹, Hyun Min Cho¹, Eunjoo Kim¹, Taeg Kyun Shin¹, Jun Seon Hong¹, Jae Cheol Kim³, John Pluske⁴, Ho-Jun Choi², and Jung Min Heo¹, ¹Chungnam National University, Daejeon, Korea (the Republic of), ²CJ CheilJedang Corporation, Seoul, Korea (the Republic of), ³AB Vista Asia Pte. Ltd., The Mezo, Singapore, ⁴Murdoch University, Murdoch, Australia.

The current study tested a hypothesis that broilers fed a lower protein (LP) diet supplemented with crystalline amino acids (CAA) would sustain growth performance and maintain makers of intestinal integrity compared with those fed a higher protein (commercial) diet. One hundred and 44 one-day-old Ross 308 broiler chicks (male) were allocated into 3 dietary treatments to give 6 replicates per treatment (8 birds per pen) in a completely randomized design. Dietary treatments were (1) high protein diet (HP: 23.5% CP to meet or exceed the requirement for Ross 308 broilers), (2) low protein diet (LP: 18.0% CP that

is lower than nutrient requirement for Ross 308 broilers), and (3) LP supplemented with CAA (LPAA: 18.0% CP with supplementation of limiting essential AA and glycine+serine to meet the ideal AA pattern for Ross 308 broilers). Average daily gain (ADG), feed intake (FI), and feed conversion ratio (FCR) were measured weekly. On d 14, one bird close to the median body weight was selected from each cage for euthanasia, and ileal tissue samples were collected. Data were analyzed using one-way ANOVA of the GLM procedure in SPSS (version 24). Broilers fed a LP diet grew slower (ADG 22.3 vs 33.5g) and converted the feed less efficiently (FCR 1.61 vs. 1.19) than broilers fed a HP diet ($P < 0.001$). Supplementation of CAA to the LP diet partly recovered the performance loss ($P < 0.001$). However, broilers fed a LPAA diet grew slower (ADG 27.5g vs. 33.5g) and converted feed less efficiently (FCR 1.32 vs. 1.19) than those birds fed a HP diet ($P < 0.01$). Broilers fed a HP diet ate more feed ($P < 0.05$) compared with broilers fed either LP or LPAA diet (FI 39.8, 35.7 and 36.7g, respectively), which partly explains the superior performance of the HP-fed birds. Interestingly, birds fed a LPAA diet did not increase feed intake while grew faster than birds fed a LP diet. This finding suggest that CAA supplementation may fulfilled the AA requirement of birds but other dietary factors that may reduce feed intake have influenced performance response between the 2 groups. Broilers fed a LP diet upregulated zonula occludin 1 (ZO 1) expression in the ileum compared with broilers fed a HP diet ($P < 0.05$), while broilers fed a LPAA diet showed intermediate expression of ZO 1. This results might reveal that broilers fed LP compensatively expressed the ZO 1 to maintain intestinal integrity in response to external stimuli such as diet composition or commence bacteria load in the gut compared with broilers fed a HP diet. In conclusion, broilers fed a LP diet supplemented with CAA partly restored the growth performance (ADG, FCR) loss of the broilers fed a LP diet without altering gut integrity for 14 d post-hatching.

Key Words: broiler, crystalline amino acids, growth performance, lower protein diet, tight junction gene

220 Effect of dietary different oil sources on laying performance, egg quality, and serum antioxidant activity in laying hens.
Yifeng Zhu*, Ping Wang, Ping Bai, Mei Ding, Qiufeng Zeng, Yue

Xuan, Wei Su, and Ying Zhang, *Sichuan Agricultural University, Chengdu, China.*

This study was conducted to compare the effect of different oil sources on laying performance, egg quality, and serum antioxidant activity in laying hens. A total of 360 40-wk-old Lohmann pink-shelled laying hens were randomly assigned to 4 treatments (6 replicates of 15 hens each). Four isoenergetic and isonitrogenous diets supplemented with 3% different oil sources, including soybean oil (control group, SBO), crude rice bran oil (RBO), oxidized rice bran oil (ORBO), and poultry fat (PF). The trial lasted for 12 weeks. The differences between dietary treatments were analyzed using one-way ANOVA. Differences between the treatment groups were considered statistically different at $P \leq 0.05$. In term of egg production performance, PF group had a higher ADFI than RBO group in laying hens during 1 to 4wk ($P = 0.01$), and SBO group had a higher ADFI than PF group during 9 to 12wk ($P = 0.01$). There were no difference in egg production, average egg weight, FCR, dirty egg rate, and broken egg rate among the different oil sources treatment ($P > 0.05$). The SBO group had a higher eggshell thickness than RBO, and PF had a lower eggshell thickness than ORBO group ($P < 0.01$). SBO group had a higher yolk color than ORBO group at wk4 ($P = 0.05$), and ORBO group had the lowest yolk color than other 3 oil sources group at wk12 ($P < 0.01$). RBO had a higher yolk relative weight than PF group at wk4 ($P = 0.03$), while PF group had a higher albumen relative weight than other 3 sources of oil at 4 wk ($P = 0.03$). PF group had a trend to decrease serum GST content at 12 wk ($P = 0.08$). Dietary oil sources had no effect on serum concentrations of T-AOC, SOD, GSH-PX activity, and MDA content at wk12 ($P > 0.05$). The results indicated that compared with SBO group, dietary supplementation with PF and ORBO reduce ADFI, and the thickness of eggshell and yolk color of were better than the RBO, ORBO, and PF group.

Key Words: oil sources, laying hens, egg production performance, egg quality, serum antioxidant activity

221 Not Presented

222 Not Presented

Metabolism and Nutrition: Feed Additives I

223 Natural multifunctional solutions (Alquermix) to improve productivity in broilers. David Díez¹, Carlos Domenech¹, Claudia Espinoza², Connie Gallardo Vela², Júlia Pié Orpí^{*1}, and Manuel Rosemberg², ¹Biovet, S.A., Constantí, Tarragona, Spain, ²Universidad Científica del Sur (UCSUR), Lima, Peru.

Background: Alquermix line is composed of natural multifunctional products that contain a patented mycotoxin binder, feed preservatives, enzymes, probiotics, and active botanical molecules that optimize the physiology of the organs. These solutions are designed to replace additives commonly used in farms. **Objective:** An experiment was conducted to evaluate whether Alquermix products could replace several additives in broilers through the analysis of growth performance and carcass quality. **Methods:** The trial was carried out in an experimental poultry unit with 248 one-day-old male broilers (Cobb 500). Chickens were raised for 42 d and allotted to 2 treatments: 1) CN (control diet with organic acids as feed preservatives, aluminosilicates as mycotoxin binders, diclazuril as a coccidiostat, bacitracin as an antibiotic growth promoter, a multivitaminic and an enzyme with protease, amylase, β -mannanase, xylanase, β -glucanase, cellulase, pectinase, phytase and probiotics); and 2) AX (basal diet without the mentioned additives + Alquermix products at 4 kg/ton, continuously during all the trial). There were 4 replicates per treatment and 31 broilers per replicate. Body weight, feed conversion rate (FCR) and mortality were evaluated weekly. Uniformity was evaluated on d 10, 20 and 42. Carcass characteristics and lesions in organs (macroscopically and microscopically) were evaluated at the end of the trial. Data were analyzed using PROC GLM procedures of SAS 9.2 and P value less than 0.05 was set as statistically significant. **Results:** Weight in AX was significantly higher on d 7 and numerically higher on d 42 (24 more grams per bird). FCR was a 2.61% better in AX on d 42 ($P = 0.054$), this improvement was significant in the first growing stage (d 1–10). Weekly mortality was lower in AX ($P < 0.001$) compared with the control (2.99% vs. 4.57%, at the end of the trial). Uniformity was higher in AX group (83.7% vs. 78.0%, $P < 0.05$). AX significantly improved breast yield, decreased abdominal fat and breasts had better appearance than those of the CN. Animals in CN showed more lesions in the gizzards and more prevalence of fatty, friable livers and intestinal lesions related to coccidia. Histology findings were correlated with those in the macroscopic observation. **Conclusions:** It is possible to replace conventional (chemical) additives with natural ones. Replacement of feed additives with Alquermix multifunctional products can better productive parameters and carcass quality in broilers. Results suggest birds fed Alquermix were more protected against coccidia and mycotoxins.

Key Words: feed additives, broilers, growth performance, gut efficiency, ABF

224 Good indication for enhanced performance and elimination of low intestinal inflammation and hyperemia through a natural synergistic approach in broilers raised without medication under experimental conditions. Alireza Khadem^{*1}, Milena Sevastyanova², Ricardo Sahagun², Christos Gougoulis², and Jamal Al-Saifi², ¹Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, ²Innovad® NV/SA, Antwerpen, Belgium.

Social concern and government regulations related to potential development of antibiotic resistance have increased the need for effective non-antibiotic growth promoters. A combination of several active ingredients acting synergistically with multifunctional activity on pathogenic

bacteria, intestinal epithelial layer integrity and inflammation may hold the best promise. The objective of this study was to assess the use of a commercial blend of esterified fatty acid, essential oils and plant extract (Lumance®) as an effective antibiotic replacement in broiler chickens. Using a 2-way ANOVA model, 32 pens with 62 Cobb 500 male chicks were split into 4 groups: control (T1), coccidiostat (T2), antibiotic + coccidiostat (T3) and Lumance® (T4). Body weight (BW), feed intake (FI) and FCR ratio were determined on d 7, 14, 21 and 28. On d 22, gut morphology and morphometry parameters were determined. The litter from the previous flock was used as natural challenge to the birds. Overall, T4 and T3 fed chicks showed a significantly higher BW and lower FCR compared with the control group. No differences were found between T3 and T4 concerning BW, FI and FCR. No differences were found between T2 and T1 groups for all measured parameters. No lesions due to coccidiosis were reported in the intestinal contents of all 4 groups. The thickness and fragility of intestine were not affected by the dietary treatments including the control. Moreover, intestinal hemorrhage, abnormal contents and excessive mucus production were not observed in any treatments. Despite the addition of bird litter from the previous flock, this study failed to produce significant challenges to the birds as evidenced by the absence of lesions in all treatments including the control group. However, there was a significant difference between the treatments with regards to intestinal inflammation and hyperaemia ($P = 0.015$) at d 22. More specifically, no intestinal inflammation and hyperaemia were observed for the T4 and T3 whereas, the control (T1) and T2 groups demonstrated moderate levels of abundance of intestinal inflammation and hyperemia. The lack of intestinal inflammation and hyperemia in T4 and T3 groups was in good agreement with their improved performance when compared with the control at D21 in terms of BW and FCR. In conclusion, Lumance® demonstrated an ability for enhanced performance and use in broilers diets by eliminating any signs of inflammation and hyperemia. At the same time Lumance® demonstrated a fully effective replacement application to routine medication alternatives, proving thus a long-term environmentally sustainable approach with no risks to antibiotic resistance and its detrimental consequences.

Key Words: non-antibiotic growth promoters, inflammation, hyperaemia, Lumance®, feed additives

225 Effects of various probiotic product types on broiler performance in antibiotic-free conditions. Chasity Pender^{*1}, Shelby Curry¹, Raj Murugesan¹, Daniel Petri², Audrey Duff³, and Lisa Bielke³, ¹Biomin America, Inc., Manassas, Virginia, United States, ²Biomin Holding GmbH, Getzersdorf, Austria, ³The Ohio State University, Wooster, Ohio, United States.

As consumer and regulatory opinions evolve, industry experts and researchers are actively seeking alternative solutions to reduce usage of antibiotics in poultry production. Probiotics have been receiving increased attention for their ability to improve enteric health in poultry. However, the probiotic products available on the market vary in their composition and mode of action, thus may have different performance outcomes. Therefore, the objective of this study was to evaluate different types of probiotic products on broiler performance. A total of 480, day-old Ross 708 male chicks were allocated to 1 of 4 treatment groups: 1) a standard control diet, 2) a diet supplemented with a *Bacillus* spp. based probiotic product (BAC), 3) a diet supplemented with a live multi-strain probiotic product (LIVE), or 4) a diet supplemented with a yeast-based

product (YST). Each group consisted of 8 replicate pens with 15 birds per pen. The trial was conducted over a 35-d period and performance variables were calculated weekly as cumulative measures. Data were analyzed using the GLIMMIX procedure of SAS with significance reported at $P \leq 0.05$. At the end of first week, all supplemented groups demonstrated significantly greater feed intake (FI) compared with the control, but were not different from each other. There was a trend ($P = 0.085$) for increased BW gain in the LIVE and YST groups compared with the control, with the BAC group being intermediate. By d 14, YST had higher BW gain than the control, while the other supplemented groups had intermediate BW gain. By d 28, LIVE birds had significantly higher FI than the control and BAC birds; the YST group had higher FI than the BAC group, but was similar to other groups. Both the LIVE and YST groups had augmented BW gain when compared with the control. For the overall period (0–35 d), there was a trend ($P = 0.083$) for LIVE and YST birds to have increased FI and the LIVE group had significantly improved BW gain than the BAC group, though it was not significantly different from other groups. Overall, these results suggest that broiler performance may vary depending on the probiotic product used and its mode of action, thus further understanding the classification of probiotics (i.e., single or multi-strain, live or spore forming, yeast, or mixed product) may be warranted.

Key Words: probiotics, bacillus, yeast, lactic acid bacteria, performance

226 Effect of levels of butyrate formulation in broiler starter feeds on performance enhancement. Bart Boomsma*¹, Krzysztof Kozłowski², Ashley Wagner³, Jan Jankowski², Bertrand Medina³, and Ivan Gerard³, ¹Palital Feed Additives, Velddriël, Netherlands, ²University of Warmia & Mazury, Olsztyn, Poland, ³Probiotech International, Saint Hyacinthe, Canada.

The beneficial effects of butyric acid supplementation in broiler diets on epithelial cell health and bird performance have been well documented and thus adapted by many in the commercial broiler industry. The objective of this study was to test the dose efficacy in the starter feed of a new commercially available butyrate product on the performance of broilers. Healthy 1 d old Ross 308 male broilers ($n = 450$) were allocated to 5 treatments with 10 pens each, and 9 birds per pen. Four treatments contained a form and level of butyrate as described: Intest-Plus Aeon (Palital Feed Additives), a 50% matrix encapsulated combination of Ca- and Na-butyrate with a design for slow release, was offered at 3 levels (T1:1000; T2:600; T3:300 g/T) for the first 14 d, followed by 300g/T for all treatments until the end of the trial (d 35). The positive control (PC) offered Intest-Plus CC80 (Palital Feed Additives), 80% unencapsulated Ca-butyrate, at 600g/T for 14 d, then 400g/T to d 35. The fifth treatment was a negative control (NC) that did not contain butyrate. Growth performance parameters which included live weight (LW), average daily gain (ADG), feed intake (FI), and feed conversion ratio (FCR) were measured at d 7, 14, 21, and 35. Feed and water were available throughout the trial *ad libitum*. Data was analyzed by ANOVA with pen as experimental unit. Statistical significance was set at $P < 0.05$. At d 7, LW and ADG was greater ($P < 0.05$) in butyrate treatments compared with NC (LW: 125.4 g; ADG: 12.6 g); however, there was no difference in LW ($P > 0.05$; PC: 132.9; T1: 131.9; T2: 131.9; T3: 132.5 g) or ADG ($P > 0.05$; PC: 13.6; T1: 13.5; T2: 13.5; T3: 13.6 g) between butyrate treatments. There was no effect ($P > 0.05$) of treatment on FI or FCR at d 7, or any performance parameter at d 14 or 21. There was an improvement ($P < 0.05$) of d 35 LW and ADG for all butyrate treatments compared with NC (LW: 1.960 kg; ADG: 54.9 g). The only difference demonstrated ($P < 0.05$) within butyrate treatments was between T1 (2.103 kg) and T2 (2.019 kg) as the heavi-

est and lightest birds, respectively. Subsequently, the same pattern ($P < 0.05$) was seen for ADG within butyrate treatments, where gains were greatest for T1 (59 g) compared with T2 (56.8 g). There was no effect ($P > 0.05$) on d 35 FI or FCR. The results at d 7 translated to d 35 and are consistent with the notion that dietary butyrate inclusion enhances broiler performance. However, these results also indicate that butyrate form may have a lesser impact on broiler performance than inclusion level in the starter feed. More research may be warranted to determine if encapsulation has a greater impact on performance in gut health challenged models.

Key Words: broiler, performance, butyrate

227 Effect of probiotics and essentials oil on growth performance of broilers: Meta-analysis approach. Amal Rouissi*¹, Frederic Guay¹, Martine Boulianne², and Marie-Pierre Létourneau-Montminy¹, ¹Laval University, QC, Quebec, Canada, ²Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, Quebec, Canada.

The development of antibiotic resistance has led many countries to reduce their use, and even ban them as growth promoters in animal production. Consequently, research has been done to find alternatives to antibiotic growth promoters. The objective of this study was to quantify the effects of probiotics and essentials oil (EO) in comparison to an antibiotic-free control diet by compiling the relevant literature data in a database and study it with meta-analysis tool. Publications were retained only if growth performance has been measured, the diet composition provided, as well as the dietary dose of alternatives. The range of doses used for probiotics were from 10^4 to 10^9 cfu / g and for EO from 50 to 1000 mg / kg. Thirty-six publications for probiotics and 79 for EO published between 2000 and 2017 were included in the database. For EO, the database included supplements of oregano oil (21.5%), thyme (20.5%), blends with oregano oil (20.5%), blends without oregano (10.5%) and other essential oil (27%). For probiotics, only 2 supplements were included Bacillus (67%, from 10^4 to 10^9 cfu/g) and Lactobacillus (33%, from 10^6 to 10^8 cfu/g). Dietary level of metabolizable energy (ME) and crude protein (CP) has been recalculated based on feed composition and expressed relative to the requirement of the genetic line and tested as X variables as well as the dose of alternatives. Only negative control without antibiotics and alternative treatments were used. All statistical analyses were carried out using the MIXED procedure of SAS 9.4 software with the random effect of the trial. All variables found significant ($P < 0.05$) were retained and the interactions between these variables were tested. Neither CP nor ME showed significant effect on the response of growth performance to EO and probiotics. Also, for both databases, the effect of EO type or the type of probiotic was never significant. Results for EO, showed no effect on ADFI, while ADG increased (quadratic effect, $P < 0.001$) ($R^2 = 0.998$) with increasing the dose. Similarly, FCR also decreased (quadratic effect, $P < 0.001$) ($R^2 = 0.936$) with doses of essential oils. Results for probiotics showed no effect on ADFI while ADG linearly increased ($P < 0.01$; $R^2 = 0.997$) and FCR linearly decreased ($P = 0.04$; $R^2 = 0.979$) with doses of probiotics. Models for FCR, showed maximum reduction of 4% (300–400 mg / kg for EO) with EO considering the quadratic effect while that of probiotic evolve linearly leading to 8% (10^6 and 10^8 cfu/g) effect at the maximal dose tested. This meta-analysis helps to quantify the effects of antibiotic alternatives on broiler growth performance.

Key Words: broiler, essential oil, probiotics, meta-analysis

228 Effects of butyrate, manna oligosaccharide, *Bacillus subtilis* and β -glucans from naked oat on growth performance of broilers. Amal Rouissi^{*1}, Frederic Guay¹, Martine Boulianne², and Marie-Pierre Létourneau-Montminy¹, ¹Laval University, QC, Quebec, Canada, ²Faculty of Veterinary Medicine, University of Montreal, Saint-Hyacinthe, Quebec, Canada.

Nowadays, there is a need for alternative approaches to maintain feed efficiency and broilers health in the absence of Antibiotic growth promoters. Given the plenty of products available, we first used meta-analysis tool to quantify their effects on growth performance and highlight modulating factors on organic acid, prebiotics, and probiotics sub-database. The most used and efficient products has been identified and then tested in terms of growth performance and gut health. Three thousand-2-hundred and 20 male day-old Ross 308 chicks were fed in 3 phases: starter (0–10 d), grower (11–21 d) and finisher (22–34 d) with one of the 7 treatments: T1) Control diet, T2) T1+coated butyric acid (Novyrate C; 1.0, 0.5 and 0.25 kg/t in starter, grower, and finisher respectively), T3) T1+ mannan oligosaccharide (Actigen; 0.8, 0.4 and 0.2 kg/t in starter, grower, and finisher respectively), T4) T1+ *Bacillus* (Gallipro, DSM 17299; 0.125 kg/t), T5) T2+T3+T4, to assess synergy or antagonist effects, T6) a portion of corn-soybean meal replaced by 30% naked oat (β -glucans), and T7) T6+butyric acid, manna oligosaccharide and *Bacillus* to test positive effect of adding alternatives in naked oat diet. All diets were formulated to fulfill 96% of metabolizable energy and digestible lysine. Broilers were randomly distributed to 10 replicate pens of 45 broilers per treatment in a complete randomized block design. Using MIXED procedure (SAS Institute 9.4), contrasts were used to study: 1) T1vs T2, 2) T1vs T3, 3) T1vs T4, 4) T1 vs T5, 5) T1 and T6 and 6) T6 and T7. During the starter phase, there was an increase of ADFI (6%), ADG (15%) and decrease of FCR (9%) ($P < 0.001$) in T6 and T7 (naked oat diets) in comparison to T1. In grower phase, ADG remained higher and FCR lower in T6 and T7 ($P < 0.001$) and T4 (*Bacillus*) increased FCR in comparison to T1 (respectively, 3%; $P < 0.001$). In the finisher phase, butyric acid was the best alternatives with increase by 4% of ADG and decrease by 5% of FCR ($P < 0.01$). On the overall experiment, lower ADG was observed in T4 ($P < 0.01$) and highest FCR in T4 and T7 ($P < 0.001$). Finally, no significant effect of the treatment on mortality. In summary, the replacement of part of the corn-soybean meal by naked oat, which considered as β -glucans, improves growth performance especially during the starter phase; butyric acid improves growth performance while *Bacillus subtilis* reduced it from the finisher phase in comparison to control. Results of gut health parameters will help understanding the mode of action of these additives and better using them in broilers diet.

Key Words: broiler, performance, alternatives, growth promoters, naked oat

229 Phytogetic feed additive offers an alternative to antibiotic growth promoters in broiler diets. Abiodun Bello^{*1}, Shi Bai², Yueming Dersjant-Li¹, Wenting Li¹, and Leon Marchal¹, ¹DuPont Animal Nutrition, Nutrition & Biosciences, Leiden, South Holland, Netherlands, ²Sichuan Agricultural University, Chengdu, Sichuan, China.

The drive to reduce the risks of antibiotic residue in poultry meat necessitates the reduction or removal of antibiotic growth promoters (AGP) in poultry diets. The effect of a phytogetic feed additive (Enviva® EO; minimum of 4.5% cinnamaldehyde and 13.5% thymol) as an alternative to AGP in poultry diets was evaluated in broilers raised in non-experimentally challenged conditions. Three dietary treatments were randomly assigned to 750 male Ross 308 broilers (25 birds per floor

pen \times 10 pen replications per treatment) in a completely randomized design. A nutritionally adequate basal diet was used to formulate the 3 test diets: 1) with AGP (enramycin at 47.2 g/ton and salinomycin at 544 g/ton, BD+AGP), 2) without AGP (BD-AGP), 3) with EO at 100 g/ton (BD+EO). Diets were based on corn-soybean meal and contained DDGs, cottonseed meal, rapeseed meal, and rice bran with 1000FTU/kg phytase in all diets and fed *ad lib* in 4 dietary phases as crumble (0–10d) and pellet (11–21, 22–35, and 35–42d). Body weight gain (BWG), feed intake and feed conversion ratio (FCR) were measured in each phase. At d 21, 5 birds per pen were sampled for apparent ileal digestibility (AID) of protein, neutral and acid detergent fibers. Data were analyzed using JMP 14.1. Protein digestibility tended to be increased ($P = 0.071$) by the BD+AGP (77.1%) and BD+EO (76.9%) compared with BD-AGP (73.9%), AID of neutral and acid detergent fibers were each increased ($P < 0.05$) by BD+EO (33.7 and 16.9%) relative to BD+AGP (28.1 and 12.8%) and BD-AGP (26.7 and 13.5%), respectively. There was no diet effect on 1–42 d BWG and feed intake between BD+AGP (2,612 and 4,306 g), BD-AGP (2,594 and 4,264 g), and BD+EO (2,613 and 4,264 g), respectively. Similarly, no diet effect was seen on 0–42d mortality and culled birds percentage. However, the 1–42 d FCR was improved ($P < 0.05$) in the BD+EO (1.632) than in BD+AGP (1.664) birds and intermediate in BD-AGP (1.653) birds. The results indicated that the phytogetic feed additive supplementation may have positive effect on protein and fiber digestion by modulating the microbiota diversity, population and/or colonization in the ileum and ceca to benefit protein digestion and fiber degradation, which can be linked to the improved feed efficiency in the EO-supplemented broilers. The results imply that the phytogetic feed additive may be used as alternative to AGP in broiler diets, with improved nutrient utilization and feed efficiency.

Key Words: broilers, nutrient digestibility, essential oil, feed efficiency, phytogenics

230 *Bacillus amyloliquefaciens* CECT 5940 helped overcoming negative effect of heat stress in broiler chickens. Juliano Cesar De Paula Dorigam, Kiran Doranalli, and Nadia Yacoubi^{*}, *Evonik Nutrition & Care GmbH, Hanau, Germany.*

Heat stress (HS) has an important impact on animal performance and welfare in many regions of the world. Heat stress leads to a decrease in feed intake and consequently to a decrease on animal growth because of reduced access to critical nutrients for optimal growth. To overcome the negative effect of HS in animals it is common to use feed with higher nutrient concentration or to use feed additives. Therefore, the present study was conducted with a hypothesis that feeding a *Bacillus amyloliquefaciens* CECT 5940 probiotic would improve the performance under thermo-neutral (TN) conditions and that the effects would be more pronounced under HS conditions. A total of 624-d-old Arbor Acres Plus male broiler chick were allocated to 4 treatments. Dietary treatments were arranged in a 2x2 factorial design, where factors were stress (TN vs HS) and probiotic supplementation (with or without *B. amyloliquefaciens* CECT 5940) were the main factors. All diets were formulated to meet the breed's requirements. Treatments with probiotics were supplemented with 500g/ton of the probiotic (2×10^9 cfu/g). The HS birds were under continuous high T° ($32^\circ\text{C} \pm 2^\circ\text{C}$) from day one while the TN birds were raised under T° as per breeder's recommendation. Final body weight (FBW), body weight gain (BWG), feed intake (FI) and feed conversion (FCR), nitrogen retention (N.R.), and histology analysis of the GIT were assessed. Blood samples were analyzed for immune biomarkers. Data was submitted to ANOVA followed by duncan's test at 5% probability. Heat stress had a significant impact on animal performance. At the end of the trial (d 35), HS reduced significantly ($P <$

0.05) FBW and BWG by 214 g/bird as compared with the birds raised under TN conditions. Heat stress significantly decreased ($P < 0.05$) FI by 252g/bird and significantly increased ($P < 0.05$) FCR (1.631 vs. 1.587). Supplementing *B. amyloliquefaciens* CECT 5940 significantly ($P < 0.05$) improved animal performance under TN conditions and HS. Probiotics supplementation improved significantly ($P < 0.05$) FBW and BWG by 3.7%, increased FI by 2.6%, and improved FCR by 1.2% at d 35. Heat stress significantly decreased N.R. ($P = 0.003$), while *B. amyloliquefaciens* CECT 5940 was able to increase N.R. by 1.5%. The HS significantly decreased ($P < 0.05$) the CD4 T-cells and immunoglobulin (IgG) in the blood while supplementation with *B. amyloliquefaciens* CECT 5940 was able to restore CD4 T-cells ($P < 0.05$) and IgG values to similar level as the TN-reared birds. In this study, HS had a negative impact on the broilers immune system that resulted in a decrease in animal performance. The addition of the probiotic was able to counteract the negative of HS and improve animal performance.

Key Words: heat stress, broiler, probiotic

231 Effects of apple cider vinegar on weight gain and carcass weight in Cornish cross broilers. Mackenzie Weldy^{*1}, Madison Allison¹, Bei Li Martin¹, Toree Williams², and Zac Williams², ¹University of Findlay, Findlay, Ohio, United States, ²Michigan State University, Lansing, Michigan, United States.

The issue of gut health, specifically in regards to disease and weight gain, has traditionally been solved utilizing antibiotics. Recently, more natural feed supplements have increased in popularity as a source of antimicrobial properties. Furthermore, frequent and increased utilization of antibiotics have also encouraged the growth of antibiotic resistance. Previous research examined the effects of combining various organic acids, like fumaric and acetic acid, on growth and weight gain in broilers, but effectiveness of acids yielded conflicting reports. Some studies support the idea of fumaric and acetic acid decreasing the pH of the gut, thus inhibiting growth of harmful bacteria and indirectly increasing feed utilization and overall weight gain. Past studies have also presented neutral results pertaining to organic acids having no effect on weight gain. The objective of the current study was to evaluate the effects of organic acids, specifically acetic acid in raw apple cider vinegar (RACV) form, as opposed to medicated feed, on weight gain and carcass weight of Cornish cross broilers. Forty-eight broilers were randomly separated into 4 treatment groups ($n = 12$): treatment 1- non-medicated feed and untreated water; treatment 2- non-medicated feed and water treated with RACV; treatment 3- medicated feed and untreated water; treatment 4- medicated feed and water treated with RACV. Basal diet of Keller non-medicated feed, in treatment groups 1 and 2, and Keller medicated feed in treatment groups 3 and 4 met N.R.C standards for broilers. RACV supplementation in water occurred in treatment groups 2 and 4 (14.8 cc of RACV per 3.78 L of water). Broilers were allowed ad libitum access to feed and water for the 7 wk duration of the study. Broilers were fed and housed as a group. Cumulative feed and water intake was recorded twice daily for all treatment groups to ensure proper nutrition requirements. Each coop were weighed once a wk, following a 2 wk growth period, and weight was recorded. A completely randomized design was utilized and differences in weight gain and carcass weight were analyzed using SPSS at $P < 0.05$. At the conclusion of the experiment, results indicated neither RACV or medicated feed had an effect

on weight gain, slaughter weight, or carcass weight in all 4 treatment groups throughout the 5 wk duration (wk 1 $P = 0.054$, wk 2 $P = 0.0642$, wk 3 $P = 0.887$, wk 4 $P = 0.758$, wk 5 $P = 0.432$, Slaughter Weight $P = 0.522$, and Carcass Weight $P = 0.577$ respectively). Based on results of the current study, RACV may not be a viable alternative to antibiotic use in broiler production to increase weight gain and carcass weight.

Key Words: vinegar, growth promoter, broiler production, antibiotics, organic acids

232 Effects of apple cider vinegar versus medicated feed on Coccidiosis in Cornish crosses. Madison Allison^{*1}, Bei Li Martin¹, Mackenzie Weldy¹, Toree Williams², and Zac Williams², ¹University of Findlay, Maplewood, Ohio, United States, ²Michigan State University, Lansing, Michigan, United States.

Due to increased demands of antibiotic free poultry products and an increase in antibiotic resistance, antimicrobials are becoming more favored. Previous studies on antimicrobial activity demonstrated that the decrease of pH in the gut creates an uninhabitable environment for *Coccidiosis* growth. Organic acids have been identified as possible alternatives to antibiotics, however, few studies have investigated acetic acid specifically. The objective of the present study was to determine the effects of raw apple cider vinegar (RACV) supplemented in water on levels of *Coccidiosis* in broilers. Before the study, 48 Cornish cross chicks were fed DuMOR starter feed crumble. At 2 wk of age, chicks were designated to 1 of 4 treatment groups utilizing a completely randomized design. Each of the 4 treatment groups contained 12 chicks ($n = 12$): treatment 1 – non-medicated feed and untreated water; treatment 2 – non-medicated feed and RACV water; treatment 3 – medicated feed and untreated water; treatment 4 – medicated feed and RACV water. Treatments 1 and 2 were fed basal rations of Keller non-medicated feed, which was synonymous to medicated feed in Treatments 3 and 4 and met N.R.C feed requirements for broilers. Treatments 2 and 4 were supplemented with RACV diluted in water (14.8 cc of RACV per 3.78 L of water) and all treatments were allowed feed and water *ad libitum*. Weights of feed and water were recorded twice daily to ensure broilers received proper nutrition. Fecal samples were collected for a duration of 5 wk, every 7 d utilizing sterile swabs directly following defecation. Four treatments were separately tested for *Coccidiosis* via McMaster method. Each fecal matter sample was diluted in saturated Epsom salt solution and ejected into the McMaster slide and each chamber of the grid was observed for oocyte presence. Data was analyzed via SPSS with $P < 0.05$. Results from all 5 test d concluded no difference between treatment groups 1, 2 and 4 in terms of *Coccidiosis* presence. During the last 2 wk of testing, treatments 1, 2 and 4 indicated coccidia oocytes were present, however no oocytes were detected in treatment 3 throughout the duration of the study, specifically wk 4 ($P = 0.491$) and wk 5 ($P = 0.491$). *Coccidiosis* oocytes were expected to be detected in treatment 1 thus, detection of oocytes was unexpected in Treatment 4, as medicated feed and RACV were provided. Results indicated that RACV and anticoccidial components of medicated feed have no effect on *Coccidiosis* presence due to the development of oocytes. Further research into antimicrobials should be perused in the interest of consumer preference.

Key Words: coccidiosis, broiler, vinegar, organic, alternative

Physiology and Reproduction

233 Physiological mechanisms regulating metabolic efficiency in commercial broilers. Laura Ellestad*¹, Samuel Latzsch¹, Michael Rothrock², and Jean Guard², ¹University of Georgia, Athens, Georgia, United States, ²USDA-ARS, Athens, Georgia, United States.

Recent implementation of the Veterinary Feed Directive and increased demand from the public for food produced without antibiotics have created a need for development of strategies to maintain broiler production efficiency in the absence of antibiotic growth promoters. As feed accounts for almost 70% of total broiler production cost, understanding basic mechanisms regulating efficiency of feed nutrient use is crucial to developing these strategies and preserving production margins, particularly in antibiotic-free systems. In any given population of broilers raised under the same conditions, there is a natural variation in metabolic efficiency as measured by feed conversion ratio [FCR; g feed intake (FI)/g body weight gain (BWG)]. The objective of this study was to identify physiological parameters associated with hormonal regulation of nutrient uptake and utilization differing between high efficiency (low FCR) and low efficiency (high FCR) commercial broiler chickens. Male Ross 708 broilers (108 birds) were reared in single-bird battery pens between post-hatch day (D) 7 and D35, and BWG, FI, and FCR were calculated on an individual bird basis from D7- D35. On D36, blood, liver, and muscle were collected from the 6 highest (HE) and lowest (LE) efficiency birds based on FCR for measurement of circulating hormones and expression of genes associated with hormonal action and nutrient partitioning (n = 6). Data were analyzed with a 2-tailed Student's *t*-test. While there were no differences between HE and LE groups in terms of D35 final body weight, BWG, or FI, LE birds had a significantly higher FCR than HE birds (1.69 ± 0.03 vs 1.41 ± 0.007; *P* < 0.0001). Circulating levels of thyroxine (T₄) were higher in the LE group (17.4 ± 0.42 vs 13.6 ± 0.62 ng/mL; *P* < 0.05), and levels of corticosterone (CORT; 558.8 ± 70.0 vs. 364.1 ± 72.8 pg/mL; *P* = 0.0787), growth hormone (GH; 1532 ± 251.9 v. 1043.9 ± 35.3 pg/mL; *P* = 0.1030), and insulin (INS; 7.0 ± 1.0 vs. 4.61.69 ± 0.4 μIU/mL; *P* = 0.0788) tended to be higher in LE birds. Plasma levels of insulin-like growth factor 1 (IGF1) and triiodothyronine did not differ between groups. In muscle, mRNA levels of IGF1 receptor (2-fold), cationic amino acid transporter 1 (*CAT1*; 5-fold), and glucose transporter (GLUT) 5 (2-fold) were higher in HE birds (*P* < 0.05). Levels of mRNA for *CAT1* (2-fold) and *GLUT2* (1.2-fold) were also higher in the liver of HE birds (*P* < 0.05), while expression of sodium-glucose transporter 1 (1.8-fold) was higher in LE birds (*P* < 0.05). These data suggest that plasma T₄, and to a lesser extent CORT, GH, and INS, may contribute to improved feed efficiency by altering expression levels of key amino acid and sugar transporters in metabolically important tissues such as liver and muscle.

Key Words: feed conversion ratio, nutrient partitioning, metabolism, liver, muscle

234 Intestinal microbial ecology associated with metabolic efficiency in commercial broilers. Michael Rothrock*¹, Laura Ellestad², and Jean Guard¹, ¹USDA-ARS, Athens, Georgia, United States, ²University of Georgia, Athens, Georgia, United States.

In the absence of antibiotic growth promoters, the broiler industry needs to develop new strategies to maintain broiler production efficiency. To better develop these strategies, a basic understanding of the microbiome effects on broiler feed efficiency is needed, with the hope that this knowledge can be leveraged to make production strategies more efficacious.

There is a natural variation in metabolic efficiency as measured by feed conversion ratio [FCR; g feed intake (FI)/g body weight gain (BWG)] in a given population of broilers. The objective of this study was to identify microbiological constituents associated with high efficiency (low FCR) and low efficiency (high FCR) commercial broiler chickens. Male Ross 708 broilers (108 birds) were individually reared between post-hatch day (D) 7 and D35, and on D36, jejunum, ileum, duodenum, and cecum were collected from the 6 highest (HE) and lowest (LE) efficiency birds based on FCR for microbiome analysis (n = 6). For phenotypic microbiome analysis, tissue samples were homogenized in tryptic soy broth (1:3 wt/vol dilution), filtered, normalized to a 1.0 OD600, and used to seed Biolog EcoPlates to assess the microbial communities' ability to utilize 31 different C-sources. For genotypic microbiome analysis, genomic DNA was extracted from homogenized tissue samples using the QIAamp DNA Stool kit, and 16S rDNA sequencing was performed using the Illumina MiSeq platform and processed using the QIIME2 pipeline. There were definite differences between gastrointestinal tract segments, regardless of feed efficiency, with the cecal microbial loads being more than 1 log higher than duodenal samples and more than 3 logs greater than ileal and jejunal segments. EcoPlate data indicated that microbial respiration was significantly higher, according to a 1-tailed, paired *t*-test, in LE birds (*P* < 0.0001) in cecal, duodenal, and jejunal segments, with metabolism of C-sources such as glycogen, α-D-lactose, glucose-1-phosphate, and γ-hydroxyl-butyric acid differing between groups. Genotypic microbiome analysis showed that while gastrointestinal segment was the greatest driver of richness, diversity, and community structure within these samples, there were significant differences in taxa within each segment between HE and LE broilers, including those within the dominant *Firmicutes* and *Bacteroidetes* phyla. These data suggest that microbial signatures are associated with feed efficiency differences that can be linked on a genotypic and a phenotypic community-scale level. These microbial signatures need to be studied further, as they represent potential targets for future strategies to maintain or even improve current production efficiencies within the poultry industry.

Key Words: feed efficiency, microbiome, gastrointestinal tract, carbon metabolism

235 The effect of an advanced growth curve for early photostimulation at 15 wks using two different feeding regimes during rearing on egg production through 40-wks of age. Andrew Benson*, Muslah Ahammad, Charles Meeks, Zachery Jarrell, Adam Davis, and Jeanna Wilson, University of Georgia, Athens, Georgia, United States.

A 2X2 factorial designed experiment was completed to compare the effects of (a) 2 rearing growth curves/photostimulation ages (15 or 21 wks) with (b) 2 different feeding regimens, every-day spin feeding (EDS) vs skip-a-day (SAD), on broiler breeder production through 65 wks of age. For this experiment, 2,400 females and 360 male Cobb-500 broiler breeder chicks were randomly divided into one of 4 treatments, with 3 replicates per treatment. From 2 wks of age until photostimulation, birds were fed either on a SAD basis to reach target body weight (2.1 kg) for photostimulation at 15 wks (15-SAD) or 21 wks (21-SAD) with chain feeders; or EDS basis to reach target body weight at 15 wks (15-EDS) or 21 wks (21-EDS). Following attainment of target body weight, at either 15- or 21-wks, the birds were transferred to breeding pens with 9 replicate pens (38 hens and 4 roosters each) per treatment, and photostimulated (14L:10D). The weight profile of the birds that were moved

into the breeding pens mirrored the weight profile of the same population at the end of the rearing period. Weight at age of photostimulation for the different groups were 2.02 (15-EDS), 2.09 (15-SAD), 2.03 (21-EDS), 2.11 (21-SAD) kgs. All data were analyzed by GLM using SPSS (version 25) with significance considered at $P < 0.05$. During rearing, the pullets fed on the advanced growth curve for photostimulation at 15 wks had significantly better body weight uniformity than the 21-week growth curve treatments when measured at 8 and 14 wks of age. Both 15-week rearing feeding regimens reached 5% egg production at 20 wks of age, while both 21-week rearing feeding regimens reached 5% egg production at 24 wks of age. The weight at 5% egg production of the 15-week photostimulated hens was greater than that of the 21-week hens. This increase in weight during early production may have led to the significant increase in double yolk (DY) eggs in the 15-week birds (2.4% DY) when compared with the 21-week birds (0.9% DY) through 40 wks of age. The 15-week birds had peak egg production at 26 wks (75.3% for 15-SAD and 75.7% for 15-EDS) while the 21-EDS birds peaked at 30 wks with 85.3% egg production, and the 21-SAD birds peaked at 32 wks with 83.1% egg production. Through 40 wks of age, the average number of eggs/hen was greater for the 15-week birds (82.8 eggs/hen) than the 21-week birds (77.6 eggs/hen). In addition, hens reared with the EDS treatments had greater average eggs/hen than the SAD birds at both 15-wks (83.8 vs 81.6 eggs/hen) and 21-wks (78.5 vs 76.6 eggs/hen) of age. In conclusion, attainment of target body weight for early photostimulation at 15 wks advanced age at first egg by 4 wks and increased egg production through 40 wks of age.

Key Words: photostimulation, broiler breeder, egg production, rearing, pullets

236 Germ cell dynamics during nest breakdown and formation of primordial follicles in the domestic turkey (*Meleagris gallopavo*). George Hall*¹, Julie Long², Ben Wood^{1,3}, and Gregory Bedecarrats¹, ¹University of Guelph, Guelph, Ontario, Canada, ²Beltsville Agricultural Research Centre USDA, Beltsville, Maryland, United States, ³Hybrid Turkeys, Kitchener, Ontario, Canada.

Within the avian ovary post-hatch, the breakdown of germ cell nests and the formation of primordial follicles marks a pivotal time in cellular depletion and reorganization. Since upon sexual maturation, birds lack a germinal bed with self-renewing oogonium, consequently, this cellular change heavily influences the finite number of ova available throughout their reproductive life span. This study aimed to histologically assess the different subpopulations of germ cells within the cortex of the left ovary during germ cell nest breakdown in the domestic turkey. This was accomplished by measuring the size and density of pre-follicular germ cells and primordial follicles within ovaries collected from poults at 1, 3, 5, 7, 9, 11, 13, 15 and 21 d post-hatch. Whereas, the total germ cell count was determined from ovaries at 5, 9 and 15 d post-hatch. Four ovaries were analyzed per age group. Ovaries were fixed, sectioned (5 μ m) and stained (H&E) for imaging. For size and density measurements, 6 to 16 sections per ovary were evaluated; for total germ cell counts, the entire ovary was assessed. Measurements were averaged for each ovary before analyses. Among age groups, a one-way ANOVA was used to test for significance ($P \leq 0.05$); followed by a post-hoc test (Tukey) to determine which ages differed. Based on our results, germ cell nest breakdown was initiated 5 - 7 d post-hatch, as shown by the first decrease ($P \leq 0.001$) in the pre-follicular germ cell density. This was accompanied by the appearance of primordial follicles on d 7. Initiation of nest breakdown is temporally followed on d 9 by the first increase ($P \leq 0.05$) in size of both pre-follicular germ cells and primordial follicles. This growth ended by d 13, with no further change in size ($P > 0.05$). The

majority (>90%) of nest breakdowns concluded by d 15, as evidenced by a lack of change in both pre-follicular germ cell and primordial follicle densities in older ovaries. Before the initiation of germ cell nest breakdown (d 5) and after the majority had broken down (d 15), the total germ cell count was $1,057,402 \pm 194,627$ and $169,578 \pm 31,594$ (mean \pm SEM), respectively, which equates to an 84% decrease in the total germ cell population. The presence of pre-follicular germ cells at 21 d post-hatch suggests that a further decrease in the overall germ cell population may occur. It appears that, in the domestic turkey, the loss of germ cells during nest breakdown is comparable to the domestic chicken, but exceeds the average 2-thirds lost in mammalian species.

Key Words: pre-follicular germ cell, primordial follicle, nest breakdown, ovary, turkey

237 Biostrong® Libido improves semen quality of male breeders and increases female reproductive performance. Megan Koppen*¹ and Jan Dirk van der Klis², ¹Delacon USA, Inc., Carlisle, Pennsylvania, United States, ²Delacon Biotechnik GmbH, Steyregg, Austria.

Male fertility is critical in turkey breeding as it directly correlates to hatchability. Crucial for sperm quality and male fertility is an effective antioxidant system to counteract sperm cell membrane lipid peroxidation and reduction of free radical formation responsible for sperm DNA fragmentation. Phytochemicals can increase resilience of spermatocytes by stimulating cellular antioxidant capacity thus upregulating cellular antioxidant response element (ARE), which results in increased production of antioxidant enzymes. On top, essential oils and saponins can support hormonal regulation of spermatogenesis and increase testosterone production. Biostrong® Libido (BSL) is a proprietary blend of essential oils, flavonoids, and quillaja saponins developed to improve male breeder fertility. A field trial was conducted to evaluate the efficacy of BSL on semen quality and quantity in turkey toms; and consequently, reproductive performance in female breeders. BSL was supplemented at 750 g/MT to 1,200 male turkey breeders fed corn-soy based diets starting at 22 weeks of age, immediately following selection (5 weeks before milking). Packed cell volume (PCV) and mLs per milked tom was evaluated over a period of 32 weeks. The weekly averages of the test barn were compared with a 9-cycle-average. Female reproductive performance was also evaluated. A house of female breeder turkeys (approximately 20,000 hens) was inseminated with semen from the BSL supplemented toms. Measurements gathered from these hens were from the first week of lay through end of production (over a period of 24 weeks). Reproductive performance was measured based on percentage of fertile eggs and were compared with 4 control barns. Due to trial design, statistics were unable to be performed. Percent differences among treatments were identified and used to demonstrate potential product efficacy in a commercial setting. BSL fed toms showed a 6.5% increase in PCV compared with the 9-cycle-average over the 32-week period. Additionally, mLs per milked tom increased by 5% compared with the control flocks. Turkey hen breeders inseminated with semen of toms fed BSL compared with the average of 4 control barns showed percentage of fertile eggs in the first half of production (1–13 weeks of lay) increased by 0.43% and by 0.19% during the second half of production (14–24 weeks). Female production for the entire 24-week period averaged 0.35% more fertile eggs in the BSL inseminated hens. Based on the results of this field trial with turkey breeders, dietary supplementation with BSL may improve semen quantity and quality and exert positive effects on the reproductive performance of females.

Key Words: fertility, semen quality, turkey, phytochemicals, antioxidant

238 Transcriptional regulation of albumen biosynthesis and eggshell biomineralization in the oviduct of laying hens. Birendra Mishra*, Nirvay Sah, Donna Kuehu, Sanjeev Wasti, and Rajesh Jha, *University of Hawaii at Manoa, Honolulu, Hawaii, United States.*

Besides environmental, nutritional, and pathological conditions, oviductal functions also govern the egg production and quality. Recently, we identified several novel genes associated with albumen formation in the magnum, and eggshell biomineralization in the shell glands. The objectives of this study were to determine the enriched biological process and pathways, and solute carrier (SLC) genes that mediate the 1) albumen synthesis and secretion in the magnum, and 2) eggshell biomineralization in the shell glands. Segments of oviducts were collected from Hy-line laying hens at 3 h post-ovulation (p.o.) and 15–20 h p.o., molter, and non-laying hens. Total RNAs isolated from the magnum of laying hens at 3 h p.o. vs. non-laying hens (n = 3/group), from the shell gland of laying hens at 15–20 h p.o. vs. non-laying hens (n = 3/group), respectively, were subjected to RNA-Sequencing. Biological pathways and SLC genes obtained from the RNA-Seq data were further validated in all the experimental groups (n = 6/group) using real-time PCR (qPCR). In the magnum, biological processes (L-serine biosynthetic process, regulation of immune system process, and proline transport) and metabolic pathways (superpathways of serine and glycine biosynthesis I, matrix metalloproteases inhibitors, asparagine biosynthesis I, asparagine degradation I and choline degradation I) were enriched. Using qPCR, we further confirmed that Interstitial Collagenase, Gelatinase B, Cingulin, and several family members of SLCs related to anion and cation exchanger, amino acid transporter were significantly higher in laying hens at 3 h p.o. Identified biological process and pathways may increase the bioavailability and transport of essential ions, amino acids and immune-related molecules in the lumen of magnum for albumen biosynthesis. In the shell gland, biological processes (serine biosynthetic, cellular sodium ion homeostasis) and pathways (calcium signaling, pantothenate, and CoA biosynthesis) were the most-enriched. Using qPCR, we further confirmed calcium signaling pathways (Otopetrin 2, Calcitonin, Stanniocalcin 2, and Plasma membrane Ca²⁺ transporting 2) and SLCs genes related to sodium/hydrogen exchanger, sodium bicarbonate cotransporter, zinc long-chain fatty acids transporter, and glucose-6-phosphate exchanger were significantly higher in shell gland at 15 h p.o. Identified biological process and pathways may increase the bioavailability, mineralization, and remodeling of calcium for the eggshell formation. In conclusion, this study identified the biological pathways involved in the albumen biosynthesis and eggshell formation, and can potentially be used to enhance the egg production traits in chickens.

Key Words: RNA sequencing, gene expression, biological pathways, magnum, shell gland/uterus

239 Organic matrix protein profile associated with changes in crystallographic texture and mechanical properties in guinea fowl eggshell. Nathalie Leroy¹, Alejandro Rodriguez³, Lucie Combes-Soia², Aurelien Brionne¹, Valerie Labas², Yves Nys^{*1}, and Joel Gautron¹, ¹Boa, IN.R.A, Université de Tours, Nouzilly, France, ²UMR PRC IN.R.A85 CN.R.S 7247, Université de Tours, Nouzilly, France, ³University of Granada, Departamento de Mineralogía y Petrología, Granada, Spain.

Guinea fowl eggshells have a particular ultrastructure and crystallographic arrangement that is different from other birds and is at the origin from its exceptional mechanical properties. It is a 2-layer structure with different microstructure: the inner layer (1/3) is constituted of columnar calcite crystal units which progressively develop preferred orientation

as observed in hens but the outer layer is made of calcite microcrystals with varying crystallographic orientations and interlocking boundaries. The study of early shell mineralization in Guinea fowl revealed the formation of a transient amorphous calcium carbonate as recently observed in hens, then formation of larger crystals issued from space completion and elongation of the crystals due to inhibition by the organic matrix of faces parallel to the C axis. The shift from larger to smaller crystal is suspected to be controlled by the shell organic matrix proteins and proteoglycans. That was confirmed by a quantitative proteomic study (nanoLC-MS/MS) of the eggshell matrix of shell sampled before and after the changes in crystallography of guinea fowl shell (8 birds sampled /stage; The comparison of protein abundance carried out using weighted spectral count and the eXtracted Ion Chromatogram combined with Anova and Hierarchical clustering). The level of 149 proteins was quantified at 5 calcification stages. Neognathae (chicken, Guinea fowl, zebra finch...) have in common 54 proteins including 4 proteins related to the biomineralization process (Nucleobindin-2, extracellular serine/threonine protein kinase, Milk Fat Globule-EGF factor 8 protein and Calbindin D-28K. Among the 15 most abundant Guinea fowl proteins at the early stage of shell formation, 10 proteins were analog to the chicken (OVAL, OVM, OVOT, HPX, LYZ C, OC-17-like, HAPLN3 (Hyaluronan and proteoglycan link protein 3), CST3, HBAA and CLU). Nine proteins were unique to the Guinea fowl eggshell including 2 acidic proteins with putative calcium-binding domains LOC110408336 and CGREF1 and Dromaiocalcin-1-like, the homolog to Ovocleidin-17. 61 matrix proteins were present in the shift period. Among them are calcium binding proteins (NPNT-X1, CALBP1, Protein S100-A6, ANXA1 and 2, CDH2...), core proteins of proteoglycans (TSKU, GPC4...), and other proteins regulating the activity of proteins driving the mineralization (SSP1, OC-116GDF6...). The identification and quantification of the proteins of the eggshell matrix should allow a better understanding of the mechanisms of shell formation and the control of its mechanical properties. It might be used for determination of biological markers for the genomic selection of chicken layers with improved shell mechanical properties.

Key Words: guinea fowl, eggshell, crystallography, proteomic, shell matrix protein

240 The effect of cooling rate of broiler hatching eggs on embryonic development, hatchability and hatch time. Serdar Özlü* and Okan Elibol, *Ankara University, Faculty of Agriculture, Ankara, Diskapi, 06110, Turkey.*

The effect of the egg cooling profile of broiler hatching eggs after oviposition on embryonic development and hatchability of fertile eggs was studied. Hatching eggs were obtained from Ross 308 broiler breeders at 35 wk (prime) of age in Exp 1, and at 28 wk (young) and 64 wk (old) of age in Exp 2, respectively. A total of 120 eggs were collected and then randomly placed to cardboard egg trays in Exp 1. The eggshell and internal egg temperatures were measured intervals 40 min during egg cooling process. To measure the internal egg temperature, a 4-mm diameter hole was drilled into the top of the each egg after measured the eggshell temperature. In Exp 2, a total of 3,150 eggs that had been laid within a 15 min period were collected and then randomly assigned to 2 temperature controlled chambers with either control (360°- 480°) or rapid (45°-120°) cooling to 24 and 18°C EST, respectively. Eggs were remained in the chambers until the EST of both cooling groups were similar, then eggs were transported to hatchery and were stored for 6 d at 16°C and 75% RH. Each tray of 150 eggs was considered to be a replicate and there were 5 replicate trays per cooling profile treatment in each flock age. Some (25 embryos in each batch) of the eggs

were opened before and after cooling profile treatment to determine the stage of the blastoderm. The eggs were randomly set in a single commercial incubator in Exp 2. The CORR procedure of SAS was utilized to determine correlation coefficient for eggshell temperature relative to internal temperature in Exp 1. Data from the completely randomized design were subjected to ANOVA using the GLM procedure of SAS in Exp 2. A significant ($P < 0.0001$) positive correlation ($r = 0.995$) existed between eggshell temperature and internal egg temperature. The stage of embryonic development was advanced by control cooling and by the older flock. In younger flock eggs, fertile hatchability was significantly decreased by rapid cooling due to higher early and late embryonic mortality ($P \leq 0.05$). However, early embryonic mortality and percentage of second grade chicks was reduced ($P \leq 0.05$) and fertile hatchability was numerically higher by rapid cooling compare with control in older flock eggs. In conclusion, the data from this study demonstrated that rapid cooling after lay retarded the stage of blastoderm development in eggs from both young and old broiler breeder flocks. This was apparently detrimental, as indicated by higher early and late embryonic mortality, in the case of the young flock but beneficial in the case of the old flock. The hatchability differences between young and old flock eggs by rapid cooling rate might depend on the differences of embryonic development at oviposition.

Key Words: hatching broiler egg, egg temperature, egg cooling rate, hatchability, hatch time

241 Development of a chicken intestinal organoid culture and its characterization. Mohan Acharya¹, Annie Donoghue¹, Joshua Lyte¹, and Narayan Rath*², ¹University of Arkansas, Fayetteville, Arkansas, United States, ²USDA/ARS, Fayetteville, Arkansas, United States.

Organoids are 3 dimensional constructs of tissues that can simulate tissue functions hence, have been studied for the purpose of regenerative medicine, analyzing physio-pathology of tissues and interaction with different agents including microbes. However, most advances in this area has been limited to mammalian species with few studies in avian

models. Optimum maintenance of gut health and protection against pathogenic organisms is an impending issue in poultry production due to the restrictions in antibiotics growth promoters. To identify factors that may improve intestinal competency for growth and protection against pathogens, we sought to develop chicken enteric organoid culture for screening of selective agents and to examine the interaction with pathogens. We isolated intestinal villi from day old chicks, purified, and cultured in DMEM-F12 media containing antibiotic/antimycotic, 10% fetal bovine serum, bovine pituitary extract, insulin, selenium, transferrin, and, polyamine supplements which favor the repair of the severed ends of villi to spheroids within 24 h of incubation. The structural changes in the organoids over 3 d were verified using scanning electron microscopy which showed these organoids budding and proliferating 3 dimensionally and contained vascular tissues. We examined the organoids by immunofluorescent staining with several different antibodies, lectins, and antigen ligands, which showed the presence of several epithelial antigens including cadherin, keratin, Na-K ATPase, mucin, and showed the cells with polarized localization of β -actin. The organoids could be infected with Salmonella, expressing green fluorescent protein (GFP). We tested the effect of selective agents that are known to affect intestine such as dextran sulfate (inducer of colitis), retinoic acid (vitamin A), 2,4 dinitrophenol (a weight reducing agent), deoxycholic acid a (bile acid), capsaicin and lipopolysaccharide, both, inflammatory agents, phorbol 12-myristate 13-acetate (a protein kinase C activator), β -glycophate (a pesticide), and indomethacin (a prostaglandin inhibitor), for their effect on organoids. We are currently evaluating the effects of enteric neurochemicals, such as serotonin, on organoid response to pathogens of relevance to the poultry industry. Our results show that these chemicals differentially affect the organoids some of which cause severe damage whereas others, showed little to no change. These results suggest that the intestinal organoids have potential to screen for chemicals and factors which can improve gut health, serve as antibiotic alternatives, provide a template to study nutrient absorption, host bacterial interactions, and other physiological mechanisms.

Key Words: chicken intestinal organoids, structure, immunohistochemistry, *Salmonella*

Processing and Products

242 Salt-uptake characterization of whole carcasses during chilling. Stephanie Richter*¹, Daniel Sabo¹, Nicole Bartenfeld Josselson², Comas Haynes¹, and Richard Jeff Buhr², ¹Georgia Tech Research Institute, Atlanta, Georgia, United States, ²USDA-ARS, Athens, Georgia, United States.

This study supports our multiyear investigation of ice slurry for poultry immersion chilling. The study focus is to analyze the penetration of salt into poultry product, as it is a component of ice slurry. High salt presence in final meat product has producer labeling and consumer avoidance effects. Poultry skin is assumed to act as a barrier to limit water and salt uptake during the processing chilling phase. This experiment examines salt uptake tendencies of whole carcasses (WOGs) during immersion chilling at different salinities. All sample WOGs were collected post-evisceration and kept at temperature (28–35°C). WOGs were chilled for 60 min by one of 5 methods; ambient air (23°C), air chill (4°C), chilled water (5°C), 4.5% salinity chilled water (5°C) or 15% salinity chilled water (5°C). Chilled salt water was used to maintain constant and consistent chloride concentrations throughout the experiment. This avoided dilution effects of ice melting and assumed the worst case scenario of salt concentration. Chilling temperatures were selected to coincide with temperatures typical in current ice slurry applications. Pre- and post-chill, all WOGs were weighed and deep breast meat temperatures were logged. Post-chill, 3 samples were collected per carcass: breast skin, breast meat, and drumstick meat. This initial investigation included 3 replicates (n = 75 carcasses; total 225 samples). Carcass samples were analyzed for salt concentration, specifically chloride. Salt was recovered from the skin and meat samples using a USDA-approved plant dry-ash method. The total chloride concentration was determined using an ion chromatograph (IC). Statistical analysis was corrected from IC data to account for sample weight and dilution factor. Chloride concentration was calculated as ppm/gram. One-way ANOVA test were used to compare chloride concentrations given groups defined by chilling treatment and carcass skin/meat sampling type, considering mean ± SD (p-value = ≤ 0.05). Initial results show, of all 15 groups (5 chilling types * 3 sample types), 4.5% salinity and 15% salinity skin samples were the only statistically significant group (P = < 0.0001) in chloride ppm/g. 4.5% salinity skin samples averaged 524 ppm/g and 15% salinity skin samples averages 2275 ppm/g. Results show no statistical significance (P > 0.05) between white and dark meat samples between the chilling forms. Results indicate that salt concentrations increase in the skin and does not affect white or dark meat. Further testing will examine a post-rinse to see if the chloride can be removed during processing.

Key Words: chloride, salt, brine, immersion chilling

243 Influence of carbon dioxide controlled atmosphere and electrical stunning methods on broiler carcass blood loss and heart beat cessation. Dianna Bourassa*, Ileana Berganza, Ranjit Boyal, Ella Lawley, and Jessica Starkey, Auburn University, Auburn, Alabama, United States.

Consumer attitudes toward improving animal welfare have led to a shift from electrical to controlled atmosphere stunning (CAS) for broiler chickens. Current CAS systems are multi-phase with increasing concentrations of carbon dioxide between the induction, transition, and completion phases. In some systems, oxygen (O₂) is added during the induction phase to minimize aversion to CO₂. To assess the impact of

a multi-phase CAS system on blood loss and cessation of heart beat, 36 broiler chickens were stunned by one of 4 methods: 1) no stunning, 2) electrical waterbath, 3) multi-phase CAS with O₂ added during the induction phase, and 4) multi-phase CAS without added O₂. For non-stunned (n = 6) and electrically stunned birds (n = 6), a unilateral neck cut was conducted on all birds. For CAS birds with (n = 12) or without (n = 12) added O₂, half of the birds received a neck cut and half did not. Weight of blood lost over time was recorded on an individual bird basis and calculated as a percentage of live weight. During each stunning method, birds were equipped with cutaneous electrodes, electrocardiograms (ECG) recorded with a DSI PhysioTel telemetry device, and data analyzed using Ponemah software. The main effect of stunning method on blood loss and time of heart beat cessation was statistically analyzed using the ANOVA procedure of SAS with means separated by Tukey's HSD test. Birds non-stunned or electrically stunned lost more blood (2.1, 1.6%) within the first 30 s compared with CAS birds with or without O₂ (0.6, 0.4%; P < 0.0001). At 2 min non-stunned or electrically stunned birds lost more blood (2.7, 2.2%) compared with CAS birds with or without O₂ (1.3, 1.0%; P < 0.0001). However, the final weight of blood loss at 6 min for non-stunned, electrical, and CAS with or without O₂ was not significantly different (2.8, 2.4, 2.3, 2.0%; P = 0.3559). For all stunning methods, heart rate was suppressed (<180 beats per min) within 100 s following stunning. Heart beat cessation occurred more quickly for non-stunned and electrically stunned birds (589, 574 s) compared with CAS with or without O₂ (375, 354 s; P = 0.0040). However, this may have been due to suppression of heart rate that occurs during CAS. When the CAS stunning duration of 280 s was considered, there were no significant differences between methods (569, 574, 655, 634 s; P = 0.4036). For CAS birds, application of neck cut did not impact cessation of heart beat (654 s cut, 632 s non-cut; P = 0.5061). Although stunning broiler chickens using a CAS system initially slows blood loss, blood loss at 6 min was not affected. The use of CAS did not alter heart beat cessation in comparison to electrically or non-stunned birds.

Key Words: controlled atmosphere stunning, blood loss, electrocardiogram, electrical stunning

244 Effect of spaghetti meat abnormality on the muscle protein composition of broiler breast meat. Brian Bowker*, Giulia Tasoniero, and Hong Zhuang, USDA-ARS, U.S. National Poultry Research Center, Athens, Georgia, United States.

Recent data suggests that the spaghetti meat (SM) myopathy observed in broiler *pectoralis major* muscle impairs meat quality and functionality; however, the impact of the myopathy on muscle protein composition is unknown. The objective of this study was to determine the effects of SM on protein characteristics and profiles of broiler breast meat. Breast fillets (30 normal (N), 30 SM) were collected from a commercial processing plant at 3 h post-mortem and stored overnight at 4°C. Exudate was collected and analyzed for protein content and SDS-PAGE protein profiles. Fillets (10 N, 10 SM) were cut into superficial and deep layers. Muscle protein fractions (myofibrillar and sarcoplasmic) were isolated and analyzed using SDS-PAGE. Protein solubility and content were measured. The effects of muscle condition, layer, and their interaction were analyzed using a 2-way ANOVA. SM fillets had lower protein content than N fillets (P < 0.0001) in the superficial layer of the muscle. Myofibrillar protein solubility was lower in SM fillets than N fillets (P < 0.001). Sarcoplasmic protein solubility was lower in the superficial layer

of the muscle compared with the deep layer ($P < 0.0001$). Although the protein concentration of the exudate from N and SM fillets was similar, the SM fillets lost more protein due to having a greater ($P < 0.05$) drip loss than SM fillets ($n = 1.22$ vs SM = 1.44%; $P = 0.0131$). Electrophoretic profiles of exudate proteins were generally similar between N and SM fillets; however, the relative abundance of protein bands corresponding to 34 kDa (lactate dehydrogenase), 47 kDa (enolase), 67 kDa, and 71 kDa were different between N and SM fillets ($P < 0.05$). With regards to the sarcoplasmic protein fraction, muscle layer influenced ($P < 0.05$) relative abundance of 5 proteins. The SM condition did not have a strong impact on the sarcoplasmic protein profile. For the myofibrillar fraction of the muscle, SM exhibited a lower relative abundance of a 73 kDa protein ($P < 0.01$). Muscle layer significantly influenced the relative abundance of myofibrillar protein bands corresponding to 25 kDa and a high molecular weight protein (>250 kDa). In N fillets the relative abundance of the protein band corresponding to 100 kDa (α -actinin) was similar between the deep and superficial layers of the muscle. However, in SM fillets the relative abundance of the 100 kDa myofibrillar protein band was lower ($P < 0.05$) in the superficial layer of the muscle compared with the deep layer. Overall data suggest that although the SM condition influences the total amount and functionality of proteins in the muscle, the sarcoplasmic and myofibrillar protein profiles in SM are similar to normal breast meat.

Key Words: broiler chicken meat, spaghetti meat, myofibrillar protein, sarcoplasmic protein, exudate protein

245 Texture characteristics of wooden breast fillets deboned at different times. Giulia Tasoniero^{*1}, Brian Bowker², and Hong Zhuang¹, ¹U.S. National Poultry Research Center, USDA-ARS, Athens, Georgia, United States, ²USDA-ARS, Athens, Georgia, United States.

The aim of this study was to understand whether deboning time contributes to the altered texture attributes of Wooden Breast (WB) fillets. A total of 30 unaffected (NORM) and 30 severely affected (WB) carcasses were sampled during 2 trials. For each trial, carcasses were selected at 15 min PM based on palpable hardness of the breast fillets and allotted into treatments with different deboning times: trt A (5 NORM + 5 WB; right fillets deboned at 15 min PM), trt B (5 NORM + 5 WB; right fillets deboned at 3 h PM), and trt C (5 NORM + 5 WB; right fillets deboned at 6 h PM). Left fillets from each carcass were deboned at 24 h PM and considered as a controls for their right counterparts. Multiple instrumental texture analyses were performed on the cranial-middle portion of the *Pectoralis major* muscles. Irrespective of the deboning time, all fillets were subjected to a single 30% compression in the raw state at 24 h PM (1 measurement/fillet). Thereafter, all fillets were cooked (76 C at core) and let sit at room temperature overnight before being subjected to shear force assessments. Cooked fillets were sheared first with MORS and B-MORS methods. Thereafter, a strip of meat not previously punctured was removed and 2 Warner-Bratzler shear force measurements were taken. Using an ANOVA mixed model, deboning time was evaluated as a fixed effect within muscle condition (sampling session and sample ID as random effects). Compression tests indicated that raw WB meat experienced texture changes from 15 min to 24 h PM, with the highest force required to compress fillets deboned at 6 h PM (15 min = 35.4 N; 3 h = 30.9 N; 6 h = 48.0 N; 24 h = 30.6 N; $P < 0.05$). Differently, deboning time had no effect ($P > 0.05$) on raw NORM sample compression values. As for the shear analyses of cooked fillets, MORS test did not detect any texture change due to deboning

time for NORM breast. However, the other 2 shear methods showed that hot deboned NORM fillets possessed the highest shear values followed by a gradually reduced shear force trend through the 24 h PM (15 min = 16.4 N; 3 h = 15.6 N; 6 h = 12.8 N; 24 h = 12.8 N, $P < 0.05$ for B-MORS and 15 min = 65.9 N; 3 h = 55.1 N; 6 h = 44.1 N; 24 h = 37.7 N, $P < 0.05$ for Warner-Bratzler shear). On the contrary, no changes ($P > 0.05$) in shear values due to different deboning time were observed in cooked WB meat regardless of shear method. These results suggest that early postmortem changes in breast muscles and their influence on meat texture are different between normal and WB fillets.

Key Words: wooden breast, deboning time, instrumental texture attributes, Warner-Bratzler shear, MORS

246 Sodium formate (Amasil® NA), monobutyryl (SILO-Health® 104) and bacitracin methylene disalicylate (BMD) effect on intestinal and processed parts bacteria count when fed to broiler chickens challenged with mild coccidia and clostridium perfringens (Cp). Michael Coelho¹, Peter Ader^{*2}, and James McNaughton³, ¹BASF Corporation, Humble, Texas, United States, ²BASF SE, Lampertheim, Germany, ³AHPharma, Hebron, Maryland, Germany.

The Objective of the trial was to evaluate the efficacy of SILOHealth 104 (SH) and Amasil Na (NA) as an antibiotic (Coban + BMD) replacement, when broilers are reared on built-up litter floor bedding (from 3-previous trials) under a mild coccidia and *Clostridium perfringens* (Cp) challenge. A total of 3,744 Ross 708 birds were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block as the random effect (52 birds/pen x 8 treatments x 9 replications). Birds were blocked by weight and sex. The treatments were T1 = PC, no additive, no Cp; T2 = NC, no additive, Cp; T3 = NC +4/4/4 kg/MT monobutyryl (SH) in starter/grower/finisher; T4 = NC+ 4.0/2.5/1.5 kg/ MT SH; T5 = NC+2.5/1.50/0.75 kg/MT SH; T6 = NC+8/8/8 kg/MT Amasil NA (NA); T7 = NC+2.5 kg SH + 4.0 kg/MT NA/ 2.5 kg SH + 4.0 kg/MT AN/2.5 kg SH + 4.0 kg/MT NA; and T8 = NC+ Coban+50 g/MT BMD. Least significant difference was used to compare means of treatment groups. T1–8 had litter inoculated on D0 with 2,500 oocytes of *E. acervulina* and *E. maxima*/bird, and T2–8 had litter inoculated with 5×10^4 cfu/bird of *E. coli* and 5×10^4 cfu/bird of Cp. On D21 and 42, 3 birds from each pen (27 birds/treatment) were sacrificed and data collected on Small Intestine (SI) and cecal (C) bacteria. At 43–45 d of age, 10 birds (5M and 5F) were processed from each replicate (9-replicates), to determine lesion scores and bacteria count. D21 SI lesion scores (0–3) (0.204, 1.667, 1.019, 1.037, 1.333, 0.778, 0.667 and 0.796, $P < 0.05$, respectively); D21 SI *Clostridium* (CL) (3.595, 4.519, 4.209, 4.136, 4.388, 3.954 and 3.683 cfu log 10, $P = < 0.05$, respectively); D21 SI *Campylobacter* (CA) (3.343, 4.489, 4.243, 4.074, 4.414, 3.834, 3.576 and 3.956, $P < 0.05$, respectively); D42 SI CL (3.526, 4.534, 4.200, 4.074, 4.168, 3.973, 3.733 and 4.035 cfu log 10, $P < 0.05$, respectively); D42 SI CA (3.329, 4.276, 3.93, 3.957, 4.088, 3.829, 3.531 and 4.002 cfu log 10, $P < 0.05$, respectively); cecal CL (3.480, 4.485, 4.277, 4.059, 4.165, 3.975, 3.678 and 4.042 cfu log 10, $P < 0.05$, respectively); cecal CA (3.330, 4.243, 3.940, 4.055, 3.778, 3.521 and 3.930 cfu log 10, $P < 0.05$, respectively); D45 processing breast CA (1.482, 2.850, 2.496, 2.655, 2.482, 2.094, 1.920 and 2.753 cfu log 10, $P < 0.05$, respectively); breast salmonella incidence (1.111, 25.556, 13.333, 18.889, 15.556, 10.000, 7.778 and 6.667%, $P < 0.05$, respectively). In conclusion, monobutyryl, sodium formate and BMD significantly reduced bacteria count in small intestine, cecal and pro-

cessing breast versus NC. The monobutyryn, sodium formate reduced bacteria count in small intestine, cecal and processing breast similarly to the BMD treatment.

Key Words: monobutyryn, sodium formate, broilers, processed parts bacteria, *Clostridium perfringens*

247 **Not Presented**

Immunology, Health, and Disease I

248 Non-Mhc background genes increase Rous sarcoma progression in major histocompatibility (B) complex genotype B24B24. Robert Taylor*¹ and Renee Kopulos², ¹West Virginia University, Morgantown, West Virginia, United States, ²Northern Illinois University, DeKalb, Illinois, United States.

Major histocompatibility (B) complex genes are key in controlling Rous sarcoma virus (RSV)-induced tumor growth. Additional genes affect such tumor growth to a lesser degree. Numerous Mhc haplotypes have been characterized for their effect on tumor outcome which may be either regression or progression. The objective of the current study was to examine RSV tumor growth in the progressive B24B24 genotype in 2 different genetic backgrounds. Congenic Line 6.15-5 (B5B5) sires were crossed to B24B24 dams from Line UNH 105 New Hampshire chickens. The F₁ B5B24 sires were backcrossed to B24B24 dams. Resulting B24B24 progeny were designated as UNH 245 having a genetic composition of 75% Line UNH 105 and 25% Line 6.15-5. In 2 trials, 6 wk old UNH 105 and UNH 245 chicks were injected in the wingweb with Bryan high-titer RSV subgroup A pseudotype (20 pfu). Tumor size was assessed using a defined scoring system 6 times over a 10 wk period after inoculation. A tumor profile index (TPI) for each bird was assigned based on the 6 tumor scores over the 70 d experimental period. ANOVA was employed to assess statistical differences. The 2 stocks were compared for mean tumor size within a time period and rank transformed TPI. Tumors grew to comparable size through 3 wk post-inoculation in both groups. From that time, tumor size was significantly larger in UNH 245 (n = 52) compared with UNH 105 (n = 38) at 6, 8 and 10 wk post-inoculation. A size plateau was found at 6 wk post-inoculation in UNH 105 but at 8 wk post-inoculation in UNH 245. The higher TPI of UNH 245 birds (2.92 ± 0.17) was significant compared with the TPI found in UNH 105 (2.21 ± 0.13). Mortality in UNH 105 = 7/38 (18.4%) was lower ($\chi^2 = 12.8$, $P < 0.001$) than that found for UNH 245 birds = 29/52 (55.8%) Non-Mhc genes influenced the tumor growth. The 25% genetic composition from Line 6.15-5 in UNH 245 was sufficient to introduce genes that added to the negative effect of Mhc genotype B24B24.

Key Words: MHC, Rous sarcoma virus, oncogene, tumor, progression

249 Marek's disease-vaccines induced differential expression of microRNAs in the primary lymphoid organ of bursa at 26th day post inoculation. Huanmin Zhang*¹, Lei Zhang^{1,2}, Chen Zhu⁴, Kunzhe Dong³, and Mohammad Heidari¹, ¹USDA-ARS, East Lansing, Michigan, United States, ²Chinese Academy of Agricultural Sciences, Changchun, Jilin, China, ³Augusta University, Augusta, Georgia, United States, ⁴Michigan State University, East Lansing, Michigan, United States.

MicroRNAs (miRNAs) are small noncoding RNAs, which are typically 17–24 nucleotides in length. Recent studies showed this epigenetic factor, miRNAs, along with long non-coding RNAs, drives phenotypic changes in both immunity and malignant cell development. MicroRNA alters the properties and behavior of cells through regulating gene expression and the accessibility of chromatin that determines cell fate. Those reports also showed cancer vaccine induces epigenetic changes. Our previous studies demonstrated that 2 highly inbred lines of White leghorns convey drastically different protective efficacy in response to Marek's disease (MD) vaccines, such as HVT and CVI99/Rispens, against vv+ MD virus challenge. This study was designed to profile

miRNAs present in the primary lymphoid organ, bursa, of the 2 inbred lines of chickens at 26th day post vaccination and to identify differentially expressed miRNAs induced by the vaccines. Chicks sampled from both MD-resistant line 6₃ and susceptible line 7₂ were inoculated on the day of hatch at a uniform dose of 2,000 FPU per bird for HVT or Rispens. A non-vaccinated control group was also included per line under the same conditions. Bursa tissues were taken on the 26th day post hatch from all treatment groups including the controls. Total RNA samples were extracted and subjected to small RNA sequencing on an Illumina HiSeq 4000 system. The miRDeep* package (v3.8) was run to identify miRNAs from the sequencing data. A total of 829 unique miRNAs was identified from all the treatment groups, including 621 novel miRNAs. HVT induced 4 and zero while Rispens induced 14 and 1 differentially expressed miRNAs in the line 6₃ and 7₂ birds, respectively. Within line, Rispens induced 29 and 3 differentially expressed miRNAs over HVT in lines 6₃ and 7₂, respectively; between the lines, Rispens induced 32 differentially expressed miRNAs in line 6₃ over line 7₂, and HVT induced 35, in line 6₃ over line 7₂. These results suggest CVI988/Rispens induced a stronger response in line 6₃ birds than in the line 7₂ birds. Pathways depicting target genes of the differentially expressed miRNAs will be discussed.

Key Words: Marek's disease vaccines, vaccine efficacy, host genetics, microRNAs

250 Marek's disease virus infection induced mitochondria changes in chickens. Jiuzhou Song*¹ and Qin Chu², ¹University of Maryland, College Park, Maryland, United States, ²Institute of Animal Husbandry and Veterinary Medicine, Beijing, Beijing, China.

Mitochondria are crucial cellular organelles and participate in many cell processes including immune response, growth development, and tumorigenesis. Marek's disease (MD), caused by an avian α -herpesvirus, commonly known as Marek's disease virus (MDV), is characterized with lymphomas and immunosuppression. In this research, we hypothesize mitochondria may play roles in response to MDV infection. Mitochondrial DNA (mtDNA) abundance and gene expression in 3 immune organs including Bursa of Fabricius, thymus and spleen were ascertained in 2 well-defined chicken lines, the MD-susceptible line 7₂ and the MD-resistant line 6₃. The samples were collected at 5, 10 and 21 d post-infection (dpi) from 5 birds per line, per group. Relative contents of mtDNA over 3 time points were determined using qPCR analysis of the mitochondrial gene ND2 and the nuclear gene β -actin as a control. Regression analysis was used to do the analysis. Meanwhile, the gene expression was studied using RNA sequencing in all 3 tissues at 21 dpi. Though RNA-seq data analysis, we found that MDV challenge had little impact on mitochondria contents in chickens of the MD-resistant line, but the mitochondrial abundance and gene expression level were obviously altered in spleen of MD-susceptible chickens at the transformation phase. At the transformation phase (21 dpi), mitochondrial DNA contents decreased significantly in spleen of the MD-susceptible line 7₂ birds in contrast to the MD-resistant line 6₃. While the mtDNA-genes and the nucleus-genes relevant to mtDNA maintenance and transcription were significantly upregulated. Furthermore, we found that gene *POLG2* may play a potential role that leads to the imbalance of mtDNA copy number and gene expression alteration. MDV infection induced imbalance of mitochondrial contents and gene expression, demonstrating the indispensability of mitochondria in virus-induced cell transformation and subsequent lymphoma formation, such as MD development in

chicken. This is the first report on relationship between virus infection and mitochondria changes in chicken, which will provide important insights to advance the understanding on pathogenesis and tumorigenesis due to viral infection.

Key Words: mitochondria, gene expression, Marek's disease, immune response, T cell transformation

251 Protection efficacy of a recombinant vaccine with double deletion of Marek's disease virus-encoded oncogenes. Lakshmi Sunkara, John Dunn, and Mohammad Heidari*, *USDA-ARS, East Lansing, Michigan, United States.*

Marek's disease virus (MDV), a highly cell-associated oncogenic α -herpesvirus, is the etiological agent of Marek's disease (MD), a contagious lymphoproliferative disease of domestic chickens. MDV causes immunosuppression, neurological disorders, chronic wasting, blindness, bursal/thymic atrophy (BTA), and fatal T cell lymphomas in susceptible chickens that infiltrate lymphoid tissues, visceral organs, and peripheral nerves. Non-oncogenic strains of MDV as well as recombinant mutant viruses have been used to control MD. A recombinant virus lacking both copies of MDV-encoded Meq oncogene, rMd5 Δ Meq, has been shown to provide better protection against highly pathogenic strains of MDV than CVI988/Rispens, the gold standard vaccine for MD. The recombinant vaccine, however, induces lymphoid organ atrophy like that of the parental virus, rMd5. To improve the safety of the recombinant vaccine by eliminating or reducing its BTA-associated negative feature, we deleted one or both copies of viral telomerase (vTR), a second virus-encoded oncogene, in addition to Meq, rMd5 Δ Meq Δ vTR. To also test the effect of vTR deletion on MDV pathogenicity and replication in the skin of infected chickens, we deleted both copies of vTR gene from the cloned parental virus, rMd5. Unpaired Student's 2-tailed *t*-test was used to determine the statistical significance between the genome copy number of the challenge virus and those of the mutant constructs. The data was considered statistically significant when $P \leq 0.05$. The double deletion mutant virus, rMd5 Δ Meq Δ vTR, was highly protective against very virulent pathotypes of MDV. Deletion of both copies of vTR in rMd5 reduced the viral replication significantly in the skin and tumor development in the visceral organs. Single or double deletion of vTR in rMd5 Δ Meq reduced the viral genome copy numbers in the skin of infected chickens by more than 3.5 and 26 folds at 20 dpi, respectively. Deletion of vTR in rMd5 Δ Meq, reduced the bursal atrophy but did not eliminate it completely. In summary, the recombinant vaccine with double deletion of MDV-encoded oncogenes was highly protective against very virulent pathotypes of MDV with minimal effect on bursal atrophy.

Key Words: Marek's disease (MD), Marek's disease virus (MDV), Meq oncogene, viral telomerase (vTR), bursal/thymic atrophy (BTA)

252 Evidence of reduced virulence patterns of avian pathogenic *E. coli* (APEC) in broilers from enviva CSI bacillus strains following inoculation from breeder hens. Jordon Gruber*, Elizabeth Kim, Alastair Thomas, and Terry Parrott, *Danisco US Inc., DuPont Animal Nutrition, Cedar Rapids, Iowa, United States.*

Pathogen transfer from breeder hens to chicks is a growing area of focus because of both the linear route of infection and the subsequent implications on the formation of the chick microbiome. Avian Pathogenic *E. coli* (APEC) is one such pathogen with an established relationship of transfer from breeder hens to progeny increasing risk of disease. Three separate complexes (2 different commercial integrators - both geographically

separated), volunteered for sampling. In total, 180 breeders, 90 eggs, and 120 chicks were sampled all through their linear relationships (breeders, eggs from same breeders, and chicks from hatched eggs followed from original breeder samplings). From either the ileal samples of birds or total surface of eggs, total *E. coli* was isolated on selective agar plates. From each sample, 5 random colonies were selected for multiplex-PCR to determine combination of genes. Together, samples were processed using established primer sets and a Qiaxcel machine with internal imaging technology. Using virulence genes as a marker for pathogenicity, the relationship between APEC in breeder hens and downstream chicks was investigated following isolation from both gastrointestinal tracts and physical surfaces within the hatchery. Breeder hens, hatchery, and broilers all maintained APEC as resident bacteria, while the hatchery maintained the highest APEC density followed by breeder hens and then broilers. This relationship in density was also reflected in distribution of virulence genes, whereby isolates from physical locations within the hatchery contained an average combination of 6 to 7 direct virulence genes, while breeder hens averaged a combination of 5 genes. However, in downstream broilers containing Enviva CSI in their diets, the average gene combination of APEC was 2 genes. Virulence gene combinations included *cvaC/iss*, *irp2/cvaC/tsh/iucC/iss*, and *tsh/iucC/iss* - highlighting both specific toxin production, iron sequestration and host-survival capability. Virulence factors play an important role in establishing an infection, but may also come at a cost of competition within bacterial networks. To this end, virulence factors undergo a selection process during transmission from breeders to hatchery and chicks, while further competition from probiotics may force bacteria to select for alternative fitness strategies. Together, this research helps to further demonstrate the direct relationship of pathogen transfer from breeders to broilers, while also showing a successful strategy in reducing virulent strains through a customized DFM plan.

Key Words: APEC, fitness, breeders, microbiome, hatchery

253 Immunohistochemistry for CPA and NetB toxins in an intestinal ligated loop model used to reproduce necrotic enteritis in chickens. Martine Boulianne*, *Faculté de Médecine Vétérinaire, Université de Montréal, St. Hyacinthe, Quebec, Canada.*

Even though necrotic enteritis is an economically important disease, the pathogenesis of this disease is still poorly understood. Intestinal ligated loop models using *Clostridium perfringens* (CP) have been used in various animal species and have been shown to be a useful model to further our understanding of the disease. Recently we have developed and validated such a model for necrotic enteritis (NE) associated CP in chicken. Our objective was to compare the virulence of mixed and single CP strains inocula from previous surgeries, using immunohistochemistry (IHC) for CPA and NetB toxins. All surgeries were conducted with the Animal Care Committee's approval. Specific pathogen free chickens were pre-medicated then anaesthetized. Nine 2 cm-loops were created and inoculated to compare up to 5 different CP strains and 4 negative controls (brain heart infusion media). Birds were closely monitored during a 7 h post-surgical anesthesia then humanely euthanized. Histological lesion scoring, IHC using NetB and CPA antibodies, and CP isolation were performed for each sampled loop. Our model showed no NE related lesion in the BHI inoculated loops while typical NE lesions were observed in the CP inoculated loops. Histological lesions varied in severity depending on the CP strain used. In the case of mix inocula, one strain usually becomes predominant. There was an association of NetB positive CP strains with in situ localization of the bacteria as well as CPA and Net toxins within necrotic enteritis lesions. IHC proved to be an interesting tool to compare strain virulence. While all CP inoculated

loops tested positive to CPA in small and discrete areas, only NetB positive CP strains showed a coloration to NetB in large areas of the lesions. IHC for NetB toxin stained enterocytes at the villous tips, Gram-positive bacterial clusters present at the villous tips as well as degenerated and sloughed enterocytes present within the luminal exudate. IHC for NetB toxin in NE lesions visually demonstrates its role in the pathogenesis of the disease.

Key Words: necrotic enteritis, pathogenesis, *Clostridium perfringens*, histopathology, immunohistochemistry

254 A proteomic view of cross-talk between intestinal microbiota and host immune system: New insights in poultry health.

Denise Russi Rodrigues*¹, Kim Wilson¹, Whitney Briggs¹, Audrey Duff¹, Kaylin Chasser¹, Johel Bielke¹, Walter Bottje², and Lisa Bielke¹, ¹The Ohio State University, Wooster, Ohio, United States, ²University of Arkansas, Fayetteville, Arkansas, United States.

Given the relevant cross-talk between the gut microbiota and host physiology, we have previously addressed the impact of intestinal pioneer colonizers on the ileal microbiome. To complement our previous study, we used a proteomics approach to predict changes in biological processes by Ingenuity Pathway Analysis software to examine ileal functional shifts in response to exposure to lactic acid bacteria (LAB) or *Enterobacteriaceae* strains *in ovo*. Embryos at 18 embryogenic days were inoculated with either saline (S), 10² cfu of *Citrobacter freundii* (CF), *Citrobacter* species (C2) or LAB (L) into the amnion. At 10 d post-hatch, 10 birds from each treatment were selected for a collection of ileum samples for proteomic mass spectrometry analysis. Our further findings indicated that the pioneer colonizers have a major impact on the modulation of immunity in young broilers. Functional analyses of proteins differentially expressed ($P < 0.1$), relative to S treatment, revealed the occurrence of ileal inflammation pathways in broilers treated *in ovo* with L (z-score = 1.80) and CF (z-score = 2.32). L treatment was associated with molecular networks involved in inflammatory response, although CF expressed evidence of downregulation of inflammation inhibitors. LAB *in ovo* exposure activated pathways that corresponded with activation and trafficking of immune cells, while CF and C2 inhibited cell movement of immune cells and expression of cytokines signaling pathways, suggestive of inhibited function of the immune system. These results demonstrated that inoculation with L affected development and maturation of immune functions during the early life of broilers. In addition, our previous microbiome analyses showed that L significantly increased the colonization of the segmented filamentous bacteria (SFB), which, in turn, might be potentially associated with the enhancement and maturation of the immune response. On the other hand, the interaction between SFB and ileal microbiota may be triggered the inflammation of ileal mucosa found in broilers treated to LAB-probiotic *in ovo*.

Key Words: enterobacteriaceae, inflammation, *in ovo* technique, probiotics, segmented filamentous bacteria

255 The use of biomarkers in the battle against mycotoxicosis in poultry. Astrid Koppenol*¹ and Luiz Caron², ¹Impextraco NV, Heist op den Berg, Belgium, ²Universidade Federal do Parana, Curitiba, Brazil.

Diagnosis of mycotoxicosis in poultry is difficult. When clinical signs are detected, it is already too late as mycotoxins are immunosuppressive creating opportunity for infectious disease to manifest. Biomarkers are great tools to find significant effects as early as possible in the animals

reaction against mycotoxicosis. To evaluate the effect of naturally contaminated feed, 96 male Cobb 500 d-old-broilers were assigned to a control or a contaminated diet until 28 d of age (48 animals over 6 pens per treatment). The contaminated diet was formulated by replacing control corn by a naturally Fusarium contaminated corn, resulting in a contamination of 17 ppm FB1+FB2. Blood was collected from 8 animals per treatment and used to quantify circulating lymphocytes through flow cytometry, activity of aspartate transaminase, gamma glutamyl transferase, alkaline phosphatase and levels of uric acid, total protein, albumin, globulin, total leucocytes count and hematocrit, as well as free sphinganine to sphingosine ratio. Blood biochemistry data were processed by 1-way-, whereas flow cytometry data by 2-way-ANOVA (ANOVA) with Bonferroni post hoc test comparing groups, considering each sample as a replicate within each treatment. Results showed that fumonisins had detrimental effects in broilers, resulting in decreased Ht, decreased TLC, increased Alb/Glb and SA:SO values in the blood. The immune response to fumonisins was also clearly demonstrated by a significant effect on the amount of circulating helper T-lymphocytes, regulatory T-lymphocytes and terminally activated cytotoxic T-lymphocytes. The number of circulating monocytes and macrophages was also significantly decreased, showing the immunosuppressive properties of fumonisins. In conclusion, the use of biomarkers clearly demonstrated significant changes in an early stage and hence are a promising tool to be used to evaluate *in vivo* effects generated by mycotoxins on health status and production losses.

Key Words: broiler chicken, mycotoxins, fumonisins, flow cytometry, immunity

256 The effects the fermentation metabolites of Original XPC have on reducing of *Salmonella* Reading using an *in vitro* turkey ceca culture model. Kristina Feye*¹, Peter Rubinelli¹, W. Evan Chaney², JA Byrd², Hilary Pavlidis², and Steven Ricke¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²Diamond V, Cedar Rapids, Iowa, United States.

Salmonella Reading is a profound human health issue facing the turkey industry today, with one death reported as of the time of the production of this paper in the United States between 2018 through the current era (March 2019). The fermentation metabolites of Diamond V Original XPC™ (XPC) have been documented to have anti-*Salmonella* effects in poultry. Ceca were harvested in a commercial turkey processing plant from birds that were fed a diet that did not contain antibiotics or XPC. Using an established *in vitro* ceca culture method at the University of Arkansas, a total of 2 trials, with 5 ceca per trial and 2 technical replicates per ceca, were tested with and without XPC for its effects on reducing *Salmonella* Reading. XPC was included at 1% (wt/vol) in a commercial grower feed. The treatment groups were: XPC alone, XPC+Ceca, XPC+Ceca (inoculated), control, control (inoculated). For the inoculated groups, after the cultures were allowed to pre-adapt, they were individually inoculated with a commercial turkey field isolate of *Salmonella* Reading or *Salmonella* Typhimurium (ST97; 1e8 cells/mL for both isolates). Samples were taken for microbial and microbiome analysis at 24 h and 48 h post-inoculation. Using linear regression, *Salmonella* load and prevalence was reduced by 24 and 48 h, with no difference between the tested serovars ($P < 0.05$; significance for all results). Therefore, without an effect of serovar, the *Salmonella* treatments were analyzed together and it indicates that effects of XPC are not serovar specific in turkeys. For detecting the percent recovered *Salmonella* within the limit of detection, the main effect of treatment was significant ($P < 0.05$), with XPC having 5-fold fewer positive plates at 24 h and 2.8-fold at 48 h as compared with the controls. *Salmonella* load decreased 5.29 Log₁₀ by

24 h, and that reduction is 2.15 Log₁₀ cfu/mL greater than the control ($P < 0.05$). By 48 h, XPC groups had an additional 1.75 Log₁₀ cfu/mL compared with the 24 h time point. Compared with the control, there was a 1.78 Log₁₀ cfu/mL reduction. Therefore, XPC is able to reduce *Salmonella* load and prevalence irrespective of the serovar tested in the in vitro turkey ceca model. As a result, XPC reduces *Salmonella* irrespective of serovar and provides evidence to suggest that implementing XPC proactively as a component of food production proactively has the potential to reduce *Salmonella* in turkeys.

Key Words: microbiome modulating, *Salmonella* Reading, XPC, chicken, food safety

257 Myofiber-type switching, and dysregulation of lipid metabolism accompanies development of wooden breast myopathy in commercial broiler chickens. Michael Babak and Behnam Abasht*, *University of Delaware, Newark, Delaware, United States.*

Several studies have been conducted to decipher the occurrence of Wooden Breast (WB), a meat quality defect in commercial broiler chickens. Prior transcriptomic studies conducted in our laboratory have shown evidence of myofiber-type switching and dysregulation of lipid metabolism associated with the development of WB. To gain a deep understanding of the occurrence and implication of these 2 biological processes in health and disease states, we examined the expression of LPL and PLIN1 (lipid-related genes) and CSRP3 and MYBPC1 (muscle-related genes) using RNA in situ hybridization on Legacy (18 d post-hatch) and Ross chicken lines (20 d post-hatch). Subsets comprising Legacy, unaffected Ross and WB-affected Ross ($n = 3$ each) targeting the 4 genes was used. Expression of LPL in the pectoralis major muscles of healthy chickens was observed in the endothelial cells of capillaries and small-caliber veins. Conversely, affected chickens revealed enhanced expression of LPL on the same sites as well as in adipocytes. PLIN1 was only expressed by affected chickens in adipocytes. MYBPC1 expression was intermittently distributed along the length of myofibers in unaffected, and homogenous in affected chickens. While CSRP3 did not show expression in Legacy chickens, the expression was multifocal in affected chickens. Additionally, CSRP3 was always co-expressed with MYBPC1, but not the other way. To our knowledge, this is the first study to show expression of LPL from the vasculature endothelium in chickens. This study also confirms the existence of myofiber-type switching and provides mechanistic insights into increased lipid uptake and metabolism in WB disease process.

Key Words: wooden breast, myopathy, commercial broiler chickens, LPL, CSRP3

258 Effects of dietary vitamin E and selenium supplementation on performance, immune response and oxidative status of broilers under heat stress. Ali Calik, Mallory White*, Nima Emami, and Rami Dalloul, *Virginia Tech, Blacksburg, Virginia, United States.*

High ambient temperature is one of the most common stressors in modern poultry production, resulting in reduced feed intake and weight gain, and increased mortality. This study evaluated the effects of Vitamin E (Vit E) and selenium (Se) supplementation on performance, rectal temperatures, and expression of heat shock proteins (HSPs), immunity and oxidative stress related genes in liver tissues of broilers exposed to daily 4-h elevated temperature during d 28–35. A total of 640 Cobb500 male birds were randomly allocated to 32 floor pens in a 2 × 2 factorial arrangement that included heat stress (optimal and high heat) and dietary treatments (basal diet or Vit E + Se). Vit E and Se were added

to the basal diet at the rate of 250 mg/kg and 1 mg/kg diet, respectively. For the heat stress groups, temperature was maintained at 35 ± 1°C and applied once daily from 10 a.m. to 2 p.m., then reduced again to 25 ± 1°C for the remainder of the day. All chicks were weighed on a per pen basis and feed intake (FI) was recorded on d 7, 14, 21, 27, and 35. Liver samples were taken on d 28 and 35 (2 birds/pen) from birds of average pen weight for qPCR analysis. The birds' (2 birds/pen) rectal temperature was monitored daily with a rectal probe during the heat stress period. Data were subjected to a 2-way ANOVA using the GLM procedure of JMP (Pro13). During the heat stress period, birds fed the diet containing Vit E and Se had significantly lower mortality (0.97% vs 3.57%) compared with control birds. Moreover, dietary supplementation of Vit E and Se significantly increased body weight gain (BWG) and FI during the overall experimental period. Heat stress significantly upregulated HSP response on d 28 and d 35. However, expression of HSP70 and HSP90 was significantly ($P < 0.001$) downregulated with Vit E and Se treatment on d 28 and 35. Expression of the oxidative stress related genes GPx and GST were significantly downregulated by Vit E and Se supplementation on d 28. However, no differences were observed in CAT, GPx, SOD, and GST expressions on d 35. Expression of TLR2, TLR4, TNF α , IFN γ , IL-1 β , IL-4, IL-10, and iNOS was not affected on d 28. However, gene expressions of TLR2, TNF α , IFN γ , IL-1 β , IL-10, and iNOS were significantly downregulated in birds fed the diet containing Vit E and Se on d 35. Also, the heat stress challenge significantly increased broiler body temperature during d 28–35. In conclusion, dietary supplementation of Vit E and Se might help alleviate the negative effects of high ambient temperature and improve broiler performance by influencing oxidative and immune related gene expression in the liver during heat stress challenge.

Key Words: broiler, heat stress, heat shock proteins, immune response, vitamin E & selenium

259 Effect of different biotic additives on noiler chickens at chick phase. Kemi Idowu¹, Olajide Adeyemi¹, Olajide Sogunle¹, and Solomon Idowu^{*2}, ¹*Federal University of Agriculture, Abeokuta, Abeokuta, Ogun, Nigeria,* ²*Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Oyo, Nigeria.*

Overtime, the amount of antibiotic used as growth promoters in live-stock industry as drastically increased. This has led to consumer and governmental efforts to reduce the administration of antibiotic in poultry production. Therefore, alternatives to the use of antibiotics must be found to enhance the growth performance, and bring out the genetic potential of the modern day chicken. Probiotic, Prebiotic, and Synbiotic are alternative sources that can improve the immune status of poultry. The focus of alternative antibiotic growth promoters (prebiotic, probiotic, and synbiotic) is to enhance the performance and health status of chicken. Noiler chicken is a hybrid of broiler and cockerel. It has the resistance traits of cockerels and meat characteristics of broilers. A total of 3 hundred and 60 d old Noiler chickens were randomly distributed into 12 treatments with 3 replicates of 10 birds each. Experimental treatments consisted of 4 different growth promoters (probiotic, prebiotic, synbiotic and antibiotic) at 3 dosage inclusion rates (0, 0.5 and 1g) into kg of feed respectively. The experiment was laid out in a 4 X 3 factorial arrangement of Completely Randomized Design. Data were collected on the growth performance characteristics and histopathology of liver. There were significant ($P < 0.05$) differences in final weight and average weight gain, across all the biotic treatments. The highest final weight (480.00g) and total weight gain (440.00g) were recorded for synbiotics treatment followed by antibiotic treatment that had (455.56g) and (415.18g) for average total final weight and average weight gain while

(428.88g; 388.66), were recorded for prebiotic and (400.00g; 353.05g) for probiotics respectively. Feed intake was significantly ($P < 0.05$) influenced differently across the treatments. Livability across the treatments was hundred percentages throughout the chicks phase. The study concluded that inclusion of symbiotic improved the weight of Noiler chickens at 1g/kg of dosage inclusion while (antibiotic) oxytetracycline tetrahydrate has a deleterious effect on the liver of Noiler chickens.

Key Words: histopathology, prebiotic, probiotic, additives, antibiotic

260 Effect of hatchery management processes on the gastrointestinal microbiota of broilers. Josh Rehberger¹, Theresia Lavergne*², Samantha Anderson¹, Rebecca Wujek¹, Eric Vang¹, Jodi Delago¹, and Alexandra Smith¹, ¹Arm & Hammer, Waukesha, Wisconsin, United States, ²Arm & Hammer Animal and Food Production, Princeton, New Jersey, United States.

To break vertical transmission of pathogenic bacteria from hen to chick, commercial hatcheries employ various strategies; fumigation of eggs with antimicrobials, use of in ovo vaccinations, as well as single stage hatchery incubators which allow for more thorough cleaning to prevent contamination across batches of set eggs. While these strategies are often effective, they might also interfere with vertical transmission of beneficial bacteria that may colonize the intestine of the young bird. The microbiota of egg contents and shell washes was studied to determine the effect of incubation stage (multi vs single) and vaccination practice (in ovo vs unvaccinated) on the colonization of broiler gastrointestinal tracts. Ten eggs were collected at placement into the hatchery to determine the microbiota of the contents and the exterior surface. Before in ovo vaccination and transfer from the setter to the hatcher, 10 eggs each were collected from a single and multi-stage incubator. Twenty 4 h after vaccination and placement, the exterior of 20 eggs, 5 eggs per treatment, were swabbed and the gastrointestinal tract of the in ovo bird was dissected. At day of hatch the gastrointestinal tract of 40 chicks, 10 from each treatment combination were collected. During grow out, at d 3, 6 and 13 post-hatch, gastrointestinal tracts of 20 broilers, 5 of each treatment combination, were collected. *Escherichia coli*, clostridia and lactic acid bacteria were enumerated for each sample. Multiplex PCRs for toxin/virulence genes were performed on all *E. coli* and clostridia isolates to determine potential pathogenicity. Bacterial DNA was extracted from each sample to amplify 16S rRNA for Illumina sequencing. *E. coli* counts at day of hatch were 5×10^5 cfu/g and were consistent through the growth period. Avian pathogenic *E. coli*, however, were 3×10^5 cfu/g at day of hatch but declined to 2×10^2 cfu/g over time. Clostridia were detected in some in ovo birds in pre- and post-transfer eggs, but spiked to 1.2×10^4 cfu/g at day of hatch before decreasing to 7.8×10^1 cfu/g. Microbiota analysis indicates that both incubation stage ($P < 0.05$) and in ovo vaccination ($P < 0.05$) affected the gastrointestinal population post-transfer and at day of hatch. This evidence demonstrates that single- versus multi-stage incubation as well as in ovo vaccination affects the gastrointestinal microbiota of broilers.

Key Words: vaccination, incubation, microbiota, illumina

261 Effect of different 16S ribosomal RNA primers on taxonomic composition and microbiome diversity in chicken. Monika Proszkowiec-Weglarz*¹, Nadia Darwish^{1,2}, Lori Schreier¹, Jonathan Shao¹, and Katarzyna Miska¹, ¹USDA-ARS, Beltsville, Maryland, United States, ²N.D. is supported by the Research Participation Program at the USDA, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between

the U.S. Department of Energy and the USDA, Beltsville, Maryland, United States.

Gut microbiota and the integrity of the gastrointestinal tract play important roles in nutrient absorption, immune system development, and disease resistance. Alterations in microbiome homeostasis may lead to a negative effect on feed efficiency, productivity, and health in chickens. Sequencing of nucleic acids is the most common method used to determine taxonomic composition and bacterial abundance. The method relies on amplifying and sequencing the 16S ribosomal RNA (rRNA) gene with specific 16S rRNA primers. The aim of this study was to determine the effect of using different 16S rRNA primers on taxonomic composition and bacterial community diversity. Cecal digesta collected from Ross 708 birds at 1, 3 and 5 weeks of age ($n = 4$) were used for bacterial DNA isolation using PowerSoil kit (Qiagen). Eight different, previously published, primer pairs targeting V1-V3 (2 different primer pairs), V3, V4, V3-V4, V4-V5, V3-V5 and V4-V6 variable region of the 16S rRNA were employed. Libraries were constructed using Nextera XT (Illumina) kits following Illumina protocol and sequenced using 600 bp v3 kit on MiSeq (Illumina). DNA sequences were analyzed using open source platform Qiime2 and Greengenes database for α and β diversity, and taxonomic composition. Differences in bacterial relative abundance due to primer set were analyzed using SAS. Average PCR amplicon size ranged from 315 bp (V3) to 769 bp (V4-V6). Alpha-diversity indexes (Evenness, Richness, Observed OTU and Shannon) were significantly ($P < 0.01$) affected by the primer sets. The V3 primer set was characterized by the lowest Evenness but the highest Richness ($P < 0.01$). The lowest Observed OTU and Shannon index were detected for V4 primer set. Beta diversity analysis based on Bray-Curtis, Jaccard, and Unweighted and Weighted UniFrac distance matrix showed separation of bacterial communities based on the selection of the primer set. Bray-Curtis, Jaccard and Unweighted UniFrac showed 4 different clusters of bacterial communities while Weighted UniFrac showed the microbiome clustered in 3 distinct populations. Significant differences in microbial communities due to primer set were also confirmed by PERMANOVA analysis ($P < 0.01$). Taxonomic composition was similar between primer pairs at the phylum, class, order, and family level but differed at genus and species level depending on primer set used. Primers targeting the V3 region (V3) detected the lowest number of genera and species. Relative abundance of bacteria at all taxonomic levels was significantly ($P < 0.05$) affected by the choice of primer pairs. Obtained results strongly suggest that the choice of 16S rRNA primers can influence microbiome analysis and data interpretation.

Key Words: microbiome, cecum, 16S rRNA, primers, chicken

328 Effects of supplemental butyrate on gene expression in the intestinal tissues of broiler chickens during *Eimeria maxima* infection. Victoria Hansen*¹, Monika Proszkowiec-Weglarz¹, Stefan Vaessen², and Katarzyna Miska¹, ¹USDA Agricultural Research Service, Beltsville, Maryland, United States, ²Perstorp Waspik BV, Waspik, NH, Netherlands.

Coccidiosis is one of the most prevalent gastrointestinal diseases seen in the poultry industry leading to excessive economic losses. Over the last several years poultry producers have begun to look for substitutes to chemotherapeutic agents to meet the demand for chicken raised without antibiotics. The addition of the short chain fatty acid butyrate has been previously reported to mitigate performance loss in chickens during coccidiosis. We hypothesized that addition of tributyrin in the form of glycerol esters of butyric acid can improve performance of *Eimeria maxima* infected broilers and change the expression of genes associated with gut performance and immune response. Ross 708 male broilers

were fed starter diet supplemented with 0 or 0.25% triglycerides of butyric acid (ProPhorce SR 130, Perstorp) from day (d) 1. On d 21, half of the birds were infected via oral gavage with 10^3 *E. maxima* oocysts. Experimental groups consisted of birds which were either infected or not infected, consuming normal or supplemented diet (n = 6/group). Tissue samples from the jejunum, ileum, and ceca were collected 7 and 10 d post-infection (PI), and RNA was extracted followed by cDNA synthesis. Relative gene transcription levels for aminopeptidase ANPEP, small nutrient transporters SLC16A1 and SLC5A8, tight junction protein ZO1, immune cytokines IL1B and IL12, and antimicrobial peptide LEAP2 were measured by quantitative polymerase chain reaction (qPCR) and normalized to 2 reference genes, ubiquitin (UB) and β -2 microglobulin (B2M). Statistical analyses for gene expression were performed using 3-Way ANOVA with Holm-Sidak correction for multiple comparisons. At 7 d PI butyrate-supplemented infected chickens showed higher weight gain and lower FCR than those eating normal diet. However, these dif-

ferences were not significant by 10 d PI. The majority of differences in gene transcription observed were between infected and non-infected samples. Consistent with previous findings, IL1B was upregulated in both ileum and jejunum ($P < 0.05$) and LEAP2 was downregulated in ileum ($P < 0.001$) and jejunum ($P < 0.001$) for infected samples. ANPEP was downregulated in butyrate-supplemented ileum ($P < 0.001$) and also infected ileum and jejunum samples ($P < 0.05$). For both ileum and jejunum SLC16A1 was significantly upregulated ($P < 0.05$) and the effect was even more pronounced on 7 d PI infected samples ($P < 0.001$). We conclude that short chain fatty acids may be a promising feed additive for broiler chickens infected with coccidia, but the downstream cellular mechanisms responsible for helping to protect the chicken gut are still unknown.

Key Words: chicken, immune response, nutrition, butyrate, Eimeria

Management and Production

262 Synthetic allicin on digestive organs weight in broiler chickens. Artemio Vargas-Galicia¹, Raúl Argüello-García⁴, Arturo Pro-Martínez¹, Fernando González-Cerón*², Lucía Martínez-Gómez², Eliseo Sosa-Montes², Anally Mata-Estrada¹, Leodan Rodríguez-Ortega³, Alejandro Rodríguez-Ortega³, and Jaime Gallegos-Sánchez¹, ¹*Colegio de Postgraduados, Campus Montecillo, Estado de México, Texcoco, Mexico*, ²*Universidad Autónoma Chapingo, Estado de México, Texcoco, Mexico*, ³*Universidad Politécnica de Francisco I. Madero, Hidalgo, Tepatepec, Mexico*, ⁴*Centro de Investigación y Estudios Avanzados, Instituto Politécnico Nacional, Ciudad de México, Mexico*.

Supplements of garlic in broiler diets have been recognized for their stimulating effect on the digestive system in birds. The current study was conducted to evaluate the effect of synthetic allicin on digestive organs weight (liver, spleen, pancreas, proventriculus, gizzard, small intestine and ceca) in broilers. Nine hundred 1-d-old mixed broiler chicks "Ross 308" were distributed among 3 treatments: 0, 1 and 2.5 mg of allicin/kg of BW (0-ALLI or control, 1-ALLI and 2.5-ALLI, respectively); 10 replicates per treatment were used. Synthetic allicin was administered by oral-esophageal via using a pediatric catheter. Treatments were randomly assigned to the experimental groups and were administered daily at the same time from d 14 to d 27. At 28, 35 and 42 d of age, 20 broilers per treatment with body weight close to the average of the pen were selected, weighed and slaughtered for evaluation of digestive organs weight. Data were analyzed as a 3 × 3 factorial arrangement (treatments and age) using the MIXED procedure of SAS. Results are reported as g/kg of BW. It was found that only liver weight was affected by treatment; broilers in the 2.5-ALLI group (22.85 ± 0.49a) showed higher ($P < 0.05$) liver weight than broilers in the other groups (0-ALLI: 21.80 ± 0.49b and 1-ALLI: 21.66 ± 0.49b). Relative weight of spleen increased ($P < 0.05$) with age (28 d: 0.86 ± 0.05b; 35 d: 1.21 ± 0.05a and 42 d: 1.31 ± 0.05a). Additionally, it was observed a reduction in proventriculus (28 d: 3.78 ± 0.09a; 35 d: 3.52 ± 0.09b and 42 d: 2.96 ± 0.09c), gizzard (28 d: 13.76 ± 0.39a; 35 d: 12.80 ± 0.39b and 42 d: 11.39 ± 0.39c), small intestine (28 d: 21.02 ± 0.47a; 35 d: 21.92 ± 0.47a and 42 d: 19.07 ± 0.47b) and ceca (28 d: 3.12 ± 0.95a; 35 d: 3.06 ± 0.95a and 42 d: 2.26 ± 0.95b) relative weight as age increased. Pancreas weight was not affected ($P > 0.05$) by treatment or age. In conclusion, administration of synthetic allicin increases liver weight without affect weight of rest of organs.

Key Words: allicin, digestive organs, broilers

263 Effects of incubator tray location and sex on broiler chicken skeletal muscle developmental characteristics and muscle stem cell heterogeneity and mitotic activity. Jessica Starkey*, Oscar Tejada, and Kathryn Meloche, *Auburn University, Auburn, Alabama, United States*.

Hatching egg incubation temperature variations of as little as 0.5°F during from embryonic d 4 to 11, which encompasses both primary and secondary myogenesis, can significantly alter broiler carcass and breast meat yields. The objective was to determine if incubator tray location (LOC) and sex interact to impact broiler muscle development and growth, thus, a randomized complete block experiment with a 3 × 2 factorial treatment structure was conducted. Broiler hatching eggs were obtained from a 40-wk-old commercial broiler breeder flock and incubated in a single stage incubator at a commercial hatchery running a

temperature profile designed to generate a 0.5°F temperature differential among trays placed in the top (TOP), middle (MID), and bottom (BOT) of the racks (n = 4 trays per rack location). From d 4 to 11, average internal egg temperatures were 99.6, 99.9, and 100.1°F in the TOP, MID, and BOT trays, respectively ($P > 0.05$). Chicks hatched from the 3 incubator tray locations (n = 160 per LOC; 80 per sex per LOC) were reared on a common diet in raised floor pens with 1 bird per pen from d 7 to 37. On d 14, 23, 28, and 37, 1 h before *pectoralis major* (PM) sampling, 12 birds per treatment were injected with 5'-bromo-2'-deoxyuridine (BrdU) to label mitotically active (BrdU+) cells. Cryohistology, immunofluorescence staining, and digital fluorescence microscopy was used to estimate PM fiber number and determine the density (cells per mm²), mitotic activity, and heterogeneity of expression of the myogenic regulatory factors (MRF) and muscle stem cell (MSC) markers, MyoD and Pax7. Data were analyzed using the GLIMMIX procedure of SAS (V9.4) and least squares means were separated using the PDIF option at $P \leq 0.05$. No significant LOC × sex interactions were observed. Chicks originating from warmer BOT trays had more muscle fibers than those from cooler MID trays ($P = 0.0176$). Not surprisingly, male (M) broilers had more fibers than females (F; 6,518,329 v. 7,326,538 ± 275,411; $P = 0.037$). On d 14, F broilers had more myonuclei than M (137 v. 107 ± 8; $P = 0.0057$). Density of MyoD+:Pax7+:BrdU+ MSC was greater in M than F on d 23 (19.6 v. 13.6 ± 1.8; $P = 0.0224$) and 28 (27.5 v. 17.2 ± 2.5; $P = 0.0052$). On d 23, 28, and 37, density of MyoD+:BrdU+ MSC was greater in M than F ($P \leq 0.0447$). Chicks incubated on BOT and MID trays had greater MyoD+:Pax7+:BrdU- MSC density on d 23 (9.1 v. 8.6 v. 4.9 ± 1.3; $P = 0.0445$) and 28 (10.3 v. 7.5 v. 5.4 ± 1.0; $P = 0.0028$) compared with those from TOP trays. Combined, these data suggest that incubator tray LOC and sex both impact muscle development and aid in explaining the mechanisms behind why M and BOT tray broilers in our previous work had greater breast meat yields.

Key Words: incubation temperature, muscle development, satellite cell proliferation, myogenic regulatory factor, broiler chicken

264 The impact of cleaning and disinfection of poultry house on broiler performance. Agabus Patu*, Jacob Hamidu, and Maxwell Okai, *Kwame Nkrumah University of Science and Technology, Kumasi, Ghana*.

The study evaluated the impact of broiler house degree of cleaning and disinfection on growth performance and microbial status. The degrees of cleaning included (dry cleaning only (T1), dry and wet cleaning with ordinary water (T2), dry and wet cleaning with detergent (T3), dry and wet cleaning with detergent and disinfection (T4), and dry and wet cleaning with detergent, disinfection and whitewashing (T5). A total of 375 Ross 308 broiler chicks were randomly allocated to each of the treatment and reared for 8 weeks. Each treatment was replicated 3 times with 25 birds each. Data recorded included feed intake, weight gain, feed conversion ratio (FCR), liveability and microbial load. Data were analyzed as one-way ANOVA using the Proc. GLM SAS® at $P \leq 0.05$. The bacterial counts were analyzed as log₁₀ cfu and ANOVA obtained my GLM. Birds from T5 had higher feed intake than birds from T1 and T2 for the period of 1–28 d but none was different from T3 and T4 ($P = 0.04$). The broiler weight gain per bird tended to be different at 49 d ($P = 0.07$) and increased with the degree of cleaning. The FCR up to 49 was lower in T4 compared with T1 ($P = 0.022$). The weekly mortality reduced with increasing degree of cleaning ($y = 1.24x^2 - 13.70x + 46.95$; $R^2 = 0.97$). Similarly, bacterial load in the litter also reduced ($y =$

$-0.99x + 8.39$, $R^2 = 0.82$ for total viable count, and $y = -0.92x + 7.64$, $R^2 = 0.82$ for *E. coli* count). It can be concluded that complete cleaning of the pens up to the application of disinfection increased broiler performance and there may not be a need to include whitewashing of the pen walls before broiler chick placement.

Key Words: cleaning, disinfection, broilers, weight gain, microbial load

265 The dose-response of phytogetic feed additives (PFA) in the starter diet phase of broilers challenge with a virulent coccidia live vaccine. Francisco Dias*², Stacie Appleton¹, and Jan Dirk van der Klis², ¹*Delacon USA, Inc., Carlisle, Pennsylvania, United States*, ²*Delacon Biotechnik GmbH, Steyregg, Austria*.

The most common challenges affecting commercial broiler gut health are coccidiosis, necrotic enteritis, and bacterial enteritis. In the past, achieving production goals under challenge conditions relied largely on the use of antimicrobial growth promoters (AGPs). However, because of legislation against the use of antibiotics (AB) as AGPs and the increasing consumer demand for AB-free animal production, the poultry industry is seeking alternatives. The objective of this study was to evaluate the efficacy of Biostrong® Protect (BP: a mixture of essential oils from the Myrtaceae and Asteraceae plant families and saponins) supplemented to broiler feed at graded dose levels up to 28 d. This dose-response experiment was conducted at Southern Poultry Research Station in Athens, GA, USA. 1,500 one-day-old male broiler chickens (Cobb 500) were randomly distributed over 5 treatments with 12 replications of 25 birds each. All experimental chickens were vaccinated with an unattenuated live vaccine (Coccivac-B52, MSD Laboratory) with virulent oocysts of *E. acervulina*, *E. mivati*, *E. maxima* and *E. tenella*. The vaccine was used at label recommended dose at the hatchery. After arrival, birds were allotted to the experimental treatments, which enabled determination of the dose-response relationship of BP (250, 375, 500, and 1000 g/MT). Basal diets were formulated to meet the nutritional requirements of broilers from 1 to 42 d of age in *ad libitum* feeding program. Weight was measured at 14, 28 and 42 d of age and body weight gain (BWG) and feed conversion ratio (FCR) per period. Mortality rate was calculated over the entire experimental period. Data were analyzed using PROC GLM of SAS. In this trial, similar ($P > 0.05$) results of body weight gain and mortality among treatments were observed. However, from 28 d onwards, a dose level of at least 400g/MT BP significantly ($P < 0.05$) improved FCR over the unsupplemented control. The maximum effect was obtained at an inclusion of 550 g/MT BP, reducing FCR from 1.70 to 1.63 for the entire 42d period. Additional studies have indicated that this effect of BP was related to its anti-inflammatory effect and quorum sensing inhibition effects. It was concluded that 375 g/MT Biostrong® Protect improved production performance after a coccidiosis vaccination.

Key Words: dose response, Biostrong Protect, phytogetic, broilers, coccidia

266 Productive performance and growth pattern of local chickens raised under confined production system in Campeche State, Mexico. Anely Mata-Estrada³, Fernando González-Cerón*¹, Arturo Pro-Martínez³, Artemio Vargas-Galicia³, Glafiro Torres-Hernández³, Jaime Bautista-Ortega⁴, Eliseo Sosa-Montes¹, and Carlos M. Becerril-Pérez², ¹*Universidad Autónoma Chapingo, Estado de México, Texcoco, Mexico*, ²*Colegio de Postgraduados, Campus Veracruz, Xalapa, Veracruz, Mexico*, ³*Colegio de Postgraduados, Campus Montecillo, Texcoco, Estado de México, Mexico*, ⁴*Colegio de Postgraduados, Campus Campeche, México, Campeche, Mexico*.

Raising local chickens (LC) is an activity that contributes to the household livelihood security of rural families of Mexico and other developing countries. It is mainly carried out by housewives. The aim of the present study was to characterize the productive performance [hatching weight, body weight (BW) at 25 weeks of age and BW gain from 0 to 25 weeks] and compare the Logistic and Richards growth models for determining the best one that fits the data of LC. This study was conducted at the experimental chicken house of the Colegio de Postgraduados-Campus Campeche, Mexico, from April to September 2017. A total of 99 chicks (43 females and 56 males) were obtained by random mating of 24 cocks and 76 hens collected from backyards of rural communities of Campeche State. LC were raised under confined production system. Chickens were fed with a starter diet (from hatching until 3 weeks of age) containing 19.0% crude protein (CP) and 3,000 kcal of metabolizable energy (ME)/kg and a grower diet (from 4 to 25 weeks of age) containing 18.0% CP and 2,800 kcal ME/kg. All LC were weighed weekly from hatching until 25 weeks of age. Productive performance was analyzed using PROC TTEST and the growth models were fitted using the PROC NLIN of SAS software. Growth models were compared using determination coefficient (R^2), Akaike' information criterion (AIC) and Bayesian information criterion (BIC). The results showed that hatching weight was similar ($P = 0.4466$) between females and males (35.9 ± 0.6 g and 36.7 ± 0.8 g, respectively); however, BW at 25 weeks of age was different ($P = 0.0001$) between sexes: $1,631.1 \pm 48.5$ g for hens and $2,365.5 \pm 53.8$ g for cocks. Additionally, females showed lower ($P = 0.0001$) BW gain ($1,595.2 \pm 48.4$ g) compared with males ($2,328.8 \pm 53.7$ g). Richards (R^2 : 0.9382 and 0.9415; AIC: 2,226.1 and 2,426.5; BIC: 2,238.6 and 2,439.2; for females and males, respectively) was the best model that describes the growth pattern of LC. The maximum relative growth was 0.0068 g/days for females and 0.0065 g/days for males. Hens and cocks reached maximum growth at 54.4 and 66.3 d of age, respectively. The asymptotic weight was higher in males than in females (2,875 and 2,012 g, respectively). In conclusion, LC males are heavier and had higher BW gain than LC females and the best fit of the data was obtained with the Richards growth model.

Key Words: local chicken, growth curves, productive performance, body weight gain, Campeche State

267 Evaluation of the combined effects of moderate house relative humidity and increased air movement on litter moisture and water activity in a commercial broiler house. Iskender Yildirim^{1,2}, Connie Mou*¹, Michael Czarick¹, and Brian Fairchild¹, ¹*University of Georgia, Athens, Georgia, United States*, ²*University of Selcuk, Konya, Turkey*.

Recent studies suggest that in addition to litter moisture, water activity (A_w) can be a good if not better indicator of microbial activity in broiler litter. Previous research in this lab has found that the provision of increased air movement at floor level with the use of "high-volume" circulation fans at an average speed of 0.76 m/s while also maintaining a moderate house relative humidity (Rh) (<60%) can result with lower litter moisture content (MC %). A secondary study was conducted to whether this combination could have an effect on water activity (A_w) of litter conditions in addition to MC %. Two field studies were conducted on 2 commercial broiler farms. On each farm, 2 12×152 m houses were used. On Farm 1, one house had no circulation fans operating (CTL) while an adjacent house was equipped with 8- 60 cm 350 W circulation fans that ran continuously (TRT). These fans were capable of circulating up to 25% of house volume each minute, providing an average air speed at floor level of 0.76 ± 0.03 m/s. On Farm 2, one house had 8- 45 cm 125 W low volume circulation fans (LVCF) (0.28 ± 0.14 m/s) while

the adjacent house had 8- 60 cm 350 W high volume circulation fans (HVCF) (0.76 ± 0.03 m/s). Both houses had fans running continuously. A_w was measured using the AquaLab® dewpoint water activity meter (model 4TE, Decagon Devices Inc., Pullman, WA, USA—measurement range 0.030–1.000 A_w , accuracy $\pm 0.003 A_w$, repeatability $\pm 0.001 A_w$). In both studies, weekly MC % and A_w were collected, measured, and analyzed. For each farm, the study was repeated over 3 flocks. From flock to flock on Farm 1, providing continuous air movement on average resulted with drier litter than CTL house (25% vs. 33%). In addition, A_w levels were lower in TRT vs. CTL (0.83 ± 0.01 vs. 0.90 ± 0.02) indicating that increased air movement may benefit with not only drier litter but also reduced microbial challenge. Similarly on Farm 2, higher air movement had a beneficial effect on litter conditions. Litter was drier in HVCF house vs. LVCF throughout the flock (23% vs. 29%). Like Farm 1, drier litter in the house with increased air movement resulted with lower A_w levels (0.75 ± 0.02 vs. 0.81 ± 0.02). Further analysis using a standard polynomial regression showed A_w levels increased non-linearly as MC % increased (MC % of 18% to 21%, A_w levels increased non-linearly from 0.81 to 0.85). This study demonstrated that the combined use of increased air movement (0.76 ± 0.03 m/s) and maintaining a moderate house Rh level (<60%) can result with drier litter and a reduction in A_w levels.

Key Words: litter management, water activity, air movement, bird health, bird welfare

268 Effects of using dry hydrogen peroxide in a commercial hatchery on hatchery performance. Brian Jordan*¹, Laura Oxford¹, Julia McElreath¹, Nelson Cox², Miguel Barrios³, and Jeanna Wilson¹, ¹The University of Georgia, Athens, Georgia, United States, ²USDA-ARS, Athens, Georgia, United States, ³Synexis Biodefense, Kansas City, Missouri, United States.

Bacteria in a commercial broiler hatchery can be detrimental to chick health and hatchery performance. Even though sanitation and disinfection occur routinely, a method of continual sanitation could be a valuable tool for commercial hatcheries. A commercially available product, gaseous dry hydrogen peroxide (DHP) from Synexis Biodefense (<https://synexis.com>) has been proposed for this purpose. Preliminary studies have shown that DHP is effective at reducing microbial load on hatching eggs and does not negatively impact hatchability or chick health. Therefore, the purpose of this study was to evaluate the effects of DHP on bacteria levels and hatchery performance in a commercial hatchery setting. For this trial, a hatchery with 2 identical sides was used. Half of the hatchery was treated with DHP, while the other half remained non-treated. Treated areas included an egg cooler, setter hall, hatcher hall, chick processing areas, and vaccine laboratories. The non-treated locations included an egg cooler, setter hall, and hatcher hall. Bacterial loads were measured by total ATP bioluminescence swab samples and static air plates using tryptic soy agar (TSA). Air samples and ATP swabs were collected for 2 weeks before treatment to establish baseline microbial load, then samples were taken bi-monthly, then weekly, then bi-weekly from similar locations on each side of the hatchery for 27 weeks for comparison. Hatchery performance data, chick quality evaluations, and residue breakout data were also collected. Data were analyzed using Prism statistical software with student's *t*-tests or 2-way ANOVA and Sidak's multiple comparisons test. Microbial load testing was affected by a "super clean" performed in the hatchery in wk 5 of the study, but reductions in microbial load as compared with baseline was seen. Chick quality data showed an increase in the percent of chicks with bacteria in the yolk-sac, but a significant decrease ($P < 0.05$) in the percent of chicks with *Aspergillus* in the lungs. Early dead, bacterially

contaminated, and *Aspergillus* contaminated embryos were numerically reduced on the treated side when compared with the non-treated side in residue breakouts. The percent hatch and hatch of fertile were numerically increased (90.2% non-treated vs. 90.9% treated HOF; $P = 0.08$) on the treated side of the hatchery as well. Three-day mortality of chicks hatched from the treated side was also numerically reduced compared with chicks hatched from the non-treated side. Taken together, this study suggests that the DHP product was beneficial as a method of continual sanitation to reduce microbial load, which in turn improved hatchery performance and production in a commercial setting.

Key Words: dry hydrogen peroxide, hatchery, hatch of fertile, microbial load, sanitation

269 A phytogetic feed additive to improve male and female reproductive performance. Jan Dirk van der Klis*¹, Stefan Hirtenlehner¹, and Megan Koppen², ¹Delacon Biotechnik GmbH, Steyregg, Austria, ²Delacon USA, Inc., Carlisle, United States.

Male fertility is of utmost importance for breeder flock performance as it is directly related to the number of day-old chicks per hen housed. One of the key factors for male fertility is sperm quality. Sperm quality is directly affected by the anti-oxidant system in the spermatozooids which reduces the concentration of intracellular free radicals that induce sperm DNA fragmentation and lipid peroxidation in the sperm cell membrane. PhytoGENICS can have direct (via polyphenols) and indirect anti-oxidant effects. The mode of action of the latter is via the Nrf2-pathway upregulating the nuclear anti-oxidant response element (ARE), which results in increased production of anti-oxidant enzymes. On top, essential oils and saponins are known to increase testosterone production. Biostrong® Libido (BSL) is a proprietary blend of essential oils, flavonoids, and quillaja saponins developed to improve male breeder fertility. This experiment was done with Hubbard breeders between 43 and 53 weeks of age, housed in cages. 54 individually housed males per treatment were fed a control diet as such or supplemented with 1.5 lb/t BSL. Males were milked for artificial insemination. Semen quality was evaluated at 43, 48 and 53 wks of age. Females (9 pens per treatment with 24 birds per pen) were inseminated with semen of the control or the BSL group. At 48 and 53 wks of age, a total of 1950 eggs per treatment were collected and set to determine fertility, hatchability and first quality day-old chicks. The number of live spermatozooids was sign. ($P \leq 0.05$) increased by 7.7%. Semen concentration (spermatozooids/ml) was increased by 3.0%. Treatment effects on semen quality were not age dependent. Fertility rate was sign. ($P \leq 0.01$) increased from 85.4 to 89.3% at 48-wks of age and from 83.8 to 88.5% at 53-wks of age. Hatchability rate (as % of fertile eggs) was increased from 93.7 to 95.5% at 53-wks of age and first quality DOC rate (as % of hatched eggs) was increased from 91.6 to 93.6% at 48-wks of age. Treatment effects on non-mentioned ages were not statistically significant. Per 100 eggs set, the number of DOC was increased by 5.1 at 48-wk breeders and by 5.7 at 53-wk breeders via supplementation of male breeder diets. It was concluded that dietary supplementation of male broiler breeder diets with Biostrong® Libido improved semen quality, resulting in higher female reproductive performance.

Key Words: semen quality, fertility, broiler breeder, phytoGENICS, antioxidant

270 Understanding how infrared beak treatment affects the beak tissue and the healing response of brown and white feathered layer pullets. Sarah Struthers*¹, Ashish Gupta², Susantha

Gomis², Eugenia Herwig¹, and Karen Schwan-Lardner¹, ¹University of Saskatchewan, Saskatoon, Saskatchewan, Canada, ²Western College of Veterinary Medicine, Saskatoon, Saskatchewan, Canada.

Despite considerable research examining the impact of infrared beak treatment (IRBT) on the production and welfare of laying hens, it is still not fully understood how IRBT affects the beak tissue during the first few days post-treatment. The objectives of this study were to understand the histology of IRBT-treated vs. untreated beaks during early life (0 to 21 d) and to examine how IRBT affects the beak length and healing response of Lohmann Brown (LB) and Lohmann LSL (LW) pullets. The experiment, a 2x2 factorial arrangement of beak treatment and strain, was performed in a completely randomized design. Pullets (n = 100 per strain) were obtained from a commercial hatchery and 50 chicks per strain were infrared beak treated post-hatch (IR). The remaining chicks for each strain were sham treated controls (C). Chicks were housed in cages from 0 to 21 d (4 per cage; 625 cm² per chick). Feed and water (360° nipple drinkers) were provided *ad libitum*. Presence of beak sloughing was recorded daily from 7 to 21 d. Beak length was measured weekly. Beak samples were collected for histology from 4 pullets per treatment every 2 d starting at 1 d of age. Histology slides were analyzed for re-epithelialization and inflammation and scored on a scale of 0 to 4, with 0 showing no lesions and 4 showing severe inflammation and necrosis. Beak length data were analyzed using PROC MIXED (SAS 9.4) with Tukey's test to separate means. Median histological scores for treated birds only were analyzed as a 1-way ANOVA using PROC NPAR1WAY (SAS 9.4). Differences were significant when $P \leq 0.05$. Sloughing of the treated beak tissue began at 10 d and was complete by 20 d. Beak treatment had an effect on beak length with C pullets having shorter bottom beaks at 1 and 7 d but longer top and bottom beaks at 21 d. LB pullets had longer top and bottom beaks than LW at 1 d but shorter top beaks at 7 d. An interaction was seen between beak treatment and strain for 14 d top beak length with LB IR pullets having longer top beaks than LW IR pullets (7.36 vs. 6.47 mm). Over the 21 d period, C pullets had more beak growth compared with IR (4.38 vs. 0.63 mm for top beak; 3.88 vs. 0.76 mm for bottom beak). LB pullets had less beak growth compared with LW over the 21 d. No differences in histology scores were found between treated LB and LW beaks; however, all treated LB beaks showed complete healing by 21 d while some LW beaks still showed moderate healing and inflammation. Overall, IRBT was effective at reducing beak length and growth post-treatment. The histology results suggest that healing begins as soon as 5 d post-IRBT and that appropriate healing responses occurred in both strains as evident by re-epithelialisation and a reduction in inflammation.

Key Words: Lohmann, histology, beak length, beak treatment

271 Photostimulation in egg production and overall performance of laying pullets under stepwise incremental lighting programme. Adekoyejo Oyegunwa*, Kassim Gbenga, and Odusina Adegoke, *Tai Solarin University of Education, Ijebu Ode, Ogun State, Nigeria.*

An 8 week study was conducted at the teaching and research farm of Tai Solarin University of Education in Ijebu Ode Nigeria to determine the effect of 30 min stepwise increase in lighting for Isa brown point of lay pullets on egg production parameters, performance characteristics and volume of waste generated. A total of 100 Isa brown pullets of 16 weeks of age were randomly divided into 4 treatments in a completely randomized design (CRD). Each treatment was further divided into 6 replicates of 4 birds each. The 4 treatments represent the duration of exposure of the birds to light on a daily basis (12hrs, 14hrs, 16hrs and 18hrs). The birds were transferred from the floor to cage at 16 weeks

of age while 2 weeks were allowed for acclimatization of the birds to the new environment. At 18 weeks of age, the birds were exposed to 30 min stepwise increase in lighting until the maximum number of hours is reached. Treatment 1 which is the control was placed under normal daylight which averaged 12 h while treatments 2, 3 and 4 received 14 h, 16 h and 18 h daily on a weekly 30 min increase. Data collected from the study were analyzed using statistical analysis software (SAS) 1999 while means were separated using the Tukey's studentized range of the same software. Available data show that birds that were reared under 14 h daylight were the first to come into lay at 19 weeks of age. Significant differences ($P < 0.05$) were observed in the feed intake per bird per week across the group. In wk 1, the highest feed intake (530g) was obtained from birds exposed to 14 h of daylight. In wk 8 however, feed intake in birds that received 12 and 14 h of light were similar (844g) but significantly higher than feed intake in birds that received 16 h (776g) and 18 h (792g) respectively. The mean values of body weight, weekly egg production and egg weight were all statistically similar. In conclusion, increased daylight did not improve egg production, body weight gain and age at first egg. However, feed intake was improved by increased daylight while volume of waste generated from birds across the treatments were not affected.

Key Words: photostimulation, pullets, incremental lighting, Isa brown, acclimatization

272 A bi-phasic curve for modeling egg production in molted flocks. Anna Wolc*^{1,2}, Jesus Arango², Ian Rubinoff², and Jack Dekkers¹, ¹Iowa State University, Ames, Iowa, United States, ²Hy-Line International, Dallas Center, Iowa, United States.

Egg production on a flock level can be summarized into several phases determined by biology of individual birds: rapid increase in production reflecting achieving sexual maturity, peak production related to maximum laying potential and gradual decrease in the rate of lay as the birds age. Mathematical models are useful for monitoring flock performance and predicting future records to optimize revenues. The goal of this study was to develop and test a mathematical formula that can accurately describe egg production in molted flocks. To do that the model of Yang et al. (1989) was extended into a bi-phasic form. The new model was tested on performance records of 44 molted flocks and 58 single-cycle flocks with production recorded until at least 90 weeks of age and cumulative egg number until 90 weeks within expected range. Those flocks represented a total of over 9.5mLn layers. The model was implemented using minpack.lm package in R. A very good fit to the real data was observed with average r-square of 0.97 in molted and 0.99 in single-cycle flocks. The difference between the fit of Yang et al. (1989) model and the bi-phasic model can be used to differentiate between single cycle and molted flocks. The bi-phasic model also adequately predicted future records up to 8 weeks in advance; longer periods were not tested because caution is recommended when predicting with regression models outside of the observed range. The newly developed bi-phasic model is an adequate tool for benchmarking egg production data from molted flocks.

Key Words: egg production curve, molt, laying hens

273 Effects of simulated transport on brown strain end-of-cycle hen blood physiology, stress, and behavior. Kailyn Beaulac*, Trever Crowe, and Karen Schwan-Lardner, *University of Saskatchewan, Saskatoon, Saskatchewan, Canada.*

Transport conditions are stressful for poultry, especially for end-of-cycle hens that are already experiencing metabolic stressors. A 5x3x2 factorial arrangement consisting of 5 temperature (T) and relative humidity (RH) combinations (-10°C uncontrolled RH (-10), $+21^{\circ}\text{C}$ 30% RH (21/30), $+21^{\circ}\text{C}$ 80%RH (21/80), $+30^{\circ}\text{C}$ 30%RH (30/30), and $+30^{\circ}\text{C}$ 80%RH (30/80)), 3 durations (D) (4, 8, and 12h), and 2 feather covers (well (WF) and poor (PF)) was used for this study. Brown hens ($n = 540$; 90 WF and 90 PF hens from 3 commercial farms) were housed for a 3–5 d adaptation period. Before exposure, hens were feed restricted (6h) and an initial blood sample was taken. Hens were moved to environmental chambers, crated (target density 54.5 kg/m^2) and exposed to the treatment combinations (tx) above. During exposure, behavior was recorded via infrared video. Post-exposure blood samples were taken (Δ) (calculated) and mortality was noted. Blood samples (3/tx) were analyzed for pH, partial pressure of CO_2 and O_2 (pCO_2 , pO_2), oxygen saturation (sO_2), sodium (Na), glucose (G), hematocrit (Hct), hemoglobin (Hb), and heterophil/lymphocyte ratio (H/L; 5/tx). Data were analyzed as a randomized complete block design via ANOVA (Proc Mixed (SAS 9.4); significance $P \leq 0.05$). Mortality occurred in -10 T/RH, with 12h resulting in higher mortality than 4h ($P = 0.01$). The $\Delta\text{H/L}$ increased in -10 more than 21/30 ($P = 0.01$). The ΔpH was higher in 30/80 than 21/80 and -10 ($P = 0.01$). The decrease in pCO_2 was larger in 30/80 than -10 , 21/30, and 21/80 ($P < 0.01$) and was larger in 12h than 4h ($P = 0.01$). For ΔpO_2 -10 was higher than other T/RH ($P < 0.01$). The ΔsO_2 was higher in -10 compared with 21/30 and 21/80 ($P < 0.01$). The ΔNa was highest in 30/30 and 30/80 compared with -10 and 21/30 ($P < 0.01$) and it rose with longer D ($P < 0.01$). The ΔG , was lowest in -10 compared with other T/RH ($P < 0.01$) and decreased more in PF hens ($P < 0.01$). The ΔHct ($P = 0.02$) and ΔHb ($P = 0.02$) increased in 12 and decreased in 4h. Ptiloerection was higher in -10 PF 4h and -10 WF 8h ($P = 0.01$). WF 30/80 hens panted most, followed by 30/80 PF and 30/30 WF ($P = 0.03$). Hens spent more time motionless in -10 , 21/30, and 21/80, followed by 30/30, then 30/80 ($P < 0.01$). Hens were most active in 30/80 compared with -10 , 21/30, and 21/80 ($P < 0.01$) and in 4 compared with 8h ($P = 0.02$). Hens shivered more in -10 compared with other T/RH and gulped more in 30/80 compared with -10 , 21/30, and 21/80. Rustling was more frequent in 30/80 and 30/30 than 21/30 ($P < 0.01$), in 4 than 8h ($P = 0.04$) and in PF than WF ($P = 0.02$). Cold T (-10) compromises welfare, as indicated by increased mortality. In addition, hens in extreme T for prolonged D exhibit signs of thermal stress as seen by ΔpH , pCO_2 , ΔpO_2 , ΔNa , and behavioral adaptations.

Key Words: feather condition, mortality, thermoregulatory behavior, thermal stress, heterophil/lymphocyte ratio

274 Upper critical ambient temperature of male growing Pekin ducks from 14 to 42 days of age. Ming Xie*, Peixin Sun, Jing Tang, and Shuisheng Hou, *Institute of Animal Sciences of Chinese Academy of Agricultural Sciences, Beijing, China.*

An experiment was conducted to examine the effects of ambient temperature on growth performance and carcass traits of male growing Pekin ducks from 14 to 42 d of age to establish the upper critical ambient temperature for these ducks. A total of 216 14-d-old male White Pekin ducks were divided randomly to 6 environment-controlled chambers with ambient temperature set at 20, 22, 24, 26, 28, and 30°C from 14 to 42 d of age, respectively. In each chamber, 36 ducks were assigned randomly to 6 raised wire-floor pens of 6 birds. The relative humidity

of all chambers was set at 60% during this period and each chamber has similar space size and pen distribution with the same pen size. At 42 d of age, the growth performance and carcass traits of ducks from each pen were measured. Data were analyzed as a completely randomized design using the one-way ANOVA procedure of SAS software with pen used as the experimental unit for analysis and means were compared by using Duncan's multiple comparison procedure when temperature treatment was significant ($P < 0.05$). Broken-line regression was used to the upper critical ambient temperature and this temperature was the inflection point temperature above which the duck response started to change. As ambient temperature increased from 20 to 30°C , the body weight decreased from 3054 to 2321 g/bird, the weight gain decreased from 85.6 to 59.4 g/bird/day, and the feed intake decreased from 236.2 to 169.8 g/bird/day, respectively, but the feed/gain increased from 2.74 to 2.87 ($P < 0.05$). According to broken-line regression, the upper critical ambient temperature during the growing period were 27.4, 27.4, and 26.0°C when body weight, weight gain, and feed / gain were served as response criteria, respectively. On the other hand, the weight of breast meat, leg meat, and abdominal fat decreased as ambient temperature increased and they were declined to minimum when the temperature increased to 30°C ($P < 0.05$). The percentage of breast meat and abdominal fat also showed decreasing response to increasing temperature but leg meat percentage increased as temperature increased and it reached maximum at 30°C ($P < 0.05$). According to broken-line regression, the upper critical ambient temperature during the growing period were 25.5 and 25.6°C when breast meat weight and percentage were served as response criteria, respectively. In conclusion, both growth performance and breast meat of growing ducks were sensitive to increasing ambient temperature and the ambient temperature during the growing period should be kept below 26°C to optimize the growth performance and carcass traits.

Key Words: ducks, temperature, growth performance, carcass trait, heat stress

275 Growth performance and physiological responses of Guinea fowl to different stocking densities in humid tropical environment. Oyegunle Oke* and O. S. Iyasere, *Federal University of Agriculture, Abeokuta, Nigeria, Abeokuta, Ogun, Nigeria.*

Commercialization of guinea fowl in Africa is still in its rudimentary stage and there is a dearth of information on the optimal stocking density of the birds. This study was conducted to evaluate the effects of stocking density on the physiological response and performance of guinea fowl. A total of 240 indigenous guinea fowl were used for this study. The birds were weighed and assigned to different stocking densities of 14, 16, 18 and 20 birds/ m^2 (SD1, SD2, SD3 and SD4, respectively) at 4 weeks of age. Feed and water were provided ad libitum. Data were collected on growth performance, hematochemical parameters, plasma triiodothyronine and rectal temperature of the birds. Data were analyzed by ANOVA with stocking density as the independent variable in a Completely Randomized Design. The results showed that there were no significant differences in the body weight of the birds at the 5th and 6th week of growth with the stocking density. From the 7th to the 13th week, birds stocked at 14 and 16 birds/ m^2 were significantly ($P < 0.05$) heavier than birds stocked at 18 and 20 birds/ m^2 , while at wk 14 and 15, body weight of the birds was in the order 14 birds/ $\text{m}^2 > 16$ birds/ $\text{m}^2 > 18$ birds/ $\text{m}^2 > 20$ birds/ m^2 . Feed conversion ratios of the birds increased

with stocking densities. Heterophil/lymphocyte ratio significantly increased with stocking density. At 7, 8, 11, 12, 13, 15 and 16 weeks, rectal temperature of the birds increased with stocking density. Plasma triiodothyronine of the birds was not significantly affected by stocking density. Most of the organ weights of the birds in SD 1 and SD2 were comparable but higher than those of SD3 and SD4. It was concluded

that stocking density of helmeted guinea fowl higher than 16 birds/m² adversely affected growth performance and welfare of the birds.

Key Words: stocking density, haematology, performance, welfare, guinea fowl

Metabolism and Nutrition: Feed Additives II

276 Effects of a commercial *Macleaya cordata* extract on turkey growth performance under a coccidiosis challenge.

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A study was conducted to evaluate the optimum inclusion level of a standardized blend of isoquinoline alkaloids extracted from *Macleaya cordata* (Sangrovit®, Phytobiotics North America, LLC) in turkeys, under a coccidiosis challenge. A total of 608 d-old Aviagen Nicholas Select females were assigned to 4 dietary treatments with 10 replicate battery cages per treatment (10 or 9 birds/cage), in a randomized complete block design. The feeding program consisted of 2 phases using corn-soybean based diets. Four dietary treatments were evaluated, including 0 (control), 60, 120, and 180 ppm of Sangrovit®. At day of hatch, all poults were orally gavaged with a commercial coccidiosis vaccine at 2X manufacturer's recommendation. Growth performance was evaluated on d 14, 21, and 28. Broken line quadratic ascending (BLQ) and quadratic polynomial (QP) models were used to determine the optimal Sangrovit® dosage which maximizes growth performance (BW and FCR). The sum of squared residuals (SSE) and R-square (Rsqr) were used to compare the models. Results show that BLQ provided lower estimation of the optimal Sangrovit® dosage and Rsqr value while higher SSE compared with QP model. Thus, QP was used to determine Sangrovit® dosage for this data set. The maximum BW and minimum FCR responses were observed at 100.4 and 78.8 ppm of Sangrovit® during 0–14 d of age, respectively. From 0 to 21d, optimal Sangrovit® levels increased to 129.3 ppm for maximum BW and 99.6 ppm for minimum FCR responses, respectively. From 0 to 28d, the maximum BW response was observed at 134.3 ppm while the minimum FCR response was observed at 119.3 ppm of Sangrovit®. These results suggest that the *Macleaya cordata* extract supports the intestinal health of the birds under a coccidiosis challenge and warrants further investigation for larger bird size as suggested by 28 d results.

Key Words: feed additive, isoquinoline alkaloids, turkeys, coccidiosis, plant extract

277 A 35-day evaluation of performance, coccidial lesions, fecal oocyst and Salmonella shedding of broilers exposed to mixed *Eimeria* species and fed diets with or without Dostofarm® Concentrate 500 or salinomycin. Michael Sims*¹, Danny Hooge², and Jess Walls³, ¹VA Diversified Research, Harrisonburg, Virginia, United States, ²Consulting Poultry Nutritionist, Pleasant Grove, Utah, United States, ³Dostofarm GmbH, Hansacker, Westerstede, Germany.

Dietary Dosto Concentrate 500, containing 50% Oregano oil (Dosto) was evaluated against Salinomycin in *Eimeria* challenged birds on a 35 d study. At study initiation, (0 d), 840 newly hatched straight run Ross 308 chicks were placed 30/pen among 7 blocks of 4 pens (28 pens total). The 4 treatments were: 1) basal (CON) diets; 2) Dosto Concentrate 500 at 25 mg/kg (Dosto 25); 3) Dosto Concentrate 500 at 75 mg/kg (Dosto 75); and 4) Salinomycin at 66 mg/kg (SAL). Dosto remained in feeds from 0 to 35d, SAL was removed from feed for 29–35 d. Data were analyzed using randomized complete block design with Tukey's LSD used to separate means when $P \leq 0.05$. Mash feeds were fed (starter, 0–14 d; grower, 15–28 d; finisher, 29–35 d). Used litter was added to each pen on 4 d to simulate top-dressed litter. On 7 d, all birds were

individually gavaged with 1 mL of mixed inoculum containing field strains of *E. acervulina*, *maxima*, and *tenella* (1×10^5 , 1×10^3 , 1.8×10^3 oocysts/mL, respectively). On 22 d, 3 birds/pen were lesion scored (0 normal to 4 severe). On 28 d, drag swab samples were used to determine salmonella shedding. The 35 d BW (kg) were 1.603^b, 1.681^a, 1.676^{ab}, 1.636^{ab} for the CON, Dosto 25, Dosto 75 and SAL groups respectively ($P = 0.052$). Mortality adjusted feed conversion values at 35 d (MAFCV) were 1.745^a, 1.685^b, 1.703^{ab}, 1.721^{ab} for the CON, Dosto 25, Dosto 75 and SAL groups, respectively ($P = 0.012$). The 35 d feed conversion values (1.776–1.731, $P = 0.490$) or mortality % (5.29–9.52, $P = 0.182$) were not affected by treatments. Cecal lesion scores were higher ($P = 0.0002$) for CON group (1.85^a) than for SAL group (0.71^b) with Dosto 25 and Dosto 75 groups (1.29^{ab}, 1.19^{ab}) intermediate. Upper intestinal lesions ($P = 0.104$) ranked highest to lowest were CON, Dosto 75, Dosto 25, and SAL. Total lesion scores were higher ($P = 0.002$) for CON (6.52^a) than for SAL (4.05^b), with both Dosto groups (5.28^{ab}, 5.19^{ab}) intermediate. Fecal oocyst counts per g at 14 d were ($P = 0.033$) 14.33^{ab}, 23.00^a, 9.33^b, 14.67^{ab} for the CON, Dosto 25, Dosto 75 and SAL groups, respectively; counts did not differ at 28 d ($P = 0.415$) or 35 d ($P = 0.828$). Litter swab salmonella cfu/mL were significantly higher ($P = 0.034$) for the CON (91.4^a) and Dosto 25 (96.3^a) groups than the Dosto 75 (17.7^b) and SAL (3.14^b) groups. The following 3 observations from this trial were noteworthy: 1) broilers of the Dosto 25 or 75 groups had cecal lesion scores that were not different from broilers of the SAL group; 2) broilers of the Dosto 25 group had 35 d BW and 35 d MAFCV significantly better than those of the CON group; 3) broilers of the SAL or Dosto 75 groups had fewer colonies (cfu/g) of salmonella found in their pens at 35 d than those of the CON and Dosto 25 groups.

Key Words: broilers, Eimeria, Salmonella, oregano, salinomycin

278 Effects of riboflavin and *Bacillus subtilis* on internal organ development in Ross 708 male broilers with or without coccidial challenge. Wei Zhai* and Sabin Poudel, Mississippi State University, Mississippi State, Mississippi, United States.

Probiotics, including *Bacillus subtilis*, may inhibit the growth of pathogenic bacteria and improve the growth of beneficial bacteria in the chicken intestine. However, some of these beneficial bacteria produce bile salt hydrolase, which breaks down the bile salts, subsequently decreases lipid absorption and impairs production. This study was conducted to test if the addition of riboflavin, as a bile salt hydrolase inhibitor, could improve broilers' growth efficiency by improving probiotic function in improving gut health and internal organ development when coccidiosis is induced. Thirteen male Ross 708 male broilers were randomly placed in each of the 96 pens in 8 replication blocks. The 12 treatments were in a $3 \times 2 \times 2$ factorial arrangement, including 3 levels of riboflavin (0.75, 6.6, and 20 ppm), with or without dietary *Bacillus subtilis* PB6 supplementation, and with or without cocci challenge. All birds were gavaged with a 20 × doses of the commercial cocci vaccine on d 14. Data were analyzed using 3-way ANOVA using Proc GLM of SAS 9.4. The relative organs weight to BW were calculated. Cocci challenge lowered BW on d 27 and 36 ($P = 0.014$, 0.013). On d 27, cocci challenge increased relative weights of proventriculus ($P = 0.001$), spleen ($P = 0.001$), duodenum, jejunum, and ileum (with all $P < 0.0001$). However, the effects of cocci challenge on spleen, duodenum and jejunum were lost on d 36. Cocci challenge decreased ($P = 0.0006$) the relative ileum weight on d 36, which was opposite as compared with d 27. In addition, on d 27, 20 ppm riboflavin lowered the relative

gizzard weight as compared with the 0.75 ppm riboflavin ($P = 0.037$). In birds not challenged with cocci, supplementation of *Bacillus subtilis* lowered the relative gizzard ($P = 0.043$) on d 27. Dietary riboflavin affected relative proventriculus and liver weights differently with different cocci challenge conditions on d 36 ($P = 0.030$ and 0.037). When birds were not challenged with cocci, riboflavin did not affect either proventriculus or liver. However, cocci challenged birds which were fed 6.6 ppm riboflavin had heavier proventriculus than those fed 20 ppm riboflavin; birds fed 0.75 ppm riboflavin had higher liver weight than those fed 20 ppm riboflavin. Among all the 12 treatments, birds fed 0.75 ppm riboflavin and *Bacillus subtilis* and challenged with cocci exhibited the heaviest duodenum weight on d 36 ($P = 0.022$). The results show that the riboflavin and *Bacillus subtilis* may affect the internal organs differently under different gut health conditions. The effect of coccidiosis on internal organ development varies with age, which may be due to the progress of coccidiosis condition in the gut.

Key Words: *Bacillus subtilis*, broiler, coccidiosis, internal organ, riboflavin

279 Impact of supplemented allicin, cinnamaldehyde and *Bacillus subtilis* on macroscopic lesions and performance under a necrotic enteritis challenge. Kim Wilson^{*1}, Corey Farmer², Tom Rijsselaere¹, Brett Lumpkins³, and Greg Mathis³, ¹*Orffa Inc, Athens, Georgia, United States*, ²*Calpis America, Inc., Peachtree City, Georgia, United States*, ³*Southern Poultry Reserch, Inc., Athens, Georgia, United States*.

Necrotic enteritis (NE) continues to be the major disease in the poultry industry in part due to limited use of AGPs. Probiotics are often included as an alternative, though their inconsistent results in the industry and in research remain. The antimicrobial properties of allicin and cinnamaldehyde have long been recognized. Nevertheless, their mode of action and proper application remains undefined. The objective of this study was to evaluate the synergistic effects of allicin and cinnamaldehyde product Alliin Plus (AP; Orffa, Inc.) and probiotic *B. subtilis*C-3102 (Calsporin; Calpis America Inc.) during an NE challenge. One-day-old male broiler chicks were fed dietary treatments (trt) in a randomized complete block design (10 cages/trt; 10 birds/cage). The 8 trt included non-challenge control (NC), challenge control (CC) while the rest of the trt included challenge plus either Calsporin 50ppm (C50), AP 1lb/ton (APH), C50 + AP 0.5lb/ton (C50APL), C50 + APH (C50APH), Calsporin 30ppm + APH (C30APH), or Virginiamycin 22ppm (V). On d 14, all trt were challenged with ~5,000 *Eimeria maxima* oocysts. On d 19–21 all but NC were challenged with *C. perfringens* orally (10^8 cfu/d). All birds were weighed on d 0, 14, 21 and remaining birds on d 28, mortalities were recorded. Feed intake (FI), body weight gain (BWG; g) and FCR were calculated at d 14 and 21. On d21, 3 birds per cage were selected to evaluate the degree of NE lesions on a 0–3 scale. The NE model, CC had 44% mortality, 45% BWG decrease and 44 pts FCR increase relative to NC ($P < 0.05$). Although there was no difference in lesion scores among feed additive trt, the BWG in C50APL and C50APH were most improved relative to CC and V from d 14–21 (166.17 \pm 4.90; 163.63 \pm 3.26; 112.48 \pm 5.67, 136.60 \pm 6.73, respectively) including the FCR (2.31, 2.21, 2.68 and 3.15, respectively) ($P < 0.05$), but never reaching the performance of the NC (BWG 355.81 \pm 9.87; FCR 1.40). The C50 and AP alone had reduced BWG relative to C50APL and C50APH (151.16 \pm 7.12; 141.18 \pm 5.27, respectively $P < 0.05$). Similar performance results occurred for d 0–21 in C50APL and C50APH (BWG: 397.24 \pm 10.91; 386.32 \pm 9.98; CC = 328.69 \pm 14.69 FCR: 1.73, 1.75, CC = 1.93, respectively $P < 0.05$). Interestingly, the mortality in C50APH was reduced by ~50% relative to C50APL (11.3% vs 21.3%, $P < 0.05$) and

only C50APH had improved FI (4.62 kg \pm 0.15 kg vs. PC 3.33 \pm 0.12 kg) and numerical improvement in BWG by 13% relative to CC from d 14–28. These preliminary results suggest the additive effect of Alliin Plus and Calsporin not only reduced the severity of NE, the proper dose may help to improve livability and performance throughout growout. Additional studies on mode of action are suggested.

Key Words: necrotic enteritis, allicin, cinnamaldehyde, probiotic

280 Feeding programs with monobutyryl (SiloHealth® 104), sodium formate (Amasil® NA) and clostridium butyricum probiotic (MiyaGold®) on performance, and small intestine and fecal pathogens when fed to broiler chickens challenged with mild coccidia and clostridium perfringens (Cp). Michael Coelho¹, Peter Ader^{*2}, and James McNaughton³, ¹*BASF Corporation, Humble, Texas, United States*, ²*BASF SE, Lampertheim, Germany*, ³*AHPPharma, Hebron, Maryland, United States*.

The Objective of the trial was to evaluate the efficacy of feeding program combinations of SILOHealth 104 (SH), Amasil Na (NA) and Miyagold probiotic (MG) as an antibiotic (Coban + BMD) replacement, when broilers are reared on built-up litter floor bedding (from 3-previous trials) under a mild coccidia and Clostridium perfringens (Cp) challenge. A total of 4160 Ross 708 birds were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block as the random effect (52 birds/pen x 8 treatments x 10 replications). Birds were blocked by weight and sex. The treatments were T1 = PC, no additive, no Cp; T2 = NC, no additive, Cp; T3 = NC +4/4/0 kg/MT SH+4/4/4 kg NA in starter/grower/finisher; T4 = NC+ 4/4/0 kg SH+0/4/0 kg NA+0/0/0.5kg MG; T5 = NC+4/0/0 kg/MT SH+4/4/0 kg NA+0/0/0.5 kg MG; T6 = NC+4/2.5/0 kg SH; T7 = NC+4/0/0/ kg SH + 0/0.5/0.5 kg/MT MG and T8 = NC+ Coban+50 g/MT BMD. Least significant difference was used to compare means of treatment groups. T1–8 had litter inoculated on D0 with 2,500 oocytes of *E. acervulina* and *E. maxima*/bird, and T2–8 had litter inoculated with 5×10^4 cfu/bird of *E. coli* and 5×10^4 cfu/bird of Cp. Birds and feed were weighed on D0, 17, 28, 35 and 42, and performance parameters were measured for each period. T3–8 had a significantly higher D17 avg body weight gain/bird (BWG) than the NC = P2 (T1 = 534, T2 = 513, T3 = 531, T4 = 527, T5 = 531, T6 = 526, T7 = 528 and T8 = 532 g, $P < 0.05$, respectively. Treatments T3–8 had a significantly lower FCR corrected than the NC (1.187, 1.224, 1.194, 1.197, 1.194, 1.198, 1.198 and 1.193, $P < 0.05$, respectively. Treatments T3–8 had a significantly lower mortality than the NC (1.538, 4.231, 1.731, 2.308, 1.923, 2.308, 2.500 and 1.923%, $P < 0.05$, respectively. Treatments T3–8 had a significantly higher D42 BWG than the NC (2574, 2456, 2536, 2526, 2525, 2494, 2421 and 2555 g, $P < 0.05$, respectively. Treatments T3–8 had a significantly lower FCR corrected than the NC (1.689, 1.748, 1.709, 1.716, 1.715, 1.731, 1.713 and 1.700, $P < 0.05$, respectively. Treatments T3–8 had a significantly lower mortality than the NC (1.957, 6.304, 2.391, 2.826, 3.261, 3.696, 3.913 and 2.609%, $P < 0.05$, respectively; Treatments T3–8 had a significantly lower D28 small intestine clostridium log 10 than the NC (3.468, 4.149, 3.568, 3.673, 3.712, 3.901, 3.759 and 3.793, $P < 0.05$, respectively). In conclusion, monobutyryl, sodium formate, MiyaGold and BMD significantly improved BWG, FCR, mortality and small intestine clostridium vs NC. Monobutyryl, sodium formate and MiyaGold performance and bacteria count were similar to the BMD treatment.

Key Words: monobutyryl, sodium formate, clostridium butyricum, coccidia, clostridium perfringens

281 Sodium formate (Amasil® NA), monobutyryn (Silo-Health® 104) and bacitracin methylene disalicylate (BMD) effect on performance and necrotic enteritis when fed to broiler chickens challenged with mild coccidia and clostridium perfringens (Cp). Michael Coelho¹, Peter Ader*², and James McNaughton³, ¹BASF Corporation, Humble, Texas, United States, ²BASF SE, Lampertheim, Germany, ³AHPharma, Hebron, Maryland, United States.

The Objective of the trial was to evaluate the efficacy of SILOHealth 104 (SH) and Amasil Na (NA) as an antibiotic (Coban + BMD) replacement, when broilers are reared on built-up litter floor bedding (from 3-previous trials) under a mild coccidia and Clostridium perfringens (Cp) challenge. A total of 3,744 Ross 708 birds were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block as the random effect (52 birds/pen x 8 treatments x 9 replications). Birds were blocked by weight and sex. The treatments were T1 = PC, no additive, no Cp; T2 = NC, no additive, Cp; T3 = NC +4/4/4 kg/MT SiloHealth 104 (SH) in starter/grower/finisher; T4 = NC+ 4.0/2.5/1.5 kg/ MT SH; T5 = NC+2.5/1.50/0.75 kg/MT SH; T6 = NC+8/8/8 kg/MT Amasil NA (NA); T7 = NC+2.5 kg SH + 4.0 kg/MT AN/ 2.5 kg SH + 4.0 kg/MT AN/2.5 kg SH + 4.0 kg/MT AN; and T8 = NC+ Coccidiostat+50 g/MT BMD. Least significant difference was used to compare means of treatment groups. T1–8 had litter inoculated on D0 with 2,500 oocytes of *E. acervulina* and *E. maxima*/bird, and T2–8 had litter inoculated with 5*10⁴ cfu/bird of *E. coli* and 5*10⁴ cfu/bird of Cp. Birds and feed were weighed on D0, 14, 21, 35 and 42, and performance parameters were measured for each period. On D21 and 42, 3 birds from each pen were sacrificed and examined and scored on the degree of severity of necrotic enteritis lesions (0–3). D14 avg body weight gain/bird (BWG) (346.39, 331.06, 340.29, 340.07, 336.20, 343.62, 344.38 and 341.37 g, $P < 0.05$, respectively; FCR corrected (1.036, 1.075, 1.051, 1.059, 1.042, 1.040 and 1.047, $P < 0.05$, respectively; mortality (1.07, 4.27, 1.92, 2.35, 1.28, 1.07 and 1.71%, $P < 0.05$, respectively; D21 BWG (789.84, 751.62, 775.16, 775.32, 764.59, 781.57, 787.17 and 777.78 g, $P < 0.05$, respectively; FCR corrected (1.283, 1.334, 1.304, 1.303, 1.317, 1.293, 1.289 and 1.297, $P < 0.05$, respectively; mortality (1.50, 4.91, 2.78, 2.56, 3.42, 1.92, 1.28 and 2.14%, $P < 0.05$, respectively; D42 BWG (2443, 2320, 2395, 2395, 2361, 2415, 2433 and 2409 g, $P < 0.05$, respectively; FCR corrected (1.76, 1.84, 1.81, 1.79, 1.79, 1.81, 1.78, 1.77 and 1.79, $P < 0.05$, respectively; mortality (1.932, 6.763, 3.865, 3.866, 4.589, 2.657, 2.174 and 3.140%, $P < 0.05$, respectively; D21 SI lesion scores (0–3) (0.204, 1.667, 1.019, 1.037, 1.333, 0.778, 0.667 and 0.796, $P < 0.05$, respectively); D42 SI lesion scores (0–3) (0.178, 1.233, 0.656, 0.722, 0.889, 0.556, 0.456 and 0.644, $P < 0.05$, respectively). In conclusion, monobutyryn, sodium formate and BMD significantly improved BWG, FCR and mortality. The monobutyryn and sodium formate improved BWG, FCR, mortality and lesion scores similarly to the BMD treatment.

Key Words: monobutyryn, sodium formate, performance, broilers

282 Efficacy of essential oils in managing coccidiosis in broilers subject to *Eimeria* challenge. Juxing Chen*, Sara Herbstreit, Frances Yan, Vivek Kuttappan, Deana Hancock, and Mercedes Vazquez-Anon, Novus International, Inc., St Charles, Missouri, United States.

Dietary essential oils (EO) are reported to enhance growth performance, modulate immunity and manage coccidiosis of broilers, however, the mechanism behind these benefits remains to be determined. The objective of this study was to evaluate the effect of EO in managing coccidiosis in broilers subject to *Eimeria* challenge. The study consisted of 3 dietary treatments – positive control (PC) without *Eimeria*

challenge, negative control (NC), and EO (NC plus 30 g/ton of EO, encapsulated blend of thymol and carvacrol, NEXT ENHANCE® 150, Novus International Inc.), each with 12 replicates of 11 birds. All birds in NC and EO treatments were orally gavaged with a coccidiosis vaccine (mixed species of *E. acervulina*, *E. tenella*, and *E. maxima*) at 20 × the recommended dose on d 14. Birds were raised in battery cages and fed corn-SBM-based diet with DDGS (5–7.5%) and meat bone meal (3%) as mild dietary challenge. Diets were provided in crumbled form in start phase and pelleted form in grower phase. Growth performance was determined on d 14, 20, and 26. On d 20 and 21, one bird per pen was sacrificed to collect serum to measure cytokines by ELISA. On d16, 19, 20, 21 and 22, fecal samples were collected to count oocyst number per gram feces (OPG). Data were subjected to one-way ANOVA and means were separated by Fisher's protected LSD test. *Eimeria* challenge caused coccidiosis with decrease of serum coloration ($P < 0.001$) and systemic inflammation as indicated by an increase of serum α -1-acid glycoprotein (AGP) on d20 ($P = 0.02$) and interferon gamma (IFN γ) on d21 ($P < 0.01$), increased fecal OPG at all time points post challenge ($P < 0.01$), and increased FCR during d21–26 by 4.7 points ($P = 0.01$). Compared with NC, EO reduced serum AGP ($P = 0.06$) on d20, IL10 ($P = 0.01$) and IFN γ ($P = 0.03$) on d21, fecal OPG ($P = 0.04$) on d20, the peak time point of oocyst shedding, and area under the curve of fecal OPG during d16–22 ($P = 0.11$), and numerically improved FCR during d21–26 by 2.5 points ($P = 0.13$). To determine the effect of EO on *Eimeria* oocysts in vitro, oocysts from *Eimeria* vaccine were incubated overnight at 41°C with EO at 0–15.36 mg/mL or monensin (COBAN®90), a commercial coccidiostat as positive control, at 0–16 mg/mL. Live oocysts after incubation were counted and analyzed by one-way ANOVA and separated by Fisher's protected LSD test. Both EO and monensin reduced live oocyst counts in a dose-dependent manner with LC50 of 3.20 and 4.94 mg/ml, respectively. In summary, under the experimental conditions, dietary EO could manage coccidiosis of broilers by reducing host inflammation and fecal oocyst shedding, the latter could be due to direct killing of *Eimeria* oocysts based on in vitro results, which was comparable to monensin.

Key Words: *Eimeria*, inflammation, essential oil, monensin, broiler

283 Effects of apple cider vinegar versus medicated feed on *Salmonella* in Cornish crosses. Bei Li Martin*¹, Madison Allison¹, Mackenzie Weldy¹, Torea Williams², and Zac Williams², ¹University of Findlay, Bradford, Ohio, United States, ²Michigan State University, Lansing, Michigan, United States.

Raw apple cider vinegar (RACV) has become increasingly popular as health effects of natural remedies, in both humans and animals, have captured consumers' attention. Products such as RACV are popular with small flock enthusiasts, yet few studies have exclusively examined the effects of acetic acid in RACV form. One theory has stated acetic acid reduces intestinal pH, thus a less favorable GI tract environment for colonization of *Salmonella*. Vinegar, supplied in water, reduced *Salmonella* populations by stimulating bifidobacteria leading to a competitive reduction of *Salmonella*. The objective of the current study was to determine the effects of RACV on *Salmonella* colonization in the GI tract of broilers. Before study, 48 Cornish Cross chicks were fed DuMOR starter feed crumble. At 14 d of age chicks were randomly allocated to 1 of 4 treatment groups designed each consisting of 12 chickens ($n = 12$): treatment 1- non-medicated feed and untreated water, treatment 2- non-medicated feed and RACV, treatment 3- medicated feed and untreated water, treatment 4- medicated feed and RACV. Treatments 1 and 2 were fed Keller non-medicated feed with similar nutritional value to the medicated feed provided in treatments 3 and 4. Treatments 2 and

4 were offered RACV diluted in water (14.8 cc of RACV per 3.78 L of water) and all treatment groups had *ad libitum* access to water and feed. Weights of feed were recorded daily to ensure birds received adequate nutrition. For a duration of 5 wk on every seventh d, fecal samples were collected immediately after defecation utilizing sterile swabs. Twelve coops were independently tested for *Salmonella* through centrifugation of twice diluted fecal matter in tryptic soy broth. Solution settled post centrifugation then was plated using XLT-4 Agar and incubated at 37°C for 48 h. A positive assessment for *Salmonella* was based on presence of black colonies. Black colonies represent 1/100 of the total population and multiplication by 100 yields cfu/g of excreta is necessary for a complete count. A completely randomized design was utilized in the current study and data was analyzed using SPSS with $P < 0.05$. Results revealed no colonization of *Salmonella* in treatments 1 through 4. Out of 4 treatment groups, treatments 1 and 3 were the most unexpected groups to test negative for *Salmonella* as neither contained RACV to create an undesirable environment for bacteria. Medicated feed contained an anticoccidial drug, therefore having no effect on *Salmonella*. Present study results revealed RACV had no effect on *Salmonella* counts, as none of the treatment groups tested positive for *Salmonella*, and cannot be deemed the sole cause of negative results.

Key Words: salmonella, chickens, alternative, vinegar, organic

284 Not Presented

285 Determination of adequate concentration of blended essential oil feed additive (Biolex) for improvement in growth performance, nutrient digestibility, and intestinal morphology in broiler chickens. Alamanda Calvert^{*1}, Saheed Osho², Nathan Horn¹, and Olayiwola Adeola², ¹BioMatrix International, Princeton, Minnesota, United States, ²Purdue University, West Lafayette, Indiana, United States.

Essential oils, prebiotics, and acidifiers have demonstrated improved poultry performance by impacting the intestinal microbiome, immune dynamics, and gastrointestinal secretions. The aim of this study was to validate the optimum dietary concentration of Biolex (BioMatrix International, Princeton, MN) on broiler growth performance, ileal digestibility and morphology. A total of 384 male broiler (Cobb 500) chicks (43.6 ± 0.9 g) were randomly allocated to 6 treatments with 8 replicate cages per treatment (8 chick per cage) in a randomized complete block design. The dietary treatments included a basal diet plus 0.0 (control), 0.25, 0.5, 0.75, 1, or 1.25 g/kg Biolex. The experimental diets were administered from 0 to 21 d post hatch. Growth performance was recorded on 7, 14, and 21 d. Ileal digesta was collected on 21 d post hatch for dry matter and energy digestibility utilizing chromic oxide as an indigestible marker. One bird at the median weight in each cage was euthanized on 21 d and the mid ileum was excised for intestinal morphology. Statistical significance was determined using the Tukey's test in Proc Glimmix (SAS 9.4). Gain and BW were improved in birds fed 0.5 g/kg compared with birds fed 1.25 g/kg Biolex ($P < 0.05$) on 14 and 21 d. Feed conversion ratio (FCR) was improved through 14 and 21 d in birds fed 0.5 g/kg compared with birds fed 1.25 g/kg Biolex ($P < 0.05$). Biolex fed at 0.25, 0.5, 1.0 g/kg improved ileal digestible energy (IDE) over control fed birds ($P < 0.05$). Birds fed 0.5 g/kg Biolex had increased villus height to crypt depth ratios compared with all other treatments ($P < 0.05$). Results from the current study indicate that dietary supplementation of 0.50 g/kg (1 lb/ton) Biolex improved BW, gain, FCR, IDE, and villus height to crypt depth ratios of broiler chickens raised in cages until 21 d.

Key Words: broiler, essential oil, prebiotic, digestibility, morphology

286 Effect of a phytogetic alkaloid extract on the growth performance of turkeys. Marcos Rostagno^{*1}, Meghan Shwartz¹, Nickki Tillman², Jason Payne³, Elle Chadwick³, and Robert Beckstead³, ¹Phytobiotics North America LLC, West Lafayette, Indiana, United States, ²Nutritional Statistics LLC, Buford, Georgia, United States, ³North Carolina State University, Raleigh, North Carolina, United States.

The objective of this study was to determine the effect of a phytogetic alkaloid extract obtained from *Macleaya cordata* (Sangrovit®, Phytobiotics North America, LLC) as a feed additive on the growth performance of turkeys. A total of 600 one-day-old male poults were divided into 30 floor pens (10 pens/treatment with 20 birds/pen) with 3 treatments: 1) Negative control; 2) Phytogetic alkaloid extract at 60 ppm; and 3) Phytogetic alkaloid extract at 120 ppm. The feeding program consisted of 5 phases using corn-soybean based diets. All poults were reared on 50% re-used litter and fed diets *ad libitum* for 18 weeks. At wk 7, all birds were moved to mimic the movement commonly done in the industry, as well as allow for proper stocking density. Data was analyzed in JMP Pro 13 using a one-way ANOVA with differences in the means found via a student's *t*-test ($P \leq 0.05$). The inclusion of the phytogetic alkaloid extract at 120 ppm resulted in significantly higher ($P < 0.05$) body weights on wk 4, 9, 12 and 15, compared with the negative control. Moreover, the phytogetic alkaloid extract at 120 ppm resulted in significantly lower ($P < 0.05$) feed conversion ratio on wk 3–4, 5–6, 7–9 and 9–12, whereas the 60 ppm inclusion rate resulted in significantly lower ($P < 0.05$) feed conversion ratio on wk 7–9, 9–12 and 12–15 when compared with the control. The results indicate that inclusion of the phytogetic alkaloid extract from *Macleaya cordata* at 120 ppm supports the growth performance of turkeys, when birds are subjected to stressors, such as moving, crowding, used litter, change from artificial to natural lighting, and frequent handling.

Key Words: turkeys, feed additives, phytogetic alkaloid, *Macleaya cordata*, growth performance

287 A commercial blend of plant extracts, fatty acids and essential oils reduces pathogenic bacteria, inflammation and intestinal barrier disruption caused by LPS in three *in vitro* experimental models. Alireza Khadem^{*1,2}, Markella Al-Saifi², Milena Sevastiyanova², Christos Gougoulis², and Jamal Al-Saifi², ¹Ghent University, Merelbeke, Belgium, ²Innovad NV/SA, Antwerp, Belgium.

A combination of different active ingredients acting synergistically with direct effects on pathogenic bacteria, immune response and intestinal barrier function may hold the most promising approach to protect and promote gut health in poultry raised without antibiotics. These studies aimed to evaluate the effects of a complex blend, combining target-release butyrate, fatty acids, plant extracts and essential oils (Lumance®, from Innovad, NV/SA Belgium) on some pathogenic bacteria (*Clostridium perfringens*, *Salmonella typhimurium* and *E. Coli*), inflammation and intestinal barrier disruption caused by LPS in 3 *in vitro* experimental models. First *in vitro* study: Lumance® was added at different concentrations to a medium inoculated with *C. perfringens*, *S. typhimurium* and *E. Coli*. The antimicrobial susceptibility testing was performed using the broth microdilution assay based on the ISO 10932/IDF 223 standard protocol. Bacterial growth was monitored by measuring the optical Density (OD) at 580 nm. Second *in vitro* study: The monocytic murine cell line RAW 264.7 was treated *in vitro* with

medium containing LPS to stimulate inflammation in the absence or presence of Lumance® and assayed for nitric oxide (NO) production. Third *in vitro* study: To investigate the effect of Lumance® on intestinal barrier integrity, the porcine intestinal epithelial cell line (IPEC-J2) was used as the experimental model and epithelial cell integrity was measured by transepithelial electrical resistance (TEER). IPEC-J2 were treated with Lumance® and challenged with LPS. TEER was measured at 0, 12, and 24 h after LPS challenge. Statistical differences between treatments were determined by one-way ANOVA using Graph pad software. In first study, Lumance® effectively inhibited the *in vitro* growth of *C. perfringens*, *S. typhimurium* and *E. Coli* in a dose-response manner. Second study, Lumance® inhibited LPS induced NO production with an IC50 value of 730 (95% CI 358, 1113) mg/L. The inhibition of NO production was not the consequence of cell death because it was established earlier using the tetrazolium salt method that cell viability was not affected by Lumance®. Third study, we observed that LPS exposure significantly decreased TEER at 24 h, indicating that LPS impaired epithelial barrier integrity. Pretreatment of IPEC-J2 with Lumance® significantly increased TEER and restore LPS-induced impairment of the intestinal barrier. This research shows that a combination of different ingredients in a well-proportioned mixture can reduce the pathogenic bacteria, inflammation and improve the intestinal barrier function. Thus, this may hold the most promising approach to promote and protect gut health and substitute antibiotics in poultry feed.

Key Words: pathogenic bacteria, inflammation, intestinal barrier function, Lumance, *in vitro* models

288 Evaluation of a specific blend of essential oils and oleoresins of spices to replace an antibiotic growth promoter program on broiler performance in a mild sanitary challenge situation. Jean-François Gabarrou*¹, Michael Sims², and Danny Hooge², ¹Phodé, Tersac, France, ²VA Diversified Research, Harrisonburg, United States.

Considering the simple removal of antibiotic growth promoters might have a negative economic impact, search for alternative additives has been incentivised. The main goal of this experiment was to evaluate the effects of a blend of essential oils and oleoresins of spices on performance of broiler between 7 to 42 d compared with usual conditions of production based on antibiotic growth promoter (AGP) in a mild challenge situation. A total of 1,200 newly hatched chicks (Ross-308) were randomly distributed (30/pen) into 3 groups with 12 (Control) or 14 (treatments) replicates in each one. All broilers received from d 0 to d 7 the same non-medicated starter. Birds did not receive any antibiotics from d 0 to d 7. At d 4 a contaminated previous litter was spread on the new litter to provide a mild sanitary challenge to chicks. Thereafter, Control group received only non-medicated feed (starter from d 0 to d 14, grower from d 15 to d 28 and finisher from 29 to 42). Birds from AGP group received 55 ppm of Bacitracin Methylene Disalicylate (BMD®) from d 8 to d 28 shuttled to 22 ppm of Stafac® from 29 to 42 d. In the tested group (OLEO), AGP were substituted by a phytogetic feed additive (Oleobiotec, Laboratoires PHODE - France) at 100 g/MT. N and P body retention were estimated by the difference between N and P intake in the feed and N and P body composition (Ross-308 datas). Statistical analysis: A completely randomized design was used ($P \leq 0.05$), and means were separated by LSD ($P = 0.05$). Statistical analysis was carried out using the General Linear Model procedure of IBM® SPSS® Statistics software. The Control group presented significantly

lower performances at d 42 compared with AGP group (1.906 vs. 2.111 kg BW; 1.933 vs. 1.765 feed conversion ratio or FCR), confirming the negative effect of AGP removal. Both groups (OLEO vs AGP) presented statistically equivalent performance on mortality rate (5.48% vs. 6.19%), growth performance (2.061 vs. 2.111 kg live weight) and feed efficiency (1.775 vs. 1.765 FCR). OLEO group presented a significantly higher N body retention compared with control (71.8% vs. 68.2%) and also a significantly higher P body retention (58.8% vs. 55.9%). Diets with the phytogetic feed additive Oleobiotec (Laboratoire PHODE), a specific blend of essential oils and oleoresins, were an effective alternative for antibiotic growth promoter (BMD®/Stafac®) diets. Further studies need to be done to investigate whether this product improves digestibility of energy and nitrogen to help explain those results.

Key Words: BMD, Stafac, essential oil, oleoresin, oleobiotec

289 Biostrong® OC100 improves organic production performance of broilers. Stacie Appleton*¹, Emily Zhou², and Jan Dirk van der Klis², ¹Delacon USA, Inc., Carlisle, Pennsylvania, United States, ²Delacon Biotechnik GmbH, Steyregg, Austria.

The US poultry industry rapidly incorporates advancements in technology, management, and nutrition for purposes such as improved animal welfare, optimized production, and to meet consumer demands. Phytogetic feed additives (PFA) have been proven effective in conventional poultry production for each of these purposes. However, availability of organically certified feed additives remains a challenge for organic poultry producers. To address this need, 3 trials were conducted to assess the efficacy of Biostrong® OC100 (OC100), an organic certified, fully phytogetic feed additive. The combined actives of essential oils, saponins, pungent and bitter substances work synergistically to enhance nutrient utilization, optimize gut health, and boost the performance of organic poultry. Trials 1 and 2 evaluated the performance of Ross 308 males from day of hatch to 42d, fed OC100 at 500g/MT, compared with a negative control (NC) with no PFA, and a conventional PFA, Biostrong® 510 (BSG510) at 150 g/MT. Trial 1 design consisted of 12 replicates per treatment with 32 birds per replicate. Data were analyzed using PROC GLM of SAS. Results of this study indicated a 2.5% increase of body weight (BW) for BSG510 and a 1.4% increase for OC100 over NC. BSG510 significantly ($P \leq 0.05$) improved FCR by 1.5% and OC100 by 0.8% in comparison with NC. Trial 2 consisted of 7 replicates per treatment with 20 birds per replicate. OC100 had a 1.6% higher BW at 42d as compared with NC and significantly ($P \leq 0.05$) improved FCR by 2.3%. BSG510 improved FCR by 1.2% in comparison with NC. Trial 3 evaluated the efficacy of graded dosage rates of OC100 compared with NC. Performance parameters and yield data for Ross 708 straight run broilers were assessed from day of hatch to 46d. The 3 treatment groups were (1) NC, (2) 375 g/MT OC100, and (3) 500 g/MT OC100. The experimental design consisted of 6 replicates of NC and 7 replicates each of treatments 2 and 3, with 180 birds per replicate. Results of this trial indicated a significant ($P \leq 0.05$) improvement of 3.6% in BW for both treatments 2 and 3 as compared with NC. In addition, yield data indicated a 3.5% and 1.5% increase of breast meat (as % live weight) for treatments 2 and 3 respectively. These results indicate an improvement in organic broiler production performance when supplemented with Biostrong® OC100 at a dosage of at least 375g/MT.

Key Words: organic, phytogetic, broiler, production, performance

Metabolism and Nutrition: Vitamins and Minerals

290 Role of 25-hydroxyvitamin D₃ and 1,25-dihydroxyvitamin D₃ in chicken embryo osteogenesis and vitamin D₃ metabolism.

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Meat-type broilers and laying hens exhibit a high incidence of bone disorders which cause economic and welfare issues. To address this challenge, maintaining adequate nutrition during embryogenesis is a novel strategy. A study was conducted to understand the bio-active form of vitamin D₃ on osteogenesis and vitamin D₃ metabolism in the chicken embryo. A total of 120 Cobb 500 fertilized eggs were used in the current study. They were incubated following the standard procedure. At the third day of incubation, PBS (C), PBS with 40ng 1,25-dihydroxyvitamin D₃ (L-1,25D), 200ng 1,25-dihydroxyvitamin D₃ (H-1,25D), 40ng 25-hydroxyvitamin D₃ (L-25D), and 200ng 25-hydroxyvitamin D₃ (H-25D) were injected into the dorsal vein of the embryo, respectively. The whole embryos were harvested at 1h, 3h and 6h post-injection for gene expression analysis (n = 8). The key osteogenesis marker genes (RUNX2, BMP2, COL1A2, BGLAP, SPP1, and ALP) and vitamin D catabolism gene (CYP24A1) were measured by using qRT-PCR. The data was normalized by the $\Delta\Delta$ CT method and analyzed using one-way ANOVA. The results showed, at 1h, no difference was found in osteogenesis gene expression. However, at 3h, the early differentiation marker, ALP, was increased by H-1,25D and H-25D. At 6h, the late differentiation marker, SPP1 was increased by H-25D only, but the BMP2 expression was downregulated by H-1,25D and 2 25D treatments compare with C. Due to the mRNA sequence of 1 α -hydroxylase is unavailable, we are not able to investigate the conversion of 25D to 1,25D in the chicken. However, by investigating CYP24A1, we can observe the 25D and 1,25D retention in the embryo. At 1h, there is no difference in CYP24A1 expression. At 3h, all the treatments upregulated CYP24A1 expression. However, at 6h, only H-25OHD group has higher CYP24A1 expression compared with all the other treatments. In conclusion, both 1,25D and 25D induced chicken embryo osteogenesis differentiation. and the higher dosage of 25D in the embryo showed a longer retention time in the embryo.

Key Words: vitamin D₃, chicken embryo, osteogenesis, metabolism, gene expression

291 25-hydroxycholecalciferol blood levels by dried blood spot (DBS) technology evaluation for broilers.

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The collection of whole blood samples by Dry Blood Spot (DBS) to evaluate 25-OH-D₃ plasma levels in animals offers several advantages over conventional plasma sampling including small sample volume, less invasive, cost-effective shipping and storage. Therefore, the aim of this study was correlate the quantification of the 25-OH-D₃ by the traditional, liquid plasma vs the Dry Blood Spot (DBS) analysis of field broilers not supplemented with 25-OH-D₃. A total of 30 broilers Cobb 500, 35 d of age were submitted the aleatory sampling study, totalizing 60 samples (30 plasma and 30 DBS samples), the samples were collected at a commercial farm in Brazil, and all broilers were fed with corn & SBM base diet, supplemented with 1,500 IU Vitamin D₃/Kg feed. Blood samples were drawn into vacutainer tubes containing anti-

coagulant (EDTA) and centrifuged at 3,000 rpm for 10 min to separate plasma. Twenty uL (approximately 1 drop) of whole blood from the brachial vein were collected and placed in one defined circlemaking of the DBS cards - Whatman® 903 (GE Healthcare, Piscataway, NJ, EUA), the procedure was repeated for the second drop of circlemaking and dried the sample card for 30 min in room temperature. The analysis was performed by liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MS/MS). Statistical analysis was performed by Pearson's Correlation. The results showed a strong significant correlation between plasma and DBS ($r = 0.836$; $P < 0.0001$). The 25-OH-D₃ levels determined by LC-MS/MS from DBS and plasma showed a good correlation. This result indicate that DBS can be an less invasive tool to determine 25-OH-D₃ levels on blood samples of broilers.

Key Words: 25-OH-D₃, dry blood spot, plasma, broilers

292 Dietary supplementation of 25-hydroxycholecalciferol increases broiler breast meat yield through the mTOR pathway.

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25-hydroxycholecalciferol [25(OH)D₃] dietary supplementation shows important effects on metabolic pathways mainly related to the growth and muscular development of broilers. The present study aimed to determine the effect of 25(OH)D₃ on broiler breast meat yield and its effect through the mTOR (mechanistic target of rapamycin) pathway. A total of 1,584 one-day-old Ross® Ap95 male chicks were distributed in a completely randomized design with 4 treatments, 9 replicates with 44 birds each one. The treatments were (T1) control diet (CD) supplemented with 3,000 IU vitamin D₃/kg of feed full cycle (1 to 46d), and experimental diets (T2) CD + 2,760 IU (69 µg) of 25(OH)D₃/kg of feed until 21 d of age, (T3) CD + 2,760 IU of 25(OH)D₃/kg of feed until 35 d of age, and (T4) CD + 2,760 IU of 25(OH)D₃/kg of feed full cycle. The parameters measured were breast meat yield, mTOR expression, breast protein deposition and 25(OH)D₃ plasma levels. Data were submitted to orthogonal contrast analysis and Pearson correlation, and deviation equations were determined when the contrast analysis was significant ($P < 0.05$). 25(OH)D₃ in broilers diet linearly increased breast meat yield in response to increasing days of supplementation ($P = 0.0267$; $Y = 0.01235x + 29.75142$). The mTOR expression ($P = 0.0025$; $Y = 0.00001965x^2 - 0.00070990x + 0.03617$), 25(OH)D₃ plasma levels ($P = 0.0001$; $Y = 0.00000018729x^2 - 0.0011x + 21,8046$) and breast protein deposition ($P = 0.0439$; $Y = 0.0031x^2 - 0.1154x + 81,888$) presented quadratic response related to supplementation periods of 25(OH)D₃. The results also showed a positive linear correlation between mTOR expression and 25(OH)D₃ plasma levels ($r = 0.59299$; $P = 0.0029$). In conclusion, dietary supplementation of 25(OH)D₃ increases broiler breast meat yield through the mTOR pathway. For maximum response it is recommended the continuous use of 25(OH)D₃ in broiler diets.

Key Words: 25(OH)D₃, breast meat, mTOR pathway, plasma

293 Effect of supplemental dietary vitamin-mineral premix withdrawal on performance and carcass characteristics of finisher broiler chickens.

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Vitamin-mineral-premix (VMP) is an obligatory component of broiler chicken diets. It is added as dietary supplement to diets throughout live cycle to sustain production. However, at the finisher stage of broiler production, dietary VMP may be withdrawn to lower cost without any consequence on production. Therefore, effect of supplemental dietary VMP withdrawal on performance and carcass characteristics of finisher broiler chickens was investigated in this study. Arbor Acre broiler chicks (n = 240) were from day one to 20 fed standard commercial broiler starter diets. At d 21, the chicks weighing 435.90 ± 37.74 g were randomly allotted to 2 dietary treatments in the deep litter housing system. Each treatment was replicated 10 times and a replicate comprised 12 chickens. Diet 1 contained supplemental VMP while diet 2 contained no supplemental VMP. Both groups were fed the diets for another 21 d. At d 42, 2 chicks with weight closest to the group mean weight of each replicate were selected and slaughtered. They were carefully scalded, eviscerated and dissected into primal cuts. The design of the experiment was a completely randomized design. Data on performance and carcass characteristics were subjected to descriptive statistics and *t*-test. The treatment means were separated at $\alpha_{0.05}$. Final weights (1536.02 ± 99.40 g) and weight gain (1102.82 ± 78.48 g) of chicks on Diet 1 were significantly higher ($P < 0.05$) than 1411.04 ± 138.67 g and 975.24 ± 116.62 g, respectively in Diet 2. However, chicks in both treatments had similar ($P > 0.05$) livability (98.32 ± 3.54 vs. 99.16 ± 2.65) and feed conversion ratio (0.40 ± 0.03 vs. 0.39 ± 0.03). Also, dressed weights (g) relative weights of all primal cuts, relative weights of internal organ and external offal were not significantly different ($P > 0.05$). In conclusion, withdrawal of dietary supplement of VMP resulted in lowered performance but without any effect on carcass indices of finisher broiler chickens.

Key Words: supplemental vitamin-mineral premix withdrawal, carcass primal cuts, finisher broiler chickens, Arbor Acre broilers performance, relative organ weights

294 Micro mineral amino acid complexes supplementation in broiler breeder hens: Effects on egg production and incubation. Thiago Noetzold¹, Bernardo Xavier¹, Raquel Horn¹, André Favero¹, Alba Fireman², Liris Kindlein¹, and Sergio Vieira¹, ¹University of Rio Grande do Sul—UFRGS, Porto Alegre, Rio Grande do Sul, Brazil, ²Zinpro Corporation, Piracicaba, São Paulo, Brazil.

A study was conducted to evaluate the effect of the partial and total micro minerals supplemented with inorganic sources (sulfates for Zn, Mn, Cu and Fe) or sodium selenite (Se). These were replaced by amino acid complexes (AAC – Zinpro Corporation) in broiler breeder diets. Eighty-four Cobb 500 broiler breeder hens, 20 wks old, were placed in 84 cages (0.33 m width x 0.46 m length x 0.40 m height). The experimental diets were composed by 7 treatments: Inorganic Full (Zn: 100/0, Mn: 100/0, Cu: 20/0, Fe: 60/0, Se: 0.30/0 and I: 2/0 ppm); Inorganic Reduced (Zn: 60/0, Mn: 60/0, Fe: 40/0, Cu: 13/0, Se: 0.15/0 and I: 2/0 ppm); Inorganic Plus (Zn: 120/0, Mn: 120/0, Cu: 30/0, Fe: 70/0, Se: 0.40/0 and I: 3/0 ppm); AAC Partial replacement (Zn: 60/40, Mn: 60/40, Fe: 40/20, Cu: 13/7, Se: 0.2/0.15 and I: 0/2 ppm); AAC Plus replacement (Zn: 60/60, Mn: 60/60, Fe: 30/40 Cu: 13/12, Se: 0.2/0.2 and I: 0/3 ppm); AAC Reduced (Zn: 0/40, Mn: 0/40, Fe: 0/20 Cu: 0/7, and Se: 0.15 and I: 2 ppm) and AAC Extra (Zn: 0/60, Mn: 0/60, Fe: 0/40 Cu: 0/12, Se: 0/0.2 and I: 0/3 ppm). Dietary treatments did not impact overall hens' productive performance. Nevertheless, contrasts between total replacement and partial replacement of AAC versus inorganic sources

showed an increase of 4.5 eggs on settable egg production ($P < 0.05$). Partial replacement treatments presented higher hatchability of fertile eggs over inorganic sources by 4.53% ($P < 0.05$). Egg weight before incubation was lower for the total replacement treatments compared with inorganic sources and partial replacement treatments ($P < 0.05$). However, this difference has not been sustained for the hatching weight of chicks. Chick's length was higher in total replacement treatments of AAC compared with other 2 groups. In conclusion, diets supplemented with AAC led to improvements in settable egg production, hatchability of fertile egg of broiler breeder hens and chicks' length.

Key Words: micro mineral, broiler breeder, organic mineral, eggshell, progeny

295 Zinc amino acid complex supplementation mitigates negative effects of cyclic heat stress on immune competence and oxidative metabolism of broilers. Alvaro Burin Junior¹, Débora B. Moretti¹, Ana Beatriz Oliveira¹, Diana Suckeveris¹, Marcos Nascimento Filho¹, Alba Fireman², and José Fernando Machado Menten¹, ¹University of Sao Paulo, ESALQ, Piracicaba, Brazil, ²Zinpro Corporation, Eden Prairie, United States.

The objective of the study was to determine the effect of a zinc amino acid complex (ZnAA; Availa[®]Zn) on immune parameters and oxidative metabolism of broilers submitted to heat stress. Ninety-six 21-d old Cobb male broilers were randomly allotted to 4 dietary treatments (0, 20, 40 and 60 ppm of zinc) and 2 environments, resulting in a 4×2 factorial scheme. The environmental conditions consisted of thermoneutral (TN) and chronic cyclic heat stress (HS). The unsupplemented basal corn-soybean meal diet contained 30 ppm of Zn. Birds were fed experimental diets from 1d up to 21d, and from 21d to 42d housed in 3 wired-cages/treatment (4 birds/cage). This experiment was repeated over time, resulting in 12 replicates. Weight of lymphoid organs were assessed as percentage of body weight. Quantification of γ -globulins, and oxidative parameters as malondialdehyde (MDA), total antioxidant capacity (TAC) and antioxidants enzymes activity were assessed at 42d in blood. Data were subjected to 2-way ANOVA in a 4×2 factorial scheme. The repetition over time was considered a random effect. Polynomial contrasts were used to determine linear and quadratic responses to dietary ZnAA levels, within HS and TN groups or over main effect. HS birds presented reduced relative weight of cloacal bursa, spleen and thymus compared with TN birds ($P < 0.05$). However, increasing levels of ZnAA had a positive linear impact on relative weight of cloacal bursa ($P < 0.05$). For γ -globulins quantification there was a positive quadratic response as ZnAA increased ($P < 0.05$). These indicators are directly correlated with the health status and immune competence of birds. Exposure to heat stress increased lipid peroxidation, as a consequence of the increased generation of ROS, as indicated by the increased concentration of MDA ($P = 0.053$). The antioxidants enzymes can diminish detrimental effects of ROS and, to do that, their activity is usually increased, as seen for glutathione peroxidase (GPx) ($P < 0.05$). In spite of that, no changes in superoxide dismutase (SOD) and catalase (CAT) were observed regardless of the environment. In turn, ZnAA supplementation has linearly decreased MDA quantification ($P < 0.05$), along with a linear decrease on SOD ($P < 0.05$) and a negative quadratic response for GPx ($P < 0.05$). Contrarily, a positive quadratic response has been observed for TAC ($P < 0.05$), while CAT activity linearly increased ($P < 0.05$) as supplementation increased. In conclusion, HS negatively affected immune and oxidative parameters, while ZnAA supplementation increased immune competence and diminished oxidative stress of broilers regardless of environmental condition.

Key Words: zinc, stress, immunity, antioxidant enzymes

296 The effect of physicochemical properties of ZnO on the bioavailability of zinc in broilers. Denise Cardoso*^{1,2}, Agathe Romeo¹, Nathalie Mème², Yves Chevalier³, and Agnès Narcy², ¹ANIMINE, Tours, France, ²IN.R.A, Nouzilly, France, ³University of Lyon, Lyon, France.

The bioavailability of a trace mineral is related to its in vivo solubility, which in turn is determined by its physicochemical properties. It is still not clear which characteristics are more relevant in affecting solubility and bioavailability of minerals. Zinc oxide (ZnO) is a common feed additive used to supplement zinc in poultry diets. However, different sources have shown different responses on bioavailability. This study hypothesized that different sources of feed grade ZnO have various physicochemical features that lead to distinct bioavailability values. Over 40 samples of ZnO have been collected from the feed industry worldwide. Samples were analyzed for density, tapped density, particle size, shape, specific surface area and dissolution kinetics. A principal component analysis (PCA) was performed to define the most relevant characteristics and categorize the samples into groups. An in vivo trial to measure the effect of their characteristics on the zinc bioavailability was performed. Seven samples were selected according to different properties and/or according to their market importance. 135 one-day-old male Cobb broilers were used in a dose-response experiment from 8 to 23 d of age. Treatments consisted of a basal diet with 23 ppm of zinc and 14 diets supplemented with 6 or 12 ppm of Zn in the form of different oxides or sulfate. Each treatment had 9 replicates. Bone zinc was used to determine zinc bioavailability. The experimental data were analyzed by ANOVA. The dose-response relationship between Zn intake and tibia Zn was used to estimate bioavailability. The slope of the Zn sulfate source was considered as the reference to estimate the relative bioavailability of ZnO sources. The bioavailability of the different sources varied from 49 to 160% ($P < 0.05$) considering zinc sulfate as the reference. Aggregate size and specific surface area explain a large part of the variability observed on values of bioavailability. In conclusion, physicochemical properties of ZnO can partly explain the variability observed in terms of Zn biological value.

Key Words: ZnO, bioavailability, physicochemical, properties, broiler

297 Determination of zinc requirements for broilers to 35 and 56 days of age. Leonardo Linares*¹, Jeffre Firman², Cristiano Bortoluzzi³, Marco Rebollo¹, and Duarte Neves¹, ¹Zinpro Corporation, Eden Prairie, Minnesota, United States, ²University of Missouri, Columbia, Missouri, United States, ³University of Georgia, Athens, Georgia, United States.

Zinc (Zn) is an essential trace element for poultry, as it participates in many biochemical reactions in the body. Zinc is crucial for protein synthesis, as well as carbohydrate and energy metabolism, and has a special role in tissue growth and repair, and bone mineralization. Zinc requirement of broilers has been set at 40 mg/kg of feed by the N.R.C (1994), however a large body of literature has shown that varied performance traits have improved with higher levels of supplementation. Conversely, much of this research has been done with inorganic Zn sources, some with organic Zn, but few with both sources together. The objectives of this study were to determine the requirement of zinc, evaluating the combined effect of Availa[®]Zn (AvZn) alone or in combination with Zn hydroxy-chloride (ZnOHCl) for broilers on productive performance, carcass yield, and tibial mineral deposition. A total of 3360 10-d-old male Ross 708 broiler chicks were randomly assigned across 14 treatments, with 10 replicates each, and 20 chicks/pen, in a complete randomized block design, from 11 to 56 d. Zinc supplementation ranged from 0 to

120 ppm from AvZn and 0 to 200 ppm from AvZn+ZnOHCl in equal amounts, from a base of 28 ppm Zn. Data were analyzed using GLM procedure of SAS and quadratic regression. Pen served as experimental unit for all analyses. Significant results of AvZn titration quadratic curve identified the supplemented requirement of Zn to be 62.5 ppm for feed intake from 10 to 21d ($P < 0.08$); 82.5 ppm for body weight gain (BWG) from 10 to 49d ($P < 0.02$); 73 ppm for BWGG from 35 to 49d ($P < 0.04$); 100 ppm for carcass yield (CY, $P < 0.06$), 64 ppm for total breast meat yield ($P < 0.01$), 67 ppm for *P. Major* yield ($P < 0.01$), 100 ppm for *P. Minor* yield ($P < 0.06$), and 100 ppm for wing yield ($P < 0.09$) at 56d processing; 58.5 ppm for tibia ash Ca % at 56d ($P < 0.01$). There were linear responses of increasing levels of dietary Zn for tibia ash Zn ppm content at 35d ($P < 0.03$) and 56d ($P < 0.09$). Significant results of AvZn+ZnOHCl titration quadratic curve identified the supplemental requirement for Zn to be 90 ppm for BWG from 10 to 56d ($P < 0.07$) and 112 ppm for CY at 35d ($P < 0.04$); 75.8 and 50 ppm for tibia ash Mn ppm content at 35d ($P < 0.08$) and 56d ($P < 0.08$), respectively. There were linear responses of increasing levels of dietary Zn on tibia ash Zn ppm content at 35d ($P < 0.04$) and 56d ($P < 0.02$). There were no differences ($P > 0.05$) in tibia ash % on any processing day. In summary, this study provided values of total Zn requirements, basal diet value included, based on live performance of broilers from 11 to 56d between 90.5 to 110.5 ppm, and carcass traits between 92 and 128 ppm, with linear response of increasing levels of dietary Zn on tibia ash Zn content.

Key Words: broiler, performance, requirement, tibia, zinc

298 Levels of copper and zinc in hydroxychloride form on performance, blood parameters and apparent ileal digestibility of broiler chickens. Tatiane Santos*¹, Karolina Augusto², Yanming Han³, Juliana Célia Denadai¹, Maria Márcia Sartori¹, Raimundo Ferreira Netto¹, Julianna Batistioli¹, Maria Claudia Sucupira⁴, and José Roberto Sartori¹, ¹UNESP, Botucatu, SP, Brazil, ²Trouw Nutrition, Campinas, Brazil, ³Trouw Nutrition, Amersfoort, Netherlands, ⁴USP, São Paulo, Brazil.

In recent years, a new concept of trace minerals on poultry nutrition was elucidated, being the hydroxychloride, which are less reactive and more stable in the diet and in gastrointestinal tract conditions when compared with inorganic sources. Thus, the objective of the present study was to evaluate the effect of different sources (sulfate and hydroxychloride) and different levels of Cu and Zn on performance (1-to 35-d-old), blood parameters and apparent ileal digestibility (AID). A total of 1,792 male Cobb 500 1-d-old were randomly distributed among 8 dietary treatments with 8 replicate per treatment. The mineral sources used were: copper sulfate pentahydrate (CSP), zinc sulfate monohydrate (ZSM), hydroxychloride - Intellibond C (IBC) or Intellibond Z (IBZ). Treatments were based on 2 levels of Cu (15 mg/kg and 150 mg/kg), 3 levels of Zn (80 mg/kg, 100 mg/kg and 120 mg/kg) and 2 control diets (15 mg/kg of CSP + 120 mg/kg of ZSM and 150 mg/kg CSP + 120 mg/kg ZSM). On d 42, 2 birds per pen were used to evaluate mineral content in plasma and ceruloplasmin in serum; and 3 birds per pen were slaughtered to collect ileum digesta and evaluate Cu and Zn AID. Data were analyzed in a factorial design 2x3+2 with the statistical software Minitab 16, and the means compared by the Tukey's test ($P < 0.05$). There was significant interaction on FCR ($P = 0.0001$) in broiler chickens during 1- 35 d of age, and birds fed with diets containing 150 mg/kg of IBC + 80 mg/kg of IBZ or 150 mg/kg of IBC + 100 mg/kg of IBZ showed better FCR than those fed 15 mg/kg of IBC + 80 mg/kg IBZ or 15 mg/kg of IBC + 100 mg/kg of IBZ. Furthermore, the inclusion of 150 mg/kg of IBC improved the BW ($P = 0.003$) and WG ($P = 0.003$) of broil-

ers compared those supplemented with 15 mg/kg of IBC. The Cu and Zn content in plasma was not affected by different sources and levels of mineral supplementation. On the other hand, the ceruloplasmin was higher ($P = 0.010$) in broilers that received 150 mg/kg of CSP + 120 mg/kg of ZSM than those fed 15 mg/kg of CSP + 120 mg/kg of ZSM. The AID of Cu was higher in broilers supplemented with 150 mg/kg of IBC + 100 mg/kg of IBZ compared those 15 mg/kg of IBC + 100 mg/kg of IBZ. Otherwise, the Zn AID increased in broiler chickens fed with 15 mg/kg of IBC + 80 mg/kg of IBZ or 15 mg/kg of IBC + 100 mg/kg of IBZ than those 150 mg/kg of IBC + 80 mg/kg of IBZ or 150 mg/kg of IBC + 100 mg/kg of IBZ. The results from this study indicate the levels of 150 mg/kg of IBC combined with 100 mg/kg of IBZ is a potential alternative to improve the performance without affect the levels of minerals in bloodstream, and reduces the mineral excretion. Trouw Nutrition, Fapesp (2017/00338–2), Capes (Financial Code - 001)

Key Words: mineral nutrition, trace minerals, feed conversion ratio, ceruloplasmin

299 Not Presented

300 Comparison of organic selenium forms efficacy in broiler studies. Timothée Guillou¹, Michele De Marco³, Rob Shirley², Alissa Welsher², and Mickael Briens*¹, ¹Adisseo France S.A.S., Commen-try, France, ²Adisseo USA INC., Alpharetta, United States, ³Adisseo France S.A.S., Antony, France.

The role of Selenium (Se) in metabolism is well established, as is the higher efficacy of organic vs. inorganic Se; however, the comparative availability between organic Se sources remains controversial. Based on tissue Se accretion, the following 2 broiler experiments (Exp1 = Ross PM3; Exp2 = Ross 308) were performed to define the availability between Se sources. From 1 to 7 d-of-age, birds were fed a control diet (CT; no supplemental Se), the CT diet supplemented with sodium selenite (SS), Se-yeast (65% or 35% of Se as selenomethionine; SY65, SY35), pure forms of selenocysteine (methylselenocysteine, SeMSC; selenocystine, SeC) or hydroxy-selenomethionine (HMSeBA). Endogenous Se level of the CT was 0.15 and 0.11 mg Se/kg in Exp1 and 2, respectively. In Exp 1, SS, SY65, SeMSC, SeC and HMSeBA were supplemented at 0.3 mg Se/kg. In Exp2, SS, SY65, SY35 and HMSeBA were also supplemented at 0.3 mg Se/kg; however, an additional treatment of SY65+SeC was fed, with both sources added at 0.3 mg Se/kg each to reach a supplemental level of 0.6 mg Se/kg. In Exp 1, on d 7, the Pectoralis major from 6 birds per treatment were collected and analyzed for total Se. Data were submitted to ANOVA and the means were compared by the Tukey test with significance at 5%. Results expressed in mg Se/kg of muscle (dry matter basis) were: CT, 0.56; SS, 0.73; SeC, 0.70; SeMSC, 0.68; SY65, 1.52; HMSeBA, 1.65 ($P < 0.001$). In Exp2, the same muscle on d 7 was collected on 9 birds per treatment, and Se accretion results were: CT, 0.34; SS, 0.53; SY35, 0.84; SY65, 1.22; SY65+SeC, 1.18; HMSeBA: 1.33 ($P < 0.001$). Statistically, organic Se forms (SY35, SY65, HMSeBA) had higher availability compared with SS, and HMSeBA had a higher availability compared with SY65 and SY35. SeC and SeMSC treatments had the same efficacy as SS, and SY65+SeC was equivalent to SY65 alone ($P > 0.05$). These data indicate that the availability of Se in yeast is dependent upon the degree by which Se is incorporated into SeMet, not selenocysteine.

Key Words: selenium, broiler, hydroxy-selenomethionine, transfer efficacy

301 Anatomical incremental changes in body composition of growing chicken between 7 and 42 days of age. Manel Hamdi* and Marie-Pierre Létourneau-Montminy, *Faculté des Sciences de l'Agriculture et de l'Alimentation, Université Laval, Québec, Québec, Canada.*

To develop models that predict precisely and in a robust way nutrient requirement of modern broilers we need information about their carcass composition in protein, lipid and ash. An experiment was conducted to measure the changes in anatomical body compartments weights and their chemical composition in protein, lipid, phosphorus (P) and calcium (Ca) in broilers from 7 to 42 d of age. A total of 65 one-day-old male broiler (Cobb500) received a control diet that fulfill all nutrients requirements. On d 7, 14, 21, 28, 35 and 42, 10 birds were fasted for 5 h to empty the gastro-intestinal tract, euthanized, scalded, feather picked, frozen and then dissected in 6 anatomical compartments: skin, fat, muscle, viscera, bones, and miscellaneous (head, neck, rest of feet, and rest of wings). After grinding and mixing, each fraction was analyzed for dry matter, Ca, and P. Protein and lipids were analyzed only in skin, fat, muscles, and viscera. Results were analyzed with MIXED procedure of Minitab 18 with the age as X variable. Regarding proportion of the 6 pools into whole body, muscle increased linearly ($P = 0.002$) from 26% at d7 to 50% at d42 while that of miscellaneous decreased ($P < 0.001$) from 21% at d7 to 13% at d42 and that of viscera decreased in a linear and quadratic manner ($P < 0.001$; 18 to 5% from d7 to d42). There was no change with age for skin, fat and bone. The distribution of protein in different pools increased linearly and quadratically with age in muscle ($P < 0.001$) while it decreased linearly in fat and skin ($P < 0.001$) and linearly and quadratically ($P < 0.001$) in viscera; at d 7, the protein is found at 46% in muscle, 39% in viscera, 13% in skin and 2% in fat in comparison to 82% in muscle, 10% in viscera, 6% in skin, and 2% in fat at d42. Lipid is mainly found in fat (45%) and skin (31%) and it evolves with age ($P < 0.001$) only in viscera with 20% at d7 and then stabilized to 8%. As expected body Ca is found mainly in bone (>65%) and miscellaneous (32%) and change only with age ($P < 0.001$) for skin and viscera with higher values at d7. For P, about 50% was found in bone, followed by 26% in miscellaneous, and 16% in muscle and values increased with age in muscle and decreased in skin, miscellaneous and viscera ($P < 0.001$). Finally, the Ca:P body ratio increased quadratically ($P < 0.001$; d7 = 1.20, d28 = 1.5, d42 = 1.4) while that of bone is of 1.7 at d7 and then quickly stabilized to 1.9. When looking at whole body protein, lipid and ash deposition in g/d, ash and protein evolve quadratically with the maximum deposition reached at 31 and 36 d respectively while lipid deposition is linear until 42 d. These results will be useful to develop mechanistic model of the growth of main body component in broilers.

Key Words: anatomical composition, broilers, calcium, phosphorus

302 Evaluation of the potential for direct-fed microbials to enhance utilization of phosphorus in broiler chickens. Joseph Donkor*^{1,2}, ¹Meharry Medical College, Nashville, Tennessee, United States, ²Tennessee State University, Nashville, Tennessee, United States.

Feed accounts for about 60–68% of the total cost of poultry production. Chicken cannot efficiently absorb organic or phytin-bound phosphorus, thus about 70–80% of dietary or plant-based phosphorus is excreted in the manure of broiler chickens. The goal of this research was to identify microbes with the potential to improve utilization of a plant source of phosphorus in the gastrointestinal tract (GIT) of broilers. A total of 8 dietary treatment's consisting of, LEB-0 the control diet-0mg/kg feed of equal amount of Lactobacillus (0%), Enterococcus (0%) and Bifidobacteria (0%), LEB-50, 50mg/kg feed of equal amount of Lactobacillus

(16.67%), Enterococcus (16.67%) and Bifidobacteria (16.67%)., LEB-100, 100mg /kg feed of equal amount Lactobacillus (33.34%), Enterococcus (33.34%) and Bifidobacteria (33.34%)., LEB-150, 150mg /kg feed of equal amount of Lactobacillus (50%), Enterococcus (50%), and Bifidobacteria (50%)., LEB-200, 200m/kg feed same amount of Lactobacillus (66.67%), Enterococcus (66.67%), and Bifidobacteria (66.67%), L-100, 100mg / kg feed of Lactobacillus (100%). E-100, 100mg of/kg feed Enterococcus (100%)., B-100, 100mg of /kg feed Bifidobacteria (100%). The results showed a total of 8,082 sequences were obtained using a metagenomic approach, with 61% of those sequences representing 5,030 species of various bacterial organisms. The highest proportion of bacteria was Massilia which represented 46% of the total dominant microbial population, Bacteroides (9%), Streptomyces (6%), Bacillus (6%), and 18 different species each constituting less than 5% of these dominant microbes. Three microbes Lactobacillus, Enterococcus, and Bifidobacterium (LEB) with the potential to hydrolyze free phosphorus

were isolated and characterized. The isolated microorganisms maintained the ability to grow at all the different pH ranges (1–5), and bile concentrations of 0–3.5%. Also, the ability of the bacteria to hydrolyze free phosphorus was evaluated in vitro. In conclusion, broiler chickens fed probiotic bacteria at the rate of 100 or 150 mg/kg of feed consumed 12.0% and 17.8% more feed, respectively, and increased body weight gain by 5.9% and 8.4%, respectively, when compared with the control birds. Broiler chickens fed diets containing the probiotics at 100 or 150 mg/kg of feed retained 15.2% and 17.5% of phosphorus as against 8.6% for the birds on the diets without the bacteria. Except for birds on dietary treatment LEB-150, which had a higher mortality (7.3%), the remaining 6 dietary treatments had mortality ranging from 2.0 to 3.3% which was less than that of the control's birds (4.5%).

Key Words: probiotics, poultry, bacteria, phosphorus, feed

Microbiology and Food Safety

196 Effectiveness of a molecular approach to study *Salmonella* dynamics along critical steps of a turkey slaughter process. Marie-Lou Gaucher*¹, Selmane Boubendir¹, Alexandre Thibodeau¹, Philippe Fravallo¹, Julie Arsenaault³, Sylvain Fournaise², and Sylvain Quessy¹, ¹Université de Montréal, St-Hyacinthe, Quebec, Canada, ²Olymel s.e.c., St-Hyacinthe, Quebec, Canada, ³Swine and Poultry Infectious Diseases Research Centre, St-Hyacinthe, Quebec, Canada.

In spite of multiple intervention procedures at the slaughter plant level, poultry meat products contamination by *Salmonella* remains one of the biggest challenges for poultry processors. *Salmonella* ranks within the top 3 bacterial pathogens causing human foodborne diseases in Canada and contaminated turkey meat has been linked to disease outbreaks during these last years. The exact contribution of live birds and the possible competitive advantage of specific *Salmonella* serovars on the final product contamination remain to be better described. The objective of the current study was to investigate the relationship between the detection of a specific *Salmonella* genotype on carcasses at the end of the slaughter process (W) and the detection of the same genotype on i) carcasses of the same flock, but at previous critical steps of the process, or iii) carcasses of previously slaughtered flocks. Seventy-one turkey flocks were surveyed using a carcass rinse approach on one randomly selected turkey carcass sampled at 2 critical steps of the slaughter process: after bleeding (B) and after water-immersion chilling (W). Collected samples were screened for the presence of *Salmonella* and recovered isolates were submitted to a HRM-typing protocol targeting *yohm*, *CR1* and *CR2* genomic regions from which HRM genotypic profiles were established. A logistic regression was used to model the carcass status at W for specific genotypes, with standard errors adjusted for clustering within visits. The B critical step showed the highest *Salmonella* prevalence with 84.5%. Schwarzengrund and Heidelberg were the most prevalent serovars, representing between 25% and 52% of all the serovars identified. Six HRM genotypes were obtained for both *S. Schwarzengrund* and *S. Heidelberg*, with the Schwarzengrund genotype 2-2-2 and the Heidelberg genotype 1-1-3 representing between 61% and 83% of the genotypes identified at both B and W critical steps. The detection of either the Schwarzengrund genotype 2-2-2 or the Heidelberg genotype 1-1-3 on turkey carcasses at W was strongly associated with the detection of the same genotype on carcasses from the same flock sampled at B (odds ratio = 19.9, $P < 0.01$; odds ratio = 6.0, $P = 0.04$, respectively). The final carcass status for the Schwarzengrund genotype 2-2-2 or for the Heidelberg genotype 1-1-3 was also significantly associated with the detection of the same genotype in the previously slaughtered flock (odds ratio = 7.6, $P < 0.01$; odds ratio = 23.1, $P < 0.01$, respectively). These results suggest that a molecular characterization approach represents a valuable tool in estimating the relative role of the *Salmonella* flock status on the final meat product contamination.

Key Words: turkey, slaughter, *Salmonella*, genotype, final carcass status

197 Estimating salmonella from live birds to processing. Charles Hofacre*¹, Virginia Baxter¹, Roy Berghaus², Kalen Cookson³, and Manuel DaCosta⁴, ¹Southern Poultry Research Group, Inc., Watkinsville, Georgia, United States, ²University of Georgia, Athens, Georgia, United States, ³Zoetis, Lawrenceville, Georgia, United States, ⁴Zoetis, Durham, North Carolina, United States.

The processing plant has been the focus of USDA-FSIS for *Salmonella* reduction since the initiation of the HACCP program in 1996. This

focus has been reflected in the poultry industry by having the majority of the interventions found inside the processing plant. This program has been successful in reducing *Salmonella* positive carcasses from 20% in 1996 to 6.5% in 2011. However, as the USDA-FSIS has lowered the performance standards, the poultry industry has had to begin *Salmonella* interventions on the farm. Understanding the load of *Salmonella* coming to the processing plant becomes critical in evaluating the effectiveness of on-farm interventions for success in meeting the FSIS Performance Standards. Two studies were performed measuring live bird samples (bootsoc swabs) to processing samples (ceca and carcass rinse) with a *S. heidelberg*(S.H.) in one study and a *S. kentucky*(S.K.) challenge in the second study. The S.H. study was 32 pens of 25 broilers with 13 birds challenged at 4 d of age. Birds were sampled to evaluate the prevalence and load (MPN) of S.H. in bootsocks (10 and 39 d) and ceca (42 d) and hot rehang carcass rinses (42 d). The second study had 48 pens of 25 broilers with 13 birds challenged with S.K. at 4 d of age. Birds were sampled to evaluate prevalence and load of S.K. in bootsocks (14 and 39 d), ceca (42 d) and whole bird feathers on rinse (42 d). In the S.H. study, comparing individual bird ceca to that birds' carcass rinse had a slight correlation; also, comparing pen level prevalences for ceca and carcass rinse samples gave a slight to moderate positive correlation. There was a significant positive correlation between bootsoc MPN's and MPN's in ceca samples. There was a significant positive correlation between bootsoc MPN's and pen level S.H. prevalences in ceca samples. Bootsocs for S.K. challenge study were significantly positively correlated to ceca prevalence and MPN's. There was also a positive correlation between bootsoc MPN's and pen level S.K. carcass rinse prevalence and MPN/carcass. In summary, these 2 studies demonstrated that bootsocs can be used to correlate the S.H. or S.K. load and prevalence to ceca samples at processing or hot rehang/ feathers on carcass rinses in the processing plant.

Key Words: *Salmonella* number, prevalence, farm, carcass rinse, ceca

198 Synbiotic supplementation to decrease cecal *Salmonella* load and carcass contamination in broilers. Revathi Shanmugasundaram*¹, Mohamad Mortada¹, Douglas Cosby³, Manpreet Singh¹, Todd Applegate¹, Basharat Syed⁴, Chasity Pender², Shelby Curry², Raj Murugesan², and Ramesh Selvaraj¹, ¹University of Georgia, Athens, Georgia, United States, ²BIOMIN America Inc, Overland Park, United States, ³USDA-ARS, Athens, United States, ⁴BIOMIN Holding GmbH, 3131 Getzersdorf, Austria.

In vitro and in vivo experiments were conducted to study the effects of synbiotic supplementation on *Salmonella enterica* ser. Enteritidis (SE) proliferation, broiler cecal colonization, and carcass contamination. *Lactobacillus reuteri*, *Enterococcus faecium*, *Bifidobacterium animalis*, and *Pediococcus acidilactici* culture supernatants decreased ($P < 0.05$) the in vitro proliferation of SE at 1:1 supernatant: pathogen dilution. A total of 240 Cobb-500 broiler chicks were randomly allotted to 3 treatment groups (8 replicates/group with 10 birds/replicate): control (basal diet), antibiotic (Virginiamycin at 20 mg/kg feed), synbiotic (PoultryStar[®] ME at 0.5 g/kg feed containing *L. reuteri*, *E. faecium*, *B. animalis*, and *P. acidilactici*) from day of hatch. At 21 d of age, all birds in experimental groups were individually inoculated orally with 250 μ L of 1×10^9 cfu SE. A one-way ANOVA was used to examine the effects of antibiotic and synbiotic supplementation on dependent variables. When the main effects were significant ($P < 0.05$), differences between means were analyzed by Tukey's least squares means

comparison. Antibiotic supplementation increased ($P < 0.05$) BW and feed consumption, compared with the control group, while birds in the synbiotic supplementation had intermediate BW and FC that were not significantly different from both the control and antibiotic group at 42 d of age in SE infected birds. No significant effects were observed on feed efficiency at 42 d of age among the groups. Antibiotic and synbiotic supplementation decreased ($P < 0.05$) cecal SE load by 0.90 and 0.85 log units/ml and chilled carcass SE load by 1.4 and 1.5 log units/mL of rinsate compared with the control group at 42 d of age (21 dpi). Relative abundance of IL-10, IL-1, TLR-4, and IFN γ mRNA, analyzed by quantitative real time PCR, was decreased ($P < 0.05$) in the antibiotic and synbiotic supplementation groups compared with the control birds at 42 d of age (21 dpi). It can be concluded that synbiotic supplementation decreased SE proliferation in vitro and decreased SE load in the cecal contents and chilled broiler carcass.

Key Words: *Salmonella*, probiotic, synbiotic, food safety

199 Determining the efficacy of acidified sodium chlorite for the reduction of inoculated *Salmonella* and *Campylobacter* on skin-on, bone-in chicken thighs. Dana Dittoe*, Kristina Feye, Zhao-hao Shi, Jessica Woitte, and Steven Ricke, *University of Arkansas, Fayetteville, Arkansas, United States.*

As *Salmonella* and *Campylobacter* spp. are the most frequently clinically isolated foodborne pathogens in the world, it is imperative to increase antimicrobial strategies during poultry processing such as the implementation of poultry part dips and sprays. Therefore, the objective of the current study was to compare the efficacy of commercially available peracetic acid (PAA) and acidified sodium chlorite (ASC) as 10 s antimicrobial part dips on skin-on, bone-in chicken thighs. No more than 24h in advance, poultry thighs were obtained from a commercial processing facility. At the onset of the experiment, 360 chicken thighs (9 treatments, 10 replications, 2 time points, 2 inoculums) were inoculated with either a cocktail of *S. Enteritidis*, *S. Heidelberg*, *S. Infantis*, *S. Kentucky*, *S. Typhimurium* or a cocktail containing *C. jejuni* (10^8 and 10^7 cfu/mL) and incubated at 4°C for 60–90 min. Immediately following, the thighs were independently dipped into sterile whirl-pack bags containing 400 mL of the following treatments for 10 s: Tap water (TW), 1100 and 800 ppm of ASC (pH 2.4 and 2.8), 200, 350, 500, and 650 ppm PAA. After treatment, thighs were allowed to rest 2 min and were sampled immediately (0h) or stored at 4°C for 24h. At each time point, thighs were rinsed in 150 mL of nBPW for 1 min and the rinsate was collected for downstream analysis. Rinsates were diluted to 10^{-7} in $1 \times$ PBS, dot plated onto XLD or mCCDA, and incubated either aerobically at 37°C for 24h or microaerophilically at 42°C for 48h. Subsequent counts were log-transformed and reported as log cfu of *Salmonella* or *Campylobacter* per gram chicken thigh basis (cfu/g). Data were analyzed using n-Way ANOVA in JMP14.0 with means separated by Tukey's Protected HSD ($P \leq 0.05$). In the current study, the total *Salmonella* (cfu/g) was significantly affected by a treatment \times time interaction ($P < 0.05$). At 0h, thighs treated with 800 ppm ASC and PAA had lower *Salmonella* loads than TW. Also, thighs treated with 650 ppm PAA had lower loads than TW and all ASC treatments at 0h. However, at 24h, thighs treated with 650 ppm PAA were no different than 1100 ppm ASC (pH 2.4 and 2.8) and 800 ppm ASC (pH 2.4). Simultaneously, there was a main effect of treatment and time on the total *Campylobacter* load. All thighs treated with experimental dips had lower *Campylobacter* compared with those treated with TW ($P < 0.05$). In addition, the load of *Campylobacter* was significantly greater at 24h post treatment compared with 0h (1.27 and 1.60 log cfu/g; $P < 0.05$). In conclusion, both peracetic acid and acidified sodium chlorite reduced the load of *Salmonella* and *Campylobacter* on

inoculated commercial chicken thighs with 650 ppm PAA having the greatest efficacy over time.

Key Words: broiler parts, *Salmonella*, *Campylobacter*, peracetic acid, acidified sodium chlorite

200 *Salmonella* serovar populations in broilers are influenced by pre-enrichment and enrichment procedures. Nelson Cox³, Mark Berrang³, Sandra House³, David Medina², Kimberly Cook⁴, and Nikki Shariat*¹, ¹*University of Georgia, Athens, Georgia, United States*, ²*Gettysburg College, Gettysburg, Pennsylvania, United States*, ³*USDA-ARS; US National Poultry Research Center, Athens, Georgia, United States*, ⁴*USDA-ARS, Beltsville, Maryland, United States.*

Salmonella is represented by > 2600 serovars that can exhibit different phenotypes, including virulence, antimicrobial resistance, and host restriction. Poultry is a major *Salmonella* reservoir, but current culture-based detection methodology is limited: 1) it only identifies the most abundant serovars and background serovars remain undetected, 2) different enrichment procedures may introduce bias, and 3) a one-minute carcass rinse before pre-enrichment is insufficient to release all *Salmonella* present. The inability to assess serovar diversity means that those posing a greater public health risk may be masked by abundant serovars such as ser. Kentucky, which is rarely associated with illness in the US, but is the top serovar isolated from broilers. CRISPR-SeroSeq, an amplicon-based next-generation sequencing tool, allows detection of multiple serovars by mapping the relative serovar frequencies in a single sample. To directly address the limitations listed above, CRISPR-SeroSeq was used on 8 broiler carcasses that were collected pre-chill at a commercial slaughter plant. Standard carcass rinse aliquot pre-enrichment (RAP) was compared with whole carcass pre-enrichment (WCP), both done overnight in buffered peptone water. This was followed by enrichment in either Rappaport Vassiliadis (RV) or tetrathionate (TT) broths. On average, 5 serovars were observed per carcass, including 9 on a single carcass. CRISPR-SeroSeq detected serovars that comprised as low as 0.005% of the population. For the most abundant serovars, CRISPR-SeroSeq data correlated with standard culture analysis. Ser. Kentucky, Typhimurium, and Schwarzengrund were identified on each carcass. Serovar diversity was higher in WCPs that were enriched in RV (t -test, $P < 0.05$). Ser. Schwarzengrund was present at higher frequencies in WCPs compared with RAPs (t -test, $P < 0.05$), suggesting it adheres more strongly to the carcass and not released within during the rinse. Ser. Enteritidis, the top serovar responsible for salmonellosis, was enriched 8-fold more in TT than RV, and ser. Schwarzengrund and Reading were preferentially enriched in RV. Comparison of pre-enriched and enriched samples suggest that enrichment in RV or TT was inhibitory to ser. Thompson and Reading. Collectively, this demonstrates some serovars are better adapted to particular enrichment conditions. This study addresses limitations to current *Salmonella* surveillance protocols and provides valuable information relating to *Salmonella* population dynamics. Importantly, we provide the framework and tools for additional studies on a broader scale to investigate *Salmonella* ecology in poultry and other reservoirs.

Key Words: *Salmonella* serotypes, broiler, CRISPR-SeroSeq

201 Dietary inclusion of enhanced organic acid improved performance and reduced carcass contamination in broilers challenged with *Salmonella enteritidis*. Shelby Curry*¹, Revathi Shanmugasundaram², Mohamad Mortada², Douglas Cosby³, Manpreet Singh², Todd Applegate², Basharat Syed⁴, Chasity Pender¹, Raj Murugesan¹, and Ramesh Selvaraj², ¹*BIOMIN America Inc., Overland Park, Kansas, United States*, ²*University of Georgia,*

Both consumer and regulatory pressure on the use of antibiotics in the livestock and poultry industry have created a need to develop alternatives to antibiotics. One potential alternative in the poultry industry is organic acids. Thus, an enhanced organic acid (EOA) was evaluated as a potential tool to reduced *Salmonella enterica* ser. *enteritidis* (SE) in vitro proliferation and in vivo broiler performance, cecal SE colonization, and carcass SE contamination. An in vitro minimum inhibition concentration (MIC) evaluation was conducted to determine the optimum concentration of EOA needed to reduce SE proliferation. A serial dilution from 5% to 0.04% EOA was tested and MIC of at least 0.63% EOA was able to reduce ($P < 0.05$) SE growth. To determine the in vivo effects of EOA, a total of 240 Cobb-500 broiler chicks were randomly allotted to 1 of 3 treatment groups (8 replicates/group with 10 birds/replicate): 1) CON (basal diet), 2) AB (Virginiamycin at 20 mg/kg inclusion), and 3) EOA (Biotronic PX Top3^{US} at 500 mg/kg inclusion) on day of hatch and remained on treatment for 42 d. At 21 d of age, all birds were individually gavaged with 250 μ L of 1×10^9 cfu SE (0 d post inoculation; dpi). On 3, 7, 14, and 21 dpi, 1 bird per pen was euthanized and cecal tissue collected to determine SE abundance. On 21 dpi, most probable number (MPN) of SE on hot, post-pick carcasses were determined. Data were analyzed using the GLIMMIX procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) with treatment as fixed effect and treatment means estimated by LSMEANS statement. Before challenge (d 0–21), feeding AB resulted in greater ($P < 0.05$) BWG and feed intake (FI; by 100 and 90 g, respectively) compared with CON birds. From d 0–42, both AB and EOA birds had greater ($P < 0.05$) BWG (by 240 and 170 g, respectively) compared with CON birds. In addition, AB birds had 270 g greater ($P < 0.05$) FI compared with CON birds. Overall (d 0–42), FCR was reduced ($P < 0.05$) in both AB and EOA birds (1.64 and 1.65, respectively) compared with CON birds (1.69). Both AB and EOA birds had reduced ($P < 0.05$) carcass SE by 1.37 and 1.66 log units, respectively, compared with CON birds. In addition, at 21 dpi, AB birds had reduced ($P < 0.05$) SE in the ceca by 0.9 log units compared with CON birds. These data indicate that EOA could be a potential tool to reduce carcass SE contamination and increase performance of broilers when challenged with SE..

Key Words: *Salmonella*, organic acids, food safety, broiler, performance

202 Thermal inactivation of the *Salmonella* surrogate *Enterococcus faecium* ATCC 8459 during extended feed conditioning.

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Salmonella causes a large number of foodborne disease outbreaks and is commonly associated with poultry. It can enter into the poultry product supply chain in several ways, and can be found in the environment and raw commodities. The Food Safety Modernization Act Preventive Controls for Animal Food Rule makes it crucial that US animal feed manufacturers provide scientific data proving effective reduction of *Salmonella*. Therefore, this study aimed to determine the ability of industry used retention times and temperatures during feed processing to inactivate *Salmonella* using a non-pathogenic surrogate *Enterococcus faecium* (verified as a suitable surrogate for thermal resistance of *Salmonella* in foods and feed). Treatments included a negative control without inoculum and a 3×3 factorial arrangement of 3 retention times in the HygenizerTM of 80, 160 and 240 s at conditioning temperatures 75, 85 and 95°C. *E. faecium* ATCC 8459 was grown in Tryptic Soy Broth at 35°C for 18–20 h. Cells were washed twice in Buffered Peptone

Water (BPW), added to bone meal, mixed thoroughly and left to dry in a biosafety cabinet for 24 h. On the day of processing, the inoculated meal was added to the feed mixture at a ratio of 5 pounds per ton of feed. Triplicate samples (25 g) were collected from the mixer, conditioner, HygenizerTM and after pelleting, stomached in BPW and serially diluted in 0.1% peptone. m-*Enterococcus* agar incubated at 35°C for 24 h provided total *Enterococcus* counts. To verify absence of *Enterococcus* cells in samples with no countable colonies, samples were also enriched in Enterococcosel broth before plating on m-*Enterococcus* agar. Three trials were conducted. No *Enterococcus* was detected in the negative control before or throughout the feed milling process. Data were analyzed using PROC GLM and Tukey test in SAS (V9.4). Means were considered significantly different when $P \leq 0.05$. Inoculated mash feed had *Enterococcus* levels of 5.29 ± 0.20 log cfu/g, which were decreased by all processing temperatures and retention times. The decrease of *Enterococcus* was significantly greater at 85 and 95°C compared with 75°C. At 75°C, conditioning was sufficient for ca. 2 log cfu/g reduction. The HygenizerTM significantly reduced *E. faecium* by a further 1 log to 2.19, 2.11 and 1.61 log cfu/g at retention times of 80, 160 and 240 s, respectively. Pelleting reduced *Enterococcus* counts by a further 1–1.5 log. There was no detectable *E. faecium* at any stage of processing at 85 and 95°C, indicating >5 log decrease. These results indicate that commercial feed processing times and temperatures used by genetic companies (82.3°C for 10–30 s) are sufficient to reduce the levels of *Salmonella* in feed.

Key Words: feed processing, *Salmonella*, thermal inactivation, surrogate, conditioning

203 Microbiota metabolite deoxycholic acid-modulated anaerobes attenuate chicken transmission-exacerbated campylobacteriosis in *III10*^{-/-} mice. Xiaolun Sun*, Hong Wang, Bilal Alrubaye, Mussie Abraha, Ayidh Almansour, and Mohit Bansal, University of Arkansas, Fayetteville, Arkansas, United States.

Campylobacter jejuni, a category B foodborne bacterial pathogen, is mainly transmitted from poultry and causes the prevalent campylobacteriosis. Little is known on how to intervene *C. jejuni* chicken transmission and subsequent campylobacteriosis. The hypothesis of this study was that *C. jejuni* transmitted through chickens increased virulence and deoxycholic acid (DCA)-modulated microbiota attenuated the transmission-increased infection. *C. jejuni* was isolated from ceca of birds infected with *C. jejuni* AR101 (passage 0) in the absence of transplanted microbiota (passage 1, Cj-P1) or in the presence of the transplanted DCA-modulated anaerobic microbiota (Cj-P1-DCA-Anaero). Specific pathogen free *III10*^{-/-} mice were orally gavaged with clindamycin for 7 d to deplete microbiota. The mice were infected with a single dose of 109 cfu/mouse *C. jejuni* AR101, Cj-P1, or Cj-P1-DCA-Anaero. The mice were sacrificed after 8 d post infection and host responses were determined using histopathology evaluation, real time PCR, and tissue culture. Interestingly, after only 6 d post infection, *III10*^{-/-} mice infected with Cj-P1 showed clinical sign of enteritis as diarrhea, fur ruffling, and hunching, while other groups of mice didn't. At cellular level, AR101 induced mild intestinal inflammation and increased histopathology score in *III10*^{-/-} mice, showing as crypt hyperplasia and immune cell infiltration. Notably, chicken-transmitted Cj-P1 induced more severe campylobacteriosis compared with AR101, suggesting increased *C. jejuni* infection capacity after its chicken colonization and transmission. Importantly, mice infected with Cj-P1-DCA-Anaero showed attenuation of intestinal inflammation compared with mice infected with Cj-P1. At molecular level, Cj-P1 induced elevated inflammatory mediator mRNA accumulation of *III17a*, *III1 β* , and *Cxcl1*

in the *Il10*^{-/-} mouse colon compared with AR101, while Cj-P1-DCA-Anaero reduced the inflammatory gene expression. In conclusion, our data indicate that chicken transmission exacerbates *C. jejuni*-induced enteritis, while DCA-modulated anaerobes attenuate the surge of *C. jejuni* infection ability.

Key Words: microbiome, enteritis, inflammation, pathogen transmission, histopathology

204 The effect of refined functional carbohydrates (RFCs) on cecal *Salmonella* prevalence in commercial turkeys and broilers.

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Refined functional carbohydrates (RFCs) are produced by enzymatic hydrolysis of the cell wall of *Saccharomyces cerevisiae* yeast. They are an all-natural feed ingredient with Generally Recognized as Safe status. RFCs have been demonstrated to have activity against gram-negative bacteria, such as *Salmonella*. A feeding trial was conducted at 2 commercial turkey farms and 7 commercial broiler farms to determine the effect of RFCs on the prevalence of *Salmonella* in ceca. CELMANAX SCP (Arm & Hammer Animal and Food Production, Princeton, NJ) was included in diets at a rate of 50 g/MT for 2 (Farm 1) or 3 (Farm 2) flocks placed at each of the turkey farms, and for 2 flocks placed at each of the broiler farms. CELMANAX was fed to each flock from placement to processing. Cecal samples (25 for turkeys or 10 for broilers) were collected from untreated, baseline flocks before treatment and from each of the treated flocks at processing, and evaluated for *Salmonella* prevalence. Data were analyzed by a 2 proportion comparison. For turkey farm 1, *Salmonella* was prevalent in all 25 birds that were sampled at baseline, while *Salmonella* prevalence was reduced ($P < 0.01$) and only prevalent in 7 of the 25 sampled turkeys in the first flock on CELMANAX and 5 of the 25 turkeys sampled in the second flock. For turkey farm 2, all samples from baseline and the first and third sampled flocks on CELMANAX had non-detectable levels of *Salmonella*. In the second sampled flock on CELMANAX, *Salmonella* was detected in one of the 25 turkeys. For the broiler farms, cecal *Salmonella* prevalence was reduced ($P < 0.01$) in flocks fed CELMANAX compared with their baseline prevalence. For the 7 broiler farms, there were a total of 29 of 70 broilers that were positive for cecal *Salmonella* before CELMANAX was fed. Only 7 of the 70 broilers sampled in the first flocks fed CELMANAX were positive for *Salmonella*, and 6 of the 70 broilers sampled in the second flocks fed CELMANAX were positive for *Salmonella*. Inclusion of CELMANAX in turkey or broiler diets, throughout the production cycle, may reduce or maintain low levels of *Salmonella* in ceca.

Key Words: prebiotics, refined functional carbohydrates, *Saccharomyces cerevisiae*, *Salmonella*

205 Pooling of laying hen housing system environmental swabs and efficacy of *Salmonella* spp. detection. Deana Jones*¹, Richard Gast¹, Prafulla Regmi³, Garrett Ward¹, Kenneth Anderson², and Darrin Karcher³, ¹USDA ARS, Athens, Georgia, United States, ²NC State, Raleigh, North Carolina, United States, ³Purdue University, West Lafayette, Indiana, United States.

Under the FDA Egg Rule in the US, egg producers are required to conduct mandatory environmental sampling for the detection of *Salmonella* Enteritidis (SE) at set time frames corresponding to pullet, post-peak, and post-molt production phases. The egg producer assumes the costs for the mandatory testing. Since outcomes are based on the overall presence

of SE, pooling of swabs has been discussed to reduce the fiscal burden. The current study was conducted to determine the efficacy of detecting *Salmonella* Enteritidis, Heidelberg (SH), and Typhimurium (ST) in single and pooled swabs. Laying hen flocks housed in conventional cage (scraper blade swabs) and cage-free slat floor (drag swabs) were monitored for all mandatory environmental phases. Each of the isolates utilized were 200 ppm nalidixic acid-resistant. A high (100 cfu) or low (10 cfu) dose of inoculum was introduced on a sample swab. Additional non-inoculated swabs were added to the bag resulting in pools of 1, 2, or 4 swabs ($n = 24$ pools each dose and swabs/pool combination). After non-selective pre-enrichment, aliquots were introduced into both tetrathionate (TT) and Rappaport-Vassiliadis (RV) selective enrichment. Data was sorted for isolate and swab type and Chi-squared analysis to determine difference in prevalence for the number of swabs in a pool within a selective enrichment method. The greatest recovery, regardless of number of swabs, occurred in the 100 cfu inoculum concentration. Single swabs always had the highest rate of detection. SH had the lowest level of detection in scraper blade swabs (58 – 8% detection range across all dose and swabs/pool combinations) compared with ST with the greatest (84 – 32%). The greatest detection in the 10 cfu dose occurred in single drag swabs (90 – 58%) compared with scraper blade swabs (58 – 25%). SE ($P < 0.05$) and ST ($P < 0.05$) recovery was negatively impacted by increasing swabs in the sample. The addition of swabs to sample pools reduced the rate of recovery due to the increased flora competing in enrichment procedures.

Key Words: *Salmonella* spp., environmental sampling, swabs, pooling

206 *Salmonella* Enteritidis contamination of eggs from experimentally infected laying hens of four commercial genetic lines housed in conventional cages and enriched colonies. Richard Gast*¹, Prafulla Regmi², Rupa Guraya¹, Deana Jones¹, Kenneth Anderson², and Darrin Karcher³, ¹USDA-ARS, Athens, Georgia, United States, ²North Carolina State University, Raleigh, North Carolina, United States, ³Purdue University, West Lafayette, Indiana, United States.

Eggs contaminated with *Salmonella enterica* serovar Enteritidis continue to be internationally significant sources of human illness. The ability of this pathogen to colonize reproductive tissues in infected hens leads to deposition inside the edible contents of forming eggs. Although diverse environmental influences are known to affect the persistence and transmission of *Salmonella* infections in poultry, the food safety consequences of different management and housing systems are not fully understood. The present study evaluated the deposition of *S. Enteritidis* inside eggs laid by groups of experimentally infected laying hens representing 4 commercial genetic lines (white egg lines W1 and W2 and brown egg lines B1 and B2). Groups of hens from each line were housed at 555 cm² of floor space per bird in both conventional cages and colony units enriched with access to perches and nesting areas. All hens were orally inoculated with 5.75×10^7 cfu of a mixture of 2 *S. Enteritidis* strains, and the internal contents of eggs laid 5–24 d post-inoculation were cultured to detect contamination. *S. Enteritidis* was found inside eggs laid between 7 and 21 d post-inoculation at an overall frequency of 2.47%, ranging from 0.25% to 4.38% for the 4 individual hen lines. The frequency of *S. Enteritidis* recovery from eggs was significantly higher ($P < 0.05$ in Fisher's exact test) for line W1 than for line W2, and the egg contamination frequency for line B2 was significantly lower than from any other line. The overall incidence of *S. Enteritidis* isolation from white eggs (3.38%) was significantly greater than from brown eggs (1.56%). The frequencies of egg contamination with *S. Enteritidis* did not differ significantly between the 2 housing

systems for any of the hen lines. These results suggest that *S. Enteritidis* deposition inside eggs can vary between genetic lines of infected laying hens, but housing these hens in 2 different systems did not affect the incidence of egg contamination.

Key Words: *Salmonella* Enteritidis, laying hens, eggs, housing systems, genetic lines

Animal Well-Being and Behavior

303 Use of stimulatory and protective environmental enrichments in quail breeding colonies. Rachel Dennis* and Melissa Rudo, *University of Maryland, College Park, Maryland, United States.*

Japanese quail (*Coturnix japonica*) breeding colonies are often maintained in barren environments. Those facilities that utilize environmental enrichments (EE) generally use stimulatory enrichments such as foraging mats and exploratory items, such as beads, to redirect pecking and increase the complexity of the environment. However, aggression and mating behaviors continue in these environments. We designed this study to evaluate the effectiveness of different EEs on the well-being of breeding quail. Utilizing a latin square design we provided each breeding colony cage of quail (1 male: 4 females; from 15 and 25 wks of age) with a mirror, cuttlebone, foraging mat, protective hut, or no EE for 2 weeks each (n = 16). Behaviors were recorded for and analyzed by scan sample for 3 d per week. Eggs were collected daily and incubated, hatchability numbers were analyzed by treatment. Data were analyzed by a latin square ANOVA. All EEs reduced walking compared with a barren environment ($P = 0.001$) but did not affect overall inactivity levels. Protective huts, mirrors and foraging mats reduced severe feather pecking and aggressive pecking ($P = 0.01$ and $P = 0.05$). Mountings were reduced only in cages with protective huts ($P = 0.009$), but did not alter fertility or hatchability of eggs. Standing decreased in birds provided with mats and mirrors ($P = 0.02$), while sitting was increased in these birds ($P = 0.04$). Vigilance was increased when mirrors were first introduced to colony cages ($P = 0.02$), however, after 2 wks of acclimation to the mirror, vigilance was greatly reduced compared with control birds ($P = 0.01$). These data suggest a great benefit of appropriate EEs in breeding colonies of quail. We have also shown that protective EEs such as our protective huts, can be a great benefit to reduce excessive mountings.

Key Words: quail, environmental enrichment, behavior

304 Validation of alternative behavior methodologies in young broiler chickens. Lauren Ross*, Monique Pairis-Garcia, Michael Cressman, and Mary Catherine Cramer, *The Ohio State University, Pataskala, Ohio, United States.*

Continuous sampling is the gold standard utilized across all species; however, it requires a substantial investment of time, labor, and resources. Therefore, validating alternative methodologies is critical. The objectives of this study were to validate behavioral methodologies of young broiler chickens using 1) 7 scan sampling intervals (0.5, 1, 3, 5, 10, 15 and 30 min) and 2) an automated tracking software program (EthoVision XT 14) compared with continuous behavioral observation. Ten, 19-d old Ross 708 broiler cockerels were enrolled in this study, and each broiler was the experimental unit. Behavior was recorded with hardwired IP video cameras continuously for 24 h. Data was collected using a continuous sampling methodology in The Observer XT 14. The same video files were utilized for analysis for scan sampling and automated tracking software analysis. For scan sampling validation, continuous data were converted to 1-s interval samples, and 7 instantaneous sample intervals extrapolated. Total behavior duration and bout number were calculated over an 8-h period for each interval. Linear regression was used to conduct pairwise comparisons between continuous observation and sample intervals, and intervals that met the following criteria were considered to accurately reflect the true duration

and frequency for each behavior: $R^2 \geq 0.9$, slope was not different from 1 ($P > 0.05$), and intercept was not different from 0 ($P > 0.05$). Inactive behavior duration was accurately estimated with 30-s, 1-min, and 3 min scan sample intervals. Number of inactive behavior bouts was accurately estimated with 30-s scan sample intervals. Eating behavior duration was accurately estimated with 30-s- and 1-min scan sample intervals. For validation of automated behavior tracking software, total duration for each behavior was obtained and linear regression (PROC REG) was used to conduct pairwise comparisons between continuous observation and automated tracking software. The automated tracking software was able to accurately detect true duration of inactive behavior ($R^2 \geq 0.9$, slope not different from 1, intercept not different from 0), but was unable to accurately detect active and wing flapping behavior. The results of this study suggest that high frequency and long duration behaviors can be accurately observed with instantaneous scan sampling up to 1-min intervals. Automated tracking software may be able to accurately determine broad behaviors that are based on movement of a center point of a subject, but activity detection resulting from pixel analysis requires further refinement for use.

Key Words: broiler, welfare, validation, video, scan sampling

305 Differences in behavioural time budget between conventional and slow growing broiler chickens. Stephanie Torrey*, Zhenzhen Liu, Linda Caston, Midian Nascimento dos Santos, Daniel Rothschild, and Tina Widowski, *University of Guelph, Guelph, Ontario, Canada.*

Significant improvements in genetic selection over the past 50 years have resulted in a large, lean, efficient broiler chicken. However, the welfare of conventional broiler chickens has come under scrutiny due, in part, to the birds' low levels of activity compared with slower growing strains. The ability to perform or express motivated natural or normal behavior is considered an important part of animal welfare, and so interest is being paid to the use of slow growing strains (defined as those growing $<50\text{g/d}$) in commercial broiler production. As part of a large, multidisciplinary study, we examined the behavioral time budget of 4 strains of broilers differing in growth rates (C, $\text{ADG}_{0-42} = 68.19\text{g/d}$; D, $\text{ADG}_{0-42} = 41.48\text{g/d}$; H, $\text{ADG}_{0-42} = 43.75\text{g/d}$; M, $\text{ADG}_{0-42} = 49.63\text{g/d}$). Over 3 replicates, 8–12 pens of 38–44 birds/pen (30 kg/m^2) per strain (n = 40 pens) were housed in an environmentally-controlled room and fed an antibiotic-free, all vegetarian 3-phase diet. Each pen had a round feeder, 5 nipple drinkers, wood shavings as litter, an elevated polypropylene platform (30 cm above the litter, attached to a 25° ramp), a mineral pecking stone, shredded nylon rope, and automated hanging scale. A minimum of 4 birds/pen (35–69 birds/strain; half male, half female) were observed for 5 min for all occurrences of behavior at d 27, 44 and 56 using Pocket Observer XT. Data for all birds per pen were averaged, transformed (arcsine square root) and analyzed with a general linear model, with observer, replicate and age included in the model. Age affected percentage of time spent sitting ($P = 0.037$), standing ($P = 0.029$), and walking ($P = 0.04$), with younger broilers (d27) spending less time sitting and more time standing and walking than older broilers (d44 or d56), regardless of strain. Younger broilers (d27) spent $59.86 \pm 2.54\%$ of time sitting, $12.58 \pm 1.52\%$ of time standing, and $5.23 \pm 0.80\%$ of time walking whereas older broilers (d44 and d56) spent $67.69 \pm 1.67\%$ of time sitting, $9.29 \pm 0.59\%$ of time standing and $3.31 \pm 0.27\%$ of time walking. Strain influenced percentage of time spent sitting ($P < 0.002$), standing ($P < 0.0001$) and walking ($P < 0.02$). At the same

age, C birds sat more (70.35% of time) and stood (4.63%) and walked (2.19%) less than birds in the 3 slow strains (3 strains combined: sitting: 63.07%; standing: 10.85%; walking: 4.23%). At similar weights, there was an effect of strain on sitting ($P = 0.0018$) and standing ($P < 0.05$), with C sitting for more time than D, and C standing for less time than all 3 slower strains. There were no differences in behavior among the 3 slow strains ($P > 0.10$). Analyses are on-going to determine if the differences in behavior indicate differences in temperament or welfare.

Key Words: broiler, welfare, slow grow, behaviour

306 The effectiveness of ventilation shutdown as an alternative depopulation method for broilers. Kenneth Anderson*², Krista Eberle-Krish², Michael Martin¹, Ramon Malheiros², Kimberly Livingston², and Sanjay Shah², ¹*Veterinary Division, Raleigh, North Carolina, United States*, ²*NCSU, Raleigh, North Carolina, United States*.

Differences in management practices, bird type, and house construction can make it difficult to prescribe a single depopulation method across poultry houses. Ventilation shutdown is an alternative depopulation method used when primary depopulation methods, including water-based foam and carbon dioxide (CO₂) gassing, cannot be performed within 24 h. Ventilation shutdown (VSD) is the cessation of natural or mechanical ventilation of atmospheric air in a building where poultry are housed. The intention of VSD is to quickly raise the temperature inside a poultry house to $\geq 40^\circ\text{C}$ within 30 min by sealing the house and shutting down ventilation, ultimately causing death by hyperthermia. While guidelines have been developed for use in the field, little research has been conducted to determine if and how VSD works in the various poultry species used in commercial production. This proof of concept study was designed to assess the effectiveness of VSD, alone and with supplemental heat (VSDH), on commercial broilers. Whole-house CO₂ gassing (WHG) was used as the control treatment. Ventilation shutdown, VSDH, or WHG were applied to one of 3 groups of 253 male broilers at 46 d of age. Broilers were placed into a 4.6 × 4.6 × 2.4 m room, providing a floor density of 0.09 m² and headspace volume of 0.22 m³. Time to death (TOD) was determined when all bird movement ceased. Survivorship was based on birds that recovered after the room was opened. Core body temperatures were collected pre- and post-treatment application. Ambient temperature, relative humidity (RH), and CO₂ concentrations were collected for the duration of each treatment. TOD for VSD, VSDH, and WHG were 180, 96, and 71 min, respectively. Ventilation shutdown had 0.8% and WHG 0.4% survivorship; no broilers survived VSDH. Pre-application core body temperatures averaged 42.4°C, 41.2°C and 40.4°C and post-application 44.6°C, 45.7°C, 39.5°C for VSD, VSDH, and WHG, respectively. Ambient temperatures averaged for 30.6°C VSD, 64.1°C for VSDH, and 24.2°C for WHG. Average relative humidity was 89%, 25%, and 64%, for VSD, VSDH, and WHG, respectively. The average CO₂ concentrations for VSD and VSDH were 1.8% and 0.1%; whole-house CO₂ concentrations reached 34.9%. Ventilation shutdown did not achieve the ambient temperature needed to cause 100% lethality and should only be used if other options are not available. Broilers were unable to withstand the high temperatures generated in VSDH, indicating susceptibility to heat when ventilation is shut down. The ability to maintain ambient temperatures of $\geq 40^\circ\text{C}$ and lack of survivors makes ventilation shutdown with supplemental heat a viable alternative depopulation method for commercial broilers.

Key Words: ventilation shutdown, depopulation, broiler, chicken

307 The influence of environmental stressors during simulated transportation on the behaviour and physiology for white-feathered end-of-cycle hens. Carley Frerichs*, Trever Crowe, Kailyn Beaulac, and Karen Schwan-Lardner, *University of Saskatchewan, Saskatoon, Saskatchewan, Canada*.

The impact of temperature/RH, duration, and feather cover on white-feathered end-of-cycle hen (sourced from 3 farms) behavior and physiology was examined. Three replicates of 210 hens (65–70wk) were divided into 2 feather cover (105 well feathered (WF), 105 poorly feathered (PF)), 3 duration (4, 8, 12h), and 5 temperature/RH (-10°C uncontrolled RH (-10), 21°C with 30 (21/30) or 80% RH (21/80), 30°C with 30 (30/30) or 80% RH (30/80)) in a factorial arrangement to make treatment combinations (tx). Crates (3; 1/duration) were divided in half for each feather cover (7 hens/side), and placed in an environmental chamber. Before exposure, all hens were feed withdrawn (6h), 5–6 hens/tx were orally administered a data logger to record core body temperature (CBT; baseline measures 5–20min) and were blood sampled for heterophil to lymphocyte (H/L) ratio. Blood parameters were collected on 3 hens/tx; pH, base excess in extracellular fluid (BE_{ecf}), hematocrit (Hct), bicarbonate (HCO₃), and total carbon dioxide (TCO₂). Behavior was recorded and analyzed using 5-min scan sampling. Post-exposure, a second blood sample was taken (delta (Δ) values calculated). Data were analyzed as a randomized complete block design using ANOVA (PROC MIXED, SAS 9.4; blocked by farm). ΔCBT was higher ($P = 0.05$) for hens exposed for 12h for both feather cover at 21/30, 21/80, 30/80, and 30/30 PF than -10 12h PF. ΔpH was higher ($P < 0.01$) for hens exposed for 12h at -10 than hens exposed for 12h to 21/30, 30/30, and 30/80. $\Delta\text{BE}_{\text{ecf}}$ was higher ($P = 0.05$) for hens exposed for 12h to -10 than 8h at -10 , 21/80, and 30/80, 4h 21/80, and 12h 30/80. ΔHct was higher ($P = 0.04$) for WF hens exposed to -10 and PF at 21/80 than PF hens at -10 and 30/80. ΔHCO_3 was higher ($P = 0.02$) in hens exposed to -10 WF than 30/80 PF. ΔTCO_2 was higher ($P = 0.01$) in hens exposed to -10 WF than PF in -10 and 30/80. $\Delta\text{H/L}$ ratio in hens exposed to -10 PF was higher ($P < 0.01$) than other temperature/RH and feather cover. Shivering was greater ($P = 0.03$) in -10 4h than other temperature/RH and duration. The number of hens remaining motionless was smaller ($P < 0.01$) in WF hens exposed to 30/30, 30/80 than PF 30/30, both feather cover 21/80, 21/30, and WF -10 . Panting was greater ($P = 0.02$) for WF hens exposed to 30/80 than PF in 30/30, 30/80, and both feather cover in -10 , 21/30, 21/80. Gulping was greater ($P = 0.05$) for WF hens in 4h than PF 4h and WF 12h. Therefore, exposure to extreme environmental conditions can result in metabolic stressors and thermoregulatory behaviors.

Key Words: layer, transport, blood physiology, thermoregulatory behaviour, welfare

308 Not Presented

309 Effect of myo-Inositol and phytase on laying hen behaviour and welfare. Eugenia Herwig*¹, Henry Classen¹, Carrie Walk², Michael Bedford², and Karen Schwan-Lardner¹, ¹*University of Saskatchewan, Saskatoon, Saskatchewan, Canada*, ²*AB Vista, Marlborough, Wiltshire, United Kingdom*.

Dietary myo-inositol (Myo-I), a byproduct of phytase action, is thought to positively modify the mental state and behavior of humans, but this concept has not been tested in chickens. An experiment was designed to assess the effects of Myo-I in pure form (0.16%) or derived from phytase action (3,000 FTU/kg) on laying hen behavior when fed amino acid deficient and sufficient diets. The 5 treatment diets were: high

balanced protein (HBP – 19% CP), HBP with Myo-I (HBP-I), reduced balanced protein (RBP – 16% CP), RBP with phytase (RBP-P), and RBP with Myo-I (RBP-I). Each treatment was fed ad libitum to 72 fully beaked Lohmann LSL lite hens housed in conventional cages (6 replicates per treatment), from 19 to 59 wk of age. Live scan observations and novel object testing (NO) were performed at 1, 10 and 40 wk of the trial. Mortality was collected daily. At the end of the experiment, feather cover, comb damage and skin lesions were assessed, and blood was collected to determine H/L ratios. Behavior and NO were analyzed using repeated measures in SAS 9.4. Mortality, feather cover, comb and skin lesions, and H/L ratios data were analyzed using one-way ANOVA. Four orthogonal contrasts were also performed to compare RBP-P vs. RBP-I, HBP vs. HBP-I, and the effects of protein level and Myo-I. All differences were considered at $P \leq 0.05$. Dietary Myo-I tended to reduce the performance of comfort behaviors (No Myo-I = 0.7 vs. Myo-I = 0.5%, $P = 0.097$). Reduced protein level increased inactivity level (RBP = 14.5 vs. HBP = 12.0%, $P = 0.003$) and decreased the performance of stereotypic behaviors (RBP = 2.1 vs. HBP = 2.7%, $P = 0.021$). Under RBP, Myo-I reduced stereotypic behavior when compared with RBP-P ($P = 0.022$). However, HBP-I had the highest numerical incidence of stereotypic behavior (HBP = 2.5%, HBP-I = 3.0%, RBP = 2.5%, RBP-P = 2.6%, RBP-I = 1.7%). Dietary Myo-I reduced the latency in NO testing by 300 s ($P = 0.012$). No effect of diet on H/L ratio was found with an average of 0.43 H/L. By the end of the experiment, dietary Myo-I reduced the feather cover in the vent area (No Myo-I = 2.5 vs. Myo-I = 2.1, $P = 0.038$) and increased mortality (No Myo-I = 5.6 vs. Myo-I = 18.8%, $P = 0.031$). Most hens died from cannibalism. No effect of diet on lesions was found. In conclusion, although dietary Myo-I might reduce fearfulness in hens, it also reduced the performance of comfort behaviors and increased vent pecking and cannibalism, diminishing hen welfare. However, none of the negative effects were observed in the RBP-P treatment, suggesting different modes of action of Myo-I supplemented in the diet or provided from phytase.

Key Words: inositol, phytase, cannibalism, fearfulness, stress

310 Welfare of laying hens in enriched colony cages supplemented with dietary probiotics. Marisa Erasmus^{*1}, Kailynn VanDeWater¹, Jiaying Hu¹, and Heng-wei Cheng², ¹Purdue University, West Lafayette, Indiana, United States, ²USDA, West Lafayette, Indiana, United States.

Enriched colony cage systems provide laying hens with behavioral opportunities such as perching, nesting and dustbathing. However, social stress and behavioral problems such as injurious pecking remain important animal welfare issues in these housing systems. Beak trimming reduces injuries from feather pecking and cannibalism, but is associated with pain. This study investigated whether supplementation with the probiotic, *Bacillus subtilis* (sporulin, Novus International, MO), reduces injurious pecking and social stress in laying hens, resulting in improved feather condition and hen welfare. Hy-Line Brown (brown) and Hy-Line W36 (white) chicks were randomly assigned to one of 3 treatment groups: beak trimmed and fed a standard diet (BTS), non-beak trimmed and fed a standard diet (NTS) and non-beak trimmed and fed a probiotic-supplemented diet (NTP). Birds were housed in groups of 9 in enriched colony cages (n = 12 cages/treatment) from 17 to 62 weeks of age. Birds were mixed at 26, 41 and 61 weeks to simulate social stress. Hen welfare (feather condition, feather cleanliness, keel bone damage, claw length and footpad condition; Welfare Quality® Protocol for Poultry, 2009) was assessed for 1 bird/cage at 17 weeks after transfer to the layer house. Thereafter, welfare was assessed for

1 bird/cage 2 weeks before mixing (39 and 59 weeks) and 2–3 d after mixing (41 and 61 weeks). Age-related differences in welfare measures were analyzed with the Friedman test, differences in welfare measures between strains were analyzed with the Mann-Whitney U test, and differences among treatment groups were analyzed with the Kruskal-Wallis test (SAS 9.4). Claw length was analyzed using a repeated measures ANOVA (SAS 9.4). Claw length did not differ among treatment groups, between genetic lines or due to age. Feather condition, footpad condition and keel damage worsened with age ($P < 0.0001$). Feather condition of the head ($P = 0.0002$), neck ($P = 0.006$), back ($P < 0.0001$) and rump ($P < 0.0001$), as well as footpad condition ($P < 0.0001$) and keel bone fractures and deviations ($P = 0.04$) were worse for brown hens. Treatment did not affect welfare measures. Hen welfare was affected more by age and hen genetic line than by probiotic supplementation, which had no measurable effects on the hen welfare parameters evaluated in this study. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2017–67015–26567 from the USDA National Institute of Food and Agriculture.

Key Words: laying hen, enriched cage, probiotics, welfare, beak trim

311 The effect of *Bacillus Subtilis* on Hy-line White and Brown laying hen performance under social stress. Jiaying Hu^{*1}, Kailynn VanDeWater¹, Marisa Erasmus¹, and Heng-wei Cheng², ¹Purdue University, West Lafayette, Indiana, United States, ²USDA-ARS, West Lafayette, Indiana, United States.

Laying hens exhibit dominance hierarchies (pecking order) within a group. Mixing unfamiliar hens together causes aggression and further leads to poor egg production and welfare status. Probiotic inclusion in feed provides various benefits to the animals by changing the gut microflora and related biological modifications. The objective of this study was to determine the effect of *Bacillus subtilis* supplementation (sporulin, Novus International, MO) on hen performance and egg quality in laying hens subjected to mixing-caused social stress. A total of 396 1-d-old female chicks of each line, Hy-Line W-36 and Hy-Line Brown, were housed in 2 rooms (separated by line). Hens were randomly assigned to 1 of 3 treatments (n = 12 cages/treatment/strain): B/W-BT, brown/white beak-trimmed hens fed a regular diet; B/W-R, non-beak trimmed hens fed the regular diet; and B/W-P, non-beak trimmed chicks fed the regular diet mixed with probiotic (300 ppm). Birds were mixed within each line at wk 16, 26, 41 and 61 to simulate social stress. Number of eggs, including cracked, dirty and soft membrane eggs were recorded daily. Feed utilization during a 5 d period was determined at wk 16, 17, 26, 41 and 61, respectively. BW was recorded during the same week. A tonic immobility test was performed at 25, 40 and 60 wk of age. Eight eggs per cage were collected at 24, 32, 40, 48, 58 wk of age to measure egg and eggshell quality. Data were analyzed using the MIXED model procedure of SAS. Repeated measures were used for performance traits. Egg weight increased and shell quality decreased over time ($P < 0.0001$). Between lines, Brown hens had overall higher egg production ($P = 0.01$), BW ($P < 0.0001$), feed consumption ($P < 0.0001$), heavier eggs ($P < 0.0001$), and better shell quality ($P < 0.0001$). Within each line, B/W-P hens had higher BW than B/W-BT hens ($P = 0.02$) and tended to be higher than B/W-R hens ($P = 0.07$). B-P hens had higher BW than B-R ($P = 0.01$) but not B-BT hens. W-P and W-R hens had higher BW than W-BT ($P = 0.04$ and 0.004). In addition, W-P and W-BT hens had a shorter duration of TI than W-R at 40 wk of age ($P = 0.02$), but not at other time points. Treatment did not affect feed intake, egg production, egg weight and shell quality. The results indicate that hen performance traits are mostly affected by hen age and genetic background. Probiotic

supplementation increases BW but does not significantly affect egg production and egg quality traits. (This work was supported by the USDA National Institute of Food and Agriculture, Foundation program of the Agriculture and Food Research Initiative Competitive Grants Program under the Award No: 2017-67015-26567).

Key Words: probiotics, laying hen, production, egg quality, social stress

312 The effects of embryonic Norepinephrine on quail well-being. Rachel Dennis*, *University of Maryland, College Park, Maryland, United States.*

Maternal and incubation stress can have long-term impacts on physical and behavioral development in offspring via an increase in *in ovo* catecholamine levels, including Norepinephrine (NE). In the present study we investigated the effects of elevated embryonic NE on productivity and behavior through weight, egg production, and scan sampling assessments. We hypothesize that increased embryonic NE will negatively impact behavior and production in a dose dependent manner. To determine the effects of elevated *in ovo* NE on productivity and survival-related behaviors, Japanese quail (*Coturnix japonica*) embryos were injected with 10 μ L of 0.01M or 0.05M treatment concentrations of NE or saline at ED1 (n = 130/trt). Body and organ weights were taken at d 1, wk 6, and wk 11. Daily egg production was recorded once the birds reached sexual maturity. Weekly behavioral scan samples were taken from wk 4 (juvenile) to wk 11 (sexually mature) with a rehoming stress just before the wk 7 scan samples. Our results show low dose birds had significantly lower body weights at wk 3 compared with saline birds ($P = 0.0007$). High and low dose birds exhibited reduced egg production compared with saline birds ($P = 0.0008$). Norepinephrine treatment had long-term impacts on survival-related behaviors and altered stress coping strategies in response to rehoming as evidenced by patterns of change in multiple behaviors including inactivity ($P < 0.001$), drinking ($P = 0.036$), foraging ($P = 0.004$), walking ($P < 0.001$), and other behaviors at wk 7. Scan samples showed significantly greater eating frequencies in mature high dose birds ($P = 0.0217$) and greater drinking frequencies in juvenile low dose birds compared with control birds ($P = 0.0088$). High dose birds foraged significantly less compared with low dose and saline birds ($P = 0.0199$). Norepinephrine-treated birds exhibited significantly reduced frequencies of walking compared with control birds ($P = 0.0004$) and significantly greater frequencies of inactivity compared with saline birds ($P < 0.0001$). Altered consumption and activity-related behaviors did not translate into greater weight gain or egg productivity, suggesting that increased *in ovo* NE at the start of embryonic development plays a role in programming of survival-related behaviors and stress-coping mechanisms in a dose- and age-dependent manner driven by changes in underlying adrenergic or metabolic mechanisms. Our findings highlight a need for decreasing sources of maternal, incubation, and environmental stress in a commercial setting to improve poultry well-being.

Key Words: quail, Norepinephrine, behavior, stress

313 Solving floor egg laying in aviaries: Can temporary litter restriction retrain hens without impacting welfare? Ahmed Ali and Janice Siegford*, *Michigan State University, East Lansing, Michigan, United States.*

Many producers in the laying-hen industry are transitioning to cage-free housing following customer and legislative demands. Major concerns of cage-free systems are the undesirable consequences of floor laying, such as more labor to collect eggs and more eggs lost to damage. Temporary exclusion of birds from litter areas is a common management strategy

to try to reduce egg laying in the litter. However, litter restriction may negatively affect bird welfare and prevent hens from performing key behaviors. Our objective was to investigate how excluding hens in midday from litter areas during the morning would influence nest use, incidence of floor laying and hens' physical welfare. Pullets were raised in floor pens and placed into 4 multi-tier aviary units (100 hens/unit) at 17 week of age (WOA). Hens had continuous access to the litter area until 54 WOA when ~60% of all eggs were being laid in the litter. Partial litter restriction (doors to litter automatically closed at 1:00 and re-opened at 11:30) was implemented for a 4-week period starting at 54 WOA. Egg production, damage and location, and number of hens in nests were recorded, and 15% hens/unit were randomly assessed for keel bone damage, foot health and plumage quality. Observations were conducted for 3 consecutive days at 4 successive time points: BEF = the week before litter restriction; DUR = the 4th week of litter restriction; 1WK = the week immediately after restriction ended; and 4WK = 4 weeks after restriction ended. Differences within measured parameters across time points were assessed using GLMM with Tukey's Post hoc test (α set at 0.05) in R 3.3.1. Hens occupied nests more frequently during morning observations in DUR compared with BEF (76.9, 39.6%, $P = 0.01$), while nest occupancy during 1WK (66.98%) was similar to DUR but dropped dramatically at 4WK (44.9%, $P = 0.02$). Hens laid more frequently outside nests during BEF compared with DUR (34.1% vs 8.31%, $P = 0.01$). However, there were slightly more non-nest laid eggs during 1WK (14.31%) than DUR, and by 4WK (30.11%) hens almost returned to their BEF lay pattern. Incidence of damaged eggs decreased from 26.7% in BEF to 3.7% in DUR but increased to 22.4% at 4WK. Incidence of footpad and plumage damage markedly increased at DUR (56.9, 69.9%) compared with BEF (33.7, 39.9%, all $P \leq 0.05$). Incidence of footpad and plumage damage decreased during 1WK (41.2, 56.9%) and 4WK (45.6, 45.7%, all $P \leq 0.05$) compared with DUR. Four weeks of partial litter restriction failed to permanently solve an already established floor-laying problem in a laying hen flock and reduced hens' foot health and plumage quality.

Key Words: laying hen, aviary, litter, nest use, welfare

314 Laying hens that exhibit feather pecking are capable of withholding pecks in a Go/No-Go task. Jennifer Heinsius¹, Nienke van Staaveren*¹, Isabelle Kwon¹, Angeli Li¹, Joergen Kjaer², and Alexandra Harlander¹, ¹*University of Guelph, Guelph, Ontario, Canada,* ²*Friedrich-Loeffler-Institut Federal Research Institute for Animal Health, Celle, Germany.*

Laying hens which perform repetitive pecking at the feather cover (FP) of other birds affect welfare and productivity of the flock. Much research on factors contributing to FP has focused on the social and physical environment, however the role of internal bird factors is less well understood. It was hypothesized that FP in laying hens occurs through a lack of behavior inhibition and so shows similarities to motor impulsivity in mammals. A Go/No-Go task in an operant chamber was designed to assess motor impulsivity in 20 female White Leghorn laying hens coming from a high FP line (HFP) or unselected control line (CON). Hens' behavior was observed over 3 d (20 min/day) to determine the level of FP expressed and continue the selection of hens that show high (>5 pecks) or low levels (<2.5 pecks) of FP. Hens were trained over a series of phases to rapidly and accurately respond to the cues in the Go/No-Go task until they reached a > 75% success rate over 2 consecutive sessions. All hens learned to complete the task and were subsequently tested for their ability to withhold pecks by recording the number of pre-cue responses and number of pecks performed during No-Go cues (false alarms). Hens that came from a HFP line or exhibited high levels

of FP behavior (P) showed the same success at withholding pecks during the pre-cue period and the No-Go cues in a Go/No-Go task as hens that came from the CON line or exhibited low levels of FP behavior. This suggest that impulsivity is not associated with the repetitive motor action of FP and that other internal bird factors should be considered.

Key Words: chicken, impulsivity, feather pecking, go/no-go task

Immunology, Health, and Disease II

315 Live *Salmonella typhimurium* vaccination: Reducing *Salmonella* spp. loads from the field to the processing plant. Manuel Da Costa*, Kalen Cookson, and Jon Schaeffer, Zoetis, Durham, North Carolina, United States.

Vaccination of broilers with live *Salmonella typhimurium* vaccines (Poulvac® ST) is a live-side *Salmonella* intervention strategy that has shown success in the past few years. Three controlled studies and one field trial had the objective of evaluating effects of Poulvac® ST on salmonella prevalence and load of broilers at market age. Poulvac® ST was given by spray at d 0 and boosted by water/oral gavage at 14 d in all the studies. Vaccinated birds were compared with non-vaccinated birds for all the studies. The birds in the controlled studies were raised in isolation rooms with fresh shavings as litter. Birds in study 1 were challenged with *Salmonella heidelberg* at 35 d and sampled at 49 d of age. Birds in studies 2 and 3 were challenged with *Salmonella kentucky* and *Salmonella infantis*, respectively, at 34 d, and sampled at 46 d of age. *Salmonella* prevalence and enumeration (Most Probable Number – MPN) were evaluated in cecae and liver/spleen for all 3 studies. The field trial was conducted in a 4-week on/off fashion of Poulvac® ST vaccinated birds for a total of 16 weeks (total of 8 weeks of vaccination). Sampling consisted of 6 bird rinsates pre-scald collected per day for salmonella enumeration, prevalence and salmonella serotyping. The controlled studies statistical analysis were conducted at the 0.05 level of significance (0.10 considered a trend) using 2-sided tests. The field trial was summarized using descriptive statistics. Prevalence of *Salmonella heidelberg* in cecae (–39% positive samples) and liver/spleen (–75% positive samples) was significantly reduced ($P < 0.05$) by vaccination. *Salmonella* enumeration was also significantly lowered ($P = 0.05$) in cecae by –1.5 log. In study 2, Poulvac® ST significantly reduced ($P < 0.05$) both liver/spleen prevalence (–75% positive samples) and cecae load (–0.5 log) of *Salmonella kentucky*. The *Salmonella infantis* challenge take was overall low. Yet, vaccinated birds tended ($P = 0.06$) to have lower *Salmonella* load in the cecae with concomitant numerical reduction in cecae prevalence (–75% positive samples). On the field trial, Poulvac® ST showed a 3.1% reduction in percent positives and 0.5 log on MPN. There were 12 *Salmonella* serotypes isolated from Control birds whereas Poulvac® ST birds had only 5 serotypes detected. In addition, the load of the serotypes isolated on Poulvac® ST birds were lower when compared with Control birds (0.2 to 1.2 log reduction). In conclusion, Poulvac® ST resulted in effective cross-protection of broilers against various serotypes of *Salmonella* spp. by reducing prevalence and load from the farm to the processing plant level.

Key Words: *Salmonella*, vaccination, broilers, serotype

316 The effect of direct fed microbials application in the water on performance and gut microbial composition of broiler chickens. Alexander Yitbarek*, Kirsty Gibbs, Marion Bernardeau, and Qiong Wang, DuPont Industrial Biosciences, Wilmington, Delaware, United States.

Stability of Direct Fed Microbials (DFMs) in the feed has been a challenge for non-spore forming probiotic bacteria. Waterline application is an alternative delivery that can secure the stability of DFMs lacking the intrinsic properties of spore formers. This study evaluated the effect of application of DFMs, a blend of 4 non-spore formers (2 species each of lactic acid and propionic acid producing bacteria) in water on performance and gut microbial composition of broiler chickens under

a mild-challenge condition with reused litter. One-day old Ross® 308 broiler chickens were randomly assigned to 8 treatments (12 pens per treatment and 30 chickens per pen). Treatments included a basal diet without antibiotics (NC); NC with 55 g/t of bacitracin methylene disalicylate (BMD); and DFMs administered in the water of NC chickens at the following doses; DFM-1 (0.02mL/bird/d for days (D)0–14), DFM-2 (0.03mL/bird/d for D1–3, D15–17 and D27–29), DFM3 (0.05mL/bird/d for D0–14), DFM-4 (0.08mL/bird/d for D1–3, D15–17 and D27–29), DFM-5 (0.4mL/bird/d for D0–14), and DFM-6 (0.62mL/bird/d for D1–3, D15–17 and D27–29). At D14 and 42, analysis of performance (FI, BWG, FCR and mortality) and gut microbial composition using Illumina Miseq® to sequence the V3–V4 region of 16S rRNA were conducted. The 16S amplicon paired-end reads were subjected to quality trimming and clustering at 99% identity level to obtain Operational Taxonomic Units (OTUs). Compared with BMD, NC resulted in significantly lower BWG and higher FCR at D0–14 and D0–42 ($P < 0.05$). Administration of DFMs in the water at all doses (except for DFM1) resulted in improvement of both BWG and FCR with significant differences in BWG and FCR in DFMs-4, –5 and –6 compared with NC ($P < 0.05$). All DFMs, except for DFM-1, resulted in significantly lower mortality compared with NC ($P < 0.05$), while no difference was observed compared with BMD ($P > 0.05$). Administration of DFMs in the water resulted in a healthier microbial profile compared with NC where a higher relative abundance of *Lactobacillus reuteri*, *Alistipes Shahii* and *Lactobacillus acidophilus* was observed, while potentially pathogenic microbes such as *Enterococcus cecorum* populations were significantly reduced. Therefore, findings from this study suggest that administration of non-spore forming DFMs via the water can modulate gut microbial composition of broiler chickens resulting in a positive effect on performances, highlighting potential usage of DFMs in the water as alternatives to antibiotic growth promoters.

Key Words: broilers, gut microbiota, probiotics, gut health

317 The effect of direct fed microbials in the feed on performance and gut microbial composition of broiler chickens with a mild necrotic enteritis challenge. Wenting Li, Alexander Yitbarek*, and Qiong Wang, DuPont Industrial Biosciences, Wilmington, Delaware, United States.

With the ban or limitations in the use of in-feed antibiotics, there is a growing challenge of necrotic enteritis outbreaks in the poultry industry globally. Direct Fed Microbials have been shown to be one of the promising alternatives to in-feed antibiotics. This study evaluated the effect of Enviva® Pro, a blend of 3 *Bacillus* spp. (DFM), on performance, and gut histomorphology and microbial population in chickens vaccinated with 10 × the recommended dose of a coccidiosis vaccine to induce a mild necrotic enteritis in the gut. A total of 1248 one-day old Ross® 708 broiler chickens were randomly assigned to 4 treatments: Basal diet without vaccination and no antibiotics (NC), NC with vaccination (CC), CC with 55 g/t of bacitracin methylene disalicylate (BMD) and CC fed diets supplemented with DFM at 10⁵ cfu/g of feed. At d 21 and 42, analysis of performance (FI, BWG, and FCR) was performed, while at d 21, lesion score, villus height (VH) and gut microbial composition analysis using Illumina Miseq® to sequence the V3–V4 region of 16S rRNA were performed. The 16S amplicon paired-end reads were subjected to quality trimming and clustering at 99% identity level to obtain Operational Taxonomic Units (OTUs). At D0–21, CC resulted in a significantly lower BWG compared with NC ($P = 0.0223$), while

both BMD and DFM were not significantly different from both NC and CC ($P > 0.05$). At D0–42, CC resulted in a significantly lower BWG compared with all treatments ($P = 0.003$), while there was no significant difference among NC, BMD and DFM ($P > 0.05$). At D0–21 and D0–42, FCR was significantly higher in CC compared with NC ($P = 0.0054$), while both BMD and DFM were not significantly different from NC and CC ($P > 0.05$). At D21, A significantly higher lesion score was observed in CC compared with all other treatment, while both BMD and DFM also resulted in a significantly higher lesion score compared with NC ($P < 0.0001$). Furthermore, a significantly lower VH was observed in CC compared with NC and DFM ($P = 0.0005$), while no difference was observed among other treatment comparisons ($P > 0.05$). Microbial population analysis showed that BMD treatments clustered together while DFM did not change the community composition compared with NC and CC. A significantly higher differential abundance of *Lactobacillus acidophilus* was observed in the DFM treatment compared with CC ($P = 0.0239$). In conclusion, the findings of the current study suggest that supplementation of 3-strain *Bacillus spp.* can have a positive effect on performance and gut health of chickens with mild necrotic enteritis, and can be considered as a good candidate to replace in-feed antibiotics to reduce necrotic enteritis in broiler chickens.

Key Words: broilers, necrotic enteritis, probiotics, gut health

318 Native broiler microorganisms improve intestinal inflammation in growing broilers and improve zootechnical performance, mortality and lesion scores of broilers challenged with *Clostridium perfringens*. Kevin Bolek*, Grant Gogul, Shea Grenier, Gavin John, Joshua Leffler, Adam Taylor, Allison Wells, James Gaffney, Michael Pham, Jennifer Cao, Norman Pitt, and Mallory Embree, *Ascus Biosciences, Inc., San Diego, California, United States.*

Two experiments were conducted using an endomicrobial supplement (EMS) comprised of 3 bacteria native to the broiler gastrointestinal microbiome. Strains BR16 (*Lactobacillus sp. nov.*), BR21 (*Clostridium sp. nov.*), and BR67 (*Clostridium sp. nov.*) were fed to determine their effects on (1) the intestinal immune response in broiler chickens and (2) performance of broiler chickens during a *Clostridium perfringens* challenge. In Experiment 1, 96 Cobb 500 broiler males were allocated to 6 floor pens (16 birds/pen) on day of hatch and divided into 2 treatment groups. Birds were fed a non-supplemented diet (Treatment 1) or a diet supplemented with BR16, BR21, and BR67 at a dose of 10^6 cells per bird, per day (Treatment 2). Ileal tissues were collected at 7 d and 35 d for analysis of tissue cytokine expression. Cytokine expression was analyzed using qRT-PCR and Mann-Whitney test. At 7 d, both IL-17 ($P \leq 0.05$) and IL-1 β ($P \leq 0.05$) expression were significantly lower in Treatment 2 compared with Treatment 1. The same result was observed for IL-17 ($P \leq 0.01$) and IL-1 β ($P \leq 0.01$) at 35 d. In Experiment 2, 1800 Cobb 500 broiler males were allocated to 60 floor pens at day of hatch and divided into 10 treatment groups fed daily. Treatment groups were: (1) no challenge, non-supplemented; (2) challenge, non-supplemented; (3) challenge + 10^4 cells/bird of strains BR21 and BR67; (4) challenge + 10^5 cells/bird of strains BR21 and BR67; (5) challenge + 10^6 cells/bird of strains BR21 and BR67; (6) challenge + 10^7 cells/bird of strains BR21 and BR67; (7) challenge + 10^4 cells/bird of strains BR16, BR21 and BR67; (8) challenge + 10^5 cells/bird of strains BR16, BR21 and BR67; (9) challenge + 10^6 cells/bird of strains BR16, BR21 and BR67; (10) challenge + 10^6 cells/bird of strain BR16, 10^7 cells/bird of strains BR21 and BR67. Birds in challenged treatments received *C. perfringens* via feed at 17 d. Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were measured between 0 and 42 d. Data were analyzed using a one-way ANOVA, with all treatments compared with

Treatment 2. Between 28 and 35 d BWG was increased in treatments 4, 7 and 8 ($P \leq 0.05$) while FI increased in treatments 7 and 8 ($P \leq 0.05$) and FCR lowered in treatments 4, 5 and 8 ($P \leq 0.05$). Lesion scores were reduced in treatments 1, 5, and 8 at 21 d ($P \leq 0.05$) and at 28 d in treatments 1, 4, 6, 7, 8, and 9 ($P \leq 0.05$). Between 28 and 35 d, FCR was decreased in treatments 5 and 6 ($P \leq 0.05$). Mortality was reduced in treatment 5 ($P \leq 0.05$) and mortality due to NE was reduced in treatments 1, 4 and 8 ($P \leq 0.05$) between 0 and 42 d. The data suggest that EMS reduces inflammation and improves performance, lesion scores, and mortality during a *C. perfringens* challenge.

Key Words: clostridium, broiler, microbiome, cytokine, performance

319 Proteobiotics reduce clinical expression of necrotic enteritis in broiler chickens challenged with *Clostridium perfringens*.

Daniel Lank*, Jonathon Roepke¹, and Mansel Griffiths², ¹*MicroSintesis, Charlottetown, Prince Edward Island, Canada*, ²*Canadian Research Institute for Food Safety, Guelph, Ontario, Canada.*

Objective: To determine the improvements when a commercial proteobiotic is applied at different dosages for the control of necrotic enteritis symptoms in broilers using a *Clostridium perfringens* challenge model. **Experimental design and Materials and Methods:** Commercially sourced male chicks were placed in the Colorado Quality Research facility for the purposes of this study and were vaccinated with commercial Mareks, Newcastle, and Infectious Bronchitis vaccines. Birds were randomly allocated to 44 pens with 11 pens (replicates) per treatment. The treatments were then assigned to blocks and randomized within the blocks. The treatment groups consisted of a control group (NTC), and 3 different dosages of commercial proteobiotic (Nuvio, MicroSintesis Inc.) administered from d 12–28 via the drinking water, 3 mg/ kgBW/day (N-3), 6 mg/ kgBW/day (N-6), and 12 mg/ kgBW/day (N-12). All treatments were orally challenged with *C. perfringens* (CI-15, type-A, α , and β 2 toxins) on d 17. Measurements collected throughout the period included the mortalities, bird weights on d 0, 17, 28, and 35, feed consumed for each stage, necrotic enteritis score on 5 birds / pen on d 21, and litter caking score on d 28. **Statement of statistical analysis:** The pens were assigned using RCBD and the pens were used as the statistical unit. **Results:** The study results demonstrated significant ($P = 0.0067$) differences in mortality associated with Necrotic Enteritis, for the treatment of N-12 compared with NTC, N-3 and N-6 treatment groups. Mortality for the respective groups were NTC 16.9%, SF 2.1%, N-3 20.3%, N-6 16.1%, and N12 11.2%. The average 21-d necrotic enteritis lesion scores were also improved ($P = 0.039$) for the N-12 group compared with the other 3 treatment groups. The average lesions scores for each group were NTC 1.62, N-3 1.93, N-6 1.64, N-12 1.38. When growth performance and feed conversion were evaluated, there were no statistical differences in average daily gain or feed conversion. However, the addition of proteobiotics improved the litter scores compared with the NTC treatment which had 72% caked litter. The N-12 dose had 59% caked litter ($P = 0.0019$), N-6 had 63% caked litter ($P = 0.0162$) and the N-3 does had 65% caked litter but was not statistically different ($P = 0.1039$). **Conclusions:** Necrotic Enteritis is a disease of significant importance to the poultry industry worldwide. Under the conditions of this challenge study the commercial proteobiotic (Nuvio) demonstrated the ability to reduce clinical expression of *C. perfringens* when fed at 12 mg/ kgBW/day before and during the challenge period. The data presented here indicates that Nuvio could reduce the impact of Necrotic Enteritis in the field.

Key Words: necrotic enteritis, *Clostridium perfringens*, proteobiotics, probiotic, metabolite

320 Nicotinic acid and hypoxanthine produced by *Bacillus subtilis* 29784 modulate the intestinal health of broilers. Pearl Choi*², Venessa Eeckhaut², Damien Preveraud¹, Lamyia Rhayat¹, Estelle Devillard¹, Lynn Vanhaecke², Richard Ducatelle², and Filip Van Immerseel², ¹Adisseo, Commeny, France, ²Faculty of Veterinary Medicine, Merelbeke, Belgium.

Multiple studies have shown that probiotic strains are capable of inducing beneficial effects on intestinal health and growth performance of broiler chickens. The underlying mechanisms however, are often not fully understood. Probiotics may produce metabolites that have a direct effect on the host or indirectly influence the host by stimulating microbial communities. SCFAs such as acetate, propionate and butyrate have often been the focus of interest. With the emergence of new technologies such as metabolomics, other microbial metabolites should be investigated. In this study, we used a 2-step approach to identify major metabolites produced by a *Bacillus subtilis*-based probiotic known to have beneficial effects on broiler performance and health. The first step consisted in cultures of the *B. subtilis* strain 29784 (Bs 29784) grown in Luria-Bertani and Tryptic Soy Broth culture media. After 4h, 10h and 24h, the supernatant of cultures was analyzed with UHPLC-MS to identify the metabolites produced in vitro. The second step was an in vivo study, in which 1-d old broiler chicks were continuously administered the same *B. subtilis* strain via the diet at a concentration of 10^{10} cfu/kg feed. At d13, intestinal samples from different locations were collected and analyzed via HPLC-MS for a targeted metabolite analysis. A DNA extraction was performed on the intestinal samples to determine the relative abundance of *Bacillus* species in different intestinal locations (via qPCR) to be able to find a correlation between the metabolites quantified in an intestinal section and the relative abundance of *Bacillus* species in that section. Nicotinic acid and hypoxanthine were the 2 main metabolites that were increased in the supernatant of Bs 29784 cultures. An increase in their concentrations was also measured in ileum and jejunum samples of 13-d old chickens to which the strain was administered. The increased concentrations correlated with the place where the relative abundance of *Bacillus* species was the highest in the intestinal tract. Nicotinic acid and hypoxanthine are 2 important metabolites produced by Bs 29784 that are found in increased concentrations in the intestinal content of chickens to which the strain was administered as a feed additive. Nicotinic acid serves as a vitamin B3 source and therefore plays an essential role in a multitude of cellular functions. It is an anti-inflammatory mediator acting on the receptor GPR109a, that is also used by butyrate to exert its' beneficial functions. Hypoxanthine, a purine degradation product, has been shown to improve barrier function and wound healing by increasing intracellular ATP.

Key Words: probiotic, *Bacillus subtilis*, metabolite, gut health, performance

321 The effects of Amasil NA, SILO Health 104L, and formaldehyde on the gut permeability of d 42 Cobb 700 broilers.

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The introduction of organic acids to the gastrointestinal tract (GIT) with organic acid-based feed amendments is an essential tool for producers in replacing feed-based antibiotics. Organic acid supplementation in poultry feeds offers the opportunity to deliver these acids directly to the GIT function, which has untold effects on GIT immunity and permeability. Amasil has documented increases in feed efficiency and digestibility as compared with Formaldehyde, but gut permeability is unknown.

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A production trial using Cobb700 broilers was conducted to order to determine whether Amasil NA (0.25% and 0.5%) and Silo Health 104L (0.25%) were comparable to formaldehyde (0.5%) feed amendments. On d 0, day-of-hatch, 864 male Cobb700 broilers were placed in 48-floor pens (5 treatments, 10 replicates for treatment groups, 9 replicates for NC, 18 birds per pen) with untreated pine litter. At d 42, 20 broilers per treatment were orally gavaged with fluoresceine isothiocyanate dextran (FITCD) one hour before the terminal bleed and sacrifice. At sacrifice, the blood was collected and the circulating FITC-dextran was quantified and compared between groups. Statistical analysis was conducted using SAS comparing all of the means individually against the formaldehyde group using a one-way ANOVA, followed by analysis with the Dunnett's test ($P \leq 0.05$). The control (37) and SILO health (39.7 ng/ml) fed broilers had almost half of the circulating FITCD as compared with the Amasil NA (94.2 ng/ml and 103.4 ng/ml) and Formalin fed group (71 ng/ml). Additionally, leaky gut increased numerically stepwise between the Amasil low and low groups. Evidence suggests that SILO Health 104 reduces leaky gut and may have improved gut function.

Key Words: gut health, microbiome, broiler, organic acids, feed

322 Effect of a lactic acid bacteria based probiotic on gut health and gut microbiome of broilers under commercial conditions.

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Establishment of gut microbial communities can be affected by dietary and host-related factors and becomes more complex as broilers grow older. Dietary probiotics can modulate the microbial community, which can result in beneficial changes for gut health and growth of broiler chickens. In this paired-house field trial, a lactic acid bacteria (LAB)-based probiotic was administered through drinking water to commercial broilers during the first 3 d life and for a period of 5 d spanning the feed change from starter to grower feed. Parameters measured included: overall mortality, body weight at 15 and 33 d of age ($n \geq 30$ birds/house), duodenal and ileal histomorphometry at 3, 12, and 15 d of age ($n = 10$ birds/house), gut microbiome content and diversity at 3, 12, and 15 d of age ($n = 10$ birds/house). While differences in mortality across paired houses were not clinically relevant (averaging $< 1\%$) and mean body weights between control and treatment houses were not significantly different at 15 or 33 d of life in either set of paired houses (Student's *t*-tests, $P < 0.05$), treated houses did have better uniformity at both 15 and 33 d of life as indicated by standard deviations from the mean. This indicates that the LAB-based probiotic may be beneficial in maintaining body weight uniformity in commercial broiler flocks. Additional trials will be needed to confirm this. Histomorphometry and microbiome results are pending and will be analyzed via multiple linear regression analysis to assess differences in average villus:crypt ratios and kruskal-wallis test to evaluate changes in α and β microbiome diversity.

Key Words: lactic acid bacteria, probiotic, broilers, microbiome, gut health

323 Synbiotic supplementation as alternative to anticoccidial for coccidiosis challenge in turkey poults.

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The poultry industry is actively seeking alternative solutions to reduce usage of antibiotics and chemicals as consumer and regulatory standards evolve. The objective of this study was to evaluate the effects of synbiotic (PoultryStar[®] me, combination of probiotics and prebiotics) supplementation on performance and intestinal health of turkey poults during a mixed *Eimeria* challenge. A total of 1,080 one-day old male poults were allotted to 1 of 3 dietary treatments: 1) a control diet, a diet supplemented with coccidiostat (Clinacox), or a diet supplemented with synbiotic. On d 16, half of the pens were administered a mixed *Eimeria* challenge (*Eimeria adenoides* and *Eimeria meleagridis*) via oral gavage resulting in a total of 6 groups. Each group consisted of 6 replicate pens with 30 birds per pen. The trial was conducted over a 45-d period with performance measurements recorded on d 0, 16, 21, 28, and 45. On d 21 and 28, 5 birds per pen were euthanized for intestinal lesion scoring and microbiome analysis. Fresh fecal samples were also collected daily starting on d 21 to determine oocyst shedding. Data were analyzed as an ANOVA with significance reported at $P \leq 0.05$. Body weight gain and feed conversion ratio (FCR) were improved ($P \leq 0.05$) in challenged birds administered synbiotic or coccidiostat from d 21–28 compared with challenged control. Challenged birds supplemented with synbiotic showed significantly improved ($P \leq 0.05$) body weight throughout the trial when compared with the challenged control and was similar to the challenged coccidiostat group. Synbiotic with or without challenge had better ($P \leq 0.05$) FCR than unchallenged coccidiostat group but was similar to challenged coccidiostat group, indicating consistency of the synbiotic's performance. On d 21, the percentage of birds displaying lesions in the jejunum and ileum was reduced ($P \leq 0.05$) in birds provided either synbiotic or coccidiostat. Similar to non-challenged controls, fecal samples from challenged birds supplemented with either synbiotic or coccidiostat displayed no evidence of oocyst shedding ($P \leq 0.05$) unlike the challenged control. Synbiotic supplementation increased ($P \leq 0.05$) Firmicutes phyla, *Lactobacillales* order, *Lactobacillus* spp., and *Bifidobacterium* spp., on d 28 compared with challenged control, but was similar to coccidiostat supplementation. Overall, these results suggest supplementation of poultry-specific synbiotic may be a viable alternative to anticoccidial used to alleviate negative consequences observed during a coccidiosis challenge in turkeys.

Key Words: *Eimeria*, probiotic, prebiotic, microbiome

324 Evaluation of broilers fed diets supplemented with Bastion[®] and challenged using coccidiosis or necrotic enteritis disease models. Nicholas Evans*¹, Peter Karnezos¹, and Curtis Novak², ¹PMI Nutritional Additives, Arden Hills, Minnesota, United States, ²Purina Animal Nutrition, Arden Hills, Minnesota, United States.

Two battery cage studies were conducted to compare performance, mortality, and lesion scores of broilers supplemented with Bastion[®] (a proprietary blend of phytochemicals, prebiotics, and probiotics) and challenged using coccidiosis or necrotic enteritis (NE) disease models. Study 1: Experimental treatment groups were: 1) NC (no challenge + no additives) 2) PC (challenge + no additives); 3) Bastion[®] (challenge + Bastion[®] 1.25 lbs/ton). Birds were challenged with *Eimeria acervulina* (100,000 oocyst/bird), *E. maxima* (25,000 oocyst/bird), and *E. tenella* (50,000 oocyst/bird) at age 14 d. Post-challenge and overall performance data were collected and evaluated at 14–20d and 0–20d, respectively. Oocyst per gram of fecal material (OPG) was evaluated at 120–144 h post-challenge and gross coccidial lesion scores were evaluated at age 20 d. All data were analyzed by ANOVA and when the model was significant ($P \leq 0.05$), a post-hoc analysis (LSD) was performed. The 14–20d and 0–20d feed intake (FI) for Bastion[®] was significantly lower ($P \leq 0.05$) than PC; however, weight gain (WG)

remained similar between the 2 groups. The 14–20d mortality adjusted feed conversion ratio (MAFCR) was significantly improved ($P \leq 0.05$) for Bastion[®] compared with PC. Total oocyst shedding and lesion scores were also significantly lower ($P \leq 0.05$) for Bastion[®] compared with PC. There were no significant differences in mortality between groups. Study 2: Experimental treatment groups were: 1) NC (no challenge + no additives) 2) PC (challenge + no additives); 3) Robenz[®] (challenge + Robenz[®] 33ppm) 4) Bastion[®] (challenge + Bastion[®] 0–14d: 1.25 lbs/ton and 15–28d: 1.0 lb/ton). Birds were challenged with *E. maxima* (5,000 oocyst/bird) at age 14 d and then *Clostridium perfringens* (10^8 cfu/ml) at age 19 d. Post-challenge and overall performance were collected and evaluated at 14–28d and 0–28d, respectively. Gross necrotic enteritis lesion scores were evaluated at age 21 d. All data were analyzed using the statistical methods outlined above. The 14–28d FI for Bastion[®] was similar to PC, but the 0–28d FI was significantly higher ($P \leq 0.05$) for Bastion[®] compared with PC. Additionally, the 14–28d and 0–28d WG and MAFCR was significantly improved ($P \leq 0.05$) for Bastion[®] compared with PC. As expected, Robenz[®] had significantly lower 0–28d FI and MAFCR compared with Bastion[®], but WG was similar between the 2 groups. Lesion scores were significantly lower ($P \leq 0.05$) for Bastion[®] compared with PC. There were no significant differences in total or NE mortality between groups. In these studies, Bastion[®] improved performance and reduced lesions in birds challenged using coccidiosis and necrotic enteritis disease models.

Key Words: coccidiosis, necrotic enteritis, broiler, performance, lesion scores

325 Efficacy of Exzolt[®] (1% fluralaner aqueous solution) in the treatment of infestations of *Dermanyssus gallinae* on laying hens. Gustavo Perdoncini*¹, Luis Vettorato¹, Rafael Chiummo², Emmanuel Thomas², and Luis Alves³, ¹Merck Animal Health, São Paulo, Brazil, ²Merck, São Paulo, Brazil, ³Unioeste, Cascavel, PR, Brazil.

Infestations caused by hematophagous mites in the egg industry have generated economic losses. The consequences include drop production data and susceptibility to poultry diseases. The solution to be used to control infestations must have high efficacy, easy application and zero egg withdrawal. From this perspective, was evaluated the efficacy of the systemic acaricide fluralaner to treat commercial laying hens infested with *Dermanyssus gallinae* (*D. gallinae*). The study was designed to evaluate the efficacy of Exzolt[®] (1% fluralaner aqueous solution) intended for treatment of poultry red mite (*D. gallinae*) infestation in layers in Brazil. One house was assigned to the Control Group (CG) and another one was the Treated Group (TG). In the TG the hens received the treatment via drinking water on Study Day (SD) d 0 and 7, at a dose of 0.5 mg fluralaner per kg of Body Weight (BW). The mite infestation of the groups at inclusion was assessed by mite traps placed in both houses and removed after approximately 48 h. The determination of primary efficacy was based upon the *D. gallinae* counting in the treated and the control groups pre- and post-treatment. Efficacy percentage was calculated for each post-treatment assessment time point using count mite in traps along the study. This method allows assessment of the effect of treatment on the survival of mite populations after treatment. Birds were observed once weekly for general health starting on SD 0 until SD 115. The study was stopped on request of the farmer to avoid leaving the control house untreated. Fluralaner showed an efficacy of 95.9% on SD 2 and 100% on SD 8 until the end of the study. There were no abnormal health observations before or after treatment. Due to regulatory demands, various conventional mite products have been withdrawn from European markets or banned in the past few years because they did not comply with European or national regulatory requirements with

regards to human consumer and user safety. Allied to the application of these insecticides, most of them must be sprayed directly on the close environment of the birds, thus decreasing the efficacy due to ability of mites to reach refuges in crevices and cracks. As a systemic acaricide given in the drinking water, fluralaner provides a combination of potent efficacy, bird safety, user convenience due to a ready to use aqueous solution for water administration with zero egg withdrawal time for layers. Under the described experimental conditions, Fluralaner solution (10 mg/ mL) was safe to the birds and highly effective in eliminating the infestation of *Dermanyssus gallinae* from the second day up to the 115th day after the first treatment administration. Exzolt is not approved for sale in Canada.

Key Words: egg, exzolt, fluralaner, hen, red mite

326 Reduced crude protein in starter phase diets of broiler chickens alters immune response in both starter and grower phase without affecting growth performance. Rose Whelan*¹, Victor Naranjo¹, Mohammad Kamely², Jeremy Wakaruk², Juan Bayona², Wanwei He², and Daniel Barreda², ¹Evonik Nutrition & Care GmbH, Hanau, Germany, ²University of Alberta, Edmonton, Canada.

The trial aimed to investigate the effect of a reduced crude protein starter diet on immune development and response of broiler chickens. Two starter (d 1–14) diets were formulated to be isocaloric with varying levels of supplemental amino acids to achieve standard (STD) or reduced (RCP) crude protein of 27% or 24% dry matter, respectively. Both treatments received a common grower diet (d 15–35). A total of 230 male Ross 708 broilers were allocated to one of the 2 dietary treatments with 9 pen replicates each (12 birds/pen). From this, 6 pens/treatment were used for weekly measurements of body weight, feed intake and feed efficiency, while 3 pens/treatment were used for immune measurements. On d 7, 14, 28 and 35, 10 birds/pen were euthanized to measure relative weights of femur, thymus, bursa and spleen; as well as total leukocytes and percent leukocyte subsets in bone marrow and blood. On d 14 and 35, 5 birds/pen were intraperitoneally injected with 2.5mg zymosan A (TLR2/dectin-1 agonist) to mimic an infection. The dose was optimized to induce a self-resolving inflammatory process to highlight changes in the induction and control of immune antimicrobial mechanisms. At 0, 4 and 12 h post injection (pi) total leukocyte and percent of leukocyte subsets were evaluated in the bone marrow, blood and peritoneum of 5 birds/replicate. Additionally, ROS production and cytokine/chemokine expression were measured in peritoneal leukocytes. Statistics were analyzed using 2-way ANOVA for time x diet effects with Tukey's post-hoc test in Prism6 (GraphPad Software, La Jolla, CA, USA) with significance at $P < 0.05$. There were no significant effects of diet on growth performance, or relative organ weights at any time point. RCP applied only in the starter phase increased ($P < 0.05$) total leukocytes/ml blood at d 35 compared with the STD treatment. Immune challenge at d 14 showed that RCP increased ($P < 0.05$) infiltration of total leukocytes into the peritoneum at 4 and 12 h pi. RCP did not affect ROS production or cytokine/chemokine expression in peritoneal cells pi. RCP increased ($P < 0.05$) basal total blood leukocytes at d 35 before stimulation, and this correlated with a higher capacity for total leukocyte recruitment into the abdominal cavity pi at d 35 compared with d 14. Bone marrow leukocyte populations remained unaffected at any time point before or after stimulation, suggesting no effect of starter phase RCP on hematopoiesis. The results conclude a potential improvement in RCP starter diets with additional supplemental amino acids on the immune development and response of broiler chickens, even into the grower phase.

Key Words: broiler, immune, crude protein, starter, challenge

327 Not Presented

329 Quantum blue reduces the severity of woody breast myopathy via modulation of oxygen homeostasis-related genes in broiler chickens. Elizabeth Greene*¹, Joshua Flees¹, Sina Dadgar¹, Barbara Mallmann¹, Sara Orłowski¹, Narasimhan Rajaram¹, Samuel Rochell¹, Michael Kidd¹, C. Brearley³, Carrie Walk², Sami Dridi¹, and Hayley Whitfield³, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²ABVista, Marlborough, United Kingdom, ³University of East Anglia, Norfolk, United Kingdom.

The incidence of woody breast (WB) is increasing on a global scale representing a significant welfare problem and economic burden to the poultry industry and for which there is no effective treatment due to its unknown etiology. Using diffuse reflectance spectroscopy coupled with iSTAT portable analyzer, we have evidence that circulatory- and breast muscle-oxygen homeostasis is dysregulated in chickens with WB. Blood and breast muscle samples were collected from normal and WB birds ($n = 8$ /group). Gene and protein expression were analyzed by Student "t" test, with significance at $P < 0.05$. Blood hemoglobin subunit Mu (HBM), Zeta (HBZ), and hephaestin (HEPH) expression were downregulated; however the expression of the subunit rho of hemoglobin β (HBBR) was upregulated in chicken with WB compared with normal birds. The expression of these genes was downregulated in the breast of WB compared with healthy birds. HIF-1 α mRNA and protein were induced in WB compared with normal breast confirming a local hypoxic status. Phosphorylated levels of upstream mediators AKT^{Ser473}, mTOR^{Ser2481}, and PI3K P85^{Tyr458}, as well as their mRNA levels were increased in WB muscle. To identify a nutritional strategy to reduce WB incidence, male broiler chicks (Cobb 500, $n = 576$) were assigned to 48 floor pens and subjected to 6 diets in a complete randomized design (12 birds/pen; 8 pens/treatment): nutrient adequate control group (PC), PC with 0.3% myo-inositol (PC+MI), negative control (NC) deficient in available P and Ca by 0.15 and 0.16%, NC with quantum blue (QB, ABVista, Wiltshire, UK) at 500, 1,000 or 2,000 FTU/kg of feed. Performance data and gene expression were analyzed by one-way ANOVA using the fit model platform in JMP Pro v14.0. Means were separated using non-orthogonal contrast statements and post-hoc Scheffe's adjustment. WB scores were analyzed as one-way ANOVA using the categorical platform in JMP Pro v14.0. Score means were separated using Pearson Chi-squared. Differences between the frequency of each score within diet was determined using Fisher's Exact Test. Significance was set at $P < 0.05$. PC+MI did not differ from PC diet in growth performance or WB incidence, but NC had lower body weight and WB incidence. QB-enriched diets did not affect growth performance, but reduced the severity of WB by 5% compared with the PC diet. This effect is mediated by reversing the expression profile of oxygen homeostasis-related genes (downregulation of HBBR and upregulation of HBM, HBZ, and HEPH in blood, and upregulation of HBA1, HBBR, HBE, HBZ, and PHD2 in breast muscle) compared with PC diet. Together, these data show that oxygen homeostasis is dysregulated in WB and this effect may be partially reversed by dietary phytase.

Key Words: quantum blue, hypoxia, woody breast, oxygen-sensing genes, growth performance

Metabolism and Nutrition: Enzymes I

330 A novel effect of a NSPase, Natugrain TS®, in improving the microbiome and reducing mortality in layers fed corn soy diets. Michael Coelho^{*1}, Robert Jones², and Frank Parks², ¹BASF Corporation, Humble, Texas, United States, ²Integrated Farm Research Inc, Waterloo, Georgia, United States.

This study presents a novel find of a Feed NSPase Enzyme, Natugrain TS, reducing mortality and reducing eggshell and egg yolk pathogen. A total of 240 Hy-Line 36 laying hens at 18 weeks (wks) were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block as the random effect (3 hens/cage x 5 NSPase treatments x 40 replications). data were analyzed as a randomized complete block design with means separation based on the least significant difference test. Layers were fed corn/soy mash diets and the NC was formulated to meet or exceed current Hy-Line W36 recommendations. Treatments consisted of T1 = NC +0 g/MT Natugrain TS, T2 = NC + 100 g/MT Natugrain TS, T3 = NC+125 g/MT Natugrain TS, T4 = NC+150 g/MT Natugrain TS and T5 = 175 g/MT Natugrain TS. Analysis included intestinal microbiome, viscosity, *E. coli*, lesion scores, villus height and depth, mortality, and eggshell and yolk pathogen content. Microbiota was analyzed in fresh fecal samples. At 90 wks, in the control treatment, T1 = NC+0 g Natugrain TS, there was a prevalence of *Escherichia*, *Shigella*, *Proteobacteria*, *Escherichia coli* and *Clostridium* spp, etc. In the T2 = NC+ 100 g/MT Natugrain TS treatment there was a prevalence of *Bifidobacterium*, *Peptostreptococcaceae*, *Ruminococcaceae*, *Firmicutes*, *Lactobacilli* and *Bacilli*, etc. Natugrain TS significantly decreased intestinal viscosity (T1 = 4.20, T2 = 2.30, T3 = 2.08, T4 = 2.10 and T5 = 2.11 M.Pa.s, $P < 0.05$); significantly decreased dirty eggs (6.45, 1.50, 1.18, 1.21 and 1.20%, $P < 0.05$); significantly decreased intestinal IgG (5.26, 3.80, 3.30, 3.20 and 3.56 g/l, $P < 0.05$); significantly decreased intestinal *E. coli* log₁₀ (3.67, 1.59, 1.21, 1.15 and 1.35, $P < 0.05$); Natugrain significantly decreased mortality from 3.4% to 1.0% at 135 g/MT (3.4, 1.7, 1.3, 0.8 and 2.1%, $P < 0.05$). Natugrain TS significantly decreased percent of eggshell bacteria pathogens at 100 g/MT Natugrain TS (*E. coli* in T1 = 11 and T2 = 4.2%, $P < 0.05$), *Salmonella* (4.7 and 1.2%, $P < 0.05$), egg yolk *E. coli* (14.2 and 5.7%, $P < 0.05$) and *salmonella* (10.2 and 6.0%, $P < 0.05$). In conclusion, Natugrain TS broke down arabinoxylans into smaller pieces that serve as food source to beneficial bacteria, and changed the microbiome to a prevalence of *Bifidobacterium*, *Peptostreptococcaceae*, *Ruminococcaceae*, *Firmicutes*, *Lactobacilli* and *Bacilli*, etc. Natugrain TS significantly decreased intestinal viscosity, dirty eggs, IgG, eggshell and yolk pathogens and significantly decreased mortality from 3.4 to 1.0% at 135 g/MT Natugrain TS.

Key Words: layers, NSPase, mortality, microbiome, egg pathogens

331 Production performance and flock uniformity in broilers supplemented with an exogenous protease enzyme. Glenmer Tactacan* and Derek Detzler, *Jefo Nutrition, Inc., Saint-Hyacinthe, Quebec, Canada.*

Along with phytase and non-starch polysaccharide-degrading enzymes, protease has become a standard component in broiler diets. By improving dietary protein digestibility and amino acid (AA) availability, protease may deliver positive impact on animal health and performance. One of the key indicators of animal performance is flock uniformity. A poorly uniform flock is generally characterized by delayed growth and poor feed conversion ratio (FCR) leading to high number of downgrades

at harvesting and processing. To this end, a study was conducted to evaluate the effects of protease supplementation on animal performance and flock uniformity in broiler diets. A total of 960-d-old male Cobb chickens were randomly assigned to receive 1 of 4 dietary treatments: 1) Treatment 1, a standard diet based on corn and soybean meal (T1), 2) Treatment 2, same as T1 but reduced with 2.5% digestible AA and 20 kcal/kg ME + 0.0125% Protease (T2), 3) Treatment 3, same as T1 but reduced with 5.0% digestible AA and 20 kcal/kg ME (T3), 4) Treatment 4, same as T3 + 0.0125% Protease (T4). A completely randomized design consisting of 4 treatments, 12 replicate pens, and 20 birds in each pen was used. Flock uniformity was measured by weighing birds individually at the start (d 0) and at the end of the study (d 42) and was expressed as coefficient of variation (CV) in live weight, with increased CV values synonymous with decreased uniformity. Relative to T1 and T3, protease supplementation in T2 and T4 diets increased ($P < 0.05$) body weight gain (BWG) (2.453 vs. 2.509 and 2.365 vs. 2.422 kg) and decreased ($P < 0.05$) FCR (1.758 vs. 1.736 and 1.799 vs. 1.770) at d 42, respectively. Compared with T1, reducing the levels of digestible AA and ME in T3 adversely affected BWG and FCR at d 42, however, protease supplementation in T4 diet allowed birds to restore performance similar to those birds fed with T1 (standard diet). Flock uniformity was significantly improved ($P < 0.05$) by protease supplementation in T2 diet relative to T1 (4.85 vs. 7.65) but had no effect in birds fed with T3 and T4 diets (8.55 vs 6.78). There was no difference in flock uniformity between birds fed T1, T3, and T4 diets. Overall, protease supplementation may improve or restore losses in animal performance when used in nutrient reduced broiler diets. In addition, it may also help promote better flock uniformity, thus, reducing economic losses due to increased number of downgrades at harvest.

Key Words: performance, flock uniformity, protease, amino acid, broiler

332 Mineral, amino acid and energy sparing effect of a high dose of phytase, combined with xylanase, on performance, carcass yield and carbon footprint of male broilers from 1 to 42 days. Gilson Gomes^{*1}, Tara York¹, Oliver Kinsey¹, and Christine Young², ¹AB Vista, Plantation, Florida, United States, ²AB Sustain, Peterborough, United Kingdom.

The objective of the current study was to evaluate the effects of feeding a higher phytase level in combination with xylanase on performance, carcass yield and carbon footprint of broilers. A total of 816 d-old male Cobb 500 chicks were allocated to 48 pens. Chicks were distributed on a randomized complete block design, with 4 treatments and 12 pen replicates (17 birds/pen). The treatments were: T1: positive control without enzymes; T2: same nutritional levels as T1, with inclusion of 500FTU/kg of phytase (Quantum Blue, AB Vista, Marlborough, UK) applying a mineral matrix (0.15, 0.165 and 0.035% of avP, Ca and Na, respectively) and 9,600 BXU/kg of xylanase (Econase XT, AB Vista, Marlborough, UK) applying a conservative energy matrix (50kcal/kg); T3: reduction, in comparison to T1, of 0.20%, 0.22%, 0.045%, 0.05% and 120kcal/kg of avP, Ca, Na, dLys and AME, respectively; T4: as T3 supplemented with 2,000 FTU/kg of phytase and 9,600 BXU/kg of xylanase. Pen BWG, FI and FCR were determined. Mortality was recorded daily. At 42 d, 6 pens per treatment were selected for carcass yields (CY). Carbon dioxide equivalency (CO₂e) was calculated using an accredited model (AB Sustain, 2017) and expressed as kg of CO₂ per kg carcass. Data was submitted to one-way ANOVA as a randomized complete block design. When differences were significant at an α level

≤ 0.05 means were then separated using Tukey's test. From 0 to 42 d of age birds fed the T3 diets showed poorer body weight corrected FCR (FCRw) by 36 points when compared with the T1 fed birds ($P < 0.05$). The same pattern of reduction on BWG, FI and CY were observed, with birds fed the T3 diets exhibiting poorer performance compared with T1, T2 or T4 ($P < 0.05$). No performance or CY differences were observed for T1, T2 or T4 ($P > 0.05$). When calculating the CO₂e of birds fed the different treatments, the best result was obtained by those birds fed T4, which was lower than the T1 fed birds ($P < 0.05$). Birds fed the T2 diets being intermediate among the 3 treatments. Birds fed the T3 diets presented the worst CO₂e due to impaired performance and CY ($P < 0.05$). Evaluating cost of feed per kg of broiler, birds fed the T4 diets were the lowest when compared with other treatments ($P < 0.05$) resulting in a competitive advantage while maintaining performance, and leading to a more sustainable production of broiler meat.

Key Words: phytase, xylanase, cost, performance, sustainability

333 Energy (ME) and amino acid releasing efficacy of graded levels of NSPase, Natugrain® TS in 4 and 44 weeks Hy-Line layers fed corn/soy diets. Michael Coelho^{*1}, Robert Jones², and Frank Parks², ¹BASF Corporation, Humble, Texas, United States, ²Integrated Farm Research Inc, Waterloo, Georgia, United States.

NSPases break down arabinoxylans into xyloses. When NSPases break down arabinoxylans, energy and AA are released in the process. The feed passage rate in 4 weeks old (wks) pullet is faster than in a 44 wks old layer. This study evaluated the apparent metabolizable energy (AME) and prececal amino acid (AA) release of graded levels of NSPase when fed to 4 and 44 wks old layers on mash corn/soy diets formulated to meet or exceed current Hy-Line feeding recommendations. A total of 840 Hy-Line W 36 layers were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block as the random effect (3 birds/cage x 7 treatments x 20 replications). Treatments consisted of T1 = PC, T2 = NC = PC - 200 kcal/kg -10% AA, T3 = NC+50 g/MT NSPase, T4 = NC+ 75 g/MT NSPase, T5 = NC+100 g/MT NSPase, T6 = NC+125 g/MT NSPase and T7 = NC+150 g/MT NSPase. TiO₂ was used as the undigestible marker. Feed, prececal and fecal collection at 4 or 44 wks. Feed and fecal samples gross energy determined with a bomb calorimeter. SID AA digestibility and total-tract apparent ME digestibility were calculated. AME for the 4 wks NSPase treatments was 2781, 2574, 2593, 2650, 2692, 2703 and 2704 Kcal/kg, $P = 0.02$, respectively and at 44 wks was 2776, 2586, 2613, 2670, 2715, 2723 and 2722. NSPase doses released at 4 wks 19, 76, 118, 129 and 130 Kcal/kg, respectively, and at 44 wks released 27, 84, 129, 137 and 136 Kcal/kg, $P = 0.03$, respectively, 9% higher than at 4 wks. NSPase SID CP% released at 4 wks was 0.250, 0.310, 0.368, 0.372 and 0.374% and at 44 wks 0.270, 0.317, 0.372, 0.376 and 0.374%, $P = 0.04$, respectively, 2% higher than at 4 wks. NSPase SID MET% released at 4 wks was 0.001, 0.002, 0.004, 0.005 and 0.005% and at 44 wks 0.002, 0.003, 0.005, 0.006 and 0.005%, $P = 0.03$, respectively, 12% higher than at 4 wks. NSPase SID LYS% released at 4 wks was 0.009, 0.013, 0.017, 0.018, 0.019% and at 44 wks 0.010, 0.014, 0.018, 0.019 and 0.019%, $P = 0.03$, respectively, 5% higher than at 4 wks. In conclusion, Natugrain TS at 100 g/MT released on average 124 Kcal/kg, 0.370% SID CP, 0.005% SID MET, 0.018% SID LYS, 0.032% SID ARG, 0.008% SID HIS, 0.015% SID ISO, 0.019% SID LEU, 0.013% SID CYS, 0.014% SID PHE, 0.016% SID THR, 0.005% SID TRP and 0.020% SID VAL. Birds at 44 wks released 9% more nutrients than at 4 wks, most likely due to a slower feed passage rate, increased time feed exposed to enzyme.

Key Words: layers, ME, amino acids, digestibility, NSPase

334 Effect of phytase dosage and limestone particle size fed to broiler breeders on performance of progeny. Jean Durau², Gilson Gomes^{*1}, Alex Maiorka², Josiane Panisson², Leopoldo de Almeida², and Andréia Massuquetto², ¹AB Vista, Plantation, Florida, United States, ²Universidade Federal do Paraná, Curitiba, PR, Brazil.

The aim of the study was to evaluate the effects of phytase dosage and limestone particle size when fed to broiler breeders and its effects on progeny performance. The trial consisted of 4 treatments distributed in a 2x2 factorial arrangement - 2 phytase levels (500 and 1500 FTU/kg, Quantum Blue, AB Vista, Marlborough, UK) and 2 limestone particle size, fine with GMD (geometric mean diameter) of 419µm and coarse with 2661µm. Breeders and roosters (720 females and 72 males Cobb 500, from 23 to 61 weeks of age) were distributed in a completely randomized design, and diets were offered as mash, based on corn, soybean and wheat middlings. Males diets contained the same phytase dosage as females from the same treatment, and limestone GMD employed on males diets was exclusively fine. The experimental diets were provided from 30 to 61 weeks of age. The incubation of eggs was performed at the 57th week of age. A total of 270 eggs were incubated per treatment, totaling 1080 eggs. Only eggs laid on nest were used, fumigated with formaldehyde at the hatchery, and stored for 5 d at 21°C. After storage, the eggs were subjected to 6 h of heating at 27°C, and incubated in a single stage machine at 37.5°C and 65% RH. On the 19th day of incubation the eggs were transferred to hatcher set at 36.5°C and 75% RH. The opening of the hatcher occurred with 500 h, and the chicks were sexed at the hatchery. The progeny performance was evaluated, with 12 replicates per treatment, being 6 of males and 6 of females with 13 birds per experimental unit. Birds were housed in metal cages with a density of 14.7 birds/m², until 21 d of age, receiving the same diet, formulated with 500FTU/kg phytase, during all the experimental period. Water and feed was offered *ad libitum*. During the first 10 d continuous light was used, and after that period it was used 9 h of darkness, and the temperature was maintained according to the breeders guidelines. It were evaluated feed intake (FI), weight gain (WG) and feed conversion rate (FCR) in the period of 1 to 7, 1 to 14 and 1 to 21 d of age. The data was submitted to ANOVA, and if significant, the means were compared by Tukey test (≤ 0.05). There was no interaction or difference for limestone particle size on the performance of progeny ($P \geq 0.05$). Chicks from breeders receiving 1500 FTU/kg phytase showed improved FCR from 1 to 7 d ($P = 0.04$), and had a tendency for better FCR from 1 to 14 ($P = 0.05$) and 1 to 21 ($P = 0.09$). As conclusion, the progeny of breeders fed with higher dose of phytase (1500 FTU/kg) showed improved feed efficiency.

Key Words: broiler, calcium, phytate, phosphorus, inositol

335 Investigating the interaction effect of different levels of apparent metabolizable energy, digestible amino acids, available phosphorus, arabinoxylan, phytate, with and without a multi-carbohydrase and phytase complex on growth performance in broilers. Maamer Jali^{*3,4}, Pierre Cozannet^{3,4}, Rob Shirley¹, Dan Moore², Marcio Ceccantini³, and Aurélie Preynat^{3,4}, ¹Adisseo USA, Atlanta, Georgia, United States, ²Colorado Quality Research, Wellington, Colorado, United States, ³Adisseo France SAS, Antony, France, ⁴Center of Expertise in Research and Nutrition, Malicorne, France.

A Box-Behnken design was used to investigate interactions between different levels of apparent metabolizable energy (ME, 3,125, 3,000, 2,844 kcal/kg), digestible amino acids (dig.Lys, 10.0, 9.6, 9.1 g/kg), available phosphorus (avP, 3.3, 2.4, 1.9, 1.6 g/kg) and substrate (soluble arabinoxylan/phytate, low = 5.5/8.2, high = 14.4/9.6 g/kg), with and without a multi-carbohydrase and phytase complex (MCPC, containing 1,250 xylanase, 860 b-glucanase, 4,600 arabinofuranosidase and 1,000

phytase U/kg diet) on growth performance of Ross 308 male broilers from 0 to 36 d of age. The design consisted of 36 treatments with 6 replicates of 17 birds each. Bird performance were significantly affected by diets nutrients content. Feed intake (FI) were affected primary by avP according a quadratic relationship. Mineral content subsequently affected ($P < 0.001$) body weight gain (BWG) and had detrimental effect on feed conversion ratio (FCR). ME and dig.Lys were secondarily affecting these performance criteria. Decrease of avP from 3.3 to 1.6 g/kg in control diets unsupplemented with MCPC significantly impaired the FI (-5% , $P < 0.001$), BWG (-10.3% , $P < 0.001$) and FCR ($+5.9\%$, $P < 0.001$). Performance were described by equations based on ME, dig.Lys and avP ($R^2 = 0.89, 0.90$ and 0.75 , for FI, BWG and FCR respectively, $P < 0.001$). Addition of MCPC resulted in significant improvement of animal performance by $+4\%$, $+9\%$ and -5% for FI, BWG and FCR, respectively. Significant interactions were observed between substrate level, diet nutrient content and MCPC efficacy on FI ($P < 0.01$), BWG ($P < 0.001$) and FCR ($P < 0.05$). Namely, MCPC effect was related with diet nutrient constraints and amount of released nutrient linked to dietary substrate level. Based on equation previously established between diet ME, dig.Lys and avP, MCPC supplementation allowed significant reductions in ME, dig.Lys, and avP by 90 kcal, 0.5 g and 0.14% unit per kg diet, respectively, with low level of substrate and 170 kcal, 0.7 g and 0.16% unit per kg diet, respectively, with high level of substrate. Our results demonstrated that MCPC is an effective means to release energy and nutrients. They also highlighted the need to consider the interactions between energy, nutrients and substrate (arabinoxylan and phytate) levels to better predict the efficacy of exogenous enzymes in broilers.

Key Words: broilers, multi-carbohydrase and phytase complex, performance, substrate

336 Energy (ME)-releasing efficacy of graded levels of 6-phytase, Natuphos® E, NSPase, Natugrain® TS, and a combination of phytase +NSPase in 28-day old broilers fed corn/soy/ DDGs diets. Michael Coelho¹, Robert Jones², and Frank Parks², ¹BASF Corporation, Humble, Texas, United States, ²Integrated Farm Research Inc, Waterloo, Georgia, United States.

This study evaluated the metabolizable energy (ME) release of graded levels of phytase, NSPase and a combination of phytase+NSPase when fed to 28-d old broilers on mash corn/soy/DDGs diets, with 0.24% phytate phosphorus, formulated to meet or exceed current 1994 N.R.C recommendations. A total of 6930 Ross 708 male birds were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block as the random effect. Phytase trial with 33 birds/pen x 7 treatments (treat) x 10 replications (reps), NSPase trial, with 5,600 TXU/g and 33 birds/pen x 7 treat x 10 reps and phytase + NSPase trial with 33 birds/pen x 7 treat x 10 reps. Phytase treatments consisted of T1 = PC, T2 = NC = PC- 0.17% Calcium (Ca), - 0.15% non-phytate phosphorus (nPP), -0.02% sodium (Na), -300 kcal/kg, T3 = NC +1000 FTU/kg phytase, T4 = NC+1500 FTU/kg phytase, T5 = NC+2000 FTU/kg phytase, T6 = NC+2500 FTU/kg phytase and T7 = NC+3000 FTU/kg phytase. NSPase treatments consisted of T1 = PC, T2 = NC = PC-100 Kcal/kg, T3 = NC+50 g/MT NSPase, T4 = NC+ 75 g/MT NSPase, T5 = NC+100 g/MT NSPase, T6 = NC+125 g/MT NSPase, T7 = NC+150 g/MT NSPase. Phytase + NSPase treatments consisted of T1 = PC, T2 = NC = PC- 0.17% Calcium (Ca), - 0.15% non-phytate phosphorus (nPP), -0.02% Na, -300 kcal/kg, T3 = NC+1000 FTU/kg phytase+0.125 g/kg NSPase, T4 = NC+1500 FTU/kg phytase+0.125 g/kg NSPase, T5 = NC+2000 FTU/kg phytase+0.125 g/kg NSPase, T6 = NC+2500 FTU/kg phytase+0.125 g/kg NSPase and T7 = NC+3000 FTU/

kg phytase +0.125 g/kg NSPase. Partial fecal collection was done at d 28. AME was calculated by the ratio of indigestible marker, TiO₂. Data was subjected to ANOVA with means separated with least significant difference at $P < 0.05$. ME for phytase treatments was 3249, 2932, 3011, 3057, 3086, 3131 and 3189 Kcal/kg, $P = 0.02$, respectively. ME for NSPase treatments was 3142, 3049, 3077, 3131, 3175, 3181 and 3183 Kcal/kg, $P = 0.03$, respectively. ME for phytase+NSPase treatments was 3217, 2926, 3089, 3114, 3128, 3167 and 3202 Kcal/kg, $P = 0.02$, respectively. 1000, 1500, 2000, 2500 and 3000 FTU/kg phytase released 79, 125, 154, 199 and 257 Kcal/kg, respectively. 50, 75, 100, 125 and 150 g/MT NSPase released 28, 82, 126, 132 and 134 Kcal/kg, $P = 0.03$, respectively. Phytase + NSPase doses released 163, 188, 202, 241 and 276 Kcal/kg. Combined phytase+NSPase of T3 = NC+1000 FTU/kg phytase+0.125 g/kg NSPase, T4 = NC+1500 FTU/kg phytase+0.125 g/kg NSPase, T5 = NC+2000 FTU/kg phytase+0.125 g/kg NSPase, T6 = NC+2500 FTU/kg phytase+0.125 g/kg NSPase and T7 = NC+3000 FTU/kg phytase +0.125 g/kg NSPase released 163, 188, 202, 241 and 276 Kcal/kg, respectively. In conclusion, combined phytase+NSPase released up to 276 Kcal/kg.

Key Words: broilers, phytase, NSPase, ME, corn soy DDGs diet

337 Effect of cholecalciferol vitamin D3, calcium, phytase, Natuphos® E, and NSPase, Natugrain® TS, on layer production persistency. Michael Coelho¹, Robert Jones², and Frank Parks², ¹BASF Corporation, Humble, Texas, United States, ²Integrated Farm Research Inc, Waterloo, Georgia, United States.

Layer production persistency is based on many factors, including gut, gizzard and liver health. This study evaluates the effect of different levels of mineral metabolism, immune system and organ health additives (vitamin D3 (D3)), gizzard health additives (coarse limestone, coarse feed, fat, soybean hulls), gut health additives (phytase, Natuphos E, and NSPase, Natugrain TS) on the lay persistency and egg quality from 80 to 110 weeks (wks). A total of 800 Hy-Line W36 laying hens at 80 wks laying were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block as the random effect (8 hens/cage x 10 treatments x 10 replications). Layers were fed corn/soy meal diets and the PC was formulated to meet or exceed current Hy-Line W36 recommendations. Treatments consisted of T1 = PC Hy-line W36 recommendations, except formulated with 4000 IU/kg D3, 4.50% Ca, 400 FTU/kg phytase and 50% coarse limestone (CL); T2 = PC+ 4000 IU/kg D3; T3 = PC+85% CL; T4 = PC+1500 FTU/kg phytase; T5 = PC+ 4000 IU D3 + 85% CL+1100 FTU/kg phytase; T6 = PC+2% vegetable/animal fat (VF) + 4% soybean hulls (SBH); T7 = PC+120 g/kg NSPase; T8 = PC+ 2% VF + 4% soybean hulls + 120 g/kg NSPase; T9 = PC+4000 IU D3 + 85% CL + 1100 FTU phytase + 2% VF+4% SBH, and T10 = PC+4000 IU D3 + 85% CL+1100 FTU/kg phytase + 2% VF + 4% SBH+120 g/kg NSPase. At 110 wks, feed intake, g/b/d ($P = 0.03$) was highest with T1 and lowest with T9 and T10; FCR ($P = 0.02$) was lowest with T9 and T10; Hen-day egg production, % ($P = 0.03$) was highest with T9 and T10; Egg weight, g ($P = 0.03$) was highest with T9 and T10; Egg shell, % ($P = 0.03$) was highest with T9 and T10; Egg thickness, mm ($P = 0.02$) was highest with T9 and T10; bone strength, kgf/cm² ($P = 0.03$) was highest with T9 and T10 and mortality ($P = 0.01$) was lowest for treatments containing NSPase (T7, T8 and T10). In conclusion, T9 = PC+4000 IU D3+85% CL + 1500 FTU phytase + 2% VF+4% SBH and T10 = PC+4000 IU D3 + 85% CL+1500 FTU phytase + 2% VF + 4% SBH+120 g/kg NSPase significantly extended performance persistency and increased layer performance, egg quality and bone strength of laying hens in extended production. Treatments with NSPase, Natugrain TS, decreased mortality from 2.7 to 0.4%.

Key Words: cholecalciferol, calcium, phytase, NSPase, layers

338 Impact of dietary calcium level and non-phytate phosphorus level on phytase efficacy in improving phosphorus digestibility of SBM in broilers. Qian Zhang*, Frances Yan, Ashley Provin, Mercedes Vazquez-Anon, and Deana Hancock, *Novus International, St. Charles, Missouri, United States.*

In addition to dietary calcium (Ca) level, non-phytate phosphorus (nPP) level may contribute to the variations of phytase efficacy on the improvement of phosphorus (P) digestibility of ingredients, as dietary nPP indicates the P deficient status of birds. Therefore, a battery study was conducted to evaluate the effect of dietary Ca level and nPP level on phytase efficacy in improving P digestibility of SBM in broilers. A total of 512 birds were allocated to 8 dietary treatments, with 8 replicate pens of 8 birds per treatment, in a completely randomized design. Eight dietary treatments were in a $2 \times 2 \times 2$ factorial arrangement (0.59% or 1.02% dietary Ca; SBM with 0.17% nPP or SBM-dicalcium phosphate blend with 0.30% nPP; with or without 500 U/kg phytase). The source of phytase was CIBENZA® PHYTAVERSE® (Novus International Inc., St. Charles, MO). The diets were provided to birds for 40 h feeding duration (d 19 to d 21) in mash form. On d 21, all birds were sacrificed to collect ileal digesta for nutrient digestibility. Data were analyzed by 3-way ANOVA to evaluate the main effect of dietary Ca level, nPP level, phytase and their interactions on P digestibility of SBM. Statistical difference was declared at $P \leq 0.05$. The results demonstrated no significant interaction among any of the factors on P digestibility of SBM. The addition of phytase significantly increased ($P < 0.0001$) the P digestibility of SBM from 34.8% to 67.6%. Dietary nPP level showed no effect on P digestibility of SBM, nor did it impact the phytase uplift of the P digestibility of SBM. High Ca significantly decreased ($P = 0.0003$) P digestibility of SBM from 55.5% to 46.9%, however, dietary Ca level didn't play a role on phytase uplift of the P digestibility of SBM. Briefly, at low Ca, dietary phytase increased the P digestibility from 40.2% to 70.8%; at high Ca, dietary phytase increased the P digestibility from 29.4% to 64.3%. In summary, the increase of dietary Ca but not nPP levels significantly decreased P digestibility of SBM. The addition of CIBENZA® PHYTAVERSE® at 500 U/kg significantly increased P digestibility of SBM by 32.7%, which was not impacted by increasing dietary Ca or nPP levels.

Key Words: phytase, phosphorus digestibility, SBM, calcium, non-phytate phosphorus

339 Effect of Allzyme SSF E+C on performance and body composition of broilers receiving diets with reduced metabolizable energy. Alice Murakami*, Marcia Sakamoto¹, Paulo Pozza¹, Luiz Costa e Silva², and Adriana Nascimento², ¹Universidade Estadual de Maringá - UEM, Maringá, Paraná, Brazil, ²Alltech do Brasil, Maringá, Parana, Brazil.

A study was conducted to evaluate performance and body composition of broilers receiving diets with enzymatic complex Allzyme® SSF E+C (EC, Alltech Inc.) with metabolizable energy level reduction from 1 to 42 d of age. The EC has protease, phytase, and cellulase enzyme activities. 1,080 one-d-old male broiler chicks were randomly distributed into 6 treatments with 9 replicates (20 birds/replicate). The treatments consisted of 4 levels of metabolizable energy (control, -50, -100, and -150 kcal/kg) with EC and the 2 treatments (control and -150 kcal/kg) without EC. The control treatments had 3,000 kcal/kg and 3,170 kcal/kg for the initial and growing phase, respectively. Performance, carcass and cut yields, and energy retention in the carcass were evaluated. The

birds where fasted during 36 hs and then were slaughtered to evaluated the carcass composition. The carcasses were frozen, ground, homogenized, and sub-samples were oven-dried at 55°C for 96 h. After the final drying, samples were ground again (2-mm sieve) and analyzed for the contents of dry matter, gross energy, ether extract, and crude protein. Data were analyzed by one-way ANOVA using SAS software (2009) at a significance level of 5% and means compared using the Tukey test and orthogonal contrast between the treatments with and without EC. No effect ($P > 0.05$) of treatments were observed on weight gain, feed intake, carcass and cuts yields, abdominal fat and retention of body energy. However, the reduction of metabolizable energy content affected ($P < 0.0001$) the feed conversion, except for the treatment with EC and 50 kcal reduction, showing the same feed conversion compared with the control treatment without EC, at 21 and 42 d of age. The contrast analysis demonstrated that the EC supplementation provided a lower abdominal fat deposition ($P = 0.0584$) than birds receiving the control diet without EC. The use of Allzyme® SSF E+C allowed a reduction of 50 kcal ME/kg in broiler diets, from 1 to 42 d of age, without affecting broiler's performance and body composition.

Key Words: carcass composition, energy reduction, exogenous enzyme, performance

340 Buttiiauxella phytase supplementation in a nutrient-reduced diet consistently increased apparent ileal digestibility of amino acids in broiler starters. Abiodun Bello*¹, Reza Abdollahi², Yueming Dersjant-Li¹, Leon Marchal¹, and Ravi Ravindran², ¹DuPont Animal Nutrition, Nutrition & Biosciences, Leiden, South Holland, Netherlands, ²Massey University, Palmerston North, New Zealand.

The objective of the current study was to evaluate the efficacy of *Buttiiauxella* phytase (Aextra® PHY; BP) on the apparent ileal digestibility coefficients (AIDC) of amino acids (AA) in broilers. Four experimental diets included a positive control (PC), which contained 2,975 kcal/kg ME, 1.06% dig lys, 20.4% protein, 0.37% avP, 0.84% Ca, and 0.18% Na. A negative control (NC = PC minus 68 kcal/kg ME, 3–5% dig AA and protein, 0.20% unit in Ca, 0.22% unit in avP, and 0.04% unit in Na), and the NC supplemented with BP at 500 (NC+BP500) and 1,000 (NC+BP1000) FTU/kg. Male Ross 308 broilers were randomly assigned to 32 cages (8 birds per cage) and were fed a common pre-experimental diet from 1 to 5 d and then fed one of 4 experimental diets from 6 to 15 d. The experimental diets were based on corn-soy-rapeseed meal-rice bran and contained 0.5% titanium dioxide. On d15, digesta of distal half ileal were collected by cage for the determination of AIDC of AA. The digestible AA content in the diet (dig AA_d) was calculated for individual AA and the total AA (n = 17), based on AIDC and the analyzed dietary AA content, expressed as % of DM. Data were analyzed using JMP 14.1. The AIDC was not different between the PC and NC for all AA and the average of AA, except Met and Cys. Phytase at both doses improved ($P < 0.05$) AIDC of Met, Cys, Thr, Ile, Leu, Phe, and average AA vs NC. At 1000 FTU/kg, phytase improved ($P < 0.05$) AIDC of Lys, Arg, Val, and His. Supplemental BP at 500 and 1,000 FTU/kg increased ($P < 0.05$) the total dig AA_d by 0.89 and 1.13% point, respectively, while the 1,000 FTU/kg BP increased ($P < 0.05$) dig Met_d, dig Cys_d, dig Lys_d, and dig Thr_d by 0.034, 0.031, 0.055, and 0.060% point, respectively, vs. NC. The data demonstrates the extra-phosphoric effect of BP to increase AA digestibility. BP at 1000 FTU/kg supplemented to dig AA deficient NC diet increased digestible lys, M+C, thr and total AA content to those comparable to PC ($P > 0.05$). This study indicates that when supplemented with *Buttiiauxella* phytase, diets can be formulated with reduced digestible AA, allowing for significant reductions in dig AA

and crude protein contents to further decrease feed cost and nitrogen excretion into the environment.

Key Words: amino acid, broilers, *Buttiauxella* phytase, digestibility, digestible amino acids

341 Phytase, Natuphos E®, glycinate trace minerals and antioxidant vitamins reduce woody breast in broilers on corn soy diets.

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Woody breast is initiated by inadequate blood supply and a breakdown of glycogen to lactic acid, low levels of physiological antioxidants and reduced hemoglobin synthesis. This study evaluated the effect of phytase, glycinate trace minerals and antioxidant vitamins on the development of woody breast in broilers fed pelleted corn/soy diets formulated to exceed in protein and energy current 1994 N.R.C recommendations. A total of 2310 Aviagen Ross 708 male birds were used in a randomized complete block design with means separation based on the least significant difference test, with pen as the experimental unit, treatment as the fixed effect, and block as the random effect (35 birds/pen x 6 treatments x 11 replications). Birds were blocked by weight. Treatments consisted of T1 = NC containing 20 mg/kg Vitamin E, 7 mg Riboflavin, 80 mg Mn, 30 mg Fe, 10 mg Cu, 0.3 mg/kg Se sulfate TM and 0 phytase, T2 = NC -0.15% aP, -0.17% Ca- 0.02% Na + 2000 FTU/kg Natuphos E, T3 = NC with glycinate Fe, Cu, Zn, Mn and Se yeast, replacing sulfates at the same element level. T4 = NC +80 mg Vitamin E, 5 mg/kg Riboflavin total, T5 = T2+T3 and T6 = T2+T3+T4. Birds were grown on built-up litter sources with a mild challenge (with litter obtained from a local Arkansas chicken farm), which contained *Clostridium perfringens* and other pathogenic bacteria plus mild levels of coccidia. In the current trial each pen will be top-dressed with *Eimeria acervulina* (100,000 oocysts/bird) and *E. maxima* (50,000 oocysts/bird), 5×10^4 cfu/bird of *Clostridium perfringens* and 5×10^4 cfu/bird of *E. coli*, applied to the litter. Data was collected at 17, 28 and 42 body weight, FCR feed intake, d42 glycogen breast muscle content, neutrophil phagocytosis and woody breast scores. T6 = T2+T3+T4 had a significantly higher d42 body weight than T1 (T1 = 2842, T2 = 2876, T3 = 2855, T4 = 2844, T5 = 2884 and T6 = 2897 g, $P < 0.05$); T2 with 2000 FTU/kg, T3 with glycinate, T5 = T2+T3 and T6 = T2+T3+T4 had a significantly lower FCR than T1 = NC (1.98, 1.92, 1.97, 1.99, 1.91 and 1.90, $P < 0.05$); Treatments T3, T4, T5 and T6 had a significantly higher glycogen breast muscle than T1 = NC (0.11, 0.22, 0.48, 1.03, 0.76 and 1.22 mg/g, $P < 0.05$); Treatments T3, T4, T5 and T6 had a significantly higher neutrophil phagocytosis (measure antioxidant capacity) than T1 = NC, $P < 0.05$); T2 through 6 had a significantly lower woody breast score 2 (severe) and 3 (extreme), % than T1 = NC (41, 32, 29, 34, 19 and 14, $P < 0.05$). The combination of phytase, glycinate, vitamin E and riboflavin, significantly improved d42 body weight, FCR and decreased woody breast from 42 to 14%.

Key Words: phytase, organic trace minerals, antioxidant vitamins, woody breast, broilers

342 Energy (ME) and amino acid releasing efficacy of graded levels of 6-phytase, Natuphos® E, in 28 day old broilers fed a corn/soy/DDGs diet.

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When phytases break down phytic acid, phosphorus, trace minerals are released, as well as energy and AA are released in the process. This study

evaluated the metabolizable energy (ME) and amino acid (AA) release of graded levels of phytase when fed to 28-d old broilers fed a pelleted corn/soy/DDGs diet, with 0.24% phytate phosphorus, formulated to meet or exceed current 1994 N.R.C recommendations. A total of 2904 Ross 708 male birds were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block as the random effect (33 birds/pen x 8 treatments x 11 replications). Birds were blocked by weight. Treatments consisted of T1 = PC, T2 = NC = PC - 0.17% Calcium (Ca), - 0.15% non-phytate phosphorus (nPP), -0.02% sodium (Na), -300 kcal/kg-10% AA, T3 = NC + 250 FTU/kg, T4 = NC + 500 FTU, T5 = NC + 750 FUT/kg, T6 = NC + 1000 FUT/kg, T7 = NC + 2000 FUT/kg and T8 = NC + 3000 FUT/kg. TiO₂ was used as the undigestible marker. Feed, prececal and fecal collection at 4 wks. Feed and fecal samples gross energy determined with a bomb calorimeter. SID AA digestibility and total-tract apparent ME digestibility were calculated. AME was 3152, 2865, 2918, 2944, 2957, 2984, 3048, and 3151 Kcal/kg, $P = 0.02$, respectively. 250, 500, 750, 1000, 2000 and 3000 FTU/kg phytase released 53, 79, 92, 119, 183 and 286 Kcal/kg, $P = 0.03$, respectively. Phytase SID CP% released was 0.247, 0.380, 0.524, 0.822, 1.171 and 1.311%, $P = 0.04$, respectively. Phytase SID MET% released was 0.002, 0.004, 0.009, 0.015, 0.022 and 0.028%, $P = 0.03$, respectively. Phytase SID LYS% released was 0.014, 0.028, 0.030, 0.052, 0.082 and 0.081%, $P = 0.03$, respectively. In conclusion, Natuphos E at 1000 FTU/kg released 119 Kcal/kg, 0.822% SID CP, 0.015% SID MET, 0.052% SID LYS, 0.028% SID ARG, 0.017% SID HIS, 0.049% SID ISO, 0.097% SID LEU, 0.043% SID CYS, 0.054% SID PHE, 0.048% SID THR, 0.024% SID TRP and 0.057% SID VAL, and Natuphos E at 3000 FTU/kg released 286 Kcal/kg, 1.311% SID CP, 0.028% SID MET, 0.081% SID LYS, 0.073% SID ARG, 0.036% SID HIS, 0.072% SID ISO, 0.143% SID LEU, 0.093% SID CYS, 0.081% SID PHE, 0.065% SID THR, 0.030% SID TRP and 0.080% SID VAL.

Key Words: ME, amino acids, phytase, broilers, corn soy DDGS diet

343 *Buttiauxella* phytase at 3,000 FTU/kg improved growth performance and carcass yield of heavy broilers under mild-challenged condition.

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The phosphoric effect of phytase to increase utilization of phytate-bound phosphorus (P) has been well established, however the extra-phosphoric effect of the enzyme to benefit utilization of other minerals, ME, and AA is still a topic of interest. The efficacy of *Buttiauxella* phytase (Aextra® PHY) at 3,000 FTU/kg (BP3000) was evaluated in broilers from 1 to 56 d of age. The dietary treatments were a nutrient-adequate positive control (PC), a negative control (NC = PC reduced by 74 kcal/kg ME, 0.03–0.04% digestible AA, 0.19% Ca, 0.18% avP, and 0.04% Na), and the NC supplemented with BP3000 (NC+BP3000). Male Ross 308 broilers (n = 837) were randomized to each of the 3 diets with 10 replicates for each of the PC and NC, and 11 replicates for the NC+BP3000 (27 birds/per pen). Birds were vaccinated with Paracox 5 (10 x recommended dose) on d 5 and raised on used litter from d 1 with a layer of clean litter spread on top as the trial advanced, to induce a mild challenge that mimicked commercial broiler production conditions. Diets were based on corn/wheat/soybean meal/rapeseed meal and fed *ad-libitum* in pellet form in 4 phases: 1–10 d; 11–21 d, 22–42 d and 42–56 d. Bird bodyweight (BW) and feed intake were measured per pen and mortality-corrected FCR (mcFCR) was calculated at each dietary phase and overall 1–56 d. Proc. GLM of JMP 14.1 was used

for data analyses. NC+BP3000 improved BW gain and feed intake vs NC and PC ($P < 0.05$) in all phases except for 22–42d BW gain vs PC. The d56 BW and overall 1–56 d feed intake were increased ($P < 0.05$) and mcFCR was improved ($P < 0.05$) by NC+BP3000 (4,533 g, 8,040 g, and 1.71) relative to PC (4,266 g, 7,386 g and 1.75) and NC (4,073 g, 7,151 g and 1.83), respectively. Similarly, eviscerated carcass and leg meat weights were each heavier ($P < 0.05$) in NC+BP3000 birds (3,568 and 946 g) than in PC (3,345 and 879 g) and NC (3,219 and 864 g) birds, respectively. Breast meat weight was bigger ($P < 0.05$) in NC+BP3000 birds (923 g) than in NC birds (846 g) and intermediate in the PC birds (895 g). Feed cost/kg BW gain was reduced by 2.5% for

the NC+BP3000 birds relative to the PC birds. The d56 BW was also 102 g higher for the NC+BP3000 birds relative to the BW objective performance for male Ross 308 broilers at d56. The data confirmed the extra-phosphoric effect of the *Buttiauxella sp.* phytase at 3,000 FTU/kg in broilers. Applying *Buttiauxella sp.* phytase at 3,000 FTU/kg in heavy birds allows for reduction of available P, Ca, digestible AA and energy in diet to reduce feed cost and increase production benefit.

Key Words: broilers, *Buttiauxella sp.* phytase, growth performance, carcass yield, feed efficiency

Metabolism and Nutrition: Feed Additives III

344 Growth performance of broilers fed diets supplemented with different levels of essential oils. Brunna Leite¹, Laura Ferreira¹, Lucio Araújo¹, Cristiane Araujo¹, Melina Bonato^{*2}, Liliana Borges², and Ricardo Barbalho², ¹Universidade de São Paulo, Pirassumunga, São Paulo, Brazil, ²ICC Brazil, São Paulo, São Paulo, Brazil.

The study was conducted with the objective to evaluate different essential oil inclusions in the diet of broilers from one to 42 d. For this, 900 Cobb500 broilers were housed at 1 d-old in a completely randomized design with 5 treatments: NC (Negative Control); PC (Positive Control with Zinc Bacitracin and Anticoccidian); EO600 (Essential Oil, 600 g/MT); EO900 (900 g/MT); EO1200 (1200 g/MT). The broilers were orally challenged at 7 d with a super dose of Coccidiosis vaccine and housed on reused litter obtained of the commercial farm. The challenge with *Eimeria* (sporulated and live oocysts) corresponded to 40,000 oocysts/birds (*Eimeria acervulina*, *E. maximus*, *E. tenella*, *E. necatrix*, and *E. brunetti*). At 42 d the weight gain, feed intake, feed conversion ratio, viability, and production factor, as such the carcass yield were evaluated. The data were analyzed by ANOVA and the means compared by Tukey test at 5% of significance. The lower dose of essential oil supplementation (EO600) showed statistically similar results of performance in relation to Negative Control. However, the higher dose (EO1200) provided a significantly better feed conversion ratio compared with NC and PC, 1.648; 1.661 and 1.678, respectively ($P < 0.05$). In addition, the highest weight gain was observed to EO1200 (2.868 g), while PC and NC showed 2.794 g and 2.691 g, respectively ($P < 0.05$). Also, the feed intake of broilers fed with EO1200 increased in relation to Negative Control, 4.524 g, and 4.423 g, respectively ($P < 0.05$). Had not statistical differences to viability, production factor, and yield among treatments. The 1200 g/MT of essential oil supplementation resulted in better performance of broilers at 42 d of age.

Key Words: AGP, essential oil, feed conversion ratio, poultry, weight gain

345 Evaluation of a prebiotic and essential oils as alternatives for antibiotic feeding programs on live performance in broilers raised in tropical conditions. Diego Gómez-Aillón¹, Ana Cevallos¹, Diego Chaves-Pazmiño³, Xavier Muñoz^{*1}, Gustavo Nava³, Gabriela Arteaga-Arredondo², and Hernan Cordova-Noboa², ¹Universidad Central del Ecuador, Quito, Pichincha, Ecuador, ²North Carolina State University, Raleigh, North Carolina, United States, ³Procesadora Nacional de Alimentos C. A. Pronaca, Quito, Pichincha, Ecuador.

Currently, the evaluation of potential alternatives to antibiotic use in Antibiotic-Free (ABF) Programs is an important topic in poultry industry and research. Therefore, the development of feed additives that support broiler performance in ABF programs is of interest. This study was conducted to assess the efficacy of a prebiotic (yeast-cell wall) and a blend of essential oils compounds (cinnamon, thymol, pinene and carvacrol) as alternatives for Antibiotic Free Programs and its effects on live performance and intestinal morphometrics traits, in broilers. Four dietary treatments (A to D) consisted in a basal corn, wheat and soybean meal with or without the inclusion of the corresponding feed additive (A: BMD bacitracin methylene disalicylate 1,360g/ton, B: no additive, C: yeast cell wall 500g/ton, and D: essential oils blend 130g/ton, respectively). These treatments were formulated following Cobb 500 Manual Recommendations to achieve maximum performance. A

total of 2,816 Cobb chicks were randomly placed in 64 floor pens, located inside a commercial house, in groups of 44 chicks/pen (22 females and 22 males respectively). Live performance data was collected at 7, 14, 21, 28 and 35 d of age. Additionally, intestinal pH and samples from duodenum and jejunum were obtained from 3 chicks/treatment at 14 and 35d to measure villi height and crypt depth. A randomized complete block design with 16 replicates per treatments was used. Data were analyzed using one-way ANOVA and mean separation was performed by Tukey's test with the significance level at 0.05. No differences ($P > 0.05$) were detected among treatments for feed intake and mortality throughout the study. At 35d, feeding diets with BMD improved ($P < 0.05$) the FCR up to 2 points compared with chickens fed on diets without feed additives. The inclusion of the prebiotic and essential oils resulted in intermediate responses. Villi height in jejunum and duodenum responses were influenced by dietary treatments at 14 and 35d ($P < 0.01$). However, no differences were detected for intestinal pH ($P > 0.05$). In conclusion, results from this study showed that live performance while using the yeast-cell wall or the essential oils blend, were not different compared with feeding with BMD. This response may be explained by the results observed in the intestinal morphometrics traits, supporting the potential benefits of these products as alternatives in antibiotic-free feeding programs.

Key Words: broiler live performance, essential oils, prebiotic, bacitracin methylene disalicylate, ABF

346 Effect of essential oil blend supplementation on zootechnical performance in commercial broilers. Prashant Kumar Mishra^{*}, AVT Natural SA DE CV, Queretaro, Queretaro, Mexico.

In recent years essential oils from different plant have caught a lot of attention as a replacement to different antibiotic growth promoter in animal production. Numerous studies have shown significant positive effects of blend of different essential oils (EO) on performance in commercial broiler farms. EO blend has been of great interest rather than individual EO because of the synergic effect among different EOs. By improving secretion of different digestive enzyme, digestibility and intestinal health, the blend improves the zootechnical performances. A blend of Oregano, Cinnamon and Clove Essential oils was selected to replace antibiotic growth promoter bacitracin methylene disalicylate (BMD). A total of 1440 d old chicks of Cobb broilers were randomly assigned to 3 treatments, 24 replicates with 20 birds each. T1 served as a Negative control (NC). T2 served as a Positive control at 500g/ton of feed (BMD-), T3 served as blend at 250g/ton of feed. Feed intake, body weight and mortality were recorded on d 7, 14, 21, 35 and 42. Accumulated mortality was registered. Histomorphometry was performed for one bird from each replicate. A day old chicks were housed under standard conditions, Pens with deep litter system having accessories for brooding, watering and feed were provided. The birds were fed with poultry broiler feed diet as per the schedule with pre-starter for 0 to 14 d, starter feed 15 to 28 d and finisher feed 29 to 42 d. Statistical software GraphPad Prism was used to perform one-way ANOVA comparing different treatments assuming Gaussian distribution. Tukey's multiple comparisons test is used to draw treatment difference. Feed conversion ratio (FCR) has improved by 5 units when compared with Negative control, with high significance ($P < 0.0001$). The body weight is increased by 12 g in blend against Negative control (2523 g vs 2511 g) and is significantly different ($P < 0.0048$). Mortality in blend treated group was as low as 0.6% whereas control was 2.8%. Statistical

analysis of mortality could not be performed. The lesion score was low, with increased villi length. Increased body weight gain and improved FCR (i.e. lower FCR value), reduced mortality and better intestinal health in the group treated with EO blend, clearly indicates that this particular blend can replace antibiotic growth promoters like BMD. Low inclusion of individual essential oils in the blend strengthens the idea that these molecules at lower level explore and exploit different host directed response rather than having a direct antimicrobial effect.

Key Words: antibiotic resistance, essential oils, host directed response, broilers, FCR

347 Not Presented

348 Not Presented

349 Efficacy of a plant alkaloid extract in laying hens under heat stress conditions.

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Heat stress decreases liveability, feed intake, egg production and egg quality in laying hens. Global annual losses in laying hens due to heat stress are estimated to be \$ 98.1 million. **Objective** The objective of the study was to evaluate the effects of a standardized formulation of Isoquinoline Alkaloids (IQs, applied as Sangrovit® Extra) on performance of laying hens under field conditions in Poland. During the trial period, a 4-week heat wave occurred. **Material and Methods** Lohmann Brown birds, aged 62 weeks, were used for a trial period of 9 weeks (wk 62 – 70). From wk 66 – 69, ambient temperatures were 43°C and 44°C. Two trial groups were tested: 1| Negative Control (NC): no additive (28,600 birds; n = 1); 2| IQs: NC + IQs (100 g/t; 28,600 birds; n = 1). Birds were kept in cages. Feed was based on soybean meal, corn, and wheat. **Results** Both treatment groups showed the same performance at the beginning of the trial (egg production: 83% in both groups, egg weight: 63 g in both groups). IQs improved performance in laying hens. Egg production was 9.5% units higher at the end of the trial period in IQs supplemented birds (80.3% and 70.8%, respectively). This effect was even more pronounced during the heat stress period: while egg production went down from 81% to 59% in the NC from wk 65 to wk 69, production went only down from 83% to 77% in birds fed IQs. IQs also improved for the overall period: mortality (4.69% and 5.14%, respectively), egg weight (62.7 and 60.1 g, respectively), egg mass (50.4g and 42.7, respectively), egg faults (4.6% and 5.4%, respectively), and feed intake (124 g and 111g, respectively). These effects were more pronounced during the heat stress period. **Conclusion** The inclusion of a standardized formulation of IQs improved performance in laying hens suffering from heat stress under field conditions. Therefore, IQs offer a beneficial and natural solution to support laying hens under challenging conditions.

Key Words: heat stress, laying hens, isoquinoline alkaloids, field trial, feed additive

350 Effects of microencapsulated *Enterococcus faecalis* and *Camellia oleifera* seed extract on growth performance, relative organ weight, serum parameters, and cecal microflora in *Escherichia coli* K88 challenged broilers. Yongwei Wang*, Zhenglin Dong, Dan Song, Haijiang Miao, Weiwei Wang, and Aike Li, *Academy of National Food and Strategic Reserves Administration, Beijing, China.*

The study was conducted to evaluate the effects of microencapsulated *Enterococcus faecalis* (MEF) and *Camellia oleifera* seed extract (COSE) on growth performance, relative organ weight, serum parameters, and cecal microflora in broilers challenged with *E. coli* K88. The MEF and COSE products were prepared by Academy of National Food and Strategic Reserves Administration (Beijing, China). In this study, a total of 240 one-day-old male Arbor Acres broilers were randomly allotted to 5 groups with 6 replicates per group. Birds in positive control (PC) group were fed a corn-soybean meal based diet without antibiotics, and birds in negative control (NC) group, MEF group, COSE group, MEF+COSE (MIX) group were challenged with *E. coli* K88 and supplemented with 0, 1×10^{10} cfu MEF/kg of diets, 500 mg COSE/kg of diets or their combinations, respectively. From d 7 to 14, each bird in challenged groups was orally dosed with 1 mL inoculum (10^9 cfu/mL of *E. coli* K88) per day using a polyethylene tube attached to a syringe. The birds in PC group were administered similarly with the same amount of saline solution. Feeding experiment was from d 1 to d 28. All data were analyzed using SPSS 17.0 (SPSS INC., Chicago, IL) for one-way ANOVA. Results showed that birds in NC group had lower average body weight (ABW) and greater *E. coli* counts in cecal contents than PC group ($P < 0.05$). No significant differences in ABW were observed among birds in MEF, COSE, MIX and PC groups at d 28 ($P > 0.05$). Diets supplemented with MEF significantly increased ABW, serum IgA level, T-SOD activity, cecal *Lactobacillus* counts, and reduced cecal *E. coli* counts compared with that in NC group ($P < 0.05$). Diets supplemented with COSE had greater ABW, relative liver and spleen weight, serum IgA and IgM levels, serum T-SOD and GSH-Px activities than that in NC group ($P < 0.05$). Birds in MIX group had greater ABW, relative liver and spleen weight, serum IgM levels and T-SOD activities, and had less cecal *E. coli* counts than birds in NC group ($P < 0.05$). The results indicated dietary supplementation of MEF and COSE could promote growth performance, enhance serum immune and antioxidative functions, and benefit cecal microflora in *E. coli* K88-challenged broilers.

Key Words: broiler, *E. coli* K88, probiotics, plant extract

351 Exogenous protease dose in diets varying in nutrient density and effects on hindgut microflora in broilers. Elizabeth Kim*, Jordon Gruber, Franco Mussini, Jim Wilson, Michael Perry, and Janet Remus, *Danisco US Inc., DuPont Animal Nutrition, Cedar Rapids, Iowa, United States.*

New findings on protease and its use to support nutrition indicate a multi-factorial impact that includes nutrient digestion and impairment of pathogen growth. To better understand the effects of exogenous protease, a study was generated to determine the effects on the hindgut microflora for broilers fed diets differing in nutrient composition and protease inclusion rate. On day of hatch, 600 male broilers were randomly allocated to 60 battery cages of 10 broilers each. Each of 6 dietary treatments were fed to 10 replicate cages until 35 d of age. Dietary treatments included 2 control diets; a positive control (PC) all-vegetable based diet used in NAE production systems formulated for full AME and AA, and a negative control (NC) diet which was the PC formulated with AME reduced by 44 kcal/kg and crude protein reduced by 5%. A protease (Aextra® PRO - subtilisin) was then added on top of each of the control diets at a half-dose (2000 U/kg) and a full dose (4000 U/kg) to complete 6 dietary treatments. Birds were fed experimental treatments from day of hatch, with starter diets being fed from 0 to 14 d; grower 15 to 28 d and finisher from 28 to 35 d, with nutrients adjusted for each feeding phase. On d 21, the entire gastrointestinal tract was collected from one bird per pen for quantification of total *Clostridium perfringens* in the

gut. All data was analyzed as a one-way ANOVA. Treatment means were separated using Fisher's Least significant difference. During the starter phase, the full dose of protease reduced FCR when added to the PC and NC. For the grower phase, BWG was increased and FCR was reduced for diets with the full dose of protease added. In addition to the performance measurements, total *C. perfringens* levels in the gut were found to decrease with the addition of the protease. To explain the outcomes of both improving FCR and decreasing pathogen load, it is predicted that Aextra® PRO works to reduce the nutrient stress of undigested protein, while also limiting the nutrients of pathogens in the hindgut such as *C. perfringens*. New research is also finding potential effects of exogenous proteases that reduce secreted bacterial proteins used to support fitness of the microorganisms in the system.

Key Words: protease, clostridia, gut microbiome, broilers

352 A phytogetic feed additive improves nutrient digestibility and production performance in laying hens from onset of lay to peak production. Jan Dirk van der Klis^{*1}, Manu de Laet¹, Stacie Appleton², and Andreas Mueller¹, ¹*Delacon Biotechnik GmbH, Steyregg, Austria*, ²*Delacon USA, Inc., Carlisle, United States*.

During the last decades, production performance of laying hens is continuously improving. Birds come into lay sooner, and laying persistency has increased tremendously, while body weight, feed conversion ratio and mortality have been significantly reduced. Laying hens are able to sustain this high level of performance, despite the increased maintenance requirements in the cage free systems that are predominant in Europe. Phytogetic feed additives can improve nutrient digestion and utilization, thereby supporting egg production especially from onset of lay to peak production when feed intake capacity is still developing. The effect of Biostrong® 510 (BSG), a phytogetic feed additive based on a combination of pungent and bitter substances, essential oils and saponins was tested on nutrient digestibility and production performance in Lohmann Brown laying hens from 22 to 29 weeks of age. The experiment was carried out at the University of Berlin, Germany. Layers were fed a corn (42.7%)/wheat (25.0%)/soybean meal (19.0%)-based mash diet that was supplemented with phytase and xylanase. The diet was fed as such or supplemented with 0.3 lb/t BSG. Production performance was measured weekly. At 29 weeks of age, ileal digestibility of crude protein, amino acids, fat, calcium and phosphorus were measured. The apparent ileal digestibility (AID, %) of protein was significantly ($P \leq 0.05$) improved from 70.2 to 75.4 and the sum of amino acids from 73.2 to 78.5. Moreover, numerical increases of crude fat, calcium and phosphorus digestibility was observed. Feed conversion ratio over the entire experimental period was significantly ($P < 0.01$) reduced from 2.406 to 2.312 (and between 25 and 29 wks of age from 2.261 to 2.142), which was mainly due to a significant increase in egg numbers ($P < 0.01$). Egg weight was numerically increased, resulting in a 4.6% increase ($P < 0.001$) in egg mass. These observations on nutrient digestibility corroborated previous results with broilers, which were supported by increased pancreatic enzyme activities in the intestinal lumen and transport proteins in the enterocytes. It was concluded that this phytogetic feed additive can improve nutrient digestibility and production performance of laying hens from onset of lay to peak production.

Key Words: laying hen, phytogetic, nutrient digestibility, production performance, phytogetic

353 Consequences of early life exposure of broilers to soluble xylan or antibiotic when fed viscous cereal grains. Dervan Bryan^{*1}, Michael Bedford², Nancy Fischer⁴, Juha Apajalahti³, and

Henry Classen¹, ¹*University of Saskatchewan, Saskatoon, Saskatchewan, Canada*, ²*AB Vista, Marlborough, United Kingdom*, ³*Alimetrics Group Ltd., Espoo, Finland*, ⁴*Country Junction Feeds, Wetaskiwin, Alberta, Canada*.

The digestive tract microbiota of broilers adapts to degrade and ferment indigestible dietary arabino-xylans (AX). In turn, adaptation may impact broiler performance and digestive tract characteristics linked to changes in AX fermentation. It can be hypothesized that adaptation to grain AX is affected by the nature of dietary AX, and bird age and digestive tract microbiota. Ileal digesta viscosity (VC) may provide an estimate of adaptation to grain AX. To test factors affecting adaptation, 2 experiments were conducted using grains (wheat – W, rye – R) and feed additives (xylanase, AX oligosaccharides (AXOS), penicillin) that influence the nature of the AX and digesta microbiota. Trial 1 was completely randomized with 160 broilers assigned to 16 battery cages and fed 40% R diets + or – 100 mg/kg penicillin for 21 d. Trial 2 was completely randomized with 768 birds in 48 cages. From 0 to 21 d (starter phase; S), birds were fed a W diet + or – a feed additive mixture (A; xylanase + AXOS). At d 21, cages were randomly allocated from the 2 S trts to 1 of 4 grower diets (G; W + or –A, 40% R + or –A) for 2 additional weeks. Ileal digesta VC (cps) and performance were recorded weekly in both trials. Trial 1 and 2 data (0–21 d) were analyzed as a one-way ANOVA, while trial 2 (d 21–35) data were analyzed as a 2-way ANOVA. Differences were considered significant when $P \leq 0.05$. In trial 1, penicillin reduced ileal digesta VC on d 7 (87 vs 33), and increased body weight gain (BWG, g/bird) d 7 (114 vs 102), d 14 (272 vs 246) and d 21 (439 vs 379). In trial 2 S phase, +A reduced viscosity at 14 d (3.4 vs 2.5) and increased feed intake (116 vs 121) from 0 to 7 d. The 21–28 d BWG (W–A: 543^{ab}, W+A: 592^a, R–A: 507^b, R+A: 554^{ab}) and feed to gain ratio (W–A: 1.566^b, W+A: 1.483^b, R–A: 1.685^a, R+A: 1.551^b), and 35 d body weight (g/bird; W–A: 2000^b, W+A: 2153^a, R–A: 2040^{ab} and R+A: 2130^{ab}) and 28–35 d feed intake (g/bird; W–A: 1086^b, W+A: 1139^{ab}, R–A: 1209^a, R+A: 1194^{ab}) were affected by G trt. At both 28 and 35 d, interactions between S and G trts demonstrated that VC during the G phase was affected by the S feeding program. At 28 d, S trt did not affect VC of birds fed WG, and in both cases A reduced VC (5.8 vs 2.6; 5.8 vs 2.5). A reduced VC for R fed birds derived from the S+A trt (83.5 vs 24.4), but not of those derived from the S–A trt (38.7 vs 32.1). At 35 d, VC for W fed birds derived from S+A trt was higher (11.99) than the S–A trt (6.5) and A in the G phase resulted in lower VC (2.9, 2.6). For R fed birds, VC was unaffected by G+A regardless of SA status (S+A: 27.9 vs 18.0; S–A: 35.9 vs 22.9). These data suggest that an antibiotic as well as the nature of AX affect adaptation of the digestive tract microbiota.

Key Words: wheat, rye, xylanase, arabinoxylan, antibiotics

354 Effect of an alternative nutritional proposal on performance and egg quality of brown egg layers. Carlos Lozano-Poveda^{*1}, Otavio Rech², Oscar Ortiz³, and Ramiro Delgado³, ¹*DSM Nutritional Products, Bogotá, Bogotá DC, Colombia*, ²*DSM Nutritional Products, Sao Paulo, SP, Brazil*, ³*Nutrientes Avícolas, Cali, Valle, Colombia*.

Due to genetic selection, laying hens have longer production cycles and a more precise and balanced nutrition is needed to support performance and egg quality until the end. The aim of this study was to evaluate the influence of 2 premix concepts on egg production and egg quality in brown laying hens from 25 to 50 weeks of age. A total of 1,185 25-week-old-HyLine brown laying hens were placed in an open sided house, using floor-pens with fresh litter (rice hulls) for a 25wk trial period. Birds were randomly assigned 2 treatments and placed in 11 pens of approximately

108 birds each. All hens were fed with a mash basal diet based on corn and soybean meal. Treatments were: Commercial premix (T1): basal diet supplemented with a premix containing vitamin and minerals (sulfate) levels commonly used by Colombian producers; phytase, and pigments; and a Concept premix (T2): basal diet supplemented with a premix containing optimal vitamin nutrition (OVN), 25OHD₃, carbo-amino-phospho-chelate trace minerals, enzyme blend (phytase, amylase, and xylanase), probiotics (*Enterococcus faecium* NCIMB 10415; *B. subtilis* and *B. licheniformis*), essential oils/organic acid (thymol, eugenol, piperine/benzoic acid), and pigments. Main differences in vitamins and minerals levels T1/T2, were: Vit. A 8,000/12,000 IU; 25OHD₃ 0/69 µg; Vit. E 20/60 mg; Vit. K3 2,500/3,000 mg; Vit. B2 5,500/7,000 mg; Vit. B6 4,000/5,000 mg; niacinamide, 30/50 mg; pantothenic acid 8/12 mg; folate 1,000/1,500 µg; biotin 75/150 µg; choline 180/300 mg; cooper 6/8.62 g; iron 30/43.74 g; manganese 80/56.37 g; zinc 65/43.74 g; selenium 250/340 mg. Performance parameters and egg characteristics were evaluated during the trial period. Data were analyzed using one-way ANCOVA (age as covariate) and means compared by Tukey test at 5% probability. Significance differences ($P < 0.05$) were found between treatments (T1 Vs T2) for BW (2,005 vs 1,992, g); egg weight (59.65 vs 59.47, g), egg production (95.13 vs 96.2, %), egg mass (56.96 vs 57.92, g), FCR (1.477 vs 1.459, g:g), egg shell strength (4,566 vs 4,618, KgF), egg shell thickness (0,389 vs 0,391, mm), and egg shell color index (82.6 vs 83.3). The concept premix increased laying with decreases in BW and egg weight probably due to greater reserves mobilization. The increased productivity and egg shell color could be related to the inclusion of probiotics, organic acid and essential oils that improve gut health. Egg shell strength and thickness improved probably due to the inclusion of additives that directly affect mineral metabolism (25OHD₃, and carbo-amino-phospho-chelate trace minerals). It was conclude that this concept premix positively affects performance and egg shell quality of a modern layer hen.

Key Words: vitamins, minerals, eubiotics, enzymes, laying hens

355 Not Presented

356 Growth and serum biochemical indices of broiler chicks fed betaine hydrochloride supplemented diets. Oluremi Daudu*,

Ango Abdullahi, and Ezekiel Salihu, *Ahmadu Bello University, Zaria, Kaduna State, Nigeria, Zaria, Nigeria.*

The study was conducted to investigate the effect of Betaine HCl on the growth and serum biochemical indices of broiler chicks. Betaine has 2 primary metabolic roles, it is a methyl group donor and an osmolyte that assists in cellular water homeostasis. Betaine accumulation results in an increased water-binding capacity of the intestinal epithelium thus improving the intestinal structure and function of chickens which can lead to improved performance. The design of the experiment was completely randomized design. The experiment was conducted at the Poultry Unit of the Teaching and Research Farm, Department of Animal Science, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Three hundred day old broiler chicks were assigned in a completely randomized design to 4 experimental diets supplemented with Betaine HCl at 0, 175, 325 and 475 g/100kg diet. Each treatment had 3 replicates with 25 chicks each. Initial weights of birds were taken at the beginning of the experiment and weight gain was recorded weekly. Feed intake and weight gain were measured weekly while weight gain and feed conversion ratio were computed. At the end of the fourth week, 2 mLs of blood was taken from the brachial vein of 3 birds per replicate into plain sample bottles for determination of glucose, total protein, albumin, cholesterol, triglyceride, alanine aminotransferase (ALT) and alkaline phosphatase (ALP). The data generated from this study was subjected to ANOVA (ANOVA) using the General Linear Model (GLM) procedure of Statistical Analysis Systems and significant difference between treatments means were separated using Tukey Test. Feed intake (83 - 88 g/bird/day), final weight (42 - 43.5 g/bird/day) and weight gain (39 - 40.6 g/bird/day) were higher ($P < 0.05$) for chicks fed Betaine HCl supplemented diets compared with the control. Chicks fed Betaine HCl diets also had the best ($P < 0.05$) feed conversion ratio (2.06 - 2.18) compared with the control. The glucose values for chicks fed 175 and 325 g/100 kg (10.15 - 10.82 g/l) Betaine HCl diet were higher ($P < 0.05$) than for those on other treatments. Total protein (27.67 - 43.33 g/l), albumin (12.56 - 20.89 g/l), cholesterol (1.84 - 2.53 mmol/L), triglyceride (0.91 - 1.01 mmol/L), ALT (10.78 - 20.78 U/L) and ALP (39.78 - 73.00 U/L) values were similar ($P > 0.05$) for all the birds. Inclusion of Betaine HCl up to 475g/100kg in the diets of broiler chicks improved growth performance and had no detrimental effect on serum biochemicals.

Key Words: broiler, Betaine HCl, growth, serum biochemicals, glucose

Metabolism and Nutrition: Nutrition I

357 Metabolizable energy coefficients of oil sources in broiler diets supplemented with or without lipase. Ricardo Nunes^{*1}, Vaneila Savaris¹, Tania Kholer¹, Felipe Campos¹, Cinthia Eyng¹, Edinan Cirilo¹, Cristine Kaufmann¹, Jarred Oxford², and Cleison de Souza¹. ¹Western Paraná State University, Marechal Candido Rondon, Paraná, Brazil, ²University of Georgia, Athens, Georgia, United States.

Since energy is an essential nutrient for proper bird development, adequate levels of energy is crucial for poultry feeding. Due to the difficulty of poultry to digest fat in the first weeks of life, supplementation with lipase may be an alternative to improve the digestibility of fats for broilers. The objectives of this study were to determine the values of Apparent Metabolizable Energy (AME), AME Corrected by Nitrogen Balance (AME_n), Apparent Metabolizable Coefficient (AMC), and AMC Corrected by Nitrogen Balance (AMC_n); of soybean oil (SO), palm fat (PF), soy hydrolyzed fat (SHF), and palm oil hydrolyzed fat (POH) supplemented or not with lipase. A total of 280 male broilers at 14 d of age, were randomized by weight and distributed into one of 10 treatments with each treatment containing 7 replicates of 4 birds each. Treatments consisted of diets with or without lipase and 4 different ingredients, plus 2 reference rations (RR). The RR replacement procedure was performed as proposed by Matterson et al. (1965), and energy values were determined by total excreta collection. Lipase diets were supplemented with 1,000 U kg⁻¹ lipase. Total excreta was collected and analyzed for dry matter (DM), crude energy (CE), and nitrogen (N). Serological analysis for cholesterol (COL), triglycerides (TRIG), alanine amino transferase (ALT), and aspartate amino transferase (AST) were performed at the end of the collection period. All data were analyzed using SAS – Version 9.1. The SO had higher values of AME, AME_n, AMC, and AMC_n when compared with the other lipids. The worst values of AMC and AMC_n were found for SHF (20.00 and 19.16%, respectively), followed by POH (44.19 and 43.11%, respectively). Lipase supplementation in the diet reduced AMC and AMC_n for PF and POH ($P < 0.05$). No differences were observed for blood analysis, as well as the interaction of enzyme use and fat source. However, the fat used in the diet influenced the serological values of the birds for COL, AST, and ALT. The AME and AME_n values for the evaluated ingredients were 9,746 and 9,541 kcal kg⁻¹; 7,559 and 7,356 kcal kg⁻¹; 1,983 and 1,899 kcal kg⁻¹; 3,627 kcal kg⁻¹; and 3,538 kcal kg⁻¹, for SO, PF, SHF, and OHP respectively. Lipase inclusion did not increase the AME and AME_n values of the diets and did not improve the AMC and AMC_n of any evaluated feedstuffs.

Key Words: soybean oil, palm oil, hydrogenation, lipase

358 Determination of metabolizable energy using the regression analysis method: influence of substitution levels, cereal grain type and length of adaptation to the experimental diet. Oluyinka Olukosi^{*}, University of Georgia, Athens, Georgia, United States.

Our earlier studies indicate that the influence of adaptation period on metabolizable energy (AME) of cereal grains is species- and cereal grain type-dependent. The objective of the current study was to investigate whether the influence of adaptation length on determined AME of cereal grains with different fiber characteristics is dependent on the method used for AME calculation. A total of 252 male broilers were used to study the influence of length of adaptation to experimental diets based on 2 cereal grains with different fiber characteristics, fed for different adaptation periods and assessed at multiple substitution rates in a

wheat-soybean meal reference broiler diet. At 14 d old, the birds were allocated to 14 treatments with 6 replicates of 3 birds per replicate. The experimental diets were fed for either 7 or 4 d of adaptation and excreta were collected on 20 and 21 d of age. Within each adaptation period, 7 diets comprising one basal and 6 test diets in which each of the 2 test cereal grains (corn or barley) substituted the basal diet at rates of 150, 300 or 450 g/kg were fed, corresponding to levels 1, 2 or 3, respectively. The AME of the cereal grains was calculated employing the regression method using different combinations of the substitution levels (i.e., 0, 1 and 2; 0, 1 and 3; 0, 2 and 3; or 0, 1, 2 and 3). The data were analyzed as $2 \times 4 \times 2$ factorial for the influence of adaptation length (2), combination of substitution levels (4) and cereal grain type (2). There were no significant main effect of adaptation length or substitution levels. AME determined by the regression method was greater ($P < 0.01$) for corn compared with barley. There was a trend ($P = 0.073$) for adaptation length \times cereal grain interaction on cereal grain AME value. For corn, AME was not influenced by adaptation length whereas the AME determined for barley tended to be lower ($P < 0.10$) when adaptation length was 4 d. The experimental design allowed simultaneous determination, and statistical comparison, of AME of the cereal grains by both regression and difference methods. There was method \times cereal grain interaction ($P < 0.01$) for the AME values. Generally, AME value determined by the difference method was greater ($P < 0.01$) than that determined by the regression method, and the marginal difference in AME values determined by regression and difference methods was greater ($P < 0.01$) for barley than corn. Irrespective of the method used, there was no influence of adaptation period of AME values determined for the cereal grains. It was concluded that AME values determined by the regression and difference methods may not be comparable when determined for cereal grains with different fiber characteristics.

Key Words: metabolizable energy, cereal grains, adaptation, regression, difference method

359 Heat production in broilers through the years: A meta-regression. Diego Martinez^{*1}, Katie Hilton¹, Carol Ponce-de-Leon², Jordan Weil¹, Antonio Beitia¹, Nawin Suesuttajit¹, Pramir Maharjan¹, and Chris Coon¹. ¹University of Arkansas, Fayetteville, Arkansas, United States, ²Independent researcher, Fayetteville, Arkansas, United States.

The ability of broilers to grow fast has increased over the years; however, since diets are not being formulated on a net energy basis, it is unknown how much dietary energy is lost as heat. The purpose of this study was to determine if a relationship between heat production (HP) and time exists and its tendency. Scientific papers reporting HP were collected but also unpublished data obtained at the University of Arkansas within the last 4 years were included. Data from 1960 to 2018 were considered including 100 useful registries. In addition, data reporting growth rate and breast yield through the years were collected and used in the analysis. Actual values (expressed in kcal/d/kg^{0.75}, g/d and % for HP, average daily weight gain and breast yield, respectively) were considered as effect sizes, sample sizes as indicators of precision, and the year and bird age as moderator variables. For each model, heterogeneity (HG) was quantified using mixed-effects models, τ^2 , τ , I^2 and H^2 were calculated, and 95% confidence intervals (IC95%) of slopes were determined. Relationships (slopes) were considered consistent if the bound of the IC95% had the same sign. Single and multiple linear models were constructed with moderator variables. Model evaluation

criteria were pseudo-R² and AIC and BIC information criteria. The analysis was performed with the Metafor package in R version 3.5.2. Model coefficients were tested under a permutation test with 10,000 iterations to overcome partially non-normally distributed data ($P < 0.05$). Results showed that the relationship between HP and years is significantly ($P = 0.001$) linear, that heat production in increasing 2.19 kcal/d/kg^{0.75} each year, and that the relationship is consistent, as the IC95% is completely positive (1.41 to 2.96 kcal/d/kg^{0.75}). The predictive power of the model (pseudo-R²) increased (from 24.1 to 46.6%) and the AIC and BIC information criteria improved when including bird age as a second moderator variable in the same model. As expected, average daily gain and breast yield increased through the years and were correlated ($P < 0.05$) and directly proportional to heat production. In conclusion, the heat production expressed as energy units per day per metabolic weight is increasing over the years, implying that even though the bird is gaining weight faster, it is also increasing daily energy expenditure mainly because the broiler body composition has changed to an increasing percentage of lean mass (increasing % breast meat) with less fat. These findings support the utilization of a net energy system to better account for HP differences in fast-growing broilers caused by changes in genetics, body composition, nutrition and environmental temperatures.

Key Words: heat production, energy system, net energy, metabolizable energy, efficiency

360 Effect of an enzyme-treated soy protein on the performance of broiler chickens infected or uninfected with *Clostridium perfringens*. Simone Rasmussen¹, Alfred Blanch^{*1}, Brett Lumpkins², Greg Mathis², and Christine Broekner¹, ¹*Hamlet Protein A/S, Horsens, Denmark*, ²*Southern Poultry Feed and Research, Inc., Athens, United States*.

The objective of this study was to assess the effect of enzyme-treated soy protein (ESP, Hamlet Protein A/S, Denmark), in starter feed, with or without antibiotic growth promoter (BMD), on the performance of *Clostridium perfringens* infected/uninfected chickens. A total of 1600 d-of-hatch Cobb 500 male broiler chickens were assigned to 8 treatments (8 replicates/treatment, 25 chicks/replicate): T1 (uninfected = negative control) and T2 (infected = positive control), corn-soybean meal (SBM) starter feed (d0–14); T3 and T4, partial replacement of SBM with 5% ESP; T5 and T6, corn-SBM starter feed supplemented with 55ppm BMD; T7 and T8, 5% ESP and supplemented with 55ppm BMD. Chicks were all fed the same grower and finisher diets (d15–28 and d29–42), except T5, T6, T7 and T8 also received 55ppm BMD, whereas T1, T2, T3 and T4 were not fed BMD. Feed and water were provided ad libitum. On d 19, 20 and 21, T2, T4, T6 and T8 were challenged with *C. perfringens*. Body weight, feed intake were recorded, weight gain, feed conversion ratio (FCR) calculated. Data were subjected to ANOVA procedure for completely randomized design and LSD comparison test by using the general linear model procedures using statistix 10 software with a P value of 0.05 to determine the level of significance. From d0 to d 14, chickens fed the corn-SBM diet showed significantly lower ($P < 0.05$) weight gain and higher ($P < 0.05$) mortality adjusted FCR than the chickens fed 5% ESP, 55ppm BMD or 5% ESP and 55ppm BMD. The weight gain (kg) at d28 was 1.078ab (T1), 0.830d (T2), 1.077ab (T3), 0.998bc (T4), 1.135a (T5), 1.005bc (T6), 1.111ab (T7) and 0.949c (T8), $P < 0.05$. FCR was 1.571b (T1), 1.704a (T2), 1.509bc (T3), 1.663a (T4), 1.544bc (T5), 1.552b (T6), 1.460c (T7) and 1.524bc (T8), $P < 0.05$. At d42, weight gain was 2.306a (T1), 2.089b (T2), 2.297a (T3), 2.295a (T4), 2.350a (T5), 2.307a (T6), 2.360a (T7) and 2.336a (T8), $P < 0.05$. FCR was 1.790bc (T1), 1.858a (T2), 1.753d (T3), 1.803b (T4), 1.746d (T5), 1.763cd (T6), 1.709e (T7) and 1.762cd (T8), $P < 0.05$.

Necrotic enteritis mortality (%) at d42 was 0.00b (T1), 4.76a (T2), 0.00b (T3), 5.40a (T4), 0.00b (T5), 2.98ab (T6), 0.00b (T7), and 3.57a (T8), $P < 0.05$. In conclusion, the addition of ESP in starter diets, with or without BMD throughout the production cycle, equals the weight of the birds infected with *Clostridium perfringens* and their FCR to that of uninfected chickens.

Key Words: enzyme-treated soy protein, necrotic enteritis, performance, mortality, protein

361 Effects of two rates of protein digestion kinetics and two sources of dietary fiber on broiler growth performance from 0 to 36 days of age. Julio Diaz Berrocoso^{*3}, Jennifer Ellis², Neil Jaworski¹, Greg Page², and Ana Isabel Garcia Ruiz³, ¹*Trouw Nutrition, Boxmeer, Netherlands*, ²*Trouw Nutrition, Guelph, Canada*, ³*Trouw Nutrition, Casarrubios del Monte, Spain*.

An experiment was conducted to test the hypothesis that broilers fed diets formulated to contain a greater concentration of fast digestible protein (soybean meal-corn-wheat based diets) would have greater performance compared with those fed a diet containing more slow digestible protein (soybean meal-canola meal-soy protein concentrate based diets), but that the addition of oat hulls to slow digestible protein diets would ameliorate the reduced performance. A total of 1,920 one-day-old, male Ross 708 broiler chickens were randomly allocated and housed in 48 floor pens (40 birds/pen). Four dietary treatments were arranged in a completely randomized block design using a 2 × 2 factorial arrangement with 2 protein digestion rates (fast or slow) and 2 sources of dietary fiber [3% oat hulls or sugar beet pulp (SBP)] and their interaction as main effects from 0 to 36 d of age. All diets were formulated to be isocaloric and isonitrogenous. Pen was the experimental unit for all variables studied (12 replicates/treatment). Data were analyzed using the MIXED procedure of SAS. There were 3 experimental feeding phases; starter (from d 0 to d 15), grower (from d 15 to d 28), and finisher (from d 28 to d 36). Results indicated that broilers fed diets containing more fast digestible protein had 4% greater ($P < 0.05$) average daily gain (ADG) and a 5% lower ($P < 0.05$) feed conversion ratio (FCR) throughout the experiment, most notably after the starter. The diets containing 3% oat hulls increased ($P < 0.05$) average daily feed intake (ADFI) and ADG in the starter phase compared with broilers fed diets containing 3% SBP. However, this was not reflected in better FCR as resistant fiber fed broilers had worse FRC compared with fast fiber fed birds. Broilers fed the fast digestible protein and 3% SBP diet had the greatest ADG and best FCR during the grower and finisher phases and over the entire experimental period. The ADG and FCR of broilers fed the diet containing slowly digestible protein was improved to the level of broilers fed the fast digestible protein and 3% SBP diet by the addition of 3% oat hulls. This confirms the hypothesis that the addition of oat hulls to slowly digestible protein diets may potentially increase passage rate and gastrointestinal development and, thereby, improve protein digestion rates to levels similar as a fast digestible protein diet. It is concluded that starter diets should be formulated to contain a high concentration of fast digestible protein, but if this is cost prohibited then 3% oat hulls can be used to increase ADFI and ADG, and protein digestion rates to reduce FCR.

Key Words: broilers, growth performance, dietary fiber, nutrient kinetics, protein degradation

362 Influence of type of mill and particle size of the corn on productive performance of broilers from 7 to 35 days of age. E. Vinyeta^{*2,3}, Lewis Aguirre¹, G. Fondevila¹, E. van Eerden⁴, S.

Stegh fer², Jan Dirk van der Klis⁴, and Gonzalo Mateos¹, ¹UPM, Madrid, Madrid, Spain, ²B hler AG, Uzwil, Switzerland, ³Trouw Nutrition, Nutreco BV, Amersfoort, Netherlands, ⁴Schothorst Feed Research BV, NA Lelystad, Netherlands.

An experiment was conducted to study the effects of the type of feed mill (hammer vs. roller) and particle size of the main cereal of the diet (4 grinding strategies used in commercial practice) on growth performance of Ross 308 broilers from 7 to 35 d of age. The feeding program consisted of 2 periods (7 to 21 d and 22 to 35 d) and the experimental diets were based on corn and soybean meal. All diets were fed as mash. When the corn was ground using a hammer mill, the screen size (mm) and the rotation speed (Hz) used were 3 and 50, 6 and 50, 8 and 35, and 8 and 25, respectively. When the roller mill was used, the gaps (mm) between the first and second pair of rollers used were 0.8 and 0.5, 1.2 and 0.7, 2.0 and 1.0, and 2.4 and 1.2, respectively. The geometric mean diameter (GMD) of the final feeds was 415, 536, 853, and 1,127 μm for the hammer-milled diets and 805, 935, 1,196, and 1,273 μm for the roller-milled diets, respectively. Each treatment was replicated 6 times and the experimental unit was a floor pen with 25 chicks. The following comparisons were performed: a) mill type effect (hammer vs. roller), b) linear and quadratic effect of the GMD of the hammer-milled diets, c) linear and quadratic effect of the GMD of the roller-milled diets, and d) interaction of the linear effect between particle size and mill type on the variables studied. Diet affected the growth performance of the birds, with effects that were more pronounced from 22 to 35 d of age than from 7 to 21 d of age ($P < 0.001$ for the interaction). Feed conversion ratio however, was not affected by treatment. At both ages, broilers ate more feed and grew faster when the feed was roller-milled than when the feed was hammer-milled ($P < 0.01$). Feed intake and BW gain increased linearly ($P < 0.05$) as the GMD of the diet increased, when the corn was hammer-milled, but no effects were observed when the cereal was roller-milled ($P < 0.01$ for the interaction). In summary, the use of the roller mill might benefit growth in broilers fed mash diets, especially when the BW gain is the main production goal. Within the range of values studied, the GMD produced by the use of the roller-mill did not affect chick growth but coarser grinding is recommended when the cereal is hammer-milled.

Key Words: corn, broiler growth performance, hammer mill, particle size, roller mill

363 Effects of corn kernel hardness, grain drying temperature, and amylase supplementation on broiler live performance.

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Drying temperature of corn can influence its nutritional quality, but whether this is influenced by endosperm hardness is not clear. An experiment was conducted to investigate the effects of 2 corn hybrids with average and hard kernel, dried at 3 temperatures (35, 80, and 120 C) without or with, amylase (Ronozyme HiStarch[ ]) supplementation (0 vs. 133 g/ton) on live performance of Ross 708 male broilers raised up to 3.3 kg. A total of 1,920 male-chicks were randomly allocated to 96 floor pens (20 chicks/pen). Twelve dietary treatments resulted from a 2x3x2 factorial arrangement of 2 corn kernel hardness, 3 drying temperatures and amylase supplementation respectively. Data were analyzed using 3-way ANOVA in a randomized complete block design. Mean separation was performed using Tukey's test. Results obtained at 14d showed

an interaction effect of treatments between drying temperature and enzyme supplementation on BW gain and BW ($P = 0.07$). Chicks fed non-supplemented diets with corn dried at 35 C were lighter (507 g) than chicks fed diets supplemented with amylase and containing corn dried at 35 C and 120 C (517 g). At 28d a 3-way interaction ($P < 0.05$) was detected on BW gain. Only for chickens fed corn with average kernel hardness and dried at 35 C, the addition of amylase resulted in greater BW gain than broilers fed non-supplemented diets (1,770 vs. 1,699 g). At 40 d, interaction effects ($P < 0.05$) of drying temperature and amylase supplementation were detected on BWG, BW, FCR, and flock uniformity. Supplementation with exogenous amylase resulted in heavier broilers, better FCR and flock uniformity, only in the diets based on corn dried at 35 C. Additionally, interaction effects were observed on FCR due to kernel hardness and drying temperature ($P < 0.01$), kernel hardness and enzyme supplementation ($P < 0.001$), and drying temperature and amylase supplementation ($P < 0.05$). Exogenous amylase addition to the diet based on corn with an average endosperm hardness improved FCR up to 2 points (1.49 vs. 1.51 g:g) whereas there was no effect of amylase on FCR of birds that received the diet based on corn with a hard endosperm. The FCR of broilers fed diets based on corn with average endosperm hardness was improved by drying at 120 C (1.49 vs. 1.51 g:g) whereas opposite effect was observed for corn with a hard endosperm (1.50 vs. 1.49 g:g). Feed intake was not affected ($P > 0.05$) by treatments throughout the whole experiment. It can be concluded that corn endosperm hardness, drying temperature and exogenous amylase can influence the live performance of broilers. However, these factors are not independent and so must be manipulated strategically to improve broiler performance.

Key Words: drying temperature, kernel hardness, corn, amylase, performance

364 Effects of steam conditioning manipulations to feed manufacturing parameters and d1-28 poult performance.

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Various strategies to improve milling efficiency, pellet quality, and bird performance can be utilized in turkey feed production. Two experiments were conducted. The objective of experiment 1 (EXP1) was to determine how feed manufacturing strategy affected pelleting efficiency and phytase activity retention. The objective of experiment 2 (EXP2) was to determine how poult performance was affected by feed manufacturing strategy. A common, commercially formulated diet containing commodity grains was batched and utilized in EXP1. This mash feed was subjected to either standard (STD) or thermally-aggressive (TA) processing techniques. Standard processing consisted of steam conditioning at 76.7 C for 15 s followed by 45 s residence in a hygieniser barrel without heat. Thermally-aggressive processing consisted of steam conditioning at 85 C for 15 s followed by 45 s residence in a hygieniser barrel at 82.2 C. Pelleted feed was then cooled and crumbled, creating 2 dietary treatments. Each treatment was provided to 24 replicate floor pens of 55 Hybrid hen poults from d1-28 in EXP2. One batch of feed represented the experimental unit (EU) in EXP1 while one pen of 55 Hybrid hen poults represented the EU in EXP2. Body weight (BW) and live weight gain (LWG) were measured from d1-7. Feed intake (FI), BW, LWG, and mortality corrected feed conversion ratio (FCR) were collected from d8-28. On d28 3 birds per pen were randomly selected and euthanized for tibia excision. Data were analyzed using the GLM procedure of SAS. Results from EXP1 explain that motor load lessened ($P = 0.0007$) while hot pellet temperature increased ($P = 0.0047$) with

TA processing. However, phytase activity retention was not different ($P = 0.9896$) when considering either processing technique. Pellet quality decreased with STD processing ($P < 0.05$) in EXP1 but feed was crumbled to eliminate feed form effects in EXP2. During d1–7 of EXP2, TA treatment fed poult tended to have reduced BW, LWG, and LWG per poult ($P > 0.10$). Interestingly, performance differences were not apparent from d8–28 ($P > 0.05$). Poults that were selected for tibia excisions differed in average poult weight. Poults selected from the STD treatment were 46 g per bird heavier than those selected from TA treatments ($P = 0.0081$). Therefore, bird weights were considered, and results represent mg of tibia ash per kg of BW. Difference in mg of tibia ash per kg of BW were not apparent ($P = 0.2324$). In conclusion, feed manufacturing efficiency and pellet quality can improve with thermally-aggressive processing strategies. More importantly, maintaining poult performance and bone mineralization is feasible when applying thermally-aggressive processing strategies to improve manufacturing efficiency.

Key Words: poult, steam conditioning, pelleting, performance, tibia ash

365 Fasting influences intestinal function to enhance feed energy digestibility of broilers. Youli Wang*, Jing Chen, Yuqin Wu, Xiaorui Guo, and Jianmin Yuan, *China Agricultural University, Beijing, Beijing, China.*

To evaluate feed digestible energy in poultry, traditionally, a same period of fasting before and after 2 d's feeding is needed to empty the digestive tract. However, more and more published data shows that fasting period influences the development of intestine, such as short-term fasting may promote the intestinal stem cells function while long-term fasting may impair the intestinal functions. So, it is necessary to investigate the impact of fasting period on feed energy utilization. In this study, a total of 5 hundred and 20 newly hatched broiler chicks were distributed into 40 cages with 13 birds in each cage. All the birds were fed same maize-soybean meal-based diet. At 23 d, one hundred and 75 broilers were transferred to metabolic room to evaluate feed energy utilization. These birds were divided into 5 treatment groups: ad libitum feeding, 12 h fasting, 24 h fasting, 36 h fasting, 48 h fasting. Each treatment had 7 cages with 5 broilers in every cage. The remaining birds left were divided in a similar way as the birds in metabolic room but each treatment had 8 replicates, which were used for tissue sampling. Excreta was collected daily, freeze-dried and tested for each day's energy and amino acid digestibility along with metabolic energy through classical total collection method. At the end of the first time fast and after one day refeeding, one broiler from each cage was killed to collect liver, spleen and intestinal mucosa samples. Intestinal morphology, mucosa and spleen enzyme activity, intestinal nutrient relative transporter, proliferation and apoptosis of intestinal epithelium were tested. Results showed that fasting for 48 h decreased the body weight by about 10%, while fasting for 12, 24 and 36 h increased ($P < 0.05$) the feed intake. Likewise, fasting enhanced ($P < 0.05$) the energy digestibility after one day refeeding and 12 h fasting improved ($P < 0.05$) the apparent metabolic energy significantly, but decreased ($P < 0.05$) the enzymatic activity in the brush border. Digestibility of most amino acids tended to increase slightly after 12 h fasting, however, it was reduced ($P < 0.05$) after 36 h fasting. Broilers fasted for 36 h and 48 h exhibited lower ($P < 0.05$) intestinal villus height and crypt depth, which can be attributed to increased expression of apoptosis marker gene *capase3* and decreased proliferation of cells in crypt ($P < 0.05$). After one day refeeding, higher expression ($P < 0.05$) of glucose and amino acid related genes in the jejunum was noticed. It can be concluded that fasting affects the intestinal functions and influences the value of metabolic energy in broilers.

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Key Words: broiler, fast time, metabolic energy, digestibility, intestinal development

366 Not Presented

367 Effect of the origin of the bean on the chemical composition, protein quality, and nutritive value of the soybean meal. L. Cámara¹, Lewis Aguirre¹, G. Fondevila¹, C. Dapozza², and Gonzalo Mateos*¹, ¹UPM, Madrid, Madrid, Spain, ²Evonik Nutrition and Care, GmbH, Barcelona, Spain.

Commercial samples of soybean meals (SBM) from USA (n = 42), Brazil (BRA; n = 51), and Argentina (ARG; n = 64) were collected from 2016 to 2018 to study the influence of the origin of the beans on chemical composition, nutritive value, and protein quality, including amino acid content per unit of protein of the SBM. All samples were collected in Europe by personal of the Quality Control Department of key feed mills and integrators. The SBM samples were either processed in the country of origin of the beans and then exported to Europe or crushed in Europe from identity preserved beans. Data were analyzed as a completely randomized design using the GLM procedure of SAS (SAS Institute Inc., 1990). Year of the crop was considered as a random effect and was not including in the model. When the model was significant, the Tukey test was used to make pairwise comparisons between treatment means. Brazilian meals had more CP than USA and ARG meals ($P < 0.001$) and less neutral detergent fiber ($P < 0.05$) than BRA and ARG meals, but an opposite effect was observed raffinose ($P < 0.001$). Lys, Met, Cys, and Trp content per unit of protein was higher for the USA meals than for the BRA and ARG meals ($P < 0.001$). Mineral content varied with the origin of the beans, with the ARG meals having more K, Mn, and Cu than the USA and BRA meals ($P < 0.001$). BRA meals had more Mg, Zn, and Fe than the USA and ARG meals ($P < 0.001$). Phosphorus content was higher for the ARG and USA meals than for the BRA meals ($P < 0.001$). Protein quality indicators were affected by the origin of the beans. Protein dispersibility index, KOH protein solubility, and trypsin inhibitor activity were higher ($P < 0.001$) for the USA meals than for the BRA and ARG meals. Heat damage indicator was lowest for the USA meals ($P < 0.001$). The data reported confirm that chemical composition, amino acid profile, protein quality, and nutritive value of the SBM varied with the country of origin of the beans. Consequently, bean origin should be considered by the nutritionists when evaluating the nutritive value of commercial soybean meals.

Key Words: amino acid profile, chemical composition, protein quality indicators, soybean origin

368 Influence of nutrient density and feed form on the growth performance and nutrient utilization in broilers fed wheat-based diets. Fifi Zaefarian*, Obright Hamungalu, Reza Abdollahi, and Ravi Ravindran, *School of Agriculture and Environment, Massey University, Palmerston North, New Zealand, Palmerston North, New Zealand.*

The interaction between dietary nutrient density and feed form on the growth performance, coefficient of apparent ileal digestibility (CAID) of nutrients and energy utilization in broilers fed wheat-based diets was investigated in a 35-d trial. A total of 10 dietary treatments were arranged in a 5×2 factorial arrangement with 5 nutrient densities, VL, very low nutrient density (2800 and 2900 kcal/kg AMEn, 13.44 and 11.46 g/kg lysine); L, low nutrient density (2900 and 3000 kcal/kg AMEn, 13.92

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and 11.85 g/kg lysine); M, medium nutrient density (3000 and 3100 kcal/kg AMEn, 14.40 and 12.25 g/kg lysine); H, high nutrient density (3100 and 3200 kcal/kg AMEn, 14.88 and 12.65 g/kg lysine) and VH, very high nutrient density (3200 and 3300 kcal/kg AMEn, 15.36 and 13.04 g/kg lysine), in starter and finisher phases, respectively, and 2 feed forms, mash vs. pellet. The first batch of each diet was used as unconditioned mash and the second batch was steam-pelleted. 480 d-old male broilers (Ross 308) were allocated to 60 cages (8 birds/cage) and cages were randomly assigned to the 10 dietary treatments. A significant ($P < 0.01$) interaction between nutrient density and feed form was observed for weight gain and feed intake during the whole grow-out period. While birds fed pelleted diets outperformed those fed mash diets at each nutrient density, the pellet-associated benefits were more pronounced at the lowest nutrient density. Increasing nutrient density lowered ($P < 0.001$) feed conversion ratio from 1.540 in VL to 1.381 in VH diets. Increasing dietary nutrient density progressively increased ($P < 0.001$) the CAID of nitrogen and fat. Feeding pelleted diets, regardless of nutrient density, lowered the CAID of starch ($P < 0.01$) and the absolute weight of gizzard compared with mash diets. The gizzard pH of birds fed pelleted diets was higher ($P < 0.001$) than those fed mash diets. The current results show that pellet-induced benefits on growth performance are reduced as dietary nutrient density increases and that the dietary nutrient density must be considered to achieve maximum benefits associated with pelleting.

Key Words: broilers, feed form, nutrient density, nutrient digestibility, performance

369 Feeding high-tannin or zero-tannin faba bean grain cultivars hulled or dehulled on growth performance, carcass traits and yield of saleable cuts of broiler chickens.

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Color-flowered, high-tannin faba bean (*Vicia faba*) cultivars are more tolerant to frost around harvest time than white-flowered, low-tannin cultivars. Tannins concentrated in the bean hull reduce both starch (40%) and protein (25%) digestibility. We therefore evaluated feeding 2 high-tannin (Fabelle, Malik) and 2 zero-tannin (Snowbird, Snowdrop) faba bean grain cultivars available in western Canada and the effect of dehulling to reduce tannin content on broiler chicken performance, dressing and carcass cuts. Male chicks (Ross 708; n = 585) housed in 64 pens were fed the 4 faba bean cultivars either hulled or dehulled included in starter (12%, 0 - 12d), grower (24%, 13 - 25d) and finisher (36%, 26 - 41d) diets. Faba bean grain replaced SBM and wheat in mash phase diets that provided 12.6, 12.9 and 13.2 MJ AMEn/kg and 1.02, 0.89 and 0.76 g SID Lys/MJ AMEn for the starter, grower and finisher phases, respectively. Individual weight of broilers at the end of each growth phase and before slaughter, chilled carcass and cut out saleable portion weights were measured on d 42. Data were analyzed as 4x2 factorial and contrasted to controls fed SBM only, using GLIMMIX (SAS 9.4) with 7 replicate pens per feeding regimen. Overall ADFI was greatest ($P < 0.05$) for broilers fed Snowbird and Fabelle, and lowest for Malik; Snowdrop was intermediate. ADG was greater ($P < 0.01$) for broilers fed Fabelle than other cultivars; greater ($P < 0.05$) for controls than broilers fed faba bean grain. Gain/feed was greatest ($P < 0.01$) for broilers fed Fabelle and lowest for Snowbird; Snowdrop was not different from Fabelle or Malik, and the latter was not different from Snowbird. Gain/feed was greater ($P < 0.001$) for controls than broilers fed faba bean grain. Antemortem WT was greater ($P < 0.01$) for broil-

ers fed Fabelle than Snowbird or Malik; Snowdrop was intermediate. Carcass WT and dressing % were greater ($P < 0.05$) for controls than broilers fed faba bean grain likely because of pulse dietary fiber. Breast meat yield was greater ($P < 0.01$) for broilers fed Snowbird, lower for those fed Fabelle; Malik was lower but not different from Snowbird or Snowdrop; Snowdrop was not different from Fabelle. Dehulling faba bean grain lowered ($P < 0.01$) breast meat yield by 8 g/kg. The results of this experiment indicate that feeding the low-vicine/covicine grain cultivar Fabelle to broilers resulted in slightly better growth performance but lower breast meat yield than feeding zero-tannin Snowbird or Snowdrop or high-tannin Malik cultivars. Dehulling faba bean grain to reduce tannin content was not effective in improving growth performance or dressing % of broilers fed faba bean to the level of control broilers fed SBM only.

Key Words: broiler chickens, carcass cuts, dehulling, faba bean grain cultivars, growth performance

370 Effect of oat hulls incorporated in the diet or fed as free choice on growth performance, digesta pH, organ weights and carcass yield of broiler chickens.

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To maintain productivity and health of chickens raised without antibiotics, there is an urgent need to investigate and develop suitable alternatives. High fiber ingredients have been shown to have prebiotic effects and their use in poultry production could promote gut health and growth performance and reduce production costs. Thus, the purpose of this study was to investigate the effect of oat hulls (OH) incorporated in the diet or fed as free choice on growth performance, organ weights, digesta pH and carcass yield of broiler chickens. Day old broiler chickens were randomly assigned to 4 dietary treatment groups consisting of a corn-soybean meal-wheat based diet (Basal), Basal + bacitracin (Antibiotic), Basal + 3% OH and Basal + free choice OH. Each group had 6 replicate pens of 27 birds and were raised on floor pens for 36 d using a phase-feeding program (starter, d 0 to 14, grower, d 14 to 24 and finisher, d 24 to 36). Diets were formulated on digestible amino acids and AME bases and met or exceeded N.R.C (1994) requirements. Feed consumption and body weight were determined weekly. Two birds per pen were slaughtered on d 36 to determine organ weights and digesta pH and on d 39 to determine carcass yield. Data were analyzed as a completely randomized design using the GLM procedure of SAS. Overall, birds fed 3% OH and antibiotics but not the basal diet group had higher ($P < 0.05$) BW and BWG than those fed free choice OH. Birds fed 3% OH had similar ($P > 0.05$) total feed intake (FI) with those fed basal and antibiotic diets while birds fed free choice OH had lower ($P < 0.05$) FI than those fed the basal diet. During the starter phase, birds fed 3% OH had the lowest ($P < 0.05$) FCR (0.86) compared with other treatment groups (0.92–0.96). Birds fed 3% OH had the highest live weight, hot carcass weight, cold carcass weight and breast muscle weight while birds fed free choice OH had the lowest values except for breast muscle weight where the lowest value was recorded for the antibiotic diet. There was no effect of diet on ileal and cecal digesta pH and weights (g/kgBW) of spleen, liver, bursa, pancreas, and intestine. However, birds fed free choice OH had higher ($P < 0.0001$) gizzard weight and tended to have higher ceca weight ($P = 0.09$) than other treatment groups. In conclusion, inclusion of 3% OH in an antibiotic-free diet enhanced FCR and carcass yield while free choice OH increased gizzard weight but reduced FI and BWG of broiler chickens.

Key Words: broiler chickens, carcass yield, growth performance, oat hulls, organ weights

Metabolism and Nutrition: Amino Acids

371 The effects of threonine, glycine and serine on growth performance, fat deposition, nutrient utilisation and starch-protein digestive dynamics in male broiler chickens offered reduced-crude protein diets. Peter Chrystal^{*1,2}, Amy Moss³, Ali Khoddami¹, Victor Naranjo⁴, Peter Selle¹, and Sonia Liu¹, ¹University of Sydney, Camden, New South Wales, Australia, ²Baiada Poultry Pty Ltd., Pendle Hill, New South Wales, Australia, ³University of New England, Armidale, New South Wales, Australia, ⁴Evonik Nutrition and Care, Hanau-Wolfgang, Germany.

Reducing dietary crude protein (CP) significantly reduces soyabean meal use, fecal output and nitrogen (N) emissions. However, tangible reductions in CP typically result in compromised broiler performance. In the present study, reduced CP diets (maize/soyabean) were offered to Ross 308 off-sex male broilers from 14 to 35 d post-hatch and supplemented with glycine (Gly), serine (Ser) and threonine (Thr). Seven iso-energetic (12.9 MJ/kg, AME_n), 11.0 g/kg standardized ileal digestible lysine (SID Lys) diets, maintaining minimum “ideal” essential AA ratios, were offered to 336 broilers (6 birds/cage × 8 replicates) fed a common starter to 14 d. CP in diets 1 to 4 was reduced incrementally from 205 to 165 g/kg and the remaining 3 diets (165 g/kg CP) were supplemented with either Thr, Gly + Ser or Thr + Gly + Ser. Gly and Ser were supplemented in diets 6 and 7 at levels equal to the SID values of the highest CP diet (6.66 and 8.46 g/kg respectively). SID Thr was supplemented to provide a ratio of 0.75 to SID Lys in diets 5 and 7 but kept at a ratio of 0.65 to Lys for the remainder of the diets. Data were analyzed by one way ANOVA and pairwise comparisons where appropriate. Decreasing dietary CP from 205 to 165 g/kg increased ($P < 0.001$) relative fat pad weights by 75% from 7.6 to 13.3 g/kg of body weight. Addition of Thr or Gly + Ser to the 165 g/kg CP diet with had no impact on growth performance. However, supplementation with all 3 AA improved ($P < 0.025$) liveweight gain (2150 vs. 1994 g), feed intake ($P < 0.025$; 3269 vs. 3114 g), reduced relative fat pad weights ($P < 0.006$; 11.7 g/kg vs. 13.3 g/kg) and numerically increased FCE ($P = 0.315$; 640 to 658 g gain/kg feed) compared with the un-supplemented 165 g/kg diet. The supplemented 165 g/kg diet performed as well as the 205 g/kg CP diet except for higher ($P < 0.001$) relative fat pad weights (11.7 vs. 7.6 g/kg) with no impact on AME or starch digestibility coefficients at the distal jejunum or ileum. Starch disappearance rates (g/bird/day) were lower ($P < 0.001$) for the 205 g/kg CP diet compared with the reduced CP diets by an average 15.7% in the distal jejunum and ileum. Furthermore, the 165 g/kg diet supplemented with Thr, Gly and Ser resulted in a higher ($P < 0.001$) N digestibility coefficient and disappearance rate at the distal ileum (0.842 vs. 0.793 and 21.39 vs. 19.42 respectively) and thus reduced ($P < 0.001$) starch:protein disappearance rate ratios in the distal jejunum and ileum (3.85 vs. 4.24 and 2.64 vs. 3.23 respectively) that have been associated with improved broiler performance. This study demonstrated that reduced CP diets supplemented with Thr, Gly and Ser resulted in broiler performance comparable to the 205 g/kg diet.

Key Words: broiler, digestive dynamics, reduced protein, glycine equivalents, threonine

372 Statistical method to determine the efficacy of L-Methionine, DL-Methionine and OH-Methionine in broilers. Dolores Batonon-Alavo^{*1,2}, Rob Shirley², and Yves Mercier¹, ¹Adisseo France SAS, Commentry, France, ²Adisseo USA Inc., Alpharetta, United States.

Methionine (Met) is available under different forms: DL-Methionine (DL-Met), L-Methionine (L-Met) and DL-Hydroxy-Methionine (OH-Met). For decades, there has been considerable debate regarding their efficacies, but only few studies focused on the methodological aspects of this comparison. This work aims to determine the appropriate statistical method to compare the efficacy of Met sources. For this purpose, new data sets were generated through 2 experiments. Male Ross PM3 broiler chickens were reared for 36 d, divided into 3 feeding phases: 0–10 d, 11–24 d and 25–35 d. Experimental treatments consisted of a basal, sulfur amino acids deficient diet and diets supplemented at 3 equimolar doses with either L-Met or DL-Met (Trial 1) and L-Met or OH-Met (Trial 2). First, growth performance data were analyzed using ANCOVA models with the dose and the source of Met as main variables. Results showed a significant improvement in growth performance ($P < 0.05$) with the increasing dose of Met, and no significant difference between the sources in the 2 trials ($P > 0.10$). In addition, 3 exponential models were applied on the body weight gain obtained in each feeding phase, as response to (i) the theoretical doses of each Met source, (ii) the analyzed doses in free Met and OH-Met and (iii) the analyzed equivalent Met intake. The analyzed equivalent Met intake was calculated as the product of the analyzed doses and the feed intake. The best performing models were selected using the Akaike Information Criteria (AIC); the lower the AIC, the better the modeling. For all feeding phases in the 2 trials, the best models were obtained when the weight gain was expressed as function of the analyzed Met intake. Using these exponential models, the efficacy of DL-Met and OH-Met was determined relative to L-Met. DL-Met was found to be 100% equivalent to L-Met in trial 1 and OH-Met was found to be 100% equivalent to L-Met in trial 2, respectively for the starter, the grower and the finisher periods. The originality of this analysis is based on the nature of the explanatory variable, which is Met intake. This shows that the relative efficacy of the sources of methionine should be determined using the most appropriate explanatory variables.

Key Words: methionine, hydroxy-methionine, efficacy, method

373 Meta-analysis of the response of broilers to dietary valine: Impact of other branched chain amino acids . Maroua Zouaoui^{*2}, William Lambert¹, and Marie-Pierre Létourneau-Montminy², ¹Ajinomoto Animal Nutrition Europe, Paris, France, ²Université Laval, Québec, Canada.

Reducing dietary crude protein (CP) in broilers is an efficient way to reduce nitrogen excretion, improve health and reduce feeding costs. Diets with reduced CP content must be balanced in indispensable amino acids. Among them, branched-chain amino acids (BCAAs) which include valine (Val), leucine (Leu), and isoleucine (Ile) share the same unique metabolism. Therefore, BCAA must be studied together since they interfere with each other and Leu appears to be the strongest regulator of BCAAs catabolism. However, their interaction has not been investigated a lot in poultry and needs to be better quantified. The objective of this study is to quantify the response of broilers to dietary Val and the impact of the 2 other BCAAs, Leu and Ile, on this response through a meta-analysis approach. Twenty-three articles published between 1999 and 2018 and including 44 experiments and 239 treatments were used. Multiple regression models were fitted with the MIXED procedure of Minitab software with the random effect of the experiment. The Y variables were Average Daily Gain (ADG), Average Daily Feed Intake (ADFI) and Feed Conversion Ratio (FCR) expressed

in percentage relative to the highest value to make the values comparable between age and genetic line. The main X variable was dietary True Digestible Val (ValTD) and the other ones were dietary True digestible Leu (LeuTD) and True digestible Ile (IleTD). The impact of LeuTD and IleTD was assessed independently given that they are collinear in most experiments. The response of ADG and ADFI to ValTD was curvilinear ($P < 0.001$: ADG, $R^2 = 64\%$; ADFI, $R^2 = 53\%$). Results showed that increasing dietary LeuTD reduced ADG and ADFI ($P < 0.001$) but also that the response of broilers to ValTD was higher in high LeuTD diet (Interaction ValTDxLeuTD; ADG and ADFI, $P < 0.001$). The FCR responded linearly ($P < 0.001$) and quadratically ($P < 0.001$) to ValTD, while LeuTD and IleTD have no effect on FCR. Based on these models, increasing dietary ValTD from 7 to 8.5g/kg generates in wheat-based diets (wheat, soybean meal; 10 g/kg of LeuTD) an improvement of ADG of 5% and ADFI of 3% compared with 8% for ADG and 6% for ADFI in corn-based diets (14 g/kg of LeuTD). There was also an effect of IleTD showing lower ADG ($P = 0.01$) and ADFI ($P = 0.03$) in high IleTD diet, but the response of ADG and ADFI to ValTD was not modified by IleTD (No interaction). The current study showed that ADG and ADFI increases with increasing digestible Val and this effect was modulated by other BCAAs except for FCR. Results support that Leu was the most important regulator of BCAAs catabolism. This is a first approach to better quantify the response of broilers to BCAAs and their interactions.

Key Words: broiler, ADG, ADFI, valine, leucine

374 Intestinal permeability and gene expression of selected tight junctions and interleukins in broiler chickens fed reduced protein diets supplemented with arginine, glutamine and glycine.

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Maintaining intestinal barrier and controlling permeability is vital to support optimal digestive health and growth performance of broiler chickens. However, specific effects of individual amino acids on intestinal barrier function and permeability are yet to be identified particularly when dietary protein is reduced. The present study was designed to determine the effect of L-glutamine (Gln), glycine (Gly) and L-arginine (Arg) on intestinal permeability (IP) and selected genes related to tight junctions and inflammation. Male Ross 308 birds, were offered one of 4 dietary treatments: a reduced protein (RP) diet [193.9 g/kg CP in grower]; or the RP diet supplemented with either 10 g/kg Gln, or 10 g/kg Gly or 5 g/kg Arg. Each experimental diet was replicated 24 times (24 birds). On d 14, 16, 18 and 20, half of the birds in each treatment group (n = 12) were injected with dexamethasone (DEX) at 0.5 mg/kg BW to induce leaky gut. The remaining birds received sham injections (0.9% saline). Fluorescein isothiocyanate dextran (FITC-d) was used to test IP on d 21. Quantitative PCR assays were performed to study gene expression of selected barrier-forming tight junction proteins (Claudin-1 and Claudin-3), interleukin 8 (IL-8), interleukin 1 β (IL-1 β) and nuclear factor- κ B (NF- κ B), a signaling inflammation pathway, in ileal and jejunal tissues collected from birds on d 21 of age. Data were subjected to 2-way ANOVA to assess the main effects of diets, DEX, and their interaction. Treatment means were separated by Fisher's LSD test ($P \leq 0.05$ as significant). Arg tended to lower FITC-d ($P = 0.086$) regardless of DEX injections. DEX increased passage of FITC-d into

the serum ($P < 0.001$). DEX and diet interacted ($P < 0.01$) for jejunal Claudin-3 mRNA level where DEX upregulated Claudin-3 for all diets except the Arg diet. Birds receiving Arg treatment had greater ($P < 0.01$) ileal expression of Claudin-1 compared with the other diets. DEX independently increased ileal expression of Claudin-3, IL-8, and IL-1 β ($P < 0.001$). Compared with control and Arg diet, dietary supplementation of Gly and Gln upregulated expression of jejunal IL-8 only in DEX-injected birds ($P < 0.05$). Gly supplementation reduced mRNA level of NF- κ B in the ileum compared with the other 3 treatments ($P < 0.01$). In conclusion, increasing dietary concentration of Arg by approximately 140% of Ross 308 specification had positive effects on intestinal barrier function and inhibited the intestinal inflammation caused by DEX injection. While Gly and Gln had no demonstrable effect on IP or tight junction expression, Gly may possibly be involved in reducing intestinal inflammation through the NF- κ B pathway.

Key Words: intestinal barrier, permeability, reduced protein, amino acids, dexamethasone

375 Dietary bioefficacy differences of methionine sources with acidifier in diets fed to broiler chickens reared in floor system.

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This study was conducted to test the effects of supplemental DL-Methionine + Organic acid (99%DLM + OA) and methionine hydroxy analog-free acid (88% HMTBA) on growth performance, redox status, gut development, and meat quality of broiler chickens compared with DL-Methionine (99%DLM). A total of 1632 male 1-d-old Cobb 500 chickens were weighed and randomly allotted to factorial design 3 \times 5 + 1 (methionine sources \times levels + control diet) treatments for 42 d. The control diet (CD) was without industrial methionine supplementation or a CD supplemented with 5 DLM or DLM + OA or HMTBA equimolar levels (0.05; 0.10; 0.15; 0.30; 0.35%). The organic acid had the same pka of HMTBA. For bioefficacy evaluation of the test products compared with DLM data were analyses by exponential regression using weight gain and feed conversion rate. For the other parameters, data were analyzed by ANOVA. Means were separated using Tukey's test ($P < 0.05$). All data were analyzed within experiments by 2-way ANOVA with the general linear GLM procedure of SAS software (2000). Performance, redox status (liver-gut), plasma uric acid (PUA) and creatine kinase (CK), intestine pH and morphology were measured. Bioefficacy of HMTBA was 69% (1–21d) and 72% (1–42d), and use of organic acid favored DLM increasing its bioefficacy was 118% (1–21d) and 109% (1–42d) compared with DLM. Shear force had increased ($P < 0.05$) in chicks fed a diet BD and methionine deficient. Chicks fed a diet supplemented with any Met source had increased ($P < 0.05$) GSH (glutathione peroxidase), CAT (catalase), SOD (superoxide dismutase) activity and reduced ($P < 0.05$) lipid peroxidation compared with chicks fed the BD. On 21d DLM and DLM + OA had greater ($P < 0.05$) villus height: crypt depth ratio compared with chicks fed HMTBA. However, at d 42, chicks fed a diet supplemented with HMTBA and DLM + OA had greater ($P < 0.05$) villus height: crypt depth. The HMTBA and DLM + OA supplementation decreased ($P < 0.05$) gizzard and jejunum pH. At d 21 and 42 CK plasma activity declined ($P < 0.05$) in chicks fed any methionine source. Nevertheless, on d 42 HMTBA and DLM + OA CK activity was lower ($P < 0.05$) than DLM source. PUA on d 42 was increased ($P < 0.05$) in chicks with all methionine sources. Supplementation of Met served a better performance, redox status and

development of the gut of chicks compared with CD diet. The use of organic acid favored DLM, increasing its bioefficacy.

Key Words: DL-methionine, gut health, HMTBA, organic acid, redox status

376 The bioefficacy of methionine sources with acidifier in diets fed to broiler chickens reared in cage system. Edwin Mendoza*¹, Andressa Carvalho¹, Fabio Caballero¹, Sueli Costa¹, Victor Naranjo², and Antonio Bertechini¹, ¹Federal University of Lavras, Lavras, Minas Gerais, Brazil, ²Evonik Nutrition, Wolfgang, Germany.

This study was conducted to evaluate the effects of supplemental DL-Methionine + Organic acid (99%DLM + OA) and methionine hydroxy analog-free acid (88% HMTBA) on growth performance, redox status, gut development, and meat quality of broiler chickens compared with DL-Methionine (99%DLM). A total of 768 male day-old Cobb 500 chickens were weighed and randomly allotted to factorial design 3 × 5 + 1 (methionine sources × levels + basal diet) treatments for 42 d. The control diet (CD) was without industrial methionine supplementation or a CD supplemented with 5 DLM or DLM + OA or HMTBA equimolar levels (0.05; 0.10; 0.15; 0.30; 0.35%). The organic acid had the same pKa of HMTBA. For bioefficacy evaluation of the test products compared with DLM data were analyses by exponential regression using weight gain and feed conversion rate. For the other parameters, data were analyzed by ANOVA. Means were separated using Tukey's test ($P < 0.05$). All data were analyzed within experiments by 2-way ANOVA with the general linear GLM procedure of SAS software (2000). Performance, redox status (liver-gut), plasma uric acid (PUA) and creatine kinase (CK), intestine pH and morphology were measured. Bioefficacy of HMTBA was 54% (1–21d) and 60% (1–42d), and use of organic acid favored DLM increasing its bioefficacy was 129% (1–21d) and 142% (1–42d) compared with DLM. Shear force had increased ($P < 0.05$) in chicks fed a diet BD and methionine deficient. Chicks fed diet supplemented with any methionine (Met) source had increased ($P < 0.05$) liver and gut GSH (glutathione peroxidase), CAT (catalase), SOD (superoxide dismutase) activity and reduced ($P < 0.05$) lipid peroxidation compared with chicks fed the BD. On 21d DLM and DLM + OA had greater ($P < 0.05$) villus height: crypt depth ratio compared with chicks fed HMTBA. However, at d 42, chicks fed a diet supplemented with HMTBA and DLM + OA had greater ($P < 0.05$) villus height: crypt depth. On the other hand, HMTBA and DLM + OA supplementation decreased ($P < 0.05$) gizzard and jejunum pH. At d 21 and 42 CK plasma activity declined ($P < 0.05$) in chicks fed any Met source. Nevertheless, on d 42 HMTBA and DLM + OA CK activity was lower ($P < 0.05$) than DLM source. PUA on d 42 was increased ($P < 0.05$) in chicks with all Met sources. Supplementation of Met served a better performance, redox status and development of the gut of chicks compared with CD diet. Finally, the use of organic acid favored DLM, increasing its bioefficacy.

Key Words: DL-methionine, gut health, HMTBA, organic acid, redox status

377 Adequate supply of DL-methionine and DL-hydroxy methionine improves laying hens' performance under high temperature. Dolores Batonon-Alavo*^{1,2}, M. V. L. N. Raju³, D. Nagalakshmi³, B. Prakash³, Rob Shirley², Yves Mercier¹, and SV Rama Rao³, ¹Adisseo France SAS, Commentry, France, ²Adisseo USA Inc., Alpharetta, United States, ³Sri Ramadhoota Poultry Research, Kothur, India.

A study was performed to evaluate the effects of different levels of sulfur amino acids (SAA) supplied as DL-Methionine (DL-Met) or DL-Hydroxy-Methionine (OH-Met) on laying hen's performance under tropical summer conditions. Eight-hundred and 96 Babcock layers were randomly allocated based on their body weight, in 4 treatments of 14 replicates each (16 hens per replicate). Hens were offered diets supplemented either with DL-Met or OH-Met at TSAA requirement (0.65%) or 25% above (0.81%) from 19 to 31 weeks of age. Temperature (from 27 ± 3°C at 9 a.m. to 30 ± 5°C at 9 p.m.) and relative humidity (from 62 ± 20% at 9 a.m. to 51 ± 21% at 9 p.m.) varied widely each day and throughout the experimental period. Body weight was measured at wk 17, 23 and 28 whereas feed intake and laying performance were measured on a weekly basis. Egg quality was assessed at wk 22, 26 and 30. Data were analyzed (R Core Team, 2017) with repeated measures ANCOVA using the methionine source and age as main variables and the dose as a covariate. Body weight increased with age and was not significantly different between treatments. Feed intake was lower than breed standards, as influenced by the high temperature but were similar between the 2 methionine sources. Likewise, laying hen performance and feed conversion ratio were not different between DL-Met and OH-Met at any given point within the trial. There were no significant differences in the egg mass or egg weight of hens fed OH-Met or DL-Met. However, egg weight was significantly reduced in the high SAA level in comparison to the adequate SAA level group. Egg production tended to be reduced in the high SAA diets ($P = 0.07$). Feed intake, egg mass and feed conversion ratio were not affected by the level of SAA. Egg density, eggshell strength and Haugh unit were not significantly different between methionine sources or the doses. Overall, this study demonstrated that increasing the SAA level to 25% above SAA requirements during hot climate in open sided house might be too high to ensure optimal performance of laying hens. The performance of layers fed DL-Met and OH-Met were similar under high temperature.

Key Words: methionine, hydroxy-methionine, layers, high temperature

378 In vivo mixed muscle and collagen synthesis in pectoralis major for two commercial broiler strains. Pramir Maharjan*, Jordan Weil, Antonio Beitia, Katie Hilton, Nawin Suesuttajit, Casey Owens, and Chris Coon, University of Arkansas, Fayetteville, Arkansas, United States.

A study was conducted to understand *in vivo* mixed muscle and collagen fractional synthesis and degradation rates (FSR and FDR) in *Pectoralis major* in 2 commercial broiler strains, Strain A and Strain B at d 56 of age. Birds (n = 10 each strain) were infused with ¹⁵N-phenylalanine or 1-¹³C proline as tracer amino acids for mixed muscle and collagen protein turnover (PT) study. Isotopic enrichment in muscle tissue samples of ¹⁵N to ¹⁴N ratio were measured for mixed muscle using ion fragments 302 and 303 whereas ¹³C-to-¹²C ratio were measured for collagen using the fragments 287 to 286 derived from GC MS ion chromatogram. FSR (expressed as % /h) were determined using precursor-product principle. Birds were also assessed for fiber growth (fiber diameter (FD), and non-fiber space (NFS)), and collagen was quantified. The fillet of sampled birds were subjectively scored for woody breast (WB) scores. Results indicated that mixed muscle and collagen FSR were higher for Strain A than Strain B ($P < 0.05$) at d 56. Mixed muscle FSR for Strain A was 0.51 (±0.04) whereas it was 0.16 (±0.01) for Strain B. FDR values for mixed muscle (0.14 for Strain A and 0.12 for Strain B) for both the strains remained similar ($P > 0.05$). FSR for collagen was not detectable on %/h basis for Strain B whereas it was 0.02 (±0.001) for Strain A. The average FD was 86.10 μm for Strain A and 66.77 μm for Strain B and NFS per mm² of tissue was greater for Strain B as

compared with Strain A. Total collagen was significantly higher ($P < 0.05$) for Strain B than Strain A (Strain A: 4.50 vs Strain B: 12.12 $\mu\text{g}/\text{mg}$ at d 56). Micrographs revealed higher deposition collagenous tissue in endomysial and perimysial spaces in Strain B with higher degree of myodegeneration occurring. WB scores were higher ($P < 0.05$) for Strain B than Strain A. Findings suggest that higher myodegeneration occurring in Strain B (than Strain A) could be due to higher degradation rate of muscle fibers (mixed muscle) observed relative to synthesis rates. Also, Strain B tended to slow down the turnover of collagen protein at d 56 by decreased synthesis and deposition of collagen in extracellular matrix of muscle tissue, which could be part of an adaptive physiological feedback mechanism.

Key Words: mixed muscle, collagen, protein turnover, broiler strains

379 Estimation of the dietary isoleucine requirement for grower broilers. Karl Schedle², Aude Simongiovanni¹, William Lambert*¹, Jörg Bartelt³, and Thomas Flécher¹, ¹Ajinomoto Animal Nutrition Europe, Paris, France, ²University of Natural Resources and Life Sciences, Vienna, Austria, ³Kaesler Nutrition GmbH, Cuxhaven, Germany.

Reducing dietary crude protein (CP) level improves animal welfare and nutrient efficiency in broiler production. It can be achieved without any performance loss, only by balancing the indispensable amino acid profile for broiler. Isoleucine (Ile) is considered as one of the next limiting amino acid after valine in broiler diets. However, published Ile requirements highlight variability. The objective of this study was to evaluate the optimal true digestible isoleucine-to-lysine (TD Ile:Lys) level in grower broilers diet through a dose-response trial. From d-1 to d-7, a total of 438 Ross broilers were fed the same commercial starter diet (12.2 MJ AMEn, 21.0% CP, 1.25% TD Lys). At d-8, birds were randomly allocated to 6 different groups of 6 pens per replicate. The 6 diets differed in their TD Ile:Lys content (from 55 to 72%) and were designed to have a sub-limiting Lys content (1.00% TD Lys, 16.5% CP) while all the other essential AA at their requirement. During this period, average daily feed intake (ADFI), average daily gain (ADG) and subsequently, gain-to-feed ratio (G:F) were determined pen-wise. At d-21, broilers final body weight (FBW) was measured. Animals had free access to feed and water throughout the experiment. ADG, ADFI, G:F and FBW data were fitted to linear-plateau as well as curvilinear-plateau models and broiler response to dietary Ile was evaluated. However, curvilinear-plateau models were not significant or the requirements modeled were outside the tested range. Requirements were estimated, with the linear-plateau models, to be 72.2 ($P < 0.01$), 72.6 ($P < 0.01$), 69.5 ($P < 0.01$) and 69.9% ($P < 0.01$) TD Ile:Lys for maximum ADG, ADFI, G:F and FBW respectively. A decrease of 10% of the optimal TD Ile:Lys was estimated to reduce by -2.7, -1.5, -0.8 and -1.2% the ADG, ADFI, G:F and FBW respectively. Based on this study, the optimum dietary TD Ile:Lys level is evaluated to be at 71.1% on average. The response observed when inducing an Ile deficiency indicates that attention have to be paid to the dietary Ile level when formulating grower broiler diet.

Key Words: isoleucine, requirement, broilers, performance, low CP diet

380 Effect of amino acid levels during broiler breeder pullet rearing on: 3. reproductive performance up to 50 wk. Edgar Oviedo*⁴, Ivan Ospina-Rojas¹, Hernan Cordova-Noboa¹, Gustavo Quintana-Ospina¹, Maria Alfaro Wisaquillo¹, Ivan Cardenas-Garcia¹, Miguel Chico¹, Andres Ortiz¹, Yilmar Matta¹, Gherlly Buitrago¹, Juan Martinez¹, Jose Yanquen¹, Juan Hoyos¹, Viviana San Martin¹, Lina Peñuela², and Justina Caldas³, ¹North Carolina State University,

Raleigh, North Carolina, United States, ²Universidad del Tolima, Ibague, Colombia, ³Cobb-Vantress Inc, Siloam Springs, Arkansas, United States, ⁴NC State University, Raleigh, North Carolina, United States.

Dietary amino acid (AA) concentration during pullet rearing affects growth and development. Consequently, it may affect reproduction, but these effects have not been quantified recently with modern broiler breeder strains. An experiment was conducted to determine the effects of 4 dietary AA levels fed to broiler breeder pullets during the rearing phase from 5 to 24wk on egg production, hatchability and fertility. A total of 1,360 Cobb-500 slow-feathering pullets were placed in 16 floor pens (85/pen). Up to 4wk all pullets were fed one starter diet in crumbles and after 29d of age fed with 4 mash grower diets containing 4 AA levels (0.49, 0.54, 0.60, and 0.66% of dLys with balanced protein) and 2,700 kcal/kg ME. From 16wk to 5% egg production, developer mash diets with 2,800 kcal/kg ME and 0.51, 0.57, 0.63, and 0.69% of dLys with balanced protein were offered. Feed amounts varied slightly (± 3 g/d) among treatments in the developer phase to maintain BW close ($\pm 2\%$) to Cobb guideline. At 21 wk, 7 Cobb MV males and 65 females per pen were moved to each one of the breeding pens, photo stimulated, and fed separately. Onset of egg production occurred around 165d and reached 5% at 167d of age with no differences ($P > 0.05$) among treatments. After 5% egg production all hens were fed one common layer diet and feed increments were made according to egg production by treatment. Egg production and mortality were recorded daily and 180 eggs/pen were incubated 10 times from 29 to 46 wk every 2 or 3 wk. Data were analyzed in a CRD with 4 AA treatments and 4 replicate pens each. One-way ANOVA and regression analyses were conducted. Egg production was affected ($P < 0.05$) by dietary AA levels during rearing in all wk evaluated (25 to 50wk). A quadratic effect of AA levels ($P < 0.05$) on egg production was observed with the average optimum level estimated at 0.57% dLys for grower and 0.60% dLys for developer diets. The best peak of egg production (85.6 \pm 1.1%) was observed at 32wk with an optimum level estimated at 0.60% dLys for grower and 0.63% dLys for the developer diets. At 50wk, hen-housed egg production was affected ($P < 0.01$) by AA levels fed during rearing, observing averages of 110.5, 121.5, 122.6 and 115.1 eggs/hen-housed for the 4 dietary treatments evaluated in ascending order. Fertility (average 98.86 \pm 1.05%) was not affected ($P > 0.05$) by AA levels fed to pullets during rearing. A linear positive effect ($P < 0.05$) of AA levels during rearing was detected on overall hatchability. No effects ($P > 0.05$) of treatments were detected on embryo mortality. In conclusion, AA dietary levels during rearing affected egg production and hatchability. Negative effects were observed with the lowest AA levels.

Key Words: amino acids, pullet breeders, egg production, fertility, hatchability

381 Effect of amino acid levels during broiler breeder pullet rearing on: 4. feathering during lay. Edgar Oviedo*¹, Andres Ortiz¹, Yilmar Matta¹, Hernan Cordova-Noboa¹, Gustavo Quintana-Ospina¹, Ivan Ospina-Rojas¹, Maria Alfaro Wisaquillo¹, Ivan Cardenas-Garcia¹, Miguel Chico¹, Viviana San Martin¹, Lina Peñuela², and Justina Caldas-Cuevas³, ¹North Carolina State University, Raleigh, North Carolina, United States, ²Universidad del Tolima, Ibague, Colombia, ³Cobb-Vantress Inc, Siloam Springs, Arkansas, United States.

Hen feathering is a common issue in broiler breeders affecting mating and fertility. Amino acid nutrition may affect feathering as it affects growth and development of other tissues. An experiment was conducted to determine the effects of 4 dietary amino acid (AA) levels fed to

broiler breeder pullets during the rearing phase from 5 to 24wk of age on feathering condition during lay. A total of 1,360 Cobb-500 slow-feathering pullets were placed in 16 floor pens (85/pen). Up to 4wk all pullets were fed one starter diet in crumbles and after 29d of age fed with 4 mash grower diets containing 4 AA levels (0.49, 0.54, 0.60, and 0.66% of dLys with balanced protein) and 2,700 kcal/kg ME. From 16wk to 5% egg production, developer mash diets with 2,800 kcal/kg ME and 0.51, 0.57, 0.63, and 0.69% of dLys with balanced protein were offered. Total sulfur AA (Met+Cys) vary between 0.48 and 0.56% in the grower phase and between 0.50 and 0.60% in the developer phase. Feed amounts varied slightly (± 3 g/d) among treatments in the developer phase to maintain BW close ($\pm 2\%$) to Cobb guideline. After 5% egg production all hens were fed one common layer diet and feed increments were made according to egg production by treatment. At 21 wk, 7 Cobb MV males and 65 females per pen were moved to each breeding pen, photo stimulated, and fed separately. Individual BW, feathering (1–5) and fleshing scores (1–6), based on Cobb standards, were evaluated at 32, 36, 40, 46, and 50 wk. Data were analyzed in a CRD with 4 AA treatments and 4 replicate pens each. One-way ANOVA, regression, correlation, and partition analyses were conducted. Results indicated that AA levels affected ($P < 0.001$) feathering scores. Hens fed the 2 lowest AA levels during rearing had better feathering scores than the other treatments. Feathering scores got worst only after 46 wk of age. Data did not fit ($P > 0.05$) any specific model for AA levels. However, a partition analysis detected that hens fed 0.63% or higher dLys in the developer phase had worst feathering, especially when weighed less than 3,921 g at 46 wk and had fleshing score 6. Fleshing scores ($r = 0.43$) and BW ($r = 0.19$) were correlated ($P < 0.001$) with feathering scores. A multiple linear regression model ($R^2 = 0.31$) indicated that age, fleshing, BW, status as layer ($P < 0.001$), and AA levels during rearing ($P < 0.05$) contributed to feathering variation. Age and fleshing explained 95.7% of all feathering variability observed. In conclusion, dietary AA levels during rearing affected hen feathering scores and hens with lower AA intake and lower egg production had the best feathering condition during lay, but dietary AA levels were not the most important factor for feathering condition.

Key Words: amino acids, pullet breeders, feathering, laying

382 Determining amino acid requirements for broiler breeders using the nitrogen balance method. Jordan Weil¹*, Antonio Beitia¹, Nawin Suesuttajit¹, Katie Hilton¹, Pramir Maharjan¹, Diego Martinez¹, Justina Caldas-Cuevas², Shivi Rao², and CN Coon¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²Cobb-Vantress, Inc., Siloam Springs, Arkansas, United States.

A major limiting factor in the continuing development and growth of the poultry industry globally is the production of adequate hatching eggs and quality broiler chicks necessary for the poultry supply chain. The use of feed restriction programs for broiler breeders to maintain ideal body composition for reproductive function places importance on the amino acids supplied in the diet. Excess amino acids in the feed are not only costly, but may affect fertility. Therefore, an emphasis is placed on the need to understand amino acid requirements in broiler breeders to ensure optimal egg production, hatchability, and progeny performance. To understand the utilization of amino acids, the nitrogen balance technique is used to determine dietary requirements. It is necessary to determine the requirements of broiler breeders, considering the reproductive health of hens is reliant on their nutritive intake. In this experiment, a total of 648 21 wk-old Cobb 700 FF broiler breeders were placed into 648 cages, with each cage containing one breeder. A total of 8 test amino acids were studied in this experiment (10 reps/diet). Test

amino acids included the total sulfur amino acids (Met + Cys), lysine (Lys), arginine (Arg), branched chain amino acids (Val, Ile), threonine (Thr), tryptophan (Trp), and glutamic acid (Glu). The concentration of amino acids that were fed to all treatments except for the test amino acid was equivalent to 120% Primary Breeder recommended amino acid intake (Cobb-Vantress, 2012) for peak production. Birds were fed for a period of 7 weeks (2 wks adaptation; 5 wks testing). The egg production, body weight and egg weight for all breeders was evaluated during the test and recovery period. Results were analyzed using a broken-line model (JMP Pro 13.1). Average digestible amino acid requirements for egg mass were as follows: Met+Cys, Lys, Arg, Ile, Val, Thr, and Trp are 405, 723, 531, 541, 712, 474, and 169 mg/day, respectively. The ideal protein inclusion level was determined to be 20.9 g/day by feeding a series of glutamic acid levels plus digestible essential amino acids needed for optimum egg mass production. The digestible amino acid requirements were slightly higher, but comparable, to previous digestible amino acid requirements determined for Cobb 500 FF hen at peak production. Knowledge gained from this study will provide the necessary information to determine the production requirements of Cobb 700 broiler breeders.

Key Words: amino acids, requirements, egg production, egg mass, broiler breeders

383 Evaluation of the standard ileal amino acid digestibility of rapeseed meal from different sources in meat duck. Kaixin Zhang*, Feng Zeng, Ying Zhang, Shi Bai, Mei Ding, and Ping Wang, Sichuan Agricultural University, Chengdu, China.

The standard ileal digestibility of amino acid (SIDAA) is an important indicator to evaluate the digestibility and utilization of amino acids in feedstuffs or diets. To the best of our knowledge, there has not been any study on the valuation of the SIDAA of rapeseed meal from different sources in meat duck. Therefore, we selected 22 different sources of rapeseed meal (RSM) to evaluate their SIDAA in meat duck. A total of 1,168 14 d-old ducks were randomly allotted to 23 treatments (6 cages per group, 8 ducks per cage) and one nitrogen-free diet treatment (8 cages per group, 8 ducks per cage) based on body weight. In 22 of the treatments, diets contained different RSM source and the basal diet in the ratio 15:85. 0.5% titanium dioxide was included in all diets as an indigestible marker. On d18, all ducks were euthanized by CO₂ asphyxiation and digesta samples were collected from the terminal ileum. The results showed that the contents of crude fat (EE), glucosinolate (GS), isothiocyanate (ITC), oxazolidine thione (OZT) and protein solubility (PS) were variability in 22 kinds of RSM, and the coefficient of variation (CV) is 49.23%, 86.84%, 90.19%, 81.98% and 52.62%, respectively. The concentration of Lys, Met, Arg, Ile, Thr and Cys were variability in 22 kinds of RSM, and the content of them are from 1.03% to 2.71%, 0.33% to 0.65%, 1.81% to 2.89%, 1.24% to 1.97%, 1.46% to 2.90% and 0.24% to 0.60%, respectively. The SID of essential and non-essential AA of 22 kinds of RSM were significantly different ($P < 0.05$), except for Leu and Tyr. For the SID of essential amino acids, the highest SID of Met and lowest SID of Lys are 90.74% (from 81.44% to 95.93%) and 66.21% (from 54.62% to 74.43%). For the SID of non-essential amino acids, the highest SID of Glu and lowest SID of Asp are 82.91% (from 77.82% to 88.72%) and 73.97% (from 66.87% to 79.86%). In conclusion, the data from the current study indicated that different origin of RSM presented a significant difference on SIDAA in meat duck. We will further analyze which factors have a significant effect and further develop some regression equations for predicting SIDAA of RSM in meat duck.

Key Words: rapeseed meal, meat duck, standard ileal amino acid digestibility

384 Leucine requirement of female Cobb broilers from 8 to 21 days of age. Hossein Janmohammadi*¹, Saeid Amidahri¹, Akbar Taghizadeh¹, Elham Asadi Soumeh², and William Lambert³, ¹*Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, East Azarbijan, Iran (the Islamic Republic of)*, ²*The University of Queensland, Gatton College, Queensland, Australia*, ³*Ajinomoto Eurolysine S.A.S.153, Paris, France*.

Leucine as a functional AA has major effect on whole body growth. The AA stimulates muscle protein synthesis and act as an inhibitor of protein degradation in skeletal muscle. However, little is known about the dietary requirements of Leucine in young female broiler chickens. So, the purpose of this study was to estimate the ideal digestible leucine ratio (to lysine) in practical diets of female Cobb 500 broiler chicks during the starter phase (8–21 d). A total of 540 female broiler chickens of the Cobb 500 from 8 to 21 d of age, were distributed in a completely randomized design with 6 treatments and 6 replicates of 15 birds each. A basal diet (based on wheat-soybean meal) was formulated to provide of all nutrients according to Rostagno et al. (2011), with the exception of leucine and lysine levels. The basal diet contained 2,890 kcal ME/kg, and L-glutamic acid was used to make it equal in crude protein at 19% of the diet. To avoid excessive lysine, 90% digestible lysine (1.05%) was used. To obtain the different leucine to lysine ratio in treatment diets, crystalline L-glutamic acid in basal diets was gradually replaced by crystalline L-leucine, and 6 different ratios of digestible leucine (to lysine) of 0.93, 0.98, 1.03, 1.08, 1.13 and 1.18 were evaluated. To evaluate leucine requirement of female broiler chicks based on performance parameters, body weight and feed intake were recorded at the beginning and the end of the experimental period and feed conversion ratio was calculated. In the study, weight gain and feed intake were significantly ($P < 0.05$) affected with increasing the digestible leucine to lysine ratio. The quadratic broken-line model estimated requirement of digestible leucine to lysine ratio for optimum feed intake, weight gain and feed conversion ratio at 1.05, 1.1 and 1.12, respectively. Based on the current study, it may be concluded that female Cobb 500 broilers need lower ratio of leucine to optimize feed intake than weight gain and feed conversion ratio. The exist difference between the results of our study and previous reports may be due to the using of different gender (female vs male) and statistical models to describe leucine requirement.

Key Words: broiler, leucine, performance, amino acid, requirement

385 Effect of interaction between glycine and threonine in low protein diets on growth performance of male broiler chickens.

Hossein Janmohammadi*¹, Zahra Hosseinzadeh¹, Akbar Taghizadeh¹, Majid Oliaei¹, William Lambert², and Ali Hosseinkani¹, ¹*Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, East Azarbijan, Iran (the Islamic Republic of)*, ²*Ajinomoto Eurolysine S.A.S.153, Paris, France*.

There are multiple metabolic pathways that ultimately promote the synthesis of Glycine. Threonine is precursor of the synthesis of Gly. Gly supplementation increases the amount of Thr available for the physiological processes of maintenance and growth. A study was conducted to evaluate the interaction between different levels of digestible Threonine (Thr) and Glycine (Gly) in low-CP diets on performance of male broiler chickens from 8 to 35 d of age. A total of 462 8-d-old Rass-308 male broiler chickens were distributed in a completely randomized as 2×3 factorial arrangement in 7 treatments with 6 replicates and 11 birds in each. A positive control diet (PC, 20.4%CP) was formulated to be adequate in essential AAs. A negative control diet (NC) had 18.3% crude protein. The treatments consisted of 3 levels of Gly (1.41, 1.51 and 1.61%) and 2 levels of dig Thr (0.76 and 0.86%). A basal diet (based on corn-soybean meal) was formulated to provide all nutrients according to Rostagno et al., (2011). Birds were fed a commercial starter diet from 0 to 7 d and an experimental diet from 8 to 35 d. Pen means were as the experimental unit for analyzed by the R Studio Version (2009). The results of this experiment showed that Glycine supplementation in low-CP diet at level of 1.41% Gly, compared with other of Gly levels significantly decreased Feed intake ($P \leq 0.05$) at 14 d of age. At this age, body weight gain and feed conversion ratio between different levels of Gly in low-CP diet was not significant. At this age, different levels of digThr have no significant effect on body weight, feed intake and food conversion ratio. An interaction between different levels of Thr \times Gly in low-CP diet for BW, FI and FCR was not significant too. At 14 d of age, positive control diet had no difference with other treatments for FI, BW and FCR. The interactions between the level of 1.41% Gly and 0.76% Thr in low-CP diet, decreased FI significantly ($P < 0.05$) at 21 d of age. At this age, the interaction between the levels of the Gly and Thr in low-CP diets had no significant effect on FCR, but the interaction between the level of Gly 1.61 and Thr 0.86 compared with other treatments improved FCR ($P = 0.17$). At 28 d of age, the treatments had no effect on feed intake, body weight gain and feed conversion ratio in low-CP diets and the control group. At 35 d of age, the level of 1.41% Gly in low-CP diet compared with other levels of Gly decreased body weight gain of broilers significantly ($P < 0.01$). It was concluded that use Gly with 1.51% Gly in low-CP diets caused maximum body weight gain for broilers in the 35 d.

Key Words: threonine, glycine, low protein diet, performance, broilers

Metabolism and Nutrition: Enzymes II

386 Impact of phytate concentration from corn on diet Ca and P digestibility in the presence or absence of phytase. Roselina Angel^{*2}, Henk Enting³, and Wenting Li¹, ¹DuPont Animal Nutrition, Wilmington, Delaware, United States, ²University of Maryland, College Park, Maryland, United States, ³Cargill, Veldriel, Gelderland, Netherlands.

Work was done to determine the impact of increasing concentration of phytate-P (PP) from the same source on Ca and P digestibility in the presence or absence of *Buttiauxella* sp. phytase in broilers. Treatment (TRT) were based on a 4x2 factorial arrangement of 4 PP levels (0.16, 0.23, 0.29, and 0.34%) and 2 phytase (0 or 1000 U/kg) levels for 36 h from 20 to 22 d of age (4 birds/pen). By analysis, the total P was 0.20, 0.30, 0.37, and 0.45%, respectively for 0.16, 0.23, 0.29, and 0.34% PP TRT. Unequal replications (reps) of TRT determined based on planned-contrasts. At 0 or 1000 U/kg phytase, there were 8 or 7, 14, 8 or 7, and 8 or 7 reps for the 0.16, 0.23, 0.29 and 0.34% PP levels, respectively. Diets were corn based with no added SBM. Different concentrations of corn and corn germ meal were mixed to achieve desired concentration of PP from the same grain source and this basal diet was analyzed at 0.3% Ca. The same limestone (LM) with a geometric mean diameter of 0.8mm was added so the final diets all contained 0.7% Ca. One N/Ca/P free diet was used to correct for endogenous losses (n = 6). Distal ileal digesta was collected from all birds and pooled by pen. Impacts of PP and phytase levels on standardized ileal digestibility (SID) of Ca and P and digestible (Dig) values were analyzed by MIXED procedure (SAS 9.4). There was no interaction between PP and phytase on SID or Dig Ca. Irrespective of phytase, increasing PP from 0.16 to 0.34% decreased SID Ca by 29% (from 53.8 to 38.1%; $P < 0.05$). Average SID Ca in 0 and 1000 U phytase/kg was 41.5 and 51.4%, respectively, across all PP concentrations ($P < 0.05$). There were interactions between PP and phytase on SID and Dig P ($P < 0.05$). SID P was 31.1, 24.0, 20.1 and 16.3% for birds fed 0.16, 0.23, 0.29 and 0.34% PP diets, respectively, in the absence of phytase ($P < 0.05$). When phytase was added, SID P was 89.9, 87.5, 73.9 and 60.4% in 0.16, 0.23, 0.29 and 0.34% PP TRT, respectively ($P < 0.05$). Birds fed 0.16% PP w/o phytase had the lowest Dig P (0.063%; $P < 0.05$). Dig P was similar and highest in birds fed 0.23 (0.280%), 0.29 (0.294%) and 0.34% (0.290%) PP diets with 1000 U phytase/kg added ($P < 0.05$). In summary, dietary PP negatively affected SID Ca and P, regardless of phytase. Phytase inclusion improved both SID and Dig of Ca and P irrespective of PP concentration.

Key Words: phytate, phytase, calcium and phosphorus, limestone, digestibility

387 Energy (ME)-releasing efficacy of graded levels of 6-phytase, Natuphos® E, NSPase, Natugrain® TS, and a combination of phytase +NSPase in 30 weeks old Hy-Line W 36 layers fed corn/soy/DDGs diets. Michael Coelho¹, Robert Jones^{*2}, and Frank Parks², ¹BASF Corporation, Humble, Texas, United States, ²Integrated Farm Research Inc, Waterloo, Georgia, United States.

This study evaluated the apparent metabolizable energy (AME) released of graded levels of phytase, NSPase and a combination of phytase+NSPase when fed to 30 weeks (wks) old layers on a mash corn/soy/DDGs diet, with 0.22% phytate phosphorus, formulated to meet or exceed current Hy-Line W 36 recommendations. A total of 1620 Hy-Line W 36 birds were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block

as the random effect. Phytase trial with 3 birds/cage x 6 treatments x 30 replications, NSPase, with 5,600 TXU/g, trial with 3 birds/cage x 6 treatments x 30 replications, and the phytase + NSPase trial with 3 birds/cage x 6 treatments x 30 replications. Phytase treatments consisted of T1 = PC, T2 = NC = PC- 0.13% Calcium (Ca), - 0.13% non-phytate phosphorus (nPP), -0.02% sodium (Na), -200 kcal/kg, T3 = NC +300 FTU/kg phytase, T4 = NC+500 FTU/kg phytase, T5 = NC+1000 FTU/kg phytase and T6 = NC+1200 FTU/kg phytase. NSPase treatments consisted of T1 = PC, T2 = NC = PC-200 Kcal/kg, T3 = NC+50 g/MT NSPase, T4 = NC+ 75 g/MT NSPase, T5 = NC+100 g/MT NSPase and T6 = NC+125 g/MT NSPase. Phytase + NSPase treatments consisted of T1 = PC, T2 = NC = PC- 0.13% Calcium (Ca), - 0.13% non-phytate phosphorus (nPP), -0.02% Na, -200 kcal/kg, T3 = NC+300 FTU/kg phytase+0.125 g/kg NSPase, T4 = NC+500 FTU/kg phytase+0.125 g/kg NSPase, T5 = NC+1000 FTU/kg phytase+0.125 g/kg NSPase, T6 = NC+1200 FTU/kg phytase+0.125 g/kg NSPase. TiO₂ was used as the undigestible marker. Feed and bird fecal collection at 33 wks. Feed and fecal samples gross energy determined with a bomb calorimeter. Data was subjected to ANOVA with means separated with least significant difference at $P < 0.05$. ME for phytase treatments was 2850, 2675, 2740, 2758, 2817 and 2859 Kcal/kg, $P = 0.02$, respectively. ME for NSPase treatments 2850, 2737, 2761, 2824, 2871 and 2878 Kcal/kg, $P = 0.03$, respectively. ME for phytase+NSPase treatments was 2850, 2634, 2797, 2805, 2846 and 2869 Kcal/kg, $P = 0.02$, respectively. Phytase doses released 65, 83, 142 and 184 Kcal/kg, respectively. NSPase doses released 24, 87, 134 and 141 Kcal/kg, $P = 0.03$, respectively. Phytase + NSPase doses released 163, 171, 212 and 235 Kcal/kg. In conclusion, combined phytase+NSPase released up to 235 Kcal/kg ME in 30 wks old layers. The ME release of combined phytase and NSPase treatments was partially additive at 77%.

Key Words: ME, NSPase, phytase, layers, corn soy DDGs diet

388 Effects of high levels of dietary phytase on performance and egg quality of laying hens. Fernando Perazzo^{*1}, Levy Teixeira², Francine Falleiros², Leticia Bittencourt², Ana Neusa Eduarda Brito¹, Marcia das Neves Soares¹, Jorge Cunha Lima Muniz¹, Lucas Nunes¹, Isabella Naemi Kaneko¹, and Andreza Marisa Macedo¹, ¹Federal University of Paraiba, Areia, Paraiba, Brazil, ²DSM Nutricional Product, São Paulo, São Paulo, Brazil.

New concepts of phytase feed enzyme have emerged based on the extra phosphoric effect of this molecule and their action inhibiting antinutritional factors and improving nutrient availability through high levels of phytase. Thus, the aim of this study was to determine the effect of high levels of phytase on performance and egg quality of laying hens focusing on extra phosphoric effect, in addition to the phytase matrix values. A total of 960 Hy-line W-80 hens from 40 to 80 weeks-old with an average initial weight of 1.639 kg were distributed in a completely randomized design, consisting of 6 treatments with 10 replicates of 16 birds per experimental unit. The diets were composed by positive control (PC), negative control (NC) with reduction of 0.15% P and 0.17% Ca; NC + 600 FYT/kg; NC + 1200 FYT/kg; NC + 1800 FYT/kg and NC + 2400 FYT/kg. The 40-wk experimental period were subdivided into 10 cycles of 28 d. Data were submitted ANOVA and polynomial regression at 5% probability. Considering the average of the total period, phytase presented a linear response ($P < 0.0001$) for performance, egg weight and improvements on feed conversion per egg mass and per dozen eggs. Phytase showed improvements on egg quality, mainly from the cycle

6, and a linear response ($P < 0.01$) for yolk weight, Haugh Unit and eggshell quality (thickness and shell resistance). In conclusion, high levels of phytase improve productive performance and egg quality for laying hens.

Key Words: enzyme, diets, layers

389 Not Presented

390 Effect of a novel microbial muramidase on performance of broilers fed with or without antibiotic growth promoters. Carlos Lozano-Poveda^{*1}, Oscar Múnera², Pablo Aguirre², Leticia Bittencourt¹, and Dino Garcez¹, ¹DSM Nutritional Products, Sao Paulo, Brazil, ²Solla Nutrición Animal, Medellín, Colombia.

Peptidoglycans (PGNs) are bacterial cell wall components that are released to intestinal lumen during bacterial turnover. Its presence could impair gastrointestinal functionality reducing feed efficiency. Muramidases are enzymes that hydrolyzes PGNs, that can lead to a better nutrient utilization and consequently increase performance. The objective of the study was to evaluate the effect of dietary inclusion of a novel microbial muramidase (MUR) on broilers performance raised under field challenge conditions, with or without antibiotic growth promoter (AGP). A total of 4,000-d-old Ross 308AP broiler males, were distributed across 4 treatments, 10 replicates of 100 birds each (8.8 birds/m²), in a completely randomized design. Birds were placed in an open sided house, using floor-pens with fresh litter (rice hulls) for a 42d trial period. The facility is located at 770 m above sea level in Bucaramanga, Colombia where the ambient temperature has a wide range (20–36°C) and high humidity (>75%) during the day causing a constant heat stress on broilers. Broilers were fed with a mash basal diet based on corn, soybean meal and rendering meal. Feeding phases consisted a pre-starter diet (0 to 10d), starter diet (11 to 24d), and finisher diet (25 to 42d). Treatments were: Positive control (PC): Basal diet + AGP (Avilamycin 10/15 ppm and Halquinol 30 ppm); Negative control (NC): PC without AGP; Muramidase1 (M1): PC + MUR 25,000 LSU(F)/kg; Muramidase2 (M2): NC + MUR 25,000 LSU(F)/kg. Feed intake, body weight gain (BWG, kg) and feed conversion ratio adjusted to mortality (FCRa, g:g) were evaluated during the whole period. Data were analyzed using one-way ANOVA and means were compared by the Tukey test with at 5% probability. Overall, microbial moramidase supplementation resulted in better FCRa and BWG compared with PC and NC treatments ($P < 0.001$): FCRa: PC (1.59b), NC (1.61a), M1 (1.54c), and M2 (1.59b); BWG: PC (2.64bc), NC (2.61c), M1 (2.72a), and M2 (2.65b). These results, collected on broilers raised under suboptimal ambient conditions, showed that dietary microbial muramidase in broilers resulted in significant improvements in FCRa and BWG, as well as AGP, even with different modes of action. This evidence allows to conclude that, muramidase improves broilers performance supplemented or not with antibiotic growth promoters

Key Words: muramidase, peptidoglycans, antibiotic growth promoter (AGP), body weight gain, feed conversion ratio

391 Effect of different feed enzymes combinations on broiler performance and ascites mortality. Sergio Fernandez^{*1}, Silvestre Charraga¹, and Ernesto Avila², ¹DSM Nutritional Products México S.A. de C.V., El Salto, Jalisco, Mexico, ²Universidad Nacional Autónoma de México, México DF, México DF, Mexico.

To evaluate the effect of 2 feed enzymes blends (units/kg of feed); 1 *Citrobacter braakii* phytase, 1000, *Thermomyces lanuginosus* xylanase, 2000, *Nocardiopsis prasina* protease, 15000 (B1), and 2 *Buttiauxella spp* phytase, 500 (from d 1 to 21) and 1500 (from d 22 to 49), *Trichoderma reesei* xylanase, 2000, *Bacillus licheniformis* amylase, 200, *Bacillus subtilis* protease 4000 (B2), on broiler performance and ascites mortality (AM), 1,575 1-d-old-Ross 308 straight run broilers were randomly allocated to 14 treatments on factorial arrangement 2 (sex) by 7: 1. Control no enzymes addition, 2 as 1 formulated with matrix 1 (NC1), 3 as 1 formulated with matrix 2 (NC2), 4 NC1 + B1, 5 NC1 + B2, 6 NC2 + B1, 7 NC2 + B2, with 6 or 3 replicates of 25 birds each, divided in 3 blocks (blocking criteria, period on experimental facilities). To exacerbate AM birds were raised at 2200 m above sea level, and they were allowed to maximum growth by feeding them ad libitum. Data were analyzed as an unbalanced RCBD on factorial arrangement (sex by treatment). Formulation matrix 1–21-d-old/22–49-d-old (g or Mcal/MT of feed), NC1, CP; 7100/6780, Lys 234/198, SAA 294/288, Thr 354/336, Arg, 486/459, ME, 100/100, Ca 1776/1776, P 1464/1464. NC2, CP; 6590/9590, Lys 310/410, SAA 252/324, Thr 274/377, Arg, 262/365, ME, 60/85, Ca 1340/1340, P 1460/1460. Following the final body weight (FBW) males 1; 3.72^a, 2; 3.44^{cd}, 3; 3.48^{bcd}, 4; 3.64^{abc}, 5; 3.43^d, 6; 3.66^{ab}, 7; 3.6^{abcd}. Females 1; 3.15^{ef}, 2; 2.95^f, 3; 3.0^{ef}, 4; 3.19^e, 5; 3.11^{ef}, 6; 3.11^{ef}, 7; 3.07^{ef}. AM, % Males 1; 33.3^e, 2; 36.5^e, 3; 31.3^{de}, 4; 23.5^{bcd}, 5; 30.8^{de}, 6; 23.4^{bcd}, 7; 22.2^{abcd}. Females 1; 27.3^{cde}, 2; 18.3^{abc}, 3; 17.0^{ab}, 4; 12.8^a, 5; 18.4^{abc}, 6; 13.8^a, 7; 13.2^a. Both, FBW and AM were affected by treatment, showing for males, a significant decrease on FBW, and no effect on AM by formulating with NC1 and NC2 matrix values without enzymes, and a significant decrease on AM on females fed NC2. Whereas addition of B1 to NC1 or NC2 showed a significant increase on FBW and a decrease on AM ($P < 0.05$). Addition of B2 had no effect when added to NC1 ($P > 0.05$), showing a recovery on FBW and a decrease on AM when added to NC2 ($P < 0.05$). Under the experimental conditions of the present trial, a decrease on diet nutrients without feed enzymes addition, decreased the FBW but had no effect on AM for males, and a decrease on AM for females fed NC2, whereas the addition of the feed enzymes B1 and B2 showed different performance. Broilers fed B1, showed an increase on FBW and a decrease on AM ($P < 0.05$) when added to NC1 and NC2, however, broilers fed B2, showed no response ($P > 0.05$) when added to NC1, but an increase on FBW and a decrease AM when added to NC2.

Key Words: broilers, phytase, xylanase, protease, ascitis

Metabolism and Nutrition: Feed Additives IV

392 Effect of an algae-clay complex on digestive enzyme activity and performance of broiler chickens fed a corn-soy based diet. Marie Gallissot¹, Rodrigo Jacob², Luiz Fernando Albino², Horacio Rostagno², Raquel Pereira¹, Mariel Tavares¹, and Maria Angeles Rodriguez*¹, ¹Olmix, Bréhan, France, ²University of Viçosa, Viçosa, Brazil.

Improving feed digestibility is a multi-faceted issue in animal nutrition. It contributes to improve livestock farming sustainability, by optimizing the use of available resources and by reducing environmental emissions, while increasing farming profitability. This study was set up to evaluate the effect of supplementing an algae-clay complex (ACC) on digestive enzyme activity and growth performance of broiler chickens fed with a corn-soy diet. The experiment was conducted at the Federal University of Viçosa (Brazil). Six hundred and 60 1-d-old Cobb 500 male chicks were randomly distributed to 3 treatments with 10 replicates per treatment and 22 chicks per experimental unit, allocated to 1 of 3 groups receiving different diets: the standard diet (C) based on corn and soybean meal, the test diet 1 (T1), being the standard diet supplemented with 0.1% of ACC, and the test diet 2 (T2), being the standard diet supplemented with 0.2% of ACC. Animal's performance was evaluated by measuring weight gain, feed consumption and feed conversion ratio for each feeding phase. Moreover, duodenum samples were collected at 35 d, 2 h after the last feeding, from 3 chickens per replicate (30 chickens/treatment) to perform digestive enzyme activity assays. The activity of amylase (using starch) and chymotrypsin (using BTEE substrate) were measured by spectrophotometer in kinetic assay. Results showed a tendency to increase weight gain for groups T1 and T2 compared with control, though it was not statistically significant. Feed conversion ratio was decreased in both test groups compared with control during the grower period (-4% and -5% respectively, $P = 0.043$) and the whole period (-3%, $P = 0.038$). These results were comforted by an increased activity of amylase (+25%, $P < 0.01$) and chymotrypsin (+13 to 20%, $P < 0.001$) in the duodenum in T1 and T2 groups compared with the control group, reflecting an improved capacity of digestion of the diet by birds supplemented with the algae-clay complex. No dose effect of the ACC product was observed. In the end, this study shows a positive effect of the algae-clay complex on feed efficiency of broiler chickens fed a corn-soy based diet. Its use at 0.1% of the feed seems the most interesting to improve performance and profitability.

Key Words: feed efficiency, broilers, algae, corn-soy diet

393 Effect of zinc methionine and manganese methionine on live performance and breast myopathies of broilers. Wilmer Pacheco*¹, Danny Patino¹, Joe Hess¹, Ken Macklin¹, Dianna Bourassa¹, and TJ Biggs², ¹Auburn University, Auburn, Alabama, United States, ²Global Animal Products, Inc, Amarillo, Texas, United States.

Organic trace minerals have shown promise in improving broiler performance and product quality. This study was conducted to evaluate the effect of chelated Zn and Mn (ZinMet and ManMet, respectively. Global Animal Products, Inc.) on broiler performance, processing yield, and incidence of breast myopathies in broilers fed antibiotic free diets. A total of 1,280-d-old male Ross x Ross 708 broilers were randomly distributed among 4 treatments (16 replicates/treatment; 20 birds/pen). Broilers were vaccinated against coccidiosis at the hatchery, placed on used litter, and raised to 47 d of age on 1 of 4 diet regimens with or

without chelated Zn or chelated Mn and Zn. All treatment diets were formulated to include 100 ppm supplemental Mn and 100 ppm supplemental Zn and formulated to be equal in methionine concentration. Diet 1 (control) contained 100 ppm Zn and 100 ppm Mn from ZnSO₄ and MnSO₄, respectively. Diet 2 (ZinMet) replaced 40 ppm Zn from zinc methionine. Diet 3 (ZinMet + ManMet) replaced 40 ppm Zn and 40 ppm Mn from zinc methionine and manganese methionine, respectively. Diet 4 (2X ZinMet) replaced 80 ppm Zn from zinc methionine on d 1 through d 35 and 40 ppm Zn on d 36 through d 46. Birds received a 10⁶ dose of *C. perfringens* in the water on d 14. Feed consumption and BW were determined at d 14, 35, and 46. Feed conversion ratio (FCR) was adjusted by adding the weight of the mortality to the BW of live birds in each pen. A 3-phase feeding program provided 0.55 kg starter, 3.18 kg grower, and finisher to 46 d. At 47 d of age, 10 birds per pen were processed to determine chilled carcass weights and on d 48, carcasses were deboned to determine meat yield and incidence of breast myopathies. Data were analyzed as a one-way ANOVA using the GLM procedure of JMP software. Means were separated by Tukey's HSD test and considered significant at $P \leq 0.05$. Broilers fed diets with 40 ppm of chelated Zn and Mn sources (Diet 3) had a trend toward higher BW ($P = 0.056$) in comparison to birds fed diets containing only inorganic Zn and Mn sources (Diet 1). In addition, FCR was lower ($P \leq 0.05$) and carcass weight higher ($P \leq 0.05$) in broilers fed diets with 40 ppm of chelated Zn and Mn sources (Diet 3) in comparison to birds fed diets containing only inorganic sources (Diet 1). Breast meat weight was lower ($P \leq 0.05$) in broilers fed only inorganic sources (Diet 1) in comparison to broilers fed the other diets (Diet 2, 3, and 4). The incidence of breast myopathies was not influenced by the treatments. The inclusion of zinc methionine and manganese methionine improved FCR, carcass weight and breast meat weight of broilers.

Key Words: zinc methionine, manganese methionine, processing, myopathies

394 Smectite clays improve performance, immune competency and liver health of broilers during aflatoxicosis conditions. Lane Pineda*¹, V. Malathi², H. V. L. N. Swamy³, and Yanming Han¹, ¹Trouw Nutrition R&D, Amersfoort, Netherlands, ²Karnataka Veterinary Animal and Fisheries Sciences University, Bangalore, India, ³Trouw Nutrition, Hyderabad, India.

In a challenge experiment, the efficacy of smectite clays in mitigating the toxicological effects of aflatoxin B1 (AFB1) in broilers was investigated. One-day-old broiler chicks were randomly assigned to one of 3 dietary treatments with 10 replicate pens of 15 birds each. The treatments included a negative control (NC, a basal diet without aflatoxin), a positive control (PC, a basal diet contaminated with 500 ppb of AFB1) and smectite clays (MX, as PC with smectite clays). Broilers were reared on floor pens with deep litter system in an open-sided poultry house and were provided with 3-phase corn-soy based diet: pre-starter for d 1-14, a starter for d 14-28 and finisher for d 28-42. The broiler's body weight (ADG), feed consumption (ADFI) and FCR were recorded during the 42-d period of the study. The weight of organs, serum biochemical parameters and the antibody titers against Newcastle disease (ND) virus and Infectious bursal disease (IBD) virus were evaluated when broilers were 21 and 42 d old. Data were analyzed using the GLM procedure in SAS and Tukey's range test was used to determine significance between treatment means ($P < 0.05$). Over the entire experiment, the ADG and ADFI were significantly higher in the NC group ($P < 0.05$), whereas the

FCR was comparably the same in NC and MX treatments ($P > 0.05$). The ADG of broilers given PC diet was significantly reduced (42 vs 56 g), while the FCR was remarkably increased (2.32 vs 1.87) compared with the NC ($P < 0.05$). The supplementation of smectite clays (MX) mitigated the adverse effect of aflatoxin on performance and improved ($P < 0.001$) ADG by 15% and reduced ($P = 0.0003$) FCR by 13% in relation to the PC group. No difference was observed between challenged broilers on aspartate aminotransferase and gamma-glutamyltransferase, however, the MX diet significantly increased the globulin levels (1.74 vs 0.86, $P = 0.004$). The MX treatment reduced the effect of aflatoxin in the liver ($P < 0.0001$), but not on the weight of other organs ($P > 0.05$). The immuno-suppression caused by aflatoxin was ameliorated by stimulating the production of antibodies against ND at d 21 ($P = 0.04$) and IBD at d 42 ($P = 0.01$) in the MX group compared with PC treatment. The current findings indicated that smectite clay binder is effective in improving growth performance, liver health, and stimulation of antibody production during severe aflatoxin challenging conditions. The results are suggestive of protective effects of smectite clays in broiler chicken exposed to aflatoxicosis.

Key Words: mycotoxin, broilers, toxin binder, performance, liver health

395 The effect of guanidinoacetic acid supplementation on the chemical composition of breast meat in broilers. Christian Scharch², Meike Rademacher¹, Ulrike Braun³, and John Thomson^{*4}, ¹Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany, ²Feedtest, Wettin-Löbejün, Germany, ³AlzChem Trostberg GmbH, Trostberg, Germany, ⁴AlzChem LLC, Canton, Georgia.

Muscle creatine is essential for cellular energy supply, stabilization of muscle cells and lean body mass. Guanidinoacetic acid (GAA), the direct endogenous precursor of creatine, is produced from arginine (Arg) and glycine. The objective of this study was to measure the effects of supplemental GAA and total dietary Arg on broiler breast weight, lean body mass and muscle GAA and creatine during a 14 d feeding experiment (d14–27). A total of 960 male 14-d-old Ross 308 broilers were equally distributed according to their body weight to 96 pens (10 birds

per pen) and assigned to 12 dietary treatments. A commercial starter feed was fed for the first 13 d. An Arg deficient basal diet (total Arg 0.916%, SID Arg 0.8%) was formulated and half of the basal diet was supplemented with 0.06% GAA. Each batch of basal diet was supplemented with 0, 0.05, 0.10, 0.17, 0.25, or 0.35% L-Arg, so the highest level of added L-Arg met a total arginine recommendation of 1.27% (Aviagen 2007). One bird per pen closest to the pen weight average was selected for measurement of breast muscle weight and levels of GAA and creatine. Crude protein (CP) and ether extract (EE) of breast muscle were measured in the 0 and 0.35% Arg groups with and without GAA supplementation. The study was conducted as a factorial design with 2 levels of GAA and 6 levels of Arg and analyzed a 2-factor ANOVA. Breast weight increased linearly ($P < 0.001$) as Arg approached the recommended level with and without GAA supplementation. However, maximum breast meat was reached with 0.1% less Arg when 0.06% GAA was added. Lean body mass as measured by higher CP and lower EE in breast meat was only numerically affected by GAA or Arg level. Muscle GAA was lower with added dietary GAA than without and lower with increasing levels of Arg ($P = 0.032$). Muscle creatine increased ($P < 0.001$) with Arg as well as GAA supplementation, however higher muscle creatine levels were achieved from GAA supplementation resulting in a significant interaction ($P = 0.012$). Supplementation with 0.06% GAA to an Arg deficient diet resulted in creatine accretion per gram of breast meat similar to supplementation with 0.128% L-Arg. In conclusion, effective metabolic adaptation was proven by analysis of muscle GAA and creatine levels: less GAA is produced endogenously from Arg when GAA is supplemented (negative enzymatic feedback on AGAT) and rising muscle creatine levels. GAA supplementation can increase muscle creatine even at adequate dietary Arg. Based on maximum breast weight, Arg equivalence from GAA was calculated as 166%, which is in the range of maximum Arg sparing on a 1:1 weight basis (148.8%).

Key Words: broiler, guanidinoacetic acid, arginine, creatine, breast muscle

396 Not Presented

Metabolism and Nutrition: Nutrition II

397 Determination of nutritional profile and metabolizable energy value of *Tenebrio molitor* meal for broiler chickens. Marcos Nascimento Filho*, Raquel Pereira, Alvaro Burin Junior, Ana Beatriz Oliveira, Diana Suckeveris, Caio Alexandre Paes Soares, and José Fernando Machado Menten, *University of São Paulo, Piracicaba, São Paulo, Brazil.*

Determination of the nutrient composition and metabolizable energy of new ingredients are essential for their use in feed formulation. Insects are considered promising sources of animal origin ingredients for poultry diets. The objectives of this study were to determine the nutrient composition and the apparent metabolizable energy corrected for nitrogen (AMEn) of tenebrio (*Tenebrio molitor*) meal (TM) for broiler chickens. Forty-eight 21-d-old male broilers of commercial strain were weighted and placed over 12 metabolic cages (0.70 × 0.66 m) equipped with one galvanized trough feeder at the front and a linear drinker at the back in a completely randomized design. Each treatment was composed of 6 replicates (cages) of 4 birds assigned into 2 assay diets: a control diet, based on corn and soybean meal to meet nutritional requirements, and an experimental diet subsequently formulated by replacing 300g/kg (wt/wt) of the control diet with TM. The adaptation period lasted 7 d. Feed and water were available *ad libitum*. Total excreta samples were collected from d 28 to d 32, twice a day (morning and afternoon) on metal trays covered with plastic material, weighted and stored at -20°C for further analysis. Feed intake was measured per cage and 1% ferric oxide was added to the diets on the first and last day of the collection to identify precisely excreta from the test period. TM was analyzed for dry matter (DM), crude protein (CP), gross energy (GE), ether extract (EE), crude fiber (CF), calcium, phosphorus, and fatty acids. Samples of the diets, and excreta were analyzed for DM, nitrogen (N), and GE. The apparent total-tract digestibility coefficient (ATTDC) of nutrients and AMEn were calculated based on the analytical results. TM used in this study contains 50.0% CP, 6,366 kcal/kg GE, 29.7% EE, 3.8% CF, 0.07% calcium, and 0.54% phosphorus, as fed basis. Regarding fatty acids profile, TM is a rich source of oleic 45.4%, linoleic 26.2%, and palmitic acid 15.4%, showing also good content of stearic 2.3%, palmitoleic 1.9%, and linolenic acid 1.1%. The ATTDC of DM, N, and GE of TM were 70.2%, 37.3%, 79.6%, respectively. Calculated AMEn was 5,004 kcal/kg DM, indicating that TM has higher metabolizable energy value compared with common animal and vegetable food origin sources as other insect larvae meals already published in literature. The values of nutritional composition and AMEn of TM determined in this study might be applied as a guide for poultry feed formulation using this ingredient. Acknowledgment: FAPESP for the research grant (No 2017/05423-8) and MS scholarship (No 2017/19751-7).

Key Words: insect meal, *Tenebrio molitor*, metabolizability, energy, broilers

398 Effect of pre-peak feeding strategy on performance, egg quality, and tibiae mineralization of brown-egg laying hens from 27 to 62 week of age. J. García^{1,2}, Lewis Aguirre¹, G. Fondévila¹, R. Scappaticcio², N. L. Corrales¹, and Gonzalo Mateos^{*1}, ¹UPM, Madrid, Madrid, Spain, ²Camar Agroalimentaria S.L., Toledo, Spain.

We studied the influence of the nutritive value (AMEn, SID Lys, and Ca) of diets fed to pullets from 15 to 26 wk of age, on productive performance, egg quality, and tibia mineral content of brown egg-laying hens from 27 to 62 wk of age. Five feeding strategies were used. Three

of them differed in the nutrient content of the diet fed from 15 to 18 wk of age: A) a pullet diet (2,700 kcal AMEn/kg, 0.61% SID Lys, and 1.0% Ca), B) a pre-lay diet (2,750 kcal AMEn/kg, 0.78% SID Lys, and 2.5% Ca), and C) a layer diet (2,750 kcal AMEn/kg, 0.78% SID Lys, and 3.8% Ca), respectively. The other 2 feed strategies (D and E) consisted in feeding the hens from 15 to 26 wk of age a diet low in energy (2,615 kcal AMEn/kg) and high in SID Lys (0.82%) diet with a medium or a high level of Ca (2.5 vs. 3.8%, respectively). After 18 wk (diets A, B, and C) or 27 wk (diets D and E) of age all the hens received the C layer diet to 62 wk of age. Each treatment was replicated 18 times and the experimental unit was an enriched cage with 10 hens for all traits. The experiment lasted for 12 periods of 4 wk each. Hen performance (egg production, egg mass, FCR, BW gain, and mortality) was controlled by replicate by period and cumulatively. Percentage of shell-less, broken, and dirty eggs were determined in all eggs produced. Egg quality, including Haugh units, shell thickness, shell strength, and shell weights in absolute (g) and in relative (%) terms, were determined by replicate in 8 eggs chosen at random for the last 2 d of each of the 12 periods. At 62 wk of age, one hen from 8 of the replicates per treatment was selected at random and sacrificed. The ash, Ca, and P content of these tibiae were determined. Data were analyzed as a completely randomized design with feeding strategy as main effect using the MIXED procedure of SAS. The Tukey test was used to make pairwise comparisons to separate treatment means. Feeding strategy did not affect any of the productive performance traits studied, except egg weight that tended ($P = 0.079$) to be greater in hens that were fed the high energy diets during the pre-peak period. Cumulatively, all shell quality variables studied were better ($P < 0.05$) in hens fed the 3.8% Ca diets during the experimental period than in hens fed 2.5% Ca or less, but Haugh units were not affected. At 62 wk of age the pre-peak feeding strategy did not affect the ash, Ca, or P content of the tibia. In summary, the use of a layer diet with 3.8% Ca from 15 wk of age onward, might improve shell quality traits during the entire egg cycle, without showing any negative effect on egg production.

Key Words: calcium level, external egg quality, laying hens, pre-peak feeding

399 Effects of the source and level of digestible phosphorus in the diet on performance and egg quality of brown laying hens from 64 to 76 weeks of age. P. Hernández¹, A. F. de Juan¹, G. Fondévila^{*1}, J. Ben Mabrouk¹, J. García², R. Scappaticcio², and Gonzalo Mateos¹, ¹UPM, Madrid, Madrid, Spain, ²Camar Agroalimentaria S.L., Toledo, Spain.

We studied the influence of the source and level of digestible phosphorus (dP) in the diet on performance and egg quality traits of brown hens from 64 to 76 wk of age. The diets were based on corn and soybean meal and all contained 4.0% Ca. The design was completely randomized with 8 treatments arranged as a 2x4 factorial with 2 sources of P [monocalcium phosphate (MCP) and calcined bone phosphate (CBP)] and 4 levels of dP (0.27, 0.31, 0.35, and 0.39%) as main effects. Each treatment was replicated 14 times and the experimental unit was an enriched cage with 6 hens. The experiment lasted for 12 wk (3 periods of 4 wk each). Egg production and hen mortality were recorded daily. Feed intake (FI) and BW of the hens were determined by period and cumulatively. Egg weight was estimated by period by weighing all the eggs produced the last day of each week on trial. From these data, ADFI, egg mass, feed conversion ratio (FCR), and BW gain (BWG) were calculated by period

and cumulatively. Egg quality traits, including Haugh Units and shell resistance to breakage, were determined per cage in 8 eggs collected at random the last 2 d of each experimental period. In addition, the percentage of dirty, broken, and shell-less eggs was recorded in all eggs produced. Data were analyzed as a completely randomized design with source of P and level of dP of the feed as main effects, and the interaction between them was also analyzed. In addition, the effect of the level of dP on the different variables studied was partitioned into linear (L) and quadratic (Q) components. No interactions between main effects were detected for any of the traits studied and therefore, only main effects are presented. Neither source of P nor level of dP affected any of the productive traits studied, except BWG that increased (L; $P < 0.05$) as the level of dP of the diet increased. An increase in dP of the diet from 0.27 to 0.39% tended to reduce (L, $P = 0.077$; Q, $P = 0.058$) shell resistance to breakage. Haugh units decreased linearly ($P < 0.01$) as the level of dP increased. The percentage of non-sealable eggs (dirty, broken, and shell-less eggs) was higher in hens fed CBP than in hens fed MCP ($P < 0.05$). In conclusion, from 64 to 76 wk of age, hens require no more than 0.27% dP in the diet for optimal egg production and egg quality. An excess of dP ($\geq 0.39\%$) might reduce shell quality. Hens responded similarly to both sources of P but the percentage of non-sealable eggs increased with the use of calcined bone phosphate.

Key Words: digestible phosphorus, calcined bone phosphate, egg quality, laying hen, monocalcium phosphate

400 Biostrong® 510 improves performance of laying hens just after peak production. Manu de Laet* and Jan Dirk van der Klis, *Delacon Biotechnik GmbH, Steyregg, Austria.*

Just before peak production of laying hens, a rapid increase in nutrients is necessary since the birds must cover the requirement to grow to the adult body weight, to achieve peak of production and to get a rapid egg weight increase. From the start of lay until peak production, feed consumption should increase by about 40% to allow the birds to meet their requirements for egg production and growth. After peak production, feed intake is stabilized and in general increases no further. That is why another strategy can be used to increase nutrient availability in laying hens after peak production. Research has shown that the use of phytogetic feed additives, such as essential oils, pungent, and bitter substances stimulate digestive enzyme production and induce a higher secretion of bile acids. Essential oils are also used to enhance the activities of trypsin and amylase. In addition, quillaja saponins can act as a nutrient transporter. A study was undertaken to examine the effect of Biostrong® 510, a mixture of essential oils, pungent and bitter substances and quillaja saponins, on the production performance of laying hens after peak production (from 32 until 55 weeks). 375 Isa Brown laying hens are used in this trial, with 5 hens per pen and 25 repetitions per treatment. Three dietary treatments have been tested: 1) control feed before and after peak (CTR), 2) 150 g/t Biostrong® 510 added before and after peak (BSG), 3) control feed before peak and 150 g/t Biostrong® 510 added after the peak (CTR-BSG). At the start of the trial, BSG had 2% lower laying rate and + 4 points FCR compared with CTR and CTR-BSG. This effect disappeared after 2 weeks. Egg weight, egg mass and laying rate

increased as FCR decreased from 32 weeks onwards for both BSG and BSG-CTR compared with CTR. Until 40 weeks, the difference between CTR and BSG and CTR-BSG was small (-4 points FCR, +1.5% laying rate). After 40 weeks, this difference broadened with an increase of laying rate (+ 4%) and a clear decrease in FCR (- 10 points) at wk 50. Considering the full period of 32-55 weeks, an increase of laying rate from 92.4% for CTR to 94.5% for CTR-BSG and to 94.6% for BSG and a decrease in FCR from 1.967 for CTR to 1.916 for CTR-BSG and to 1.918 for BSG was achieved. These results clearly indicate that laying hens take advantage of the extra nutrients that are released with the help of phytogetic feed additives.

Key Words: phytoGENICS, digestibility, laying hens, nutrient utilization, post-peak production

401 The effects of dietary rubber seed oil on hens laying performance, egg quality, and fatty acid content and composition and cholesterol content in yolks. Zhiguo Wen*, Xiumei Li, and Peilong Yang, *Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China.*

To evaluate the application of rubber seed oil (RSO) in the production of n-3 polyunsaturated fatty acid enriched eggs, a dose-response experiment was conducted to evaluate the effects of dietary RSO levels on hens laying performance, egg quality, and fatty acid content and composition and cholesterol content in egg yolk. Three hundred and 60 30-week-old healthy Lohmann Brown laying hens, with similar laying rate and body weight, were randomly allotted to 5 groups (4 replicates, 18 birds/replicate) and fed 5 experimental diets (0, 1%, 2%, 4%, and 6% RSO) for 8 weeks. The results showed that the egg production and daily egg mass were increased in 4% RSO group ($P < 0.05$), but egg weight, yolk percentage, egg shape index, albumen height, and the contents of dry matter, lipid, and protein in yolks were not influenced by dietary treatments ($P > 0.05$). Yolk color was improved ($P < 0.05$) while Haugh units after storing at room temperature for 6 d decreased ($P < 0.05$) gradually with the increasing RSO levels. Total triglyceride and cholesterol contents of yolks were also reduced in RSO supplemental groups. The concentration of total n-3 PUFA in yolks increased ($P < 0.001$) gradually, while n-6/n-3 ratio of yolks decreased ($P < 0.001$) gradually with increasing RSO levels in diets. The enrichment of total n-3 PUFA and DNA in yolks showed a tendency that had a gradual increase and then reached a plateau during the feeding period, and more feeding days for the hens was needed to reach maximum enrichment at the low RSO levels. The 6% RSO group had highest DHA and total n-3 PUFA levels, reaching 353.74 mg DHA and 396.49 mg total n-3 PUFA per egg, respectively. In conclusion, dietary RSO supplementation enriched yolk with n-3 PUFA (especially DHA) and improved yolk color without adverse effects on performance and egg quality, and indicated the practical feasibility to produce n-3 PUFA-enriched eggs.

Key Words: N-3 polyunsaturated fatty acids, rubber seed oil, eggs, enrichment, cholesterol

402 Not Presented

Poster Session—Monday

403P Impact of extension educational programs on commercial broiler flock biosecurity. Jonathan Moyle¹, Eric Benson*², Jennifer Rhodes³, and Nathaniel Tablante⁴, ¹University of Maryland, Salisbury, Maryland, United States, ²University of Delaware, Newark, Delaware, United States, ³University of Maryland, Centerville, Maryland, United States, ⁴University of Maryland, College Park, Maryland, United States.

Commercial poultry production accounts for approximately 70% of the total economic value of agriculture in the Delmarva area (Delaware and the eastern shores of Maryland and Virginia). A disease outbreak, such as Avian Influenza (AI), on Delmarva would economically impact poultry growers, processors and allied industries. Additionally, the Delmarva Peninsula ranks as one of the top 10 largest broiler producing areas in the United States and has some of the highest poultry density in the nation. Biosecurity – any and all measures used to prevent disease – is an important risk management tool that farmers need to use to help reduce the risk of disease. To help farmers understand biosecurity and implement good practices, the University of Maryland Extension service along with colleagues at the University of Delaware, conducted several outreach programs to train commercial poultry growers in the Delmarva region (DE, MD, and VA). These programs included: traditional extension hands-on education, newsletters, factsheets, videos and on-farm field days. Before involvement in the program, growers were surveyed on biosecurity practices. Follow on assessment was conducted 3 to 4 mo after trainings and then again 2 years later. The results found both short-term and long-term impact on grower biosecurity, with 87% the growers making changes in the short term, while 75% maintained the practices 2 years later. Additionally, 2 years after the training, 92% of the growers reported they continue to train employees on biosecurity.

Key Words: biosecurity, outreach, assessment

404P Return on investment with the use of Exzolt® for *Ornithonyssus sylviarum* and *Dermanyssus gallinae* control. Gustavo Perdoncini*, Jorge Werlich, and Lucas Colvero, *Merck Animal Health, São Paulo, Brazil.*

The use of effective tools for the control of *Ornithonyssus sylviarum* and *Dermanyssus gallinae* is essential to ensure adequate production data and financial gains in egg production. The ideal choice of mite control method (registered for use in birds and with zero withdrawal period) should allow a direct analysis of the Return On Investment (ROI). It also protects the company against future losses related to intangible factors such as entrance of a pathogenic disease in the farm. The objective of this work was to evaluate the ROI with the use of Exzolt® in a flock of chickens infested by *Ornithonyssus sylviarum* and *Dermanyssus gallinae*. The effectiveness of the Exzolt® and the ROI was evaluated in a house with 68 thousand infected hens. The hens received the oral treatment via drinking water on d 0 and 7, at a dose of 0.5 mg fluralaner per kg of Body Weight (BW). Treatment was given using a tank of medication for an average period of 6 h. The determination of primary efficacy was based upon the presence of *Ornithonyssus sylviarum* and *Dermanyssus gallinae* inside the houses or on the hens pre- and post-treatment. Three days after the administration of the first dose of Exzolt® no mites live adult stage were observed. The data collected for analysis was egg production from 4 weeks prior and 6 weeks after treatment, the production and sale costs of a box with 360 eggs. The average egg production before treatment was 83.26% and the average 6 weeks

after was 85.48%. To calculate the ROI, the data used was: the cost of producing a box with 360 eggs (R\$ 75/\$20,27), box cost of sale (R\$ 90/\$24,32) and an average egg increase of 2.2% after the treatment of the hens. The cost of treatment with Exzolt per hen was R\$ 0.73/\$0,20. A hen will have to produce 2.94 eggs to pay for the treatment, recovering the amount invested after 67 d of treatment. The recovery of the investment is related to the return of the production of eggs that the hen had stopped producing before. If this productivity is maintained for 90 d, the final return on investment will be R\$ 17.560,80/\$4.746,80. The use of Exzolt® made it possible to identify a positive return on investment and it recovered the egg production. Intangible factors also should be considered when mite control is chosen and it allow to have a complete evaluation of ROI. Such as reducing the change of transmission of pathogenic microorganisms by mites and the damage caused to farm workers due to labor problems. Exzolt is not approved for sale in Canada.

Key Words: Exzolt, egg, Fluralaner, hen, return on investment

405P An update of the Virginia Tech (VT) National Institutes of Health (NIH)-funded training programs for minorities interested in pursuing research careers in science technology engineering and math (STEM). Edward Smith*, Zachary Mackey, Luke Achenie, and Eni Ramaj, *Virginia Tech, Blacksburg, Virginia, United States.*

The 2 training programs, the Initiative to Maximize Student Development (VT-IMSD) and the Postbaccalaureate Research and Education Program (VT PREP) are both research education efforts supported under the NIH's very highly competitive R25 mechanism. Now in the 16th and 12th years respectively, the VT PREP and VT IMSD programs are one of only 2 tandem R25s at land grant institutions (Missouri is the other, but in their Biological Sciences department, after North Carolina State (NC State) lost their IMSD in 2016). In the Fall of 2019, 10 new trainees (8 for PREP and 2 IMSD) will arrive on campus ready to pursue the training and developmental experiences we have developed at VT. Those experiences have produced 66 PhDs from VT (IMSD predoctoral trainees) and other institutions (PREP alums and IMSD undergrads) who are pursuing careers as tenure track faculty at Research Intensive and teaching institutions (8 including Missouri, Boston University, and Ball State) and in prestigious postdocs including at University of Pennsylvania (Provost Postdoc, an endowed position), at Stanford University, Northwestern, and NC State. Compared with other students, our trainees have received significantly more fellowships and other grants arising from a course we started in 2005 in grant writing. Examples of grants/fellowships received by our trainees include the F31 (one of our trainees was first at VT and of the 9 received at VT since 2005, 5 are from our program). Our training program and the experiences we have from 16 years of implementation show that the GRE importance in graduate admission is exaggerated, mentoring, especially community based, is the most significant for trainees earlier in their college careers (regardless of whether the student is pursuing a prebac or predoc degree).

Key Words: training, PhD, STEM, URM

406P Dust mitigation in winter and heat alleviation in summer with water sprinkling in a cage-free henhouse. Lilong Chai*¹ and Hongwei Xin², ¹University of Georgia, Athens, Georgia, ²University of Tennessee Institute of Agriculture, Knoxville, United States.

Concerns over animal welfare have led to pledges of sourcing only cage-free (CF) eggs by many US food retailers and restaurants by 2025. In November 2018, California passed a state law that requires all eggs consumed in California come from cage-free hens by January 1, 2022. However, CF housing poses many inherent environmental challenges, among which are high levels of particulate matter (PM), especially during cold weather when the henhouse has limited ventilation. In summer, PM levels in CF houses are naturally low because of higher ventilation rate; however, henhouse temperature may increase, adversely affecting hen's well-being and production performance. Poultry farms in the Midwest are generally not equipped with evaporative cooling systems. Hence, a sprinkling system offers a viable option for suppressing PM in winter and alleviating heat challenge in summer. The objectives of this study were to mitigate PM generation in winter and suppress the heat stress of birds in summer in a commercial US CF henhouse. This study was conducted with a commercial aviary CF house (50,000 laying hens) in Iowa during winter of 2017–2018 and summer of 2018. A water sprinkling system was installed in half of the experimental henhouse lengthwise (treatment section), whereas the other half of the henhouse served as the control. An optical PM sensor was used to measure PM concentrations of different particle sizes. The body surface temperature of laying hens before, during and after the water spray was monitored with FLIR thermal cameras. Hen activities on dry litter (10% - 15% moisture content) in CF houses was identified as the main reasons for high indoor PM levels. By wetting the litter, the sprinkling system reduced the PM generations by up to 50% without causing ammonia (NH₃) elevation in winter. For the test of heat stress relief in summer, data from thermal imaging measurements revealed that sprinkled hens (wet birds) on the litter floor had up to 7°C lower body surface temperature than non-sprinkled ones (dry birds) immediately after a 20-s or 30 mL m⁻² water spray. Surface temperature of the sprinkled hens was 5°C cooler than that of the non-sprinkled ones 3 min after water sprinkling. While the cooling effect for some birds lasted for up to 10 min, most dried out in shorter period under the testing conditions of 35°C temperature, 32% relative humidity, and 0.3 m s⁻¹ air velocity. Intermittent sprinkling is an effective way to maintain the cooling efficacy and cover more hens. The application frequency will likely depend on the environmental conditions, which warrants further investigation, development and testing.

Key Words: alternative hen housing, heat stress, air quality, sprinkling, animal well-being

407P The impact of various in-ovo wavelength spectrums on behaviour of Lohmann LSL Lite and Lohmann Brown pullets under mismatched photoperiods. Celma Santos¹, Tory Shynkaruk^{*1}, Bruce Rathgeber², and Karen Schwan-Lardner¹, ¹University of Saskatchewan, Saskatoon, Saskatchewan, Canada, ²Dalhousie Agricultural Campus, Truro, Nova Scotia, Canada.

In-ovo lighting spectrum has not been well studied with respect to welfare and behavior of young pullets. The objective of this study, part of a larger experiment, was to examine the impact of 4 in-ovo light treatments on behavioral expression of Lohmann LSL Lite (LW) and Lohmann Brown (LB) pullets to 4 d. Six incubators (2 per treatment) were equipped with white, red or blue LED lights (Once Innovation, Plymouth, MN) and 2 incubators remained dark. After hatch, 192 LW and 192 LB pullets were placed into 64 cages (each housing 6 pullets of one strain), located within 6 rooms, with equal distribution based on in-ovo treatment and strain. Three rooms were exposed to a near-constant (NC) brooding photoperiod (23L:1D from 0 to 3 d, then 20L:4D onward) and 3 rooms to an 18L:6D photoperiod that was switched to

intermittent lighting (18L-1) (18L:6D from 0 to 3 d, then maintained at 17L:2D:0.5L:2D:0.5L:2D). Light was provided via blue LED lights (Once Innovation, Plymouth, MN). Infrared cameras recorded behavior for 24 h per day on d 0, 2, and 4. Behavior was observed using a 20 min scan sampling technique. The data were analyzed as a nested factorial design, with strain, in-ovo wavelength, and brood lighting as fixed effects and room within brood lighting as a random effect. Proc Glimmix was used to determine if treatments differed and if interactions existed. Differences were considered significant when $P \leq 0.05$. No interactions were observed between main effects. In-ovo lighting had no effect on behavior. With regards to strain, LB pullets spent 2.6% more time walking ($P = < 0.01$), 1.2% more time running ($P = < 0.01$), 4.8% less time standing ($P = < 0.01$), and 1.4% less time drinking ($P = 0.01$) at d 0. At d 2, LB pullets spent 9.4% more time resting ($P = < 0.01$), 1.2% less time drinking ($P = < 0.01$), and 9.2% less time feeding ($P = < 0.01$). At d 4, LB pullets spent 0.9% more time running ($P = 0.04$) and 3.8% less time feeding ($P = 0.01$). Birds reared under NC light were less active at 0, 2, and 4 d, spending 10.4, 12.1, and 10.3% more time resting ($P = 0.01$, $P = < 0.01$, and $P = < 0.01$, respectively), 4.2, 5.6, and 4.4% less time at the feeder ($P = 0.04$, $P = 0.02$, and $P = 0.05$, respectively), and 2.5 and 2.2% less time walking at 2 and 4 d ($P = 0.04$ and $P = 0.04$, respectively). Overall, the data demonstrated little impact of in-ovo lighting spectrum on pullet behavior, but did show strain differences in behavioral expression. Interestingly, the lighting program which provided more darkness during the early brooding phase resulted in higher chick activity, with more time spent at feeders and drinkers.

Key Words: incubation, LED light, light colour, chicks

408P Methods to evaluate broiler welfare and environmental enrichment for research and on-farm audits. Meaghan Meyer*, Anna Johnson, and Elizabeth Bobeck, Iowa State University, Ames, Iowa, United States.

On-farm welfare auditing in the US has become a common expectation due to concerns for broiler well-being. The objectives of this study were to validate welfare measures using the National Chicken Council (NCC) guidelines, and to evaluate behavior assessment methods not included in these guidelines. Measures were evaluated in a subset of 300 Ross 308 broilers (out of 1200) housed in pens of 30 for 6 wk. Five of the pens were exposed to environmental enrichment; a novel laser device used as an example treatment to validate measures, and the other 5 were control. Pens were video recorded one d/wk during 4-min "enrichment periods," repeated 4 times/d. Five "focal birds"/pen ($n = 50$) were randomly assigned and used for on-farm assessments, behavioral analyses, and tibia quality measures. Focal birds were gait scored weekly using a novel walking platform and 0–2 scale adapted from the NCC, and examined for breast and footpad condition, a measure not required by NCC. The American Association of Avian Pathologist's paw scoring guide (pass/fail) was used for footpads, and a present/absent system where a blister was considered present if equal to or larger than 0.5 cm² was used for breast blister scoring. Video recordings quantified broiler behavior (%), latency to feed (s), and distance walked (cm), all measures not included in NCC guidelines. Images taken on wk 1 and 6 during the Human-Approach Paradigm (HAP) measured broiler response to an unfamiliar human; an additional measure not required by NCC. Focal birds were euthanized on d42 and right tibias were collected for DXA (Hologic) scanning for bone mineral density and content, and bone breaking strength analysis (Instron 3367 Test Machine), another measure that is not NCC recommended. Data were analyzed using a one-way ANOVA (Proc Mixed or Proc Glimmix; SAS Version 9.4). Lameness score, breast and footpad condition, HAP responses, and tibia quality

measures were not affected by enrichment treatment ($P > 0.05$), but were validated in the context of assessing welfare of the birds. Broiler behavior and walking distance were affected ($P \leq 0.05$), with enriched birds showing greater activity (up to a 14.49% increase, wk 2), time at feeder (up to a 27.5% increase, wk 5), and distance walked during enrichment periods (up to a 173.89 cm increase, wk 2), indicating the usefulness of these measures in assessing bird activity. In conclusion, HAP and bone quality analyses are recommended to researchers, due to impracticality in a production setting, and broiler behavior, latency to feed, walking distance, lameness, and breast and footpad scoring are recommended to researchers and in commercial audits, because they are easily adaptable and applicable to both settings.

Key Words: broiler, welfare, audit, environmental enrichment, behavior

409P Investigating factors associated with health traits in Canadian turkey (*Meleagris gallopavo*) flocks. Emily Leishman*¹, Nienke van Staaveren¹, Sarah Adams¹, Vern Osborne¹, Ben Wood^{1,2}, Christine Baes^{1,3}, and Alexandra Harlander², ¹University of Guelph, Guelph, Ontario, Canada, ²Hybrid Turkeys, Kitchener, Ontario, Canada, ³University of Bern, Bern, Switzerland.

Footpad dermatitis (FPD) and aggressive pecking are challenges that can emerge in production systems for commercial turkeys and this has both welfare and economic consequences. The prevalence of FPD in commercial flocks can vary markedly with season and management system. Similarly, injuries resulting from aggressive pecking can vary based on management, but they can also be a leading cause of culls and mortality in some turkey flocks. Little is known about the factors influencing either FPD or aggressive pecking in Canada. In this cross-sectional study, over 500 turkey farmers across Canada will receive a survey which includes a health-scoring guide and a questionnaire. Farmers will be asked to record the health status of 30 turkeys on their farms using the illustrated instructions to score head injuries, skin damage, and FPD. The information on head injuries and skin damage will provide insight into the extent of aggressive pecking within the flock. Areas on the birds will be scored according to severity using a 3-point scale where a score of zero indicates no damage and 2 indicates severe damage. Additionally, an inventory of housing and management practices will be taken on each farm using a questionnaire covering topics on bird characteristics, lighting, air quality, litter quality, feeding, and health. Multivariable linear regression will be used to determine associations between housing and management practices and the prevalence of FPD and pecking injuries in turkey flocks. The data obtained from this survey will be used to 1) estimate the prevalence of FPD and pecking injuries, 2) describe housing and management practices, and 3) identify risk factors for FPD and pecking injuries. With the information from this study we plan to develop a management tool tailored to the Canadian industry to reduce FPD and pecking injuries on Canadian farms.

Key Words: footpad dermatitis, aggressive pecking, risk factors, turkeys

410P Well-being and behaviour of Rhode Island Red laying hens: Enriched cages vs. free range under sub-humid conditions of Mexican tropic. Ciro Sanchez-Hernandez¹, Monica Vargas-Mendoza*¹, Angel Juarez-Zarate², Silvia Lopez-Ortiz¹, and Pablo Diaz-Rivera¹, ¹Colegio de Postgraduados Campus Veracruz, Veracruz, Veracruz, Mexico, ²Universidad Autonoma Chapingo, Huatusco, Veracruz, Mexico.

The objective was to compare the well-being and behavior of enriched-caged vs. free-range laying hen systems, under the conditions of a mango

orchard at a sub-humid tropical climate. A random blocks design with 2 treatments and 6 replicates was used, and 9 Rhode Island Red hens as the experimental unit, with individual mango trees as the blocking factor. The experiment was conducted from August to December 2018 in Veracruz, Mexico. One hundred and 8 laying hens of the D853 Dominant CZ Rhode Island Red hybrid, 18 weeks age, were randomly assigned to one of 2 treatments: enriched cages (EC), or free range (FR). Cages of the EC treatment had 0.25 m² of space per hen, a perch, 4 nests, 2 feeders, 2 drinkers, and a 0.160 m² sand box. FR treatment had the same conditions plus day-time free access to a range area of 64 m². All cages were set under the shade of selected mango trees; free access to water and feed; feed formula was the recommended by the breeding company (Dominant CZ). The well-being of each hen was examined at ages 20, 24, 26, 28 and 31 weeks, by recording: cleanness of body feathers; number and size of featherless spots; degree of keel deviation; number and degree of injuries in feet, body, and head; degree of tonic immobility; motion facility; and clinic signs of illness. An External Health Index (EHI) was constructed with these variables. Frequencies of daily activities of hens were recorded by visual scans in each experimental unit, once every hour, during 6 h on one day, at ages 21, 25, 28, 31 and 33. Occurrence of aggressive behaviors were observed in periods of 30 min, twice a day, during 3 consecutive days, at ages 21, 28, and 34 weeks; aggressive behaviors were rated by the level of aggression and an Aggression Index (AGI) was constructed. Treatment effect on EHI and AGI was analyzed through repeated measure ANOVA (PROC GLM, SAS). Contingency tables (PROC FREQ, SAS) were used to test for independence of daily activities from treatment. The EHI of FR hens (9.87 +/- 0.017) was higher than that of EC hens (9.47 +/- 0.017, $P < 0.0001$), with significant effects of time ($P < 0.0001$) and of treatment*time interaction ($P = 0.0017$), with treatment differences increasing over time. Frequency of daily activities were not independent on treatment ($P < 0.0001$); EC hens rested more frequently than FR ones (35% vs. 9%), but walked and foraged less frequently (7.5% vs. 40%). Treatments were not different in the AGI. This study shows evidence that FR system allowed a greater well-being of hens than the EC system and that hens' behavior respond to the environment they experience.

Key Words: laying hens, external health index, aggression index, alternative production systems

411P Effects of woody breast and white striping on plasma corticosterone, glucocorticoid receptor and 11 beta hydroxysteroid dehydrogenase 1 gene expression in liver and breast muscle of male broilers. Seong Kang, Michael Kidd*, Hakeem Kadhim, Bhuwan Khatri, Stephanie Shouse, Sara Orłowski, Joseph Hiltz, Nick Anthony, Wayne Kuenzel, and Byungwhi Kong, *University of Arkansas, Fayetteville, Arkansas, United States.*

The occurrence of Woody Breast (WB) and White Striping (WS) of broilers has been associated with fast growth rate. Recent studies suggested that localized hypoxia and metabolic stress may be involved in these 2 myopathies. The objective of this study was to investigate effects of WB and both WB/WS on stress hormone corticosterone (CORT) levels and stress response gene expression including glucocorticoid (GC) receptor (GR) and 11- β -hydroxysteroid dehydrogenase type 1 (HSD11B1). Live male birds (Ross 708) were examined by manual palpation and visual examination of the breast muscle (pectoralis major) at 56 d of age, and were grouped into control (Con), WB, and WB/WS birds ($n = 7-10$). Blood samples, via the brachial wing vein, and liver and breast muscle samples were collected. CORT levels were determined by radioimmunoassay (RIA). Gene expression levels in liver and breast muscle were determined by qPCR. Differences of CORT and gene expression

levels among groups were analyzed using one-way ANOVA followed by mean separation using the Tukey's HSD test (significance level of $P < 0.05$) using JMP Pro 14. RIA results showed that CORT levels of WB birds were significantly higher compared with Con (3 fold, $P < 0.05$). CORT levels of WB/WS birds were not significantly different compared with WB, but were significantly higher than Con (2.6 fold, $P < 0.05$), suggesting that the effects of WB and WS on CORT are not synergistic, and circulating CORT may be a significant biochemical marker involved in the pathogenesis of WB and WS. GR expression levels in liver of both WB and WB/WS birds were significantly higher compared with Con ($P < 0.05$). However, GR expression levels in breast muscle of both WB and WB/WS birds were significantly lower compared with Con ($P < 0.05$). Results suggest that high levels of plasma CORT in the WB and WB/WS birds increased GR expression in liver for the stress response, while GR expression was decreased in breast muscle, indicating the chronic stress response of breast muscle in WB and WB/WS birds. Hepatic HSD11B1 expression was higher only in the WB/WS birds compared with Con birds with no significant difference between Con and WB birds. HSD11B1 expression in breast muscle was significantly lower and higher in WB and WB/WS birds compared with Con, respectively ($P < 0.05$). Taken together, results suggest that chronic stress responses may be characteristic occurrence of breast muscle myopathy and regulation of active GCs by HSD11B1 coupled with tissue specific regulation of GR expression in the liver and breast muscle may be involved in the development of WB and WB/WS in broilers. This research was supported by Arkansas Biosciences Institute, and Arkansas Agricultural Experiment Station.

Key Words: woody breast, white striping, corticosterone, glucocorticoid receptor, welfare

412P Effects of prenatal stress on the behavior of different genetic lines of laying hens. Mariana Peixoto* and Tina Widowski, *University of Guelph, Guelph, Ontario, Canada.*

Prenatal stress can have long-lasting consequences on the development of a hen. However, its effects on different genetic strains are unclear. Here we tested how prenatal stressors affect the behavior of different strains of layers. For this, 2 stress models (Natural and Pharmacological) were tested in 5 strains of breeders: 2 commercial brown (B1 & B2), 2 commercial white (W1 & W2) and a Pure Line White Leghorn (WL). To form the Parent Stock, fertilized eggs were incubated, hatched and housed identically in 4 flocks of 27 birds (24F:3M) per strain. Each strain was equally separated into 2 groups: "Maternal Stress" where hens were subjected to a series of acute psychological stressors (e.g., physical restraint, crating) for short periods over 8 d before egg collection, and "Control," which received routine husbandry. At 3 maternal ages, fertile eggs from both treatments were collected, and additional eggs from Control were injected with corticosterone (10 ng/mL egg content) ("CORT"). A "Vehicle" treatment was included to account for effects of egg manipulation. Each maternal age comprised a replicate over time. Eggs were incubated, hatched, and offspring ($n = 1919$) brooded under identical conditions. Animal use was approved by the University of Guelph Animal Care Committee. Isolation can induce chicks to an anxiety-like state that increases stress-related behaviors, such as distress calls. Thus, we placed 5- to 10- day old birds ($n = 720$) individually into a sound-proof box and recorded their vocalizations for 5 min. The effects of the stress model, genetic line, sex and the interaction between stress model and genetic line were subjected to ANOVA using the Glimmix procedure in SAS. Random effects included pen, room and maternal age. Further pre-planned comparisons included stress model (Control vs Maternal Stress; Control vs Injections) and strain (brown vs white).

Treatment interacted with strain ($P < 0.0001$) to affect vocalization. Maternal stress decreased ($P < 0.0001$) the number of calls in W1 compared with Control. Additionally, W1 Maternal Stress vocalized less ($P = 0.0001$) than B1 and B2 from the same treatment. Contrast comparisons displayed differences between brown and white strains ($P < 0.0001$) but no effects of treatment were observed in the offspring (Control versus Maternal Stress: $P = 0.2201$; Control versus Injections: $P = 0.6385$). Results suggest that W1 was the most susceptible strain to the effects of Maternal Stress. Moreover, brown and white lines responded differently to isolation, highlighting the importance of genetics not only on the study of prenatal stress but also on their overall behavior.

Key Words: stress, layer breeder hens, offspring, behavior

413P Evaluation of the repellent effect of a characterized natural product on flies. Anne Burel*¹, Sorphon Suor-Cherer¹, Agathe Demortreux², and Pierre Chicoteau¹, ¹Nor-Feed, Beaucouze, France, ²Laboratoire Commun Feed InTech, Beaucouze, France.

Objective Flies in farm houses cause discomfort for the workers and the animals. Flies may cause some sanitary risk for the animals, their droppings increase the number of unsalable eggs and have economic consequences. The tested natural product in this study is made from lemongrass (*Cymbopogon nardus*), clove (*Eugenia caryophyllus*), oregano (*Oreganum vulgare*) and citrus extracts, which has already shown a repellent effect against red mite. To evaluate the potential of this characterized product for new applications, studies were conducted to determine its effect on flies. In a previous study, an electroantennography (EAG) test on flies (*Sarcophaga sp.*) already allowed to determine a significant response of flies to this natural product. Two different trials were coupled in this study to define the repellent effect of this natural product on flies (*Musca domestica* and *Lucilia sericata*). The first part of the study was set up in a laboratory to observe the flies repartition between 2 zones, one neutral zone and the other one with the natural repellent. Coupled to this trial, a meta-analysis with 6 poultry farms was done, to observe the effect on farms. In a first experiment, 5 plexiglass boxes with 2 different compartments were built, '30 flies' larvae were allocated per box. After hatching, 1 mL of the natural repellent in a little cup was put in one side of the box. Each hour flies repartition was measured during 3 h. At farm, the product was used either in drinking water or in feed (100 mL /1000 L of water, 500g/T of complete feed) during 15 to 30 d. Adhesive traps with an attractive odor were placed in the farms during 24h before and after the supplementation. The traps were used to count flies and estimate the infestation level before and after the treatment. The normality of the laboratory trials and the meta-analysis data were confirmed by a Shapiro-Wilk test. Both data series were analyzed by a paired *t*-test. Concerning the laboratory trials, no significant difference of flies repartition at T0 ($P = 0.58$) was observed. A significant difference of flies repartition between the neutral and the natural repellent zone at T1, T2, T3 was demonstrated ($p < 0.01$). Results of the tests on the 6 farms, showed a significant reduction in fly population ($p < 0.05$). A previous EAG study has demonstrated the sensibility of flies to a natural product. However this study did not allow determination of whether the effect was repellent or attractive. The laboratory study coupled to a meta-analysis of 6 farm trials allowed to show a significant repellent effect of this natural product on flies.

Key Words: flies, repellent effect, essential oils, characterization, natural

414P The effects of rearing Pekin ducks under high or low illuminance as measured by Clux and Lux light meters on duck stress, behavior, and growth. Gabrielle House*, Eric Sobotik,

Austin Stiewert, Jill Nelson, and Greg Archer, *Texas A&M University, College Station, Texas, United States.*

Light intensity is commonly measured as either Lux (Lx) or, more recently, Clux (cLx). cLx measurements are adapted to the sensitivity curve of chickens, while Lx simply measures illuminance. Although it is known that poultry welfare can be affected by different light intensity, limited research has been conducted to understand the effects of light intensity on Pekin duck behavior and welfare. The objective of this study was to evaluate the effects of 4 different light intensities on Pekin duck stress, behavior, and growth. Pekin ducks ($n = 768$) were reared in environments illuminated with LED lights set to 20 Lx from 0 to 35d of age (20L), 20 cLx (20cL) from 0 to 35d of age, 20 Lx from 0 to 10d and 5 Lx from 11 to 35d of age (20,5L), or 20 cLx from 0 to 10d and 5 cLx from 11 to 35d of age (20,5cL). Stress response was measured using heterophil to lymphocyte ratios (HL; $n = 160$), plasma corticosterone (CORT; $n = 160$), and physical asymmetry of 3 bilateral traits (ASYM; $n = 480$). Tonic immobility (TI; $n = 480$) and inversion (INV; $n = 480$) tests were used to measure fear response. Growth parameters included tibia bone mineral content (ASH; $n = 480$), tibia breaking strength (BS; $n = 480$), left and right eye dimensions (weight, depth, width; $n = 80$), gait score (GS; $n = 384$), and feed conversion ratio (FCR; $n = 64$). A LSD post hoc test was used for mean separation. Data were analyzed using GLM, and $P < 0.05$ was considered a significant difference. HL were higher in 20,5cL ducks (0.80) than in 20cL ducks (0.55, $P < 0.04$). However, HL for 20,5cL ducks and 20cL ducks did not differ from 20L and 20,5L ducks ($P > 0.05$). No differences were found in CORT or ASYM measurements ($P > 0.05$). No differences were found in fear response tests or growth parameters ($P > 0.05$). The results of this study indicate possible increases in stress susceptibility in ducks raised under 20,5 cL. Light intensity does not appear to affect behavioral responses or growth parameters, indicating potential insensitivity to differences in cLx and Lx illuminance measures of LED lights in Pekin ducks.

Key Words: Clux, Lux, duck, welfare, illuminance

415P The effect of different photoperiods on Pekin duck production, stress, and behavior. Gabrielle House*, Eric Sobotik, Jill Nelson, and Greg Archer, *Texas A&M University, College Station, Texas, United States.*

Manipulation of photoperiods in domestic poultry species can influence the productivity and behavior of poultry raised in modern production systems. Limited research has studied the effects of different photoperiods on Pekin duck behavior and welfare. The objective of this study was to evaluate the effects of 2 different photoperiods on duck production, stress, and growth. Pekin ducks ($n = 384$, 32 total pens) were reared in floor pens in light tight rooms under a lighting scheme consisting of 24L for day one, then 16L:8D or 20L:4D until ducks reached 35 d of age. Growth and physical parameters were obtained by measuring weight gain, pen feed conversion (FCR, $n = 32$), and gait score (GS, $n = 192$). Plasma corticosterone concentrations (CORT, $n = 80$), the heterophil to lymphocyte ratio (HL, $n = 80$), humoral immunity (NDV, $n = 80$), and physical asymmetry of bilateral traits (metatarsal length, metatarsal width, and middle toe length; ASYM, $n = 240$) were measured to determine stress susceptibility. Fear response was measured using tonic immobility (TI, $n = 240$), and inversion (INV, $n = 240$) tests. Data were analyzed using GLM, and $P < 0.05$ was considered a significant difference. FCR was poorer in 16L:8D ducks (1.43 ± 0.03) than 20L:4D ducks (1.34 ± 0.02 , $P < 0.03$). 20L:4D ducks had lower stress susceptibility than 16L:8D ducks. 20L:4D ducks had lower CORT (6558.3 ± 697.16 and 10592.2 ± 1058.01 pg/mL, respectively, $P < 0.01$), HL (0.41 ± 0.03 and 0.55 ± 0.05 , respectively, $P < 0.02$), ASYM (1.73 ± 0.07 and 2.09 ± 0.09

mm, respectively, $P < 0.01$). 20L:4D ducks had higher NDV titers than 16L:8D ducks (393.8 ± 88.19 and 191.6 ± 33.21 units/mL, respectively, $P < 0.04$). 16L:8D ducks had less intense fear responses during INV than 20L:4D ducks (3.5 ± 0.16 and 4.0 ± 0.18 flaps/s, respectively, $P < 0.03$). No differences were found between treatments for TI, GS, and final body weight. Results of this study indicated that photoperiod can alter stress levels and FCR of Pekin ducks. This emphasizes the critical role of artificial lighting in commercial duck welfare and the need for further research in this area.

Key Words: photoperiod, stress, behavior, welfare, duck

416P The effects of ultraviolet supplemented LED lighting on Pekin duck stress, behavior, and growth. Gabrielle House*, Eric Sobotik, Jill Nelson, and Greg Archer, *Texas A&M University, College Station, Texas, United States.*

Ducks and other domestic poultry species have the ability to see in the UV (UV) spectrum (320–400 nm), however there has been no research conducted studying the importance of UV light supplementation. The objective of this study was to evaluate the effects of UV supplementation on stress, behavior, and growth in Pekin ducks. Pekin ducks were reared under environments illuminated with either LED lights supplemented with UV light (UV+), or under just LED lights (UV-). Plasma corticosterone (CORT), heterophil to lymphocyte ratio (HL), and physical asymmetry of 3 bilateral traits (metatarsal length, metatarsal width, and middle toe length; ASYM) were measured to determine stress susceptibility. Fear responses were measured using inversion (INV) and tonic immobility (TI) tests. Body weight, feed conversion ratio (FCR) and gait score (GS) were measured to determine the effects of UV light supplementation on physical growth and development. Left and right eye dimensions (weight, depth, and width) were used to determine how UV supplementation affects eye development. A LSD post hoc test was used for mean separation. Data was analyzed using GLM, and $P < 0.05$ was considered a significant difference. UV+ ducks had lower CORT (6316.74 and 9242.37 pg/mL, respectively, $P < 0.03$), HL (0.43 and 0.54, respectively, $P < 0.04$), and ASYM (0.58 and 0.76 mm, respectively, $P < 0.01$). More attempts were needed to induce TI in UV+ ducks compared with UV- ducks (1.48 and 1.71 attempts, respectively, $P < 0.02$). First head movements during TI occurred quicker in UV+ ducks than UV- ducks (61.28 and 100.71 s, respectively, $P < 0.03$). Eye weight was lower in UV+ ducks than UV- ducks (1.46 and 1.53 g, respectively, $P < 0.03$). UV+ ducks had narrower eyes than UV- ducks (12.25 and 12.47 mm, respectively, $P = 0.01$). There was a nonsignificant trend for UV+ ducks righting faster during TI than control ducks. No significant differences were observed in INV testing ($P > 0.05$). These results indicate that UV light supplementation can lower stress and fear responses and impact eye development in Pekin ducks.

Key Words: duck, ultraviolet, stress, behavior, welfare

417P Evaluating fear and stress amongst different genetic strains of laying hen. Austin Brown*, Jill Nelson, Eric Sobotik, and Greg Archer, *Texas A&M University, College Station, Texas, United States.*

Fear and stress levels differ based on genetic strain. To determine differences in fear and stress levels 4 strains of cage-free laying hens were raised to 32 weeks of age in 3.05 m by 4.57 m floor pens. 60 birds were allotted to each pen with 1 strain per pen. The stocking density of each pen was 0.232 m² per bird. Three white strains were used, W-36, W-80, and LSL, and one brown strain was used, Hyline Brown (HB). Fear

and stress tests were conducted on each strain at 3, 16, and 30 weeks of age with additional testing done at 31 weeks where a 30 min period of confinement was administered to induce stress; after which blood was sampled to measure plasma corticosterone concentration (CORT) At 3 weeks of age Isolation testing (ISO, n = 60) and emergence testing (EMER, n = 60) was conducted. At 16- and 30-weeks tonic immobility (TI, n = 60), physical asymmetry of bilateral traits (metatarsal length, metatarsal width, middle toe length) (ASYM, n = 60), CORT (n = 20), and heterophil to lymphocyte ratios (HL, n = 20) were measured. Data was analyzed using GLM and fisher pairwise comparisons: a significant difference was considered $P < 0.05$. In ISO W-80 (59.0 ± 7.9 vocal/ 3 min) had the most vocalizations among all other strains ($P < 0.001$) with LSL and HB (34.3 ± 5.5 vocal/ 3 min) at an intermediate level and W-36 the lowest (12.3 ± 3.4 vocal/ 3 min). During emergence testing W-36 and LSL (39.9 ± 7.1 s.) took longer ($P < 0.001$) to leave a 18.5 L bucket than W-80 and HB (14.5 ± 3.3 s.). Righting time did not differ ($P > 0.05$) between strains in TI, however, time for each strain to alert showed a difference in first head movement ($P < 0.001$). HB (5.1 ± 2.3 s.) was quickest to alert at when compared with other strains. W-36 and W-80 (23.7 ± 7.1 s.) alerted at an intermediate level while LSL (58.475 ± 9.4 s.) was the slowest to alert. ASYM score is a composite score calculated by taking the difference in left metatarsal length and right metatarsal length. During ASYM scoring HB (1.9 ± 0.29 mm) had the greatest score ($P = 0.048$) with W-36 (1.5 ± 0.17 mm) having an intermediate score and W-80 and LSL (1.4 ± 0.13) having the lowest score. HB (0.52 ± 0.08 , 20424.3 ± 1570.2 pg/ml) had higher HL ($P < 0.001$) and CORT ($P < 0.001$) than the other strains. The other strains (0.18 ± 0.03 , 14431.1 ± 1581.0 pg/ml) did not differ from each other. After the period of stress LSL (21358.0 ± 1367.0 pg/ml) showed a trend to have higher CORT than the other strains ($P = 0.057$). HB (19059.0 ± 1225.8 pg/ml) had an intermediate level and W-36 and W-80 (16380.0 ± 16183.1 pg/ml) did not differ from each other. Results of this study suggest that fear and stress vary depending on the strain of bird used.

Key Words: stress, fear, genetics, layer

418P The effects of supplementary ultraviolet-A light on the physiology and welfare of broiler chickens. Curtis Leyk* and Aaron Stephan, *Once Inc, Plymouth, Minnesota, United States.*

Previous work has shown chickens prefer a more natural light spectrum that contains UV-A (UVA) light and an improvement in welfare parameters in both broilers and layers raised under UVA light in university pen trials was seen. The objective of this study is to look at the effects of supplemental UVA light on welfare and production parameters on a commercial scale trial. This trial was conducted at 2 farms, each with 4 barns (Farm 1: 60' x 600', Farm 2: 44' x 600'). Two of the barns at each farm were outfitted with supplemental UVA lights (ONCE Agri-shift HL-UVA, ONCE, Inc., Plymouth, MN). Barns were matched by broiler strain, source, environmental conditions, and management. Feed delivery records and settlement data were separated for UVA and control barns. Chicks were placed in all 4 barns on a farm simultaneously and raised per the normal and accepted methods of the grower and integrator. An automatic scale was utilized in one of the treatment barns and one of the control barns at one farm to continually monitor bird weight. Approximately 1–2 weeks before the birds being loaded out and processed, welfare measurements were conducted on a representative sample of the birds in each barn, including: Blood Corticosterone, Blood Heterophil:Lymphocyte ratios, Inversion Test, Isolation Test, Emergence Test, Tonic Immobility. For each parameter, the Mean, Standard Deviation, and Standard Error of the Mean were calculated and analyzed with a paired *t*-test. Mixed results between farms and between rounds of the

trial were seen. Isolation test vocalizations (Farm 1 - Round 1: 23.3 ± 4.5 vs. 33.5 ± 3.8 , $P = 0.086$, Round 2: 43.3 ± 7.1 vs. 47.0 ± 7.3 , $P = 0.716$; Farm 2: 58.05 ± 9.0 vs. 65.5 , $P = 0.534$) and Tonic Immobility times [min:sec] (Farm 1 - Round 1: $1:32 \pm 0:15$ vs. $3:29 \pm 0:55$, $P = 0.053$; Round 2: $2:16 \pm 0:22$ vs. $3:03 \pm 0:37$, $P = 0.282$; Farm 2: $1:37 \pm 0:20$ vs. $1:45 \pm 0:24$, $P = 0.79$) were numerically improved in UVA barns, but not statistically significant in all cases. Other welfare parameters showed little difference. Production parameters from the settlement data showed mixed results, with little overall difference. Birds were less active in the UVA barn as evidenced by fewer birds visiting the scale daily. This ongoing trial did not show a consistent welfare or production improvement with UVA lighting. However, this trial used a lower UVA light level than in the earlier pen trials, which may have resulted in the lack of effect on measured parameters. Future work will include significantly increasing the intensity of UVA light and determining whether any welfare benefits may be realized.

Key Words: broiler, welfare, ultraviolet light, tonic immobility, isolation test

419P Not Presented

420P Does wearing sensors affect perching behavior of laying hens? Zahira Budeguer*, Christina Rufener, and Maja Makagon, *UC Davis, Martinez, California, United States.*

Use of sensor technology is becoming wide spread in poultry research. Sensors used to monitor a hen's behavior often require that bird be fitted with a vest or backpack containing the sensor, and for the hen to acclimate to the sensor weight. It is, however, possible that the very presence of the vest and the weight of the sensor may alter hen behavior. The objective of this study was to evaluate whether wearing a jumpsuit vest with or without a sensor impacted the perching behavior of laying hens. Twenty 4 W-36 laying hens were housed individually in cages. A single PVC perch (50.8 cm wide, 3 cm diameter) was placed across the width of each cage when the hens were 39 weeks old. One week later hens were assigned to one of 3 treatment groups: no vest, a jumpsuit vest (JV), a jumpsuit vest with sensor (JVS). The jumpsuit vests were sewn from elastic fabric and fitted over the ventral and dorsal side of the bird. A small dorsal pocket served as housing for the sensors. Perching behavior was video recorded for the first 3 full days after the backpacks were fitted. We recorded whether or not each hen was sitting or standing on her perch every 3 min between 2 and 3 a.m., 9–11 a.m., 3–5 p.m., and 8–10 p.m. Data were analyzed using linear mixed effects models in R. Hens fitted with JV and JVS perched more than controls ($P < 0.0001$). On average, JV hens perched during 72.2% (estimated mean) of the observations [lower and upper confidence intervals: 61.9, 82.4], JVS hens during 65.8% [55.5, 76.0], while control hens perched during 41.2% [31.0, 51.5] of the observations. When perching JV and JVS hens sat more often than stood compared with control hens ($P = 0.005$). JV and JVS hens sat while perching in 52.7% [44.4, 61.0] and 56.2% [48.0, 56.5] of the cases, whereas control hens sat while perching during 36.0% [27.8, 44.3] of the observations. Perching behavior was not associated with observation day, either as a main effect or an interaction with treatment. Further work will investigate frequencies of transitions onto the perch, which could explain the presented results.

Key Words: laying hen, sensor, vest, perch, research

421P Using fixed and reduced light intensity effects on performance, meat quality, serum biochemical and behavior of broiler

chickens. Kyeong Seon Ryu*, Chun Ik Lim, Md. Masud Rana, Se Youn Cha, and Hyung Kwan Jang, *Chonbuk National University, Jeonju, Korea (the Republic of).*

The aim of this study was to know the impact of varying light intensity on production performance, meat quality, blood parameters and behavior of broilers. A total of 1200 one-day-old Cobb-500 broiler chicks were randomly assigned to 6 treatments in 24 floor pens measuring 1.95 × 2.15 m each, in a total area of 4.2 m². Each treatment had 200 chicks in 4 replicates of 50 chicks each. Six light intensity treatments were as follows: 5, 10, 20 and 30 lx were fixed throughout the duration of the study, treatment 30–20–10 lx and 20–10–5 lx, where light intensity was gradually reduced at the end of pre-starter (0–11 d), starter (12–25 d) and grower (26–42 d) period, respectively. Temperature was set initially in experimental house at 33°C on the first day to the seventh day of age and then gradually reduced by 3°C in the third week, and fixed at 22°C until end of the experiment. All birds were fed same commercial mash pre-starter, starter and grower diet from d 0 to 11, 12 to 25 and 26 to 42, respectively. The experiment lasted for 42 d. Body weight, feed intake, feed conversion ratio, meat quality and serum biochemical parameters were measured during the study. The behavior of the chickens was analyzed based on following categories: sitting, standing, walking, pecking, inactive behavior (sitting plus standing) and active behavior (walking plus pecking), and closed closed-circuit television (CCTV) was used to record these behavioral status. Data were analyzed using the GLM procedure of SAS (9.1, 2009). No significant differences were observed in performance parameters among light intensity treatments and as the effect between fixed and reduced light intensity treated groups. Over the entire experimental period, the biochemical properties of blood serum and meat quality traits were not affected by varying light intensity treatments, and the interaction between fixed and reduced light intensity groups. There were significant differences in sitting, standing and pecking behavior of broilers among different light intensity treatments. Significantly spent low time in an inactive behavior (sitting plus standing) when broilers kept under 30 and 20 lx while spent more time in an active behavior (walking plus pecking) under 20–10–5 lx treatment group. Whereas, no significant difference in behaviors of broiler were observed in interaction between of fixed and gradually reduced light intensity treatments. Therefore, this study demonstrates that varying light intensities had executed tiny effects on behaviors of broiler whereas no discernible differences were observed in performance, meat quality and biochemical properties.

Key Words: light intensity, performance, meat quality, behavior, broiler chickens

422P Impacts of alternative housing systems on the welfare of laying hens. Éloïse Denis*³, Martine Boulianne³, Alexandra Harlander¹, and Stéphane Godbout², ¹*University of Guelph, Guelph, Ontario, Canada*, ²*Research and Development Institute for the Agri-environment, Quebec, Quebec, Canada*, ³*Université de Montréal, Saint-Hyacinthe, Quebec, Canada*.

There are 2 categories of housing systems in laying hen husbandry used in Canada; conventional cages and alternative housing systems, which include enriched cages and single-tier or multi-tier aviaries. With most eggs coming from hens housed in conventional cages, Canadian egg producers are facing a phase-out of conventional layer housing since this system will be abolished in Canada by 2036. The objective of the study was to investigate the impact of 2 alternative housing systems, enriched cages and aviaries, on the welfare of laying hens. Twelve commercial farms in Quebec housing White Lohmann hens in aviaries (n = 6) and enriched cages systems (n = 6) were selected. Each farm

was visited at bird age of 19, 23, 27, 31, 35, 45, 55 and 65 weeks. At each visit, 12 hens were randomly selected and examined for footpad dermatitis, keel bone fracture and feather coverage. Chi-squared tests were performed to evaluate the impact of both housing systems on the measured parameters. Footpad dermatitis prevalence varied over the time and was higher in enriched cages compared with aviaries at 23 and 27 weeks ($P < 0.05$) and lower at 31, 35, 45, 55 and 65 weeks ($P < 0.05$). The highest prevalence occurred at 27 weeks in enriched cages (37.5%) and 31 weeks in aviaries (51.4%). Prevalence of keel bone fracture was similar between the 2 housing systems but was significantly higher in aviaries at 23 weeks (5.6% vs. 0%; $P < 0.043$) and 65 weeks (47.9% vs. 6.3%; $P < 0.0001$). Fractures were noticeable as soon as 19 weeks in aviaries while the first ones in enriched cages were detected at 27 weeks. No significant differences were observed in terms of feather condition through the study between enriched cages and aviaries ($P > 0.05$). A minority of hens kept an intact feather condition at 55 and 65 weeks. Early results showed that both housing systems have repercussions on the welfare of hens at different time points in the laying period and underlines the necessity to better understand the hens' environment to improve housing facilities.

Key Words: housing systems, laying hens, welfare, enriched cages, aviaries

423P Prevalence of keel bone fractures in broiler breeder hens. Yves Zounli*, Ramon Malheiros, Dimitri Malheiros, Robert Beckstead, and Prafulla Regmi, *North Carolina State University, Raleigh, North Carolina, United States*.

Keel bone fractures in cage and cage-free layers has become an important welfare issue for the poultry industry. Recently, keel bone fracture prevalence around 80% has been reported in different housing systems. Lack of sufficient breast muscle mass around the keel bone in laying hens has been hypothesized as a risk factor as the keel is exposed, making it vulnerable to trauma during flights and landing accidents. In the last few years, many studies have devoted their focus on laying hens, and not enough have been done on broiler breeder hens. Since broiler breeders have extensive pectoralis muscle mass, it would be interesting to evaluate keel fracture prevalence in these birds. The objective of this study was to evaluate the prevalence of keel bone fracture in broiler breeder hens. Ross 708 birds were housed in 2 different housing systems. House 1 (H1) had 6 pens (1.80 m by 1.10 m) with a raised slat 72cm high by the nest box. Each pen contained 11 birds (9 hens and 2 roosters) at 0.18 cm² per bird. House 2 (H2) consisted of 8 pens 4.65 m by 3.73 m with a provision of raised slat of 51 cm height. Each pen in H2 housed 68 birds (61 females and 7 males) at approximately 0.23 cm² per bird. At 56 wk age, 5 hens/pen (H1) and 4 hens/pen (H2) were randomly selected and euthanized. Immediately post-euthanasia whole breasts were excised and the breast muscles surrounding the keel bone were stripped. Complete removal of breast muscle was carried out after autoclaving. Keel bones were examined for prevalence of fracture and deviation. Fractures were further characterized as single or multiple and the location of the fracture on the cranial, middle, or caudal third of the bone was observed. Fisher's Exact procedure in SAS was used to test the association of prevalence with H1 and H2. Keel fracture prevalence was not different between H1 (85.71%) and H2 (82.76%). Majority of the fractures were multiple in nature and located in the caudal region of the keel regardless of the housing type. There were 58.33% and 66.67% multiple breaks with 83.33% and 62.50% caudal fractures respectively in H1 and H2 ($P > 0.05$). Fracture was not observed in the cranial keel in either H1 or H2 birds. Keel deviation, without fractures, was observed in 28.57% in H1 and 20.69% in H2 hens ($P = 0.55$). Together

these findings suggest that keel bone fractures are occurring in broiler breeder hens at concerning level and further studies are warranted to identify the risk of factors.

Key Words: broiler breeder, hens, welfare, keel bone, fractures

424P Scatter feeding as a viable form of enrichment for commercial broiler chickens. Brittany Wood*, Maja Makagon, and Richard Blatchford, *University of California, Davis, Davis, California, United States.*

The provision of enrichment for grow-out broilers in the US has grown as a management practice in recent years. Scatter feeding, in particular, is commonly recommended based on the assumption that it will stimulate foraging behavior and activity. However there are few studies demonstrating the efficacy of scatter feeding as an enrichment. The goal of this study was to evaluate different feed items and their ability to stimulate foraging behavior when scatter fed to broiler chickens. One of 6 feed types: shavings (SH) to test if the act of scattering stimulates foraging, standard feed (FE), whole wheat (WW), alfalfa pellets (AL), cabbage (CA), and dried mealworms (MW) was scattered in each pen (5 broilers/pen with 6 replicate pens/treatment) at the same time of day for 3 consecutive days each week from when the birds turned 2 weeks old through 6 weeks of age. Broiler behavior was observed on wk 2 and 4 during the 30-min period immediately following the first scatter feeding of the week (d 1) A second set of observations was made 5 d later (d 6), on a day when scatter fed items were not provided. Within the 30-min blocks, each pen was observed for 1-min every 5 min (6 observations/30-min block). Each bird was scored as either foraging or not within each 1 min observation. Data were summarized as the proportion of 1 min observations during which each bird was observed foraging within a given 30 min time period, and analyzed using a linear mixed effect model in R with treatment, week, day and all interactions as fixed effects. No age effect was found across wk 2 and 4. However, we found an interaction between treatment and day ($P < 0.001$), with a treatment difference on d 1 and no difference on d 6. On d 1 time spent foraging within respective pens was as follows: SH (24%), FE (28%), AL (20%), WW (28%), CA (35%) and MW (48%). On d 6 foraging activity ranged between 15% and 23% across all treatments: SH (18%), FE (15%), AL (18%), WW (22%), CA (23%) and MW (21%). Broilers foraged on days that enrichment was present, with cabbage and meal worms stimulating the greatest foraging behavior. The absence of an age effect indicates that broilers forage irrespective of age. In conclusion, scatter feeding as a method of enrichment can stimulate foraging behavior at 2 and 4 weeks of age but the presence and value of the feed item matters.

Key Words: broilers, scatter feeding, enrichment, foraging behavior, welfare

425P Behavioral analysis of alternative chick euthanasia strategies. Allison Milby*, Xi Wang¹, Dan Zhao¹, Shailesh Gurung¹, Greg Archer¹, Edgar Peebles², and Morgan Farnell¹, ¹Texas A&M University, College Station, Texas, United States, ²Mississippi State University, Mississippi State, Mississippi, United States.

Maceration is an instantaneous and humane way to euthanize neonatal chicks. However, it is not always perceived by the public to be a humane means of euthanasia. The objective of this experiment was to evaluate the effects of euthanasia methods on behavioral responses of newly hatched male layer chicks. A randomized design was applied to study 4 methods on a commercial scale. Treatments consisted of compressed

breathing air followed by decapitation (control), carbon dioxide (CO₂), nitrogen (N₂), and a commercial low atmospheric pressure stunning system. A customized gas vaporizer was used to provide approximately 1,019 standard liters per minute of N₂ and CO₂. A total of 480 d-of-hatch male layer chicks were randomly assigned to 16 replicates with 30 chicks/replicate and 4 replicates/treatment over a 2 d period. Chicks received the corresponding treatments in a commercial vacuum chamber (volume of 3,890 L). A vent plug was removed to allow air to escape as compressed air, CO₂ or N₂ was added and to prevent pressurization of the vessel. An infrared camera system was used to monitor birds. Ataxia, loss of posture, and convulsive behavior of the first and last bird in each treatment replicate group was observed. Cessation of vocalization and cessation of movement were also evaluated. Since birds in the control treatment were alive throughout the study, no behavioral data of control birds were analyzed. One-way ANOVA analysis was used to study the effects of the remaining treatments. Chicks in the N₂ treatment took a longer time to initiate and terminate all behaviors as compared with those in the CO₂ and low atmospheric pressure treatments (all $P < 0.001$). It took a longer time for birds euthanized by the low atmospheric pressure stunning system to start convulsing (156.50s vs 84.75s, $P < 0.001$) and stop vocalization (288.75s vs 94.25s, $P < 0.001$) as compared with chicks euthanized by CO₂. However, the low atmospheric pressure stunning system was statistically similar to the CO₂ treatment when comparing time to initiate (29.25s vs 22.75s) and terminate (75.00s vs 76.25s) ataxia, initiate (58.75 vs 50.75s) and terminate (101.25s vs 82.00s) loss of posture, and terminate (356.50s vs 341.00s) movement. Our data suggests that the administration of CO₂ gas or the low atmospheric pressure system euthanized neonatal chicks faster as compared with the administration of N₂ gas. Future work will examine the effects of these treatments on stress hormones from serum collected from these birds.

Key Words: behavior, carbon dioxide, euthanasia, low atmospheric pressure stunning system, nitrogen

426P Comparing platform enrichment usage between conventional Cobb 500 and Ross 708 broilers. Rodrigo Lopez*, Shawna Weimer², and Katy Tarrant¹, ¹Fresno State, Fresno, California, United States, ²University of Maryland, College Park, Maryland, United States.

As broiler producers are increasing the usage of environmental enrichments being utilized, more information is needed to determine how these enrichments should be implemented in a conventional environment. To warrant the usage of platforms as enrichments in conventional broiler systems, bird utilization of the platform must be established. Due to intensive selection of economically important production traits within genetic lines of broilers, it is possible that behavioral variations also exist between lines. This study aims to determine if behavioral differences exist between 2 lines of broilers to the extent that the lines will vary in their usage of platforms. Twelve 1.51 m² pens were set up in a conventional broiler house. Each pen encapsulated a water line, and was hand fed through a funnel feeder. A 0.91x0.25x0.05 m wooden platform was placed in an identical location within each pen. Pens alternated in designation for Cobb 500 or Ross 708 broilers. Twelve broilers were placed per pen for a total of 72 Cobb and 72 Ross birds. To determine platform usage, bird counts were performed twice daily, once at a random hour between 6:00 a.m. and 10:00 a.m., and once between 4:00 p.m. and 8:00 p.m., beginning at d 6 until d 44. The platform usage frequency was calculated as (number of birds visualized using the platform / the maximum platform frequency based the area of the platform). Frequencies were categorized as none (0%), low (>33%), medium (33% - 66%), and high (<67%) usage. Genetic lines were compared in JMP v.11 by

a Pearson chi-squared test, and significance was evaluated at $P \leq 0.05$. During evaluations, platforms use in the Cobb pens occurred 67% of the time, compared with 63% of the time in Ross pens. On d 10, Cobb pens were evaluated as having a larger frequency of usage at 50% low frequency and 50% none, compared with 100% none in Ross pens ($P = 0.045$). On d 44, Ross pens were evaluated as being 100% high usage frequency, compared with 50% high and 50% medium in Cobb pens ($P = 0.045$). All other days evaluated did not differ significantly. The data from this study suggests genetic line differences are minimal between lines, but further study is warranted. Establishing this knowledge is critical, as conclusions drawn from research studies on enrichment usage could vary based on line.

Key Words: broiler, enrichment, platform

427P Changes on stress indicators and growth parameters of broilers under two rearing densities with a functional sensory additive included in the diet. Jean-François Gabarrou^{*1}, Pedro Ciriaco², and Cynthia Bravo³, ¹Phodé, Tersac, France, ²University of La Molina, La Molina, Peru, ³Montana, Lima, Peru.

The high rearing density induces stress perception and decreases the performance of broilers. Ve0, a mixture of Functional Sensory Molecules (FSM), based on sweet orange essential oil (rich in D-Limonem, PHODE, Albi, France), was designed to mitigate the consequences of animal stress. The objective of this study was to evaluate the changes in the stress indicators and performance of broilers reared under 2 densities, when the FSM was used. One day old Cobb 500 chicks ($n = 272$ males and females) were distributed into 8 pens X 3 treatments (24 pens). Treatment 1 (T1) was the control rearing density (10/m²) and treatment 2 (T2) served as the negative control with a high rearing density (12/m²). T1 and T2 did not receive FSM. Treatment 3 (T3) received FSM in the feed (250 g/t) from d 1 to d 42 in a high rearing density environment. At 42 d, 2 broilers per pen underwent an immobility test (Gordon & Galup, 1974) to determine stress level. The birds that took more than 20 s to stand up were categorized as stressed. Stress level was also determined based on corticosterone in blood samples from one broiler per pen at d 42. Feed consumption (FC), body weight (BW) and feed conversion ratio (FCR) were determined at d 42. Statistical analyses were performed on all parameters with a one way ANOVA with "treatment" as fixed factor (IBM SPSS statistics v25 software), and a Chi2 test was used to analyze the immobility test. The frequency of animals that took more than 20 s to stand following the immobility test was higher ($P < 0.001$) for T2 (62.5%) than T1 (18.8%) and T3 (17.3%). Blood cortisol was also elevated ($P = 0.051$) for T2 (21.1 ± 2.9ng/ml) birds compared with T1 (17.3 ± 2.1ng/ml) and T3 (17.3 ± 1.9ng/ml). All the growth parameters were affected by treatments. The birds reared with high density had reduced FC and BW compared with control density and FSM treatments. FI was reduced ($P < 0.001$) in T2 birds (5290 ± 17g) compared with T1 (5537 ± 29g) and T3 (5353 ± 22g). Similarly, live BW of T2 birds was less ($P < 0.001$) in T2 (3115 ± 22g) than in T1 (3308 ± 12g) and T3 (3190 ± 35g). As a result, T2 birds (1.721 ± 0.012) demonstrated elevated ($P < 0.001$) FCR in comparison to T1 (1.697 ± 0.007) and T3 (1.701 ± 0.017). The results of the immobility test as a behavioral indicator of stress perception and the blood corticosteroid levels as a measurement of physiological stress demonstrated that the rearing density model was a stressor. Additionally, these results indicate the benefits of FSM to mitigate environmental stress. Furthermore, these benefits were demonstrated by the beneficial impact on the growth performance parameters. Additional research is warranted to examine the benefits of FSM on other stressors factors.

Key Words: rearing density, behavior, stress, sweet orange essential oil

428P Effects of functional sensory molecules, based on sweet orange essential oil, on heat growth parameters of broilers under heat stress. Jean-François Gabarrou^{*1}, Cynthia Bravo², and Juan Neisser Hernandez Bazan³, ¹Phodé, Tersac, France, ²Montana, Lima, Peru, ³University of Trujillo, Trujillo, Peru.

Heat stress is a common chronic stress in poultry production. Birds usually increase water uptake but not enough especially when heat stress period is too long in the day. As a consequence, some birds die others reduce feed intake (FI) live body weight (LBW) and by the way increase feed conversion ratio (FCR). Ve0, a Functional Sensory Molecules blend (FSM), based on sweet orange essential oil (rich in D-Limonem; PHODE; Albi, France) was designed to mitigate animal stress consequences. The objective of this study was to evaluate the FSM effect on broilers subjected to heat stress. One day old Cobb 500 chicks ($n = 3 \times 150$) were dispatched into 6 pens X 3 treatments (25 birds per 2m² pens). Heat stress was maintained at 33°C ± 3 during all the production cycle. Treatment 1 (T1) was the negative control without any FSM. Treatment 2 (T2) was supplemented with FSM at 200 ppm. Treatment 3 (T3) was supplemented with FSM at 300 ppm. FI, LBW and FCR were determined at d 42. Statistical analyses were performed on all parameters with a one way ANOVA with "treatment" as fixed factor (IBM SPSS statistics v25 software). Significance was considered at $P < 0.05$. All of the examined growth parameters were impacted by treatment. FI was significantly increased ($P < 0.001$) in T2 (107.1 g/d) and T3 (113.2 g/d) birds compared with T1 (100.9 g/d). Similarly, LBW of T2 birds was significantly higher ($P < 0.001$) in T2 (61,0 g/d) and T3 (67.8 g/d) than T1 (55.0 g/d). As a result, T2 and T3 birds demonstrated a significantly lower FCR (respectively 1.75 and 1.67) in comparison to T1 (1.86). The results of the mortality show a reduced mortality in T2 (2%) and T3 (1,3%) compared with T1 (7,3%). The main part of the mortality occurred from d 26 to d 42 when birds are more sensitive to heat stress. Additionally, these results indicate the benefits of VeO to mitigate environmental stress. Furthermore, these benefits were demonstrated by the beneficial impact on the growth performance parameters. Additional research is warranted to examine the benefits of VeO on other psycho-social stressors.

Key Words: heat stress, essential oil orange, broiler, performance

429P Evaluation of a specific formulation of curcumin to modulate IL6, IL10 and TNF- α and growth parameters of broilers. Jean-François Gabarrou^{*}, Phodé, Tersac, France.

Intensive system of production increases animals oxidative stress and sub-inflammatory status. Curcumin is well known for its anti-oxidant and anti-inflammatory properties, but curcumin is also very fragile and sensitive to oxidation. The main goal of this experiment was to evaluate the effect of a new targeted release of curcumin technology provided by Laboratoires Phodé (France) compared with a commercial standard protection. 84 Ross-308 chickens of 10 d old were randomly distributed in individual cages into 6 groups ($n = 21$). The first group receive a standard feed without any plant extract feed additive. The second one receive the same control feed but supplemented with 6.66ppm of standard protected curcumin. The 2 other feeds contained respectively 3.33ppm and 2.20ppm of formulated and vectorised curcumin. Blood interleukins IL6, IL10 and TNF- α were determined at 35 d on 10 birds per treatment by flow cytometry. Feed intake (FI), live body weight (LBW) and feed conversion ratio (FCR) were also determined at 35 d but on 21 birds per treatments. Statistical analysis was carried out using the General Linear Model procedure of IBM® SPSS® statistic v25. $P < 0.05$ was considered as significant. No significant difference is observed between groups for pre-inflammatory (IL-6) and pro-inflammatory (TNF- α)

indicators. Whatever the dosage or the presentation of the curcumin, curcumin increase significantly IL-10, suggesting that anti-inflammatory effect of curcumin is controlled by anti-inflammatory cytokines and not pro or pre-inflammatory ones. The control group presented significant lower growth parameters compared with curcumin's groups suggesting that the anti-inflammatory effect of curcumin permitted better growth performances. On the other hand, no significant difference in FI, LBW nor FCR was observed between the 3.33ppm of vectorised curcumin and the standard 6.66ppm, 126.8 vs. 127.9 g/day of FI; 2227 vs. 2263 g LBW; 1.629 vs. 1.612 FCR respectively. Vectorization of the curcumin permitted animals to exhibit the same level of performance with twice less curcumin compared with commercial standard protection.

Key Words: curcumin, inflammation, IL-10, TNF alpha, vectorization

430P In vivo efficacy of four commercial anti-mycotoxin additives during concurrent mycotoxin challenge. LeAnn Johnston*, Ed De Boer, Chris Ching, Hongyu Xue, and Dongping Wang, *Amlan International, Vernon Hills, Illinois, United States.*

Anti-mycotoxin additives are often included in poultry diets to mitigate the toxic effects of dietary mycotoxins by reducing their absorption by the gastrointestinal tract. Derived from diverse sources and having various traits, prime examples of these additives include thermally activated minerals, yeast and yeast cell wall preparations, products featuring biotransformation enzymes and biologicals and algae-modified clays. A 21-d study evaluated the ability of 4 anti-mycotoxin additives to decrease the toxic effects of concurrent aflatoxins and fumonisins on broiler performance. Five hundred 40 1-d-old male Cobb 500 broiler chicks, with an average body weight of 46.6 g, were randomly assigned to one of 6 treatments: 1) control (non-challenged, non-treated); 2) mycotoxin-challenged control (1 ppm aflatoxins, 100 ppm fumonisins [TOX]); 3) TOX + 0.5% activated calcium montmorillonite (MMT, [Calibrin®-Z, Amlan International, Chicago, IL]); 4) TOX + 0.5% biotransformation product (BIO); 5) TOX + 0.5% yeast cell wall-based product (YCW); and 6) TOX + 0.5% algae-based product (ALG). Each treatment had 9 replicates of 10 chicks/group. Dietary treatments used a corn-soybean meal basal diet that was screened for multiple mycotoxins. Chicks were weighed on d 1, 7, 14 and 21. Body weight gain and feed intake were determined weekly. Feed conversion ratio (FCR) and European Efficiency Index were calculated at 21 d. Data were subjected to one-way ANOVA. Any differences between means were compared by Bonferroni test ($P \leq 0.05$). Survival rates across treatments ranged from 94.4% to 98.9% and were not different ($P > 0.05$). Concurrent dietary exposure to aflatoxins and fumonisins negatively affected broiler feed intake; the broilers consuming the TOX diet averaged 13.4% lower feed intake than the control group ($P \leq 0.05$). Although statistically lower than the control group, the feed intake of the MMT group averaged 10.5% higher than the TOX group ($P \leq 0.05$). Broilers in the control, MMT and BIO groups had greater body weight gain ($P \leq 0.05$) compared with TOX group birds for the 21-d study. Birds in the MMT group had an FCR comparable to those of the control group (FCR: 1.39 and 1.37, respectively; $P > 0.05$) and significantly better than YCW and ALG groups (FCR: 1.47 and 1.47, respectively; $P \leq 0.05$). For the European Efficiency Index, MMT was the only anti-mycotoxin additive to deliver improved performance compared with the TOX group ($P \leq 0.05$). In conclusion, when MMT was included in broiler diets concurrently contaminated with aflatoxins and fumonisins, birds had greater final body weight and improved feed intake compared with birds in the challenged control group, and FCR equal to the control group.

Key Words: anti-mycotoxin additive, concurrent mycotoxins, activated montmorillonite

431P Leukocyte immunoglobulin-like receptors A2 and A6 are modulate Th1-, Th2-, and Th17-type cytokines by stimulating JAK-STAT, NF- κ B, PI3K/AKT, and ERK1/2 MAPK signaling pathways. Yeong Hong*¹, Anh Duc Truong¹, Yeojin Hong¹, and Hyun Lillehoj², ¹*Chung-Ang University, Anseong, Korea (the Republic of),* ²*Animal Biosciences and Biotechnology Laboratory, Beltsville, United States.*

The activating leukocyte immunoglobulin-like receptors (LILRAs) play an important role to control innate and adaptive immune response. However, there is no information on the primary structure and function of LILRA members in avian species including chickens. In this study, we have first cloned, analyzed structure, and characterized the functional of 2 LILRAs (LILRA2 and LILRA6) in chickens. Multiple sequence alignments and construction of a phylogenetic tree of chicken LILRA2 and LILRA6 with mammalian proteins revealed high conservation between chicken LILRA2 and LILRA6, and a close relationship between the chicken and mammalian proteins. The chicken *LILRA2* and *LILRA6* mRNA expression was higher in the small intestine and HD11 cells compared with that of other tissues and cells. To examine the function of LILRA2 and LILRA6 in chicken immunity, *LILRA2*- and *LILRA6*-eGFP recombinant DNA were transfected into HD11 cells. Our results indicated that LILRA2 and LILRA6 are associated with the phosphorylation of Src kinases and SHP2 that play a regulatory role in immune functions. Moreover, LILRA6 associated with and activated MHC class I, β 2-microglobulin, and induced the expression of transporters associated with antigen processing, but LILRA2 did not. Specifically, LILRA2 and LILRA6 induced and regulated the JAK-STAT, NF- κ B, PI3K/AKT, and ERK1/2 MAPK signaling pathways, and upregulated Th1, Th2, and Th17 cytokines and toll-like receptor in HD11 cells. Collectively, both LILRA2 and LILRA6 are essential for macrophage mediated immune responses, and they have the potential to complement the innate and adaptive immune system against pathogens.

Key Words: chicken, LILRA, MHC class I, cytokine, signaling pathway

432P Chicken leukocyte immunoglobulin-like receptor subfamilies B1 and B3 bind with MHC class I and β 2m and activate JAK/STAT signaling pathway. Yeojin Hong*², Anh Duc Truong², Hyun Lillehoj¹, and Yeong Hong², ¹*United States Department of Agriculture, Beltsville, Maryland, United States,* ²*Chung-Ang University, Anseong, Korea (the Republic of).*

The leukocyte immunoglobulin-like receptors (LILR) are a family of receptors possessing extracellular immunoglobulin domains exert immunomodulatory effects on a wide range of immune cells. The leukocyte immunoglobulin (Ig)-like receptor subfamily B (LILRB) acts as an important group of immunoreceptor tyrosine-based inhibitory motifs [ITIMs: I/V/L/S)XYXX(L/V)] containing receptor in their cytoplasmic tail. The present study represents the first description of the cloning and structural and functional analysis of LILRB1 and LILRB3 isolated from 2 genetically disparate chicken lines (Marek's disease resistant line 6.3 and Marek's disease susceptible line 7.2). Amino acid homology and phylogenetic analyses showed that the homologies of LILRB1 and LILRB3 in the chicken line 6.3 to those proteins in the chicken line 7.2 ranged between 97 and 99%. Chicken line 6.3 has 2 ITIM motifs of the sequence LxYxxL and SxYxxV while line 7.2 has 2 ITIM motifs of the sequences LxYxxL and LxYxxV. These motifs bind to SHP-2 that plays a regulatory role in immune functions. Our data indicate that LILRB1 and LILRB3 associated with and activated MHC class I and β 2-microglobulin, modulating the expression of components in the MHC class I pathway and thereby regulating immune responses. Furthermore, LILRB1 and LILRB3 activated JAK2/TYK2, STAT1/3,

and SOCS1 genes of signaling pathway that expressed in HD11 cells, which also induced Th1, Th2, and Th17 cytokines.

Key Words: chicken, LILRB1, LILRB3, MHC class I, cytokines

433P Comparison of upstream regulator analysis of wooden breast proteomics in commercial broilers with breast muscle proteomics in pedigree male broilers exhibiting high feed efficiency.

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The purpose of the present study was to compare global protein expression data in commercial broilers with wooden breast (WB) myopathy with proteomic data of breast muscle from pedigree male (PedM) broilers phenotyped feed efficiency (FE). Shotgun proteomics was conducted on breast muscle obtained at approximately 8 wk of age, flash frozen in liquid nitrogen, and stored at -80 C. Comparison of the data sets was facilitated using the overlay function of a commercial software program, Ingenuity Pathway Analysis (IPA) (Qiagen, CA). For this study, we focused on upstream regulator analysis that provides predictions of activation or inhibition of molecules based on expression of downstream target molecules and on the IPA scientific citation database. Some examples of these comparisons are provided below. Angiopoietin 2 (ANGPT2) exhibited the highest predicted activation Z-score of all molecules in the WB data set but the overlay of the FE proteomics data on the WB ANGPT2 upstream regulator network resulted in a prediction of inhibition. Thus, there appears to be signals to increase vascularization in WB myopathy that were opposite in the high FE PedM broiler phenotype. Whereas the transcription factor peroxisome proliferator coactivator 1 α (PGC1 α) and the closely related PPAR γ were predicted to be activated in the high vs low FE data set, overlay of the networks revealed these molecules were predicted to be inhibited in the WB data set, suggesting that there is a negative impact of WB myopathy on mitochondrial biogenesis. There was also a prediction of inhibition of complex I of the electron transport chain associated with WB which contrasts with activation of Complex I in the high FE PedM broiler. Whereas RICTOR (rapamycin independent companion of mammalian target of rapamycin) was predicted to be inhibited in both WB and FE data sets, the predictions were based on different downstream molecules. These preliminary findings suggest that there are considerable differences in protein expression observed in WB myopathy and in high FE PedM broilers that may provide additional insight into the etiology of WB myopathy.

Key Words: proteomics, woody breast myopathy, upstream regulator analysis, broiler

434P Upstream regulator analysis of proteomic data from wooden breast myopathy.

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A global protein expression study was conducted in breast muscle tissue with and without wooden breast (WB) muscle myopathy (Kuttapan et al., 2017, Poultry Sci. 96:2992–99). In that study, Ingenuity Pathway Analysis (IPA) software was used to organize data into canonical pathways and networks. A component of the program that was not utilized, however, was upstream regulator analysis. In upstream regulator analysis, an activation z score is generated based on expression of downstream

molecules in the data set and on literature citations in the program with similar (or dissimilar) relationships between the upstream regulator and downstream target molecules. The activation z score provides a prediction or inhibition of the upstream molecule and a P value based on Fischer's exact test (P value of overlap) determined. Molecules among those predicted to be inhibited in WB included 3 transcription factors associated with mitochondrial biogenesis; peroxisome proliferator activator receptor gamma-coactivator 1 α (PPARGC1A) with PPARA and PARAG. There were also predictions of inhibition of molecules or complexes associated with the mTOR (mechanistic target of rapamycin) signaling pathway, tuberous sclerosis complex (TSC) and RICTOR (RAPTOR Independent target of mTOR). Interestingly, angiopoietin 2 (ANGPT2) was predicted to be activated in WB myopathy, suggesting that factors favoring angiogenesis (e.g., tissue hypoxia) were present in WB myopathy. However, aryl hydrocarbon receptor (AHR) was predicted to be inhibited in WB. The AHR encoded protein has been identified as a β subunit of hypoxia inducible factor 1. Further examination of these upstream target molecules and their downstream target molecules may help in elucidating fundamental mechanisms of wooden breast myopathy.

Key Words: wooden breast myopathy, broiler, proteomics, upstream regulator analysis

435P Establishing a pulmonary aspergillosis model in SPF

chickens. Nickolas Holznecht*¹, Cord Brundage², and Jill Adler-Moore¹, ¹California State Polytechnic University, Pomona, San Dimas, California, United States, ²California State Polytechnic University, Pomona, Pomona, California, United States.

Objective: Pulmonary aspergillosis is an infection of poultry with limited treatment options. Presently there is no established chicken infection model to test aspergillosis treatments. The goal of this project was to use the immunosuppressant, dexamethasone sodium phosphate (dex s.p.), to establish pulmonary aspergillosis in chickens since poultry are more susceptible to this infection when they are immunosuppressed by environmental stresses. **Experimental design:** SPF male and female, 21 d old chickens were challenged with increasing doses of *Aspergillus fumigatus* along with varying doses of dex s.p. administered at different times before and after fungal challenge. **Materials/Methods:** SPF eggs were incubated for 21 d, hatched and moved to BSL2 rated cages until d20 when they were divided into 4 cages (8 chickens/cage). In the first study, each group was given a different concentration of dex s.p. (5 mg/kg or 8 mg/kg) intramuscularly from d20-d24, challenged d21 with *A. fumigatus* (3.3 or 6.3 X 10⁸ spores) via the nares and eyes, weighed daily, and tissues collected for determination of fungal burden in the lungs. In the second study, groups of chickens (8/cage) were given 8 mg/kg dex s.p. once a day or twice a day from d20-d24, challenged with 7.6 X 10⁸ *A. fumigatus* spores on d21, weighed daily, monitored for disease signs (e.g., lethargy, crop stasis, increased lay down time), until the end of the study and lungs collected for fungal burden d25. **Results:** The Mann-Whitney test was used for statistical analysis of the data. In the first study, birds given 8 mg/kg dex s.p. and 6.3 X 10⁸ spores/chicken had significantly higher cfu/g lung compared with birds given 5 mg/kg dex s.p. and the lower dose of 3.3 X 10⁸ spores/chicken ($P = 0.01$). In the second study, the cfu/g lung were significantly higher ($P = 0.003$) in birds administered dex s.p. twice daily versus once daily treatment (mean 478 cfu/g lung versus mean 154 cfu/g lung) and twice daily treatment also significantly increased disease signs ($P = 0.025$). There was no significant weight loss observed in either study. **Conclusions:** A reproducible model of pulmonary aspergillosis can be produced in 21 day old SPF chickens when the birds are given 8 mg/kg dex s.p.

twice daily, intramuscularly, with a high challenge dose of 6.3 or 7.6 X 10⁸ spores/chicken administered via the nares and eyes. This model can be used for testing the efficacy of aspergillosis treatments, such as anti-fungal drugs or *Aspergillus* vaccines.

Key Words: *Aspergillus*, chickens, dexamethasone, pulmonary, aspergillosis

436P The role of mTOR in *Salmonella* invasion of macrophages. Famatta Perry*, Casey Johnson, and Ryan Arsenault, *University of Delaware, Newark, Delaware, United States.*

Salmonella is capable of exploiting immune responses upon evasion to replicate and establish infection of host cells. We hypothesize that mTOR signaling is targeted by *Salmonella* Enteritidis (SE) and *Salmonella* Heidelberg (SH) in host macrophages to promote invasion. To study the intracellular changes in host during *Salmonella* infections, a kinome peptide array analysis was performed to show the phosphorylation states of peptides in infected chicken HD11 macrophage-like cells. A Western blot was performed at 30 min post-infection (p.i.) to confirm the presence of mTOR and its phosphorylation state during *Salmonella* infections. Metabolic flux assays measuring extracellular acidification rate (ECAR) were performed to determine the effect of *Salmonella* on the rate of glycolysis during infection with SE and SH. Colony counts were performed at 30 min and 2 h p.i. to quantify SE and SH ability to invade HD11s, after treatments with mTOR inhibitor rapamycin and activator MHY1485. P-values reported were less than 0.05 using paired *t*-test for kinome peptide array, Tukey-Kramer comparison for the ECAR results and Bonferroni correction for colony counts. The kinome peptide array showed that at 30 min p.i., phosphorylation of mTOR at residue S2448 was significantly less than control for both strains, while mTOR sites S2446 and S863 were significantly more phosphorylated than control in SE infected cells at 30 min p.i.. The Western blot analysis validated the decrease in the mTOR phosphorylation at S2448 in *Salmonella* infected cells compared with control at 30 min p.i.. ECAR readings at 30 min p.i. showed an increase in glycolysis of SE infected cells and a decrease in SH infected cells. The *Salmonella* counts showed that SE was significantly higher than SH at 30 min p.i.. At 2 h p.i., kinome peptide array revealed increase phosphorylation of S2448 for both SE and SH infected cells. ECAR readings did not show any significant difference in the rate of glycolysis nor was there a difference between 2 h p.i. plate count in SE or SH infections. Rapamycin treatment at 2 h significantly increased the counts of SE in host macrophages ($P=0.014$). In all, kinome data indicated a central role for the mTOR complex 1 in SE and SH infected macrophages at 30 min and 2 h p.i.. The results of the experiments performed at 30 min p.i. suggest that SE is more invasive than SH as shown by the increased pro-inflammatory glycolytic response and increase *Salmonella* counts compared with SH. Inhibition of mTOR during acute SE infection weakens macrophages response to SE infection, thus increasing invasiveness as indicated by rapamycin treated cells infected with SE at 2 h.

Key Words: *Salmonella* invasion, mammalian target of rapamycin (mTOR), immunometabolism, kinome peptide array, chicken

437P Low pathogenic avian influenza virus influences immune response in the digestive tract of mallard ducks. Casey Johnson*¹, Sabarinath Neerukonda², Brian Ladman¹, Bridget Aylward¹, and Ryan Arsenault¹, ¹*University of Delaware, Newark, Delaware, United States*, ²*U.S. Food and Drug Administration, Silver Spring, Maryland, United States.*

Migratory waterfowl are an important reservoir for diseases that can affect agricultural species. Here, we consider the mallard duck as a reservoir of low-pathogenic (LP) avian influenza virus (AIV). Viable influenza virus has been found in duck excreta, therefore it must pass through or replicate in the gut tissue. We hypothesize viral presence in gut tissue elicits immunological responses. Our objective here was to test for immunological responses to viral inoculation in duck gut tissue. We infected mallard ducks with a mallard duck origin H5N1 LP-AIV and collected gut samples, oropharyngeal (OP) swabs, and cloacal swabs 3 and 8 d post infection (dpi). To confirm infection, OP and cloacal swabs were tested for AIV by qRT-PCR. All inoculated OP samples tested positive for virus at both time points. Cloacal samples 3 dpi were negative for virus while 8 dpi samples tested positive. Gut samples were collected and flash frozen in liquid nitrogen before being stored at -80C. Ileum and cecum samples were used for species-specific kinome peptide array analysis. A cross-reactive peptide array was developed for mallard and black ducks, the proteome similarity was significant enough to warrant a single array design for both species. The peptide arrays gave insight into the cellular signaling at the tissue level of the species of interest. Variance stabilization and 2-tailed student's *t*-tests were used for data normalization and to determine significance. qRT-PCR was performed to test for changes in the quantity of key transcripts between the infected and control tissues. Fold-change was determined using $\Delta\Delta C_t$ for qRT-PCR. At 3 dpi, cecal tissues showed differential phosphorylation in the RIG-I signaling pathway and ileal tissues showed differential phosphorylation in the TLR signaling pathway. At 8 dpi, this response was reversed. In ileum, 3 dpi qRT-PCR results showed an increase in expression of pro-inflammatory cytokines IFN α , INF γ , IL-6, and TNF α . An increase in IL-2 and INF α was observed in cecum samples collected 3 dpi. An increase in IL-8 and TGF β was observed in 8 dpi ileal samples, while cecal samples showed an increase in IFN γ , IL-2 and IL-6. We conclude infection with LP-AIV elicits an inflammatory response as measured by kinase activity and gene expression in the ileum and cecum of mallard ducks and this response is dynamic over time.

Key Words: avian influenza, duck, kinome, qRT-PCR, immunometabolism

438P Effect of coccidiosis vaccination and dietary sotonol supplementation on production performance characteristics and immune response of chicks fed wheat based diet. L. Berriman, S. C. Mansbridge, S. P. Rose, and Vasil Pirgozliev*, *Harper Adams University, Newport, Shropshire, United Kingdom.*

The aim of the present experiment was to investigate the effect of coccidiosis vaccination and dietary sotonol (an active component of fenugreek, *Trigonella foenum-graecum*) supplementation on performance, dietary apparent metabolisable energy (AME), nitrogen (NR) and fat (FR) retention, intestinal lesion scores and cytokine gene expression in cecal tonsils of broiler chicks fed wheat based diets. Day-old Ross 308 male chicks were obtained from a commercial hatchery, weighed, and assigned to 48 pens (5 birds per pen) situated in 4 separated rooms (12 pens in a room). Birds were fed 2 mash diets; a control (C) wheat-soybean diet (containing per kg: 210 g crude protein (CP) and 12.95 MJ AME) and C + sotonol (0.2 g/kg feed) (4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one; Sigma-Aldrich Co.). The birds in 2 of the rooms were vaccinated against coccidiosis with Paracox 5 (MSD Animal Health, UK), providing 4 experimental treatments in total. The environment in the rooms followed standard industry recommendations. The weight of the birds and feed intake were recorded on d 0 and 21 d age. At the end of the study one bird per cage was slaughtered, dissected and assessed for intestinal lesions on a 1 - 5 scoring scale. A sample from the left cecal tonsil was

also collected in RNA later and analyzed for the expression of selected cytokines. Performance data were analyzed by split-plot ANOVA in GenStat (18th edition). Lesion score data were analyzed using Pearson chi-squared test to identify associations between treatments and severity of lesion score. Dietary treatments did not affect AME, nutrient utilization and intestinal lesion score severity ($P > 0.05$). Vaccination did not affect feed conversion ratio (FCR) in sotonon fed birds, but reduced FCR in sotonon free diets ($P < 0.05$). The expression (as copy numbers per reaction) of interferon gamma (IFNG) was reduced ($P < 0.05$) by 36% in vaccinated birds. The expression of interleukin (IL) 8 L1 and IL8 L2, was increased ($P < 0.05$) by 121% and 221%, respectively in vaccinated birds, confirming a likely response to vaccination. In conclusion, vaccination did not adversely affect bird growth performance and nutrient availability variables. There is an indication that sotonon may interact with vaccine, affecting FCR, justifying further research.

Key Words: coccidiosis, phytogenic, sotonon, cytokines, chicks

439P Microbiota shifts and histopathology of the turkey ceca during early *Histomonas meleagridis* infection. Elle Chadwick^{*1}, Frank Edens¹, David Ellis², and Robert Beckstead¹, ¹North Carolina State University, Raleigh, North Carolina, United States, ²Smart-Gene, Raleigh, North Carolina, United States.

Turkeys fail to mount a proper immune response to the protozoan *Histomonas meleagridis*, allowing this parasite to infect the ceca, migrate to the liver and cause mortality. It is understood that *H. meleagridis* must have bacteria as a nutrient source and that cecal tissue inflammation does occur. However, current literature neglects the course of early infection. The objective of this study was to determine microbiota and histopathology changes occurring in the ceca during infection before liver necrosis. Turkeys were reared in 3 floor pens until 25 d. The day before inoculation, 5 birds from each pen were euthanized, the ceca and liver subjectively scored for disease signs (0–4 scale), a mid-cecal section was isolated for histology and cecal contents collected. Twenty-four hours later, birds from 2 pens were inoculated cloacally with 100,000 histomonads/ bird. On d 2, 4 and 6 post-inoculation sampling occurred as previously described and samples were sent for histological staining and microbiome analysis. Cecal villi were analyzed for width measurements via H+E staining. Three birds from each time period with the same scores had goblet cells counted (3 counts/ bird) via PAS staining and had their cecal bacteria populations defined using the SmartGene® 16s microbiome analysis platform. Differences in disease signs, villi measurements and goblet cell expression (GCE) were analyzed in JMP Pro 14 with means separated via *t*-test or Dunnett's test ($P \leq 0.05$). Control birds showed no clinical signs of disease and only 1 bird had significantly larger villi width on d 4; all others did not differ from day –1 therefore various measurements were compared with day –1 values. Infected birds showed no disease signs on days –1 and 2 nor did the villi measurements differ. There was a significant increase in GCE between days –1 and 2 ($P = 0.02$). Deferribacteres phyla increased in infected birds. On d 4, infected birds had a significant increase in damage: thickening of the cecal wall gave a score of 1 ($P = 0.019$), the villi had fused precluding counting and GCE was higher ($P < 0.001$). Bacteroidetes phyla decreased while Proteobacteria increased. By d 6, the ceca had a subjective score of 3 ($P < 0.0001$) and the liver had lesions. GCE did not differ from d 4 but was significantly higher than day –1 ($P < 0.0001$). PAS staining indicated granuloma development surrounding *H. meleagridis* cells within the cecal lumen and caseous necrosis was visually apparent. Bacteroidetes population recovered while Deferribacteres decreased. This study indicates that turkeys mount an innate response

via mucin but based on bacterial population shifts, continuous villi destruction and liver damage by d 6 post-infection, other defenses fail.

Key Words: *Histomonas meleagridis*, turkey, ceca, microbiome, histology

440P Dietary raw potato resistant starch supplementation attenuates lipopolysaccharide-induced intestinal inflammatory responses in meat ducks. Weiqiang Bai^{*}, Ying Zhang, Mei Ding, Shi Bai, Ping Wang, Huanwei Peng, and Feng Zeng, *Sichuan Agricultural University, Chengdu, Sichuan, China.*

This study was conducted to investigate the effects of raw potato resistant starch (RPS) supplementation on serum cytokines concentration, intestinal inflammation and barrier function in meat duck challenged with lipopolysaccharide (LPS). A total of 108 1-d-old Cherry Valley meat ducks were randomly assigned to one of 3 treatments (6 pens/ treatment; 6 birds/pen): non-challenged ducks fed a basal diet (control group), LPS-challenged ducks fed a basal diet without RPS supplementation and LPS-challenged ducks fed a basal diet with 12% RPS supplementation. LPS challenge (2 mg/kg body weight) was performed intraperitoneally at 14 d of age, whereas the control group received physiological saline injection. Samples were collected at 4h after LPS injection. Compared with the control group, LPS challenge significantly evaluated ($P < 0.05$) pro-inflammatory cytokine concentration in intestinal mucosa (endotoxin and TNF- α), and 12% RPS administration reversed ($P < 0.05$) LPS-induced increase in pro-inflammatory cytokine concentration in intestinal mucosa. Similarly, LPS-induced tending to alterations in the serum IL-6 concentration ($P = 0.062$) and mRNA abundances of genes related to intestinal inflammation and barrier (claudin-1, $P = 0.082$) were normalized with RPS supplementation. It can be concluded that 12% RPS supplementation could attenuate LPS-induced intestinal inflammatory response and intestinal barrier damage of meat duck aged from 1 to 14 d.

Key Words: lipopolysaccharides, inflammatory responses, intestinal barriers, resistant starch, meat duck

441P Effects of rearing system and anticoccidial drug on growth, slaughter performance, gastro-intestine development and health of broilers. Lei Yan^{*1}, Sha An¹, Zunzhou Lyu¹, Zhengguo Wang¹, He Huang¹, Xin Li¹, Tingting Tang¹, Mingbin Lyu¹, Guilian Zhou¹, and Yuming Guo², ¹Shandong New Hope Liuhe Group Co., Ltd., Qingdao, China, ²China Agricultural University, Beijing, China.

This study was conducted to evaluate the effects of 3 rearing systems (FL: flooring litter rearing, MC: multilayer cage rearing, PN: plastic net rearing) and anticoccidial drug inclusion or not on growth performance, slaughter performance, gastro-intestine development and health of broilers. A total of 2400 one-day-old Ross 308 mixed-sex broilers (1:1 ratio of males and females) were used in a completely randomized design utilizing a 3 × 2 factorial arrangement of treatments, with 12 replicates in each treatment, and 34 in each floor pen, 30 birds in each cage, and 36 in each net pen, respectively. The density of each rearing system was the same (12.5/m²). Birds were fed crumble-pellet diets from d 1–12, and pellet diets from d 13–36. Broiler starter (d 1 to 12), grower (d 13 to 23) and finisher (d 24 to 36) diets were formulated to meet Ross 308 strain recommendations. Effects of treatments were analyzed as a 3 × 2 factorial arrangement by 2-way ANOVA. Experimental data were analyzed using the GLM procedure of SAS 9.3. Results showed that lower ADG (average daily gain), ADFI (average daily feed intake) and FCR (feed conversion ratio) observed for birds reared in the cage than

those of the other 2 systems from 1 to 36 d of age ($P < 0.05$). Anticoccidial drug inclusion in diets decreased ADFI and FCR significantly ($P < 0.05$). On d 37, MC rearing birds had lower breast yield than that of birds on PN and FL rearing ($P < 0.05$). Anticoccidial drug addition in diets increased thigh yield significantly ($P < 0.05$). MC and PN rearing reduced the relative weight of gizzard and increased the relative weight of proventriculus significantly ($P < 0.05$). Compared with FL rearing, MC rearing reduced the relative weight of duodenum, jejunum and ileum ($P < 0.05$). The difference of mRNA expressions of ileum IL-1 β and IFN- γ among 3 systems were significant ($P < 0.05$), and the levels in group FL tended to be higher than that of PN, and the lowest level was in the group MC. Anticoccidial drug decreased the mRNA expressions of TNF- α in ileum ($P < 0.05$). Significant interactive effects were found in gene expressions of ileum IL-1 β and IL-8 between rearing system and anticoccidial drug ($P < 0.05$). It concluded that birds reared in PN had higher body weight. MC birds had poorer breast yield and intestine development, though better FCR. FL rearing appeared to propitious for gastro-intestine development and health. Anticoccidial drug inclusion in diets improved FCR and thigh yield.

Key Words: rearing system, anticoccidial drug, slaughter performance, gastro-intestine development, broilers

442P Evaluation of *in ovo* Bacillus spp. base probiotic administration on hatching cabinet horizontal transmission of virulent E. coli in broiler chickens. Margarita Arreguin-Nava¹, Brittany Mahaffey¹, Callie Selby¹, Christine Voung¹, Kyle Teague¹, Lucas Graham¹, Juan Latorre¹, Xochitl Hernández-Velasco², Billy Hargis¹, and Guillermo Tellez*¹, ¹Eco-Bio LLC, Fayetteville, Arkansas, United States, ²UNAM, FMVZ, Mexico City, Mexico.

This study evaluated the effect of *in ovo* Bacillus spp. base probiotic administration on a virulent *E. coli* seeder challenge model in 2 independent trials. In each trial, 360 18-d-old Ross 308 broiler embryos were candled and randomly allocated into one of 2 groups and *in ovo* treated. Embryos in the control group were inoculated with 0.2mL sterile PBS *in ovo* via injection into the amnion. Embryos in the treatment group were inoculated with 10⁷ cfu/mL of Bacillus spp. base probiotic. Hatchers were housed separately to prevent possible cross contamination between treatments during hatch. On d19 of embryogenesis, seeder embryos (n = 18 seeders/hatcher or 8.45%/hatcher) were *in ovo* inoculated with respective virulent *E. coli*/tetracycline challenge and segregated into mesh hatching bags. (Seeder challenge dose was 4.5x10⁴ cfu/mL *E. coli* + 272 μ g/ml tetracycline.) On d21, dry chicks were removed from hatchers and hatchability was determined. In each trial, 12 chicks per group were euthanized at hatch and at d7 to evaluate the gastrointestinal composition of total Gram-negative or total aerobic pasteurized bacteria respectively. Chicks were weighed and allocated into floor pens (n = 30) and were provided *ad libitum* access to water and a balanced, unmedicated corn-soybean diet. In both trials, hatchability was not affected by the treatment; however, embryos inoculated with the Bacillus spp. base probiotic showed a significant ($P < 0.05$) reduction in the total number of Gram-negative bacteria at day-of-hatch and at d7. Furthermore, a significant increase in total aerobic pasteurized bacteria at day-of-hatch was observed in the 1 Bacillus spp. base probiotic group when compared with control chickens. Probiotic treatment increased body weight at hatch, d 7, and body weight gain day0-d7 when compared with control chickens. These data suggest that *in ovo* administration of Bacillus spp. base probiotic can reduce the severity of virulent *E. coli* horizontal transmission and infection of broiler chickens in the hatching cabinet.

Key Words: probiotic, *Escherichia coli*, *in ovo*, broiler, hatchers

443P Microbiota signatures of viral infections in turkeys and chickens. John Ngunjiri*, Kara Taylor, Daral Jackwood, Michael Abundo, Hana Ji, Linda Michel, Mahesh K. C., Hyesun Jang, Mohamed Elaish, and Chang-Won Lee, *The Ohio State University, Wooster, Ohio, United States.*

Infection with most poultry pathogens occur at body sites that are colonized by commensal microbial communities (microbiota). A pathogen can induce disruption of the baseline levels of microbiota and exacerbate the disease or promote secondary infections. Knowledge of what microbiota changes are associated with pathogen infection can be instrumental in prediction of flock health and production performance. The impacts of 2 poultry viruses on the baseline characteristics of bacterial microbiota in turkeys and chickens were examined in this study. Two weeks-old turkey poults were infected with avian influenza virus (AIV, H5N2 strain) and bacterial microbiota analyzed through 16S rRNA gene sequencing. AIV infection tended not to alter intra-bird (α) diversity of bacteria in the ileum, cecum and trachea, but it triggered a significant increase of species evenness (spread of the bacterial population across the species found in each bird) in the sinus during active virus shedding at 5 d post-infection (dpi). A significant increase of β -diversity (variation of species composition among birds) was observed in the ileum at 5 dpi, and in trachea and sinus at 14 dpi (long after virus clearance). AIV infection also induced changes in the baseline abundance of some core bacterial taxa: Streptococcus decrease and Pseudomonas increase in the sinus, Lactobacillus increase and Burkholderia decrease in the trachea, Enterobacteriaceae increase in the cecum, and Clostridiales decrease in the ileum. In a separate study, 4 weeks-old chickens were infected with either infectious bursal disease virus (IBDV, T1 variant strain) or AIV, or pre-infected with IBDV before superinfection with AIV. At 4 weeks of age (21 dpi of IBDV or 14 dpi of AIV), neither IBDV nor AIV had significant effect on bacterial α -diversity in trachea and sinus. However, the evenness of bacterial species was decreased in ilea of IBDV-infected birds and increased in ceca of birds infected with either or both viruses. β -diversity was unaffected. Changes in the abundance of core bacteria taxa included Streptococcus and lactobacillus suppression in the trachea by AIV, Streptococcus suppression in the sinus by both viruses and enrichment of Clostridiales in ileum by IBDV. Taken together, these data indicate that virus-specific signatures of microbiota disruption were present in the digestive tracts of turkeys and chickens during active virus shedding and long after the viral infection has cleared. Similar findings were observed in turkeys infected with arthritis reovirus. The effects of prolonged virus-induced microbiota disruption on susceptibility of poultry to new pathogens will be examined in our upcoming studies.

Key Words: microbiota disruption, influenza virus, infectious bursal disease virus, chicken microbiota, turkey microbiota

444P Evaluation of *in ovo* administration of a lactic acid base probiotic (LABP) on hatching cabinet horizontal transmission of virulent E. coli in broiler chickens. Margarita Arreguin-Nava³, Brittany Mahaffey¹, Callie Selby¹, Christine Voung¹, Kyle Teague¹, Lucas Graham¹, Juan Latorre¹, Xochitl Hernández-Velasco², Billy Hargis¹, and Guillermo Tellez*¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²UNAM, FMVZ, Mexico City, Mexico, ³Eco-Bio LLC, Fayetteville, Arkansas, United States.

This study evaluated the effect of *in ovo* administration of a lactic acid base probiotic (LABP) on a virulent *E. coli* seeder challenge model in 2 independent trials. In each trial, 360 18-d-old Ross 308 broiler embryos were candled and randomly allocated into one of 2 groups and *in ovo* treated. Embryos in the control group were inoculated with 0.2mL sterile PBS *in ovo* via injection into the amnion. Embryos in the treatment

group were inoculated with 10^7 cfu/mL of a lactic acid base probiotic (LABP). Hatchers were housed separately to prevent possible cross contamination between treatments during hatch. On d19 of embryogenesis, seeder embryos ($n = 18$ seeders/hatcher or 8.45%/hatcher) were *in ovo* inoculated with respective virulent *E. coli*/tetracycline challenge and segregated into mesh hatching bags. (Seed challenge dose was 4.5×10^4 cfu/mL *E. coli* + 272 µg/ml tetracycline.) On d21, dry chicks were removed from hatchers and hatchability was determined. In each trial, 12 chicks per group were euthanized at hatch and at d7 to evaluate gastrointestinal composition of total Gram-negative or total aerobic lactic acid bacteria (LAB). Chicks were weighed and allocated into floor pens ($n = 30$) and were provided *ad libitum* access to water and a balanced, unmedicated corn-soybean diet. In both trials, hatchability was not affected by the treatment; however, embryos inoculated with LABP showed a significant ($P < 0.05$) reduction in total number of Gram-negative bacteria at day-of-hatch and at d7. Furthermore, a significant increase in total LAB at day-of-hatch was observed in the LABP group when compared with control chickens. Probiotic treatment increased body weight at hatch, d 7, and body weight gain day0-d7 when compared with control chickens. These data suggest that *in ovo* administration of a selective LABP can reduce the severity of virulent *E. coli* horizontal transmission and infection of broiler chickens in the hatching cabinet.

Key Words: probiotic, *Escherichia coli*, *in ovo*, broiler, hatchers

445P Effect of lactic acid bacteria probiotic culture on the treatment of *Salmonella enterica* serovar Enteritidis in neonatal turkey poults. Margarita Arreguin-Nava¹, Christine Voung¹, Brittany Mahaffey¹, Callie Selby¹, Kyle Teague¹, Lucas Graham¹, Juan Latorre¹, Xochitl Hernández-Velasco², Billy Hargis¹, and Guillermo Tellez*¹, ¹Eco-Bio LLC, Fayetteville, Arkansas, United States, ²UNAM, FMVZ, Mexico, City, Mexico.

The effect of a *Lactobacillus* spp.-based probiotic on *Salmonella enterica* serovar Enteritidis (SE) recovery was evaluated in 2 separate experiments. For each trial, 40 turkey poults were randomly allocated into 2 treatments: control and probiotic. All poults were challenged with 10^4 cfu SE upon arrival. One hour after SE-challenge, poults were treated with the probiotic culture via oral gavage (10^4 cfu). At 24h post-treatment, ceca/cecal tonsils were aseptically removed for enumeration of enteric SE at 24h post-challenge on xylose lactose deoxycholate (XLT-4). In trial 1, the probiotic significantly reduced the incidence of ceca/cecal tonsil SE 24h following treatment, from 80% in control poults to 35% in treated poults ($P < 0.001$). Administration of the probiotic reduced SE recovered 24h following treatment by 2.71 log₁₀ cfu/g as compared with controls ($P < 0.05$). A similar trend was observed in trial 2, where the probiotic reduced the incidence of SE, from 75% in controls to 25% in treated poults, and a 3.03 log₁₀ SE reduction in treated poults when compared with the control group. In trial 2, control poults exhibited lower proportions of Firmicutes and Bacteroidetes, but the highest proportion of Proteobacteria. In contrast, poults that received the probiotic had the highest proportion of Firmicutes and Bacteroidetes, but lowest Proteobacteria. Results showed that application of this probiotic culture can reduce SE recovery in day-of-hatch turkey poults, although further research is needed to elucidate the mechanism of this response.

Key Words: *Salmonella*, probiotic, lactic acid bacteria, turkey poults, microbiome

446P Forskolin and butyrate act synergistically in protecting chickens from necrotic enteritis. Qing Yang*, Binlong Chen, Kelsy

Robinson, and Glenn Zhang, *Oklahoma State University, Stillwater, Oklahoma, United States.*

Modulating the synthesis of endogenous host defense peptides (HDPs) is emerging as a novel antibiotic alternative approach to disease control and prevention. Here, we investigated the effect of butyrate and forskolin (FSK), a natural labdane diterpene and adenylate cyclase signaling agonist present in the Indian *Coleus* plant (*Coleus forskohlii*), on HDP expression in chicken macrophages. Chicken HD11 cells were stimulated with butyrate, FSK individually or in combination for 24 h, which was followed by treatment of lipopolysaccharide (LPS) for 3 h. Gene expression was then analyzed. One-way ANOVA and Tukey's test were used for data analysis in the current study. Butyrate and FSK showed a strong synergy in *AvDB9* induction in the absence of LPS stimulation ($P < 0.05$). The treatment of butyrate and FSK also synergistically improved the expression of mucin-2 and claudin-1 genes ($P < 0.05$). Moreover, the augmented expression of HDP and barrier function genes by butyrate and FSK were not compromised under LPS-induced inflammation. We further tested the hypothesis that FSK and butyrate have a synergistic effect on the resistance of necrotic enteritis in chickens. Cobb male broiler chickens were fed diets supplemented with sodium butyrate (1 g/kg diet) and FSK containing *C. forskohlii* plant extract (10 mg/kg diet) individually or in combination from d 10. Chickens were challenged with *Clostridium perfringens* through d 14 to 17. Intestinal lesion scoring and intestinal content collection for *C. perfringens* quantification were performed at d 18. Results indicated that the dietary supplementation of butyrate and 10 mg/kg FSK synergistically attenuated lesions in the duodenum and jejunum of chickens ($P < 0.05$). The combination also resulted in reduced *C. perfringens* colonization in the jejunum ($P = 0.08$). Collectively, these results revealed the potential of feeding butyrate and FSK as an alternative to antibiotics for control and prevention of necrotic enteritis and likely other diseases in chickens.

Key Words: antibiotic resistance, antibiotic alternatives, host defense peptide, necrotic enteritis, chicken

447P Effect of probiotics on intestinal villus morphology and mRNA abundance of marker genes for stem and absorptive cells. Meiting Jia* and Eric Wong, *Virginia Tech, Blacksburg, Virginia, United States.*

Probiotics consist of beneficial bacteria that enhance intestinal morphology and function. However, the mechanisms responsible for this effect have not been determined. The objective of this study was to determine the effect of probiotics on intestinal villus morphology and ontogeny of intestinal stem and absorptive cells in broiler chickens. Day of hatch Cobb 500 chicks were randomly divided into 3 groups: Basal diet (Control), basal diet + Probiotic 1, and basal diet + Probiotic 2. Each treatment group contained 6 replicate cages with 11 chicks/cage. At d 2, 4, 6 and 8, all chicks were weighed and one chick from each replicate cage was randomly selected for collection of duodenum, jejunum, and ileum ($n = 6$). Intestinal stem cells and absorptive cells were identified using the RNAscope *in situ* hybridization procedure with probes for olfactomedin 4 (*Olfm4*) and peptide transporter 1 (*PepT1*). Stem cells staining for *Olfm4* mRNA were used to define the crypt. Crypt depth (CD) and villus height (VH) were measured using ImageJ v1.52a ($n = 3$, with an average of 13 to 14 measurements per sample). The mRNA abundance for *PepT1* and *Olfm4* were measured by qPCR ($n = 6$). The body weight, morphology, and mRNA abundance data were analyzed by one-way ANOVA and Tukey's test using JMP Pro v14.0. The significance level was set at 0.05. There was no difference for body weights of the broilers in this study. However, Probiotic 1 increased the VH/CD ratio in the duodenum, jejunum, and ileum at d 4 as well as the jejunum at d 2. In

contrast, Probiotic 2 increased VH/CD in the jejunum and ileum on d 2 and the ileum on d 6. These results show that Probiotic 1 mainly affects intestinal morphology at d 4, while Probiotic 2 affects morphology at early (d2) and later (d6) stages. For gene expression analysis, Probiotic 1 decreased PepT1 mRNA abundance in the duodenum on d 4, but did not affect Olfm4 mRNA abundance; while Probiotic 2 increased both Olfm4 and PepT1 mRNA abundance in the jejunum and ileum on d 4. In summary, on d 4, Probiotic 1 increased VH/CD but did not alter Olfm4 or PepT1 mRNA abundance, whereas Probiotic 2 did not affect VH/CD, but increased Olfm4 and PepT1 mRNA abundance. These results show that these 2 probiotics have different effects on intestinal morphology and expression of marker genes for intestinal stem and absorptive cells.

Key Words: probiotics, Olfm4, PepT1, intestine, in situ hybridization

448P Evaluation of a *Bacillus*-based direct fed microbial on Aflatoxin B1 toxic effects, performance, immunological status and serum biochemical parameters in broiler chickens. Bruno Solis-Cruz¹, Margarita Arreguin-Nava*², Daniel Hernandez-Patlan¹, Juan Latorre³, Xochitl Hernández-Velasco⁴, Rubén Merino-Guzman⁴, Billy Hargis³, Raquel López-Arellano¹, and Guillermo Tellez³, ¹Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autónoma de México, Cuautitlán Izcalli, Mexico, Mexico, ²Eco-Bio LLC, Fayetteville, Arkansas, United States, ³University of Arkansas, Fayetteville, Arkansas, United States, ⁴Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Mexico City, Mexico.

The aim of the present study was to evaluate the effect of selected *Bacillus* spp. as a direct fed microbial (DFM) on aflatoxin B1 toxic effects, performance, as well as biochemical and immunological parameters in broiler chickens. Ninety one-day-old Cobb 500 male broiler chicks were raised in floor pens for a period of 21 d. Chicks were neck-tagged, individually weighted and randomly allocated to one of 3 groups: Negative control (basal feed); Aflatoxin B1 (basal feed + 2 ppm AFB1) and DFM (basal feed + 2 ppm AFB1 + DFM). Each group had 3 replicates of 10 chickens (n = 30/group). Body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were calculated weekly. On d 21, all chickens were bleed, gastrointestinal samples were collected and abdominal immune-related organs were weighed. This study confirmed that 2 ppm of AFB1 causes severe detrimental effect on performance, biochemical, and immunological parameters, generating hepatic lesions in broiler chickens ($P < 0.05$). However, DFM supplementation provided beneficial effects such as the regulation in the intestinal microbiota, gut barrier function, anti-inflammatory and antioxidant activities, as well as humoral and cellular immunomodulation. Data were subjected to ANOVA as a complete randomized design using the General Linear Models procedure, and significant differences among means were determined by using Duncan's multiple range test at $P < 0.05$. Differences among median values of lesion scores of liver histopathological analysis were analyzed with the Mann-Whitney U test, with a level of significance set at $P < 0.05$. The results of the present study suggest that this *Bacillus*-DFM added at a concentration of 10^6 spores/gram of feed can be used to counteract the negative effects that occur when birds consume diets contaminated with AFB1, showing beneficial effects on performance parameters, relative organs weights, hepatic lesions, immune response, and serum biochemical variables, mitigating and decreasing aflatoxicosis problems in the poultry industry, improving food security, public health problems, and economic benefits. Future studies are required to fully elucidate the specific mechanisms by which this *Bacillus*-DFM counteracts the toxic effects of AFB1.

Key Words: direct fed microbial, *Bacillus*, aflatoxin B1, broilers, performance

449P Evaluation of cellulosic polymers and curcumin to reduce aflatoxin B1 toxic effects on performance, biochemical, and immunological parameters of broiler chickens. Bruno Solis-Cruz¹, Margarita Arreguin-Nava*², Daniel Hernandez-Patlan¹, Juan Latorre³, Xochitl Hernández-Velasco⁴, Rubén Merino-Guzman⁴, Billy Hargis³, Raquel López-Arellano¹, and Guillermo Tellez³, ¹Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autónoma de México, Mexico, Mexico, ²Eco-Bio LLC, Fayetteville, United States, ³University of Arkansas, Fayetteville, United States, ⁴Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Mexico City, Mexico.

To evaluate the effect of cellulosic polymers (CEL) and curcumin (CUR) on aflatoxin B1 (AFB1) toxic effects on performance, and the biochemical and immunological parameters in broiler chickens, 150 one-day-old male broiler chicks were randomly allocated into 5 groups with 3 replicates of 10 chickens per pen: Negative Control (feed); AFB1 (feed + 2 ppm AFB1); CUR (feed + 2 ppm AFB1 + Curcumin 0.2%); CEL (feed + 2 ppm AFB1 + 0.3% Cellulosic polymers); and, CEL + CUR (feed + 2 ppm AFB1 + 0.3% Cellulose polymers + 0.2% Curcumin). Every week, body weight, body weight gain, feed intake, and feed conversion ratio were calculated. On d 21, liver, spleen, bursa of Fabricius, and intestine from 5 broilers per replicate per group were removed to obtain relative organ weight. Histopathological changes in liver, several biochemical biomarkers, antibody titers, and muscle and skin pigmentation were also recorded. Data were subjected to ANOVA as a complete randomized design using the General Linear Models procedure, and significant differences among means were determined by using Duncan's multiple range test at $P < 0.05$. Differences among median values of lesion scores of liver histopathological analysis were analyzed with the Mann-Whitney U test, with a level of significance set at $P < 0.05$. Dietary addition of 0.3% CEL and 0.2% CUR separately significantly diminished some of the toxic effects resulting from AFB1 on performance parameters, relative organs weight, histopathology, immune response, and serum biochemical variables ($P < 0.05$); however, the combination of CUR and CEL showed a better-integrated approach for the management of poultry health problems that are related with the consumption of AFB1, since they have different mechanisms of action with different positive effects on the responses of broiler chickens.

Key Words: aflatoxin B1, broiler chickens, cellulosic polymers, curcumin, performance

450P Gut health and growth performance of broiler fed American Cranberry (*Vaccinium macrocarpon*) by-products. Quail Das*^{1,2}, Xianhua Yin², Dion Lepp², Kelly Ross³, Yan Martel Kennes⁴, Hassina Yacini⁴, Massimo Marcone¹, Keith Warriner¹, and Moussa Diarra², ¹University of Guelph, Guelph, Ontario, Canada, ²GRDC, Guelph, Ontario, Canada, ³AAFC, Summerland, British Columbia, Canada, ⁴Centre de Recherche en Sciences Animales de Deschambault, Deschambault, Quebec, Canada.

The objective of the present study was to evaluate the growth performance, intestinal health, and gut microbiota of broiler chickens receiving cranberry by-products through feed. A total of 1680 male day-old broiler Cobb 500 chicks were distributed between 42 floor pens (40 birds/pen) randomly to 6 treatments (7 pens/treatment): negative control (non-medicated basal diet); basal diet supplemented with bacitracin (55 ppm), 2 groups receiving 1% (CRP1) and 2% (CRP2) cranberry

pomaces; 2 groups receiving 150 (COH150) and 300 ppm (COH300) of cranberry ethanolic extracts. All birds were vaccinated against coccidiosis but not given any additional anticoccidials or antibiotics. Performance parameters were measured at d0, d10, d20 and d30 from each pen. At d21, necropsy and scoring of intestinal lesions due to coccidiosis and necrotic enteritis (NE) was performed on 14 randomly selected birds/treatment. Cecal contents and blood serum were collected from all sacrificed birds for 16S rRNA gene sequencing and ELISA for antibodies titers, respectively. Growth performance and relationship between treatments and the incidence of intestinal lesions were analyzed using the one-way ANOVA and the Cochran-Mantel-Haenszel's test, respectively. The 16S rRNA gene sequencing data were analyzed with QIIME and MANOVA. At d 10, the highest bodyweight ($P < 0.05$) was observed in birds fed with CRP2 and both concentrations of COH150 and COH300. At d10 and 30, COH150 and COH300 resulted in a comparable feed efficiency to those induced by bacitracin at 55 ppm ($P < 0.05$). Cranberry pomace 2% seems to promote IgY production slightly similar to bacitracin, compared with control and other treatments at d21 ($P < 0.05$). A lower prevalence ($P = 0.01$) of NE was observed in birds treated with CRP1 (21.4%) compared with bacitracin-treated (42.8%) and control (50.0%). The prevalence (7.1%) of coccidiosis lesion due to *E. maxima* was lower in birds treated with CRP1 than the control (28.6%) and the bacitracin (21.4%) treated birds. The most abundant phyla ($\geq 1\%$) in 21-d old broiler cecal contents were *Firmicutes* (88.4.4%) and *Bacteroidetes* (7.9%), while *Proteobacteria* and *Tenericutes* were present at 1.9 and 1.2% respectively. The population of *Lactobacillus* ($P = < 0.0001$) was significantly higher in CRP2 while that of *Clostridium* ($P = 0.01$) was lower in CRP1 treatment. The highest OTUs of *Lactobacillus agilis* ($P < 0.01$) known to be a probiotic bacterium was found in cranberry pomace and its ethanolic extracts compared with bacitracin treatments. This study demonstrated that cranberry product supplementation improved the intestinal health of broilers while impacting the dynamics of the gut microbiota.

Key Words: cranberry by-products, performance, gut microbiota, immunoglobulin

451P Mode of action of dexamethasone is not microbial dependent. Audrey Duff*¹, Mikayla Baxter², Kaylin Chasser¹, Whitney Briggs¹, Denise Russi Rodrigues¹, Billy Hargis², and Lisa Bielke¹, ¹The Ohio State University, Wooster, Ohio, United States, ²University of Arkansas, Fayetteville, Arkansas, United States.

Enteric inflammation and stress can negatively influence tight junction permeability and predispose broilers to a variety of diseases. Models incorporating poorly digested feedstuffs or feed restriction have been used to simulate intestinal inflammation with great success. We evaluated the effects of dexamethasone (Dex), a synthetic glucocorticoid, in feed to increase gut permeability via stress-mediated mechanisms. While decreases in body weight, lymphocytes, and lymphoid tissue weights correspond to increases in Dex dose, the exact mode of action on gut barrier function is not fully understood. The objectives of the following experiments were to evaluate intestinal permeability and potential role of bacterial populations in broilers administered dietary Dex as measured by fluorescein isothiocyanate-dextran (FITC-d) in serum and bacterial enumeration. All data were subject to an ANOVA model as a completely randomized design with differences between means determined using Tukey's HSD. Exp. 1 treatments included untreated control, Dex (0.29ppm) and BMD50+Dex with treated groups receiving dietary Dex from d7-d14. Serum FITC-d was measured on d7 and d14. On d7, serum FITC-d was lower ($P < 0.05$) in BMD50+Dex than in Dex or Control, while on d14 BMD50+Dex was higher than Dex which was

higher than Control. In Exp. 2 birds were administered Dex (0.57ppm) in feed and/or Baytril (2mg/kg) in water from day-of-hatch (DoH)-d7. Treatments included untreated control, Dex, Baytril and Baytril+Dex, and serum FITC-d, bacterial translocation (BT) to the liver (TSA) and cecal bacteria (MacConkey's) were measured on d7. Serum FITC-d was highest ($P < 0.05$) in both Dex and Baytril+Dex relative to Control and Baytril. No differences were observed in BT to liver, but lactose positive and negative (L+ and L-) bacterial recovery was higher ($P < 0.05$) in both Control and Baytril+Dex. In Exp. 3 groups included untreated control and Dex (0.57ppm) administered via feed d5-12. Bacteria were enumerated from the liver, ileum and ceca on TSA, MacConkey's, and Chromagar Orientation on d12. No differences were observed on TSA for any intestinal section. On Chromagar there was lower ($P < 0.05$) recovery of *E. coli* from the ileum of Dex treated birds, but no differences in Gram-negative bacteria from the liver or ceca. No differences were observed between L+ or L- for any intestinal section except for the ileum of Dex birds which was lower than control. Increases in serum FITC-d in antibiotic treated groups after Dex and variable BT results suggest the action of dexamethasone is not dependent on interactions with the intestinal microbiota. Therefore, further study is needed to reveal the mechanism(s) by which Dex alters intestinal permeability.

Key Words: dexamethasone, leaky gut, stress, glucocorticoid, intestinal bacteria

452P Drinking water temperature effects on laying performance, egg quality, growth hormone and insulin-like growth factor in liver and plasma of laying hens during winter season.

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A study was performed during winter period at the month of November to January to evaluate the effects of drinking water temperature on laying performance, egg quality, growth hormone (GH) and insulin-like growth factor (IGF-1) in liver and plasma. A total 216, 68-wk old Hy-line brown hens were arranged into 3 groups in a completely randomized design. Hens were housed in 3-tier cage and 9 cages were considered as one replicate in the same row (2 hens/cage). Before on set of trial, the egg production of hens was assessed individually and hens with equal production were replaced in each replicate. Three drinking water temperature treatments were 10, 20 and 30°C, respectively. Drinking water was given via nipple lines in which water was constantly circulated from insulated water tanks according to the required temperature. Mean minimum and maximum air temperature was recorded in the house 8°C and 18°C and lighting (16L:8D) was provided throughout the trial. GH and IGF-1 in liver and plasma were analyzed before beginning of the experiment and thereafter at 74 and 80 weeks of age. The experiment lasted for 12 weeks. Data were statistically compared using GLM model of SAS (2009) and Duncan's multiple range test was used to estimate significant differences ($P < 0.05$). Results showed that productive performance parameters egg production, egg weight, daily egg mass, feed intake and feed conversion ratio were not affected at 74 and 80 weeks of age by the variation of water temperature. Egg quality in terms of albumen height, haugh unit, shell weight and shell thickness had not influenced over the entire experimental period due to hens were offered different water temperature. At commencement of study, GH and IGF-1 in liver and serum were not differed each other. Afterward, GH and IGF-1 in liver was found significantly higher at 74 weeks of age as well as same trend was observed in GH and IGF-1 in liver and serum during end of the experiment (80 weeks of age) when hens provided 20°C drinking

water while there was no statistical difference between 10°C and 30°C drinking water group. According the results, providing drinking water temperature during winter period did not affect performance and egg quality in laying hens and the 20°C drinking water has stimulated to secrete GH and IGF-1.

Key Words: water temperature, winter season, performance, growth hormone, laying hens

453P Chinese chives (*Allium tuberosum*) fermentation using autochthonous *Lactobacillus plantarum* toward the production of antimicrobial poultry feed additive. Woo Do Lee*, Soo-Ki Kim, Kai-Min Niu, and Damini Kothari, *Konkuk university, Seoul, Korea (the Republic of)*.

The indiscriminate use of antibiotics in poultry has resulted in a huge economic loss pertaining to the treatment failures due to the emergence of resistant pathogens. Hence, the development of an effective antibiotic alternative without impairing the poultry production is an important area of research. The genus *Allium* exhibits promising antimicrobial activity because of high saponins, phenolics, and organosulfur compounds. Fermentation is an olden technology to improve the biofunctionalities of plant materials. Therefore, the present study aimed at the fermentation of Chinese chives (CC) (*Allium tuberosum*) juice using autochthonous microorganisms toward the development of an effective antimicrobial feed additive for poultry. A total of 9 microorganisms were isolated and identified from CC juice as potential fermentative starter cultures. The isolates were screened based on their antagonistic activities of their cell-free supernatants against poultry pathogens (*Salmonella enteritidis*, *S. paratyphi*, *S. anatum*, *S. typhi*, *S. gallinarum*, *S. pullorum*, *Staphylococcus aureus*, *E. coli*, and *Enterococcus faecalis*) using agar-well diffusion assay. Among them, *Lactobacillus plantarum* was selected based on its distinctive antimicrobial activities against all of the tested pathogens. The optimal fermentation of CC juice (25%, vol/vol) using *L. plantarum* was achieved at 30 degree following 24 h of incubation. The antibacterial activity of CC juice was also investigated before and after fermentation. The data were statistically tested on one-way ANOVA using the SPSS software. The fermentation of CC juice resulted in significantly improved antibacterial activities against all the aforementioned poultry pathogens ($P < 0.05$). The untargeted LC-MS/MS analyses revealed a distinct metabolite compositions for CC juice following fermentation. In conclusion, we construe that CC juice fermentation with *L. plantarum* may offer unconventional alternatives to develop the poultry feed additives with broad-spectrum antibacterial activities. However, many aspects of fermentation parameters, related mechanisms, and the gamut of antimicrobial compounds produced in this bioprocess needs to be explored.

Key Words: Chinese chives (*Allium tuberosum*), fermentation, probiotics, poultry, anti-bacterial activity

454P Cecal microbiota profile of Hawaiian feral chickens and pasture-raised broiler chickens. Kayla Caliboso^{1,2}, Jannel Nanquil^{1,2}, Sudhir Yadav*¹, Helmut Kae², Kabi Neupane², Birendra Mishra¹, and Rajesh Jha¹, ¹*University of Hawaii at Manoa, Honolulu, Hawaii, United States*, ²*Leeward Community College, University of Hawaii, Pearl City, Hawaii, United States*.

The complex and diverse gut microbiota has a significant impact on its host nutrient utilization, intestinal health, and overall performance. This study was conducted to determine the microbiota profile and the abundance of selected bacteria in both feral and pasture-raised broiler

chickens. For this purpose, cecal content from feral and pasture-raised broiler chickens ($n = 7$ each) was collected, and microbial DNA was isolated using a Repeated Bead Beating Plus Column Method. The denaturing gradient gel electrophoresis was used to visualize the amplified V3 region of the 16S rRNA. Further, NextGen sequencing (NGS) was performed to identify the classification and abundance of cecal microbiota. Specific bacteria studied were: *Bacteroides*, *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, *Escherichia*, and *Clostridium*. The abundance of specific bacteria was quantified using qPCR analysis. The NGS result presented around 77,000 reads per sample, with 82% and 43% classification to the genus and species level, respectively. At the phyla level, 93% of bacteria belonged to Firmicutes, Bacteroidetes, and Proteobacteria for both feral and commercial group. In feral group Firmicutes was 55.31%, Bacteroidetes was 32.69%, and Proteobacteria was 7.04%. Whereas in commercial birds, Firmicutes is 63.16%, Bacteroidetes is 24.54%, and Proteobacteria is 5.87%. High Firmicutes to Bacteroidetes gut microbial ratio in the commercial bird is an indicator for increased body fat accumulation. The NGS data also indicate differences with respect to α diversity. The Shannon Diversity index at the species level, ranged from 2.17 to 2.63, and from 2.02 to 2.28 for feral and commercial chicken microbiota, respectively. In conclusion, feral chickens have a greater percentage of the target bacteria - *Bacteroides*, *Bifidobacterium*, and *Clostridium*. These bacteria have different role in the host body such as *Bacteroides*, and *Bifidobacterium* are known as short-chain fatty acid producers where earlier produces more propionate and later produces lactic and acetic acid. Whereas, *Bifidobacterium*, and *Clostridium* deconjugate bile acids, which leads to poor fat emulsification, reduced fat absorption, and storage. Many of the *Clostridium* species are cellulolytic and benefit feral host to degrade cellulose. Within feral chickens, there were a wider variation in microbiota profile than commercial broiler chickens. This could be due to scavenging nature, diverse feed ingredients, and lack of colonization of the feral chicks by maternally derived bacteria, suggesting the role of living condition and diets on the gut microbiota profile.

Key Words: feral chicken, microbiota, NextGen sequencing, pasture raised chicken, qPCR

455P Effect of heat-stress challenge on broiler performance: A meta-analysis approach. Thomas Flécher¹, Marie-Pierre Létourneau-Montminy³, Joris Michiels², and William Lambert*¹, ¹*Ajinomoto Animal Nutrition Europe, Paris, France*, ²*Ghent University, Ghent, Belgium*, ³*Université Laval, Quebec, Canada*.

Heat stress (HS) is a major concern in the poultry sector and will continue to be so due to global warming as well as the development of broiler production in hot climate areas. Many performance studies have been published to assess the effect of HS on broiler performance. HS globally reduces feed intake, feed efficiency and, as a result, growth, and can be detrimental to survival rates. However, there is a wide variation in the results and it appears that various HS challenge protocols are applied in published papers (diverging in ambient temperature (T°), relative humidity (RH), or number of hours of HS per day). A meta-analysis was therefore conducted aiming at 1) quantifying the effect of T° on broiler performance and 2) identifying the potential effect of relative humidity (RH) and type of challenge on the magnitude of the response. Regarding the type of challenge, it is defined as “constant” if the broiler was subjected to more than 18h of high temperature per day. Other challenges are considered as “cyclic.” The database included 83 challenges from 43 papers published between 1992 and 2018. Most trials were conducted during finisher period, between 21 and 42d. One challenge was defined as 2 T° levels: a thermo-neutral group and a HS

group. Dependent variables included ADG, ADFI, and FCR. T° and RH were considered as covariates. The type of protocol applied was computed in the database for each challenge (TYPE = “constant” or “cyclic”). The database included 41 constant and 42 cyclic challenges. Mixed procedure of Minitab software was used to test the effect of T° on performance, with challenge as random effect. According to linear regression equations, ADG decreases by 2.1g ($P < 0.001$, $R^2 = 0.87$), ADFI decreases by 2.3g ($P < 0.001$, $R^2 = 0.93$) and FCR increases by 0.04 ($P < 0.001$, $R^2 = 0.90$) per incremental °C above the thermo-neutral temperature. The effect of the RH level on the aforementioned performance response was studied. Surprisingly, the effect RH as well as the effect of the T°*RH interaction on the performance parameters was not statistically significant but only 51 challenges reported RH values. In addition, an interaction was observed for T°*TYPE on ADFI ($P = 0.009$) and FCR ($P = 0.004$) but not on ADG ($P = 0.270$). ADFI was more affected by constant HS, while FCR was more affected by cyclic HS. This study indicates that the daily gain losses occurring under HS stress challenge are differentially driven by either a reduction of feed intake or a decrease in feed efficiency, depending on the type of HS. Data generated might help to adapt future nutritional solutions to alleviate HS effects for specific conditions.

Key Words: meta-analysis, heat stress, broilers, performance, challenge cyclicality

456P Development of an immunohistochemistry protocol to detect CPA and NetB toxins in intestinal histological sections of chickens infected with *Clostridium perfringens*. Lila Maduro*, Eric Parent, Audrey Charlebois, Marie-Odile Benoit-Biancamano, and Martine Boulianne, *University of Montreal, Saint Hyacinthe, Quebec, Canada.*

Necrotic enteritis (NE) is a lethal intestinal disease of poultry caused by *Clostridium perfringens*. The trend to produce chickens raised without antibiotic has seen an increase in the prevalence of this condition in the past years. If risk factors associated with NE are well understood, and controlled, the pathogenesis is still poorly understood hence the need to develop tools to further investigate this disease. Development of immunohistochemistry (IHC) has been emphasized as a critical issue in histopathology. To run an IHC test, the choice of adequate reagents as well as the close monitoring of each step of the protocol is crucial to achieve reproducible and reliable results. Our objective was to develop and validate an IHC protocol to observe the presence or absence of CPA and NetB toxins in intestinal histological sections of Specific Pathogen Free (SPF) chickens experimentally infected with pathogenic and commensal *Clostridium perfringens* (CP) strains, during a Ligated Intestinal Loop Model. Immunohistochemistry was performed with NetB antibodies (provided by Dr. John Prescott, UofGuelph) and CPA antibodies, produced in our lab after inoculating a rabbit with inactivated CPA. The histological sections revealed the presence of CPA and NetB toxins. The presence of CPA was observed in all CP inoculated segments including commensal strains, while NetB toxin was only observed in the histological sections of the loops inoculated with NetB-positive and pathogenic CP strains. In both cases, staining was mainly located in the intestinal lumen and in the case of pathogenic strains, associated with intraluminal bacterial clusters and villi tips.

Key Words: immunohistochemistry toxins, intestine histological sections

457P Impact of intestinal pioneer colonizers on inflammation in broiler chicks at ten days of age. Whitney Briggs*¹, Denise Russi

Rodrigues¹, Kim Wilson², Audrey Duff¹, Kaylin Chasser³, Johel Bielke¹, Walter Bottje⁴, and Lisa Bielke¹, ¹The Ohio State University, Wooster, Ohio, United States, ²Orffa Inc, Athens, Georgia, United States, ³Ohio State University, Columbus, Ohio, United States, ⁴University of Arkansas, Fayetteville, Arkansas, United States.

Inflammation is a multifaceted process that involves the participation of many proteins and results in activation of the innate and adaptive immune systems. Pro-inflammatory responses are mediated by the regulation of select proteins like angiotensin I converting enzyme (ACE), which promotes the conversion of angiotensin I into angiotensin II, resulting in vasoconstriction. Other proteins like high mobility group box 1 (HMGB1) and poly (ADP-ribose) polymerase 1 (PARP1) can elicit pro-inflammatory cytokine responses to signal responses by antigen presenting cells. Other proteins influenced, such as integrin subunit α 1 (ITGA1) can be upregulated, resulting in the binding with other immune factors to form proteins like the very late antigen 1 (VLA-1), as part of the adaptive immune response. As previously described, intestinal pioneer colonizers can impact the ileal microbiome, as well as the proteome at day of hatch but the impact of these colonizers through d10 has not been reported. At d18 of embryogenesis, broiler eggs were inoculated into the amnion with saline (S) or ~100 cfu of lactic acid bacteria (L), *Citrobacter freundii* (CF) or mixed *Citrobacter* species (C2), and at d10 ileal tissue from 10 chicks per group were collected for proteomic analysis by tandem mass spectrophotometry. Results were analyzed in Ingenuity Pathway Analysis software and protein regulation fold changes examined were expressed as log₂ values relative to S. Differences were considered significant at $P \leq 0.05$, and were presented as fold changes. In L, upregulation of HMGB1 (0.77) and ITGA1 (0.85) were noted, whereas the CF group had a decrease in ACE (-3.32), along with a downregulation of PARP1 (-1.72) and an upregulation of ITGA1 (0.68), while the C2 group had an upregulation of PARP1 (0.49). Upregulation of HMGB1 and ITGA1 in L may be in response to the generation of memory T cells, facilitated by the increase in ITGA1. Downregulation of ACE in CF may be an indicator of increased vasodilation, and increased PARP1 in C2 may indicate pro-inflammatory activity by antigen presenting cells. In relation to previously reported day of hatch proteomic results, L appears to have a continued increased activation of the acquired immune response, whereas CF and C2 suggest, at least low level, continued a chronic pro-inflammatory state.

Key Words: inflammation, immune response, in ovo inoculation, proteomics, pioneer colonizers

458P Exploring spore-former subpopulation in chicken gut microbiota. Bishnu Adhikari*, Deepti Samarth, Jianmin Chai, and Young Min Kwon, *University of Arkansas, Fayetteville, Arkansas, United States.*

Culture-independent method using 16S rRNA gene sequencing has significantly increased our understanding on gut microbiotas. However, it remains unknown how spore-formers are well represented in such analysis, due to the difficulty of DNA extraction from the spores. Many important members of gut microbiota in chickens are spore-formers, including the genera *Clostridium* and *Bacillus*, and the spore-formers might play a crucial role in transmission and stability of microbiota in chickens due to their superior capability to resist harsh conditions. In this study, 14 cecal samples of 41 d old healthy broiler chickens were processed in 2 different ways for 16S rRNA gene profiling to understand the spore subpopulation in the chicken gut: (1) metagenomics DNA was directly used (Direct), and (2) samples were treated with chloroform for 30 min to kill vegetative cells, and the surviving cells (putative spore-formers) were recovered on BHI agar plates supplemented with 0.1%

taurocholate under anaerobic condition and used for DNA extraction (CF-Culture). Statistical significant differences were calculated based on Wilcoxon signed-rank test (α diversity and taxonomic composition) and PERMANOVA (β diversity) at $P < 0.05$. At genus level, 24 genera were recovered from CF-Culture group, while 48 genera from Direct group after filtering out low abundant genera (< 100 total counts). We found that 14 out of 24 and 21 out of 48 genera were previously known spore formers in CF-Culture and Direct group, respectively. There was significant difference in the distribution of spore formers between the 2 groups (CF-Culture, 54.55 ± 2.88 ; Direct, 45.99 ± 0.40 ; $P < 0.05$). *Ruminococcus*, *Anaerotruncus*, *Blautia*, *Butyrivibrio*, *Clostridium*, and *Oscillospira* were common spore former genera in both groups, whereas in CF-Culture group the genera *Bacillus* and *Paenibacillus* were also recovered. Although not significant, *Clostridium* that belong to family Clostridiaceae and Erysipelotrichaceae were enriched in CF-Culture group by 17 and 8 folds, respectively, as compared with Direct group. Interestingly, genera previously known as non-spore formers such as *Lactobacillus*, *Bacteroides*, and *Enterococcus* were also enriched in CF-Culture by 4, 7, and 248 times, respectively, as compared with Direct group. Additional, Direct group revealed the microbiotas with significantly ($P < 0.001$) higher α diversity as compared with CF-Culture group as measured by observed OTUs (314 ± 10.26 vs. 24.30 ± 2.52) metric. Beta diversity analysis also showed clear separation between the 2 groups ($P = 0.001$). The results from this study indicate more diverse genera in chicken gut might have the ability to form spores than previously recognized.

Key Words: chicken, gut microbiota, 16S rRNA gene, culture, spore-former

459P Repeated intraperitoneal exposure to dexamethasone overtly retards growth, gut integrity, blood chemistry and immune organ development in young broiler chicks. Lizza Macalintal*, Kristen Brennan, Tony Pescatore, Tuoying Ao, Mike Ford, and Karl Dawson, *Alltech-University of Kentucky Nutrition Research Alliance, Lexington, Kentucky, United States.*

Exogenous chemical stressor agents including dexamethasone (DEX) have been reported to influence growth performance in growing animals. In the current study, repeated intraperitoneal (IP) exposure to DEX was used to determine the extent at which IP route can influence the wellbeing of young chicks. A total of 120 Cobb male broilers at 1-d of age were randomly divided into 2 groups each consisting of 10 replicate cages with 6 chicks/cage. Birds were equally divided into 2 equal treatments: sham-PBS injected control birds and DEX-injected birds receiving either 1mL of PBS or 2mg/kg BW DEX (in PBS) via IP injection. Birds were fed a corn-soybean meal basal diet through 21d. At d 7, 9 and 12, birds were injected IP with either PBS or DEX. Growth performance was monitored weekly, tissue samples and blood were collected from 2 birds /cage on d 13 and 20. On d 13, 2 birds/cage were randomly selected, and orally gavaged with a marker for intestinal permeability *i.e.* fluorescein isothiocyanate dextran (FITC-D, 4 kDa) at 1.1 mg/ml/bird. Data were subjected to one way ANOVA as a completely randomized design, mean differences were separated using Fisher's protected LSD when $P < 0.05$. Bodyweight, feed intake (FI) and F:G were markedly affected by IP DEX injection ($P < 0.001$). By d21, a 40% reduction in BW and a corresponding reduction in FI (1166.3 vs 703.36 g) was evident. Hypertrophy of the lymphoid organs *i.e.* spleen and bursa were observed in DEX vs PBS birds. Total small intestinal tract is longer in length and significantly lighter in weight in DEX injected birds ($P < 0.001$). The morphometrics of ileum from DEX birds showed a narrower villus surface area ($P = 0.003$). DEX treatment

resulted in enhanced absorption of plasma FITC-D levels 2.5 h. after chicks were treated with oral FITC-D (2.12 vs 1.96 ug/ml), indicating intestinal permeability is compromised. Blood PCO_2 , pH, HCO_3 , BE, TCO_2 , iCa and glucose were all significantly depressed in DEX birds ($P < 0.0001$). Reduction in blood Na level was directly correlated with severe watery diarrhea exhibited by DEX birds ($P < 0.0001$). Hence, birds that were exposed to IP DEX developed acidemia. From these results, it is evident that IP DEX grossly retards growth and development in young birds by not only affecting the immune organ development, small intestine profile including gut barrier integrity but also the acid-base and electrolyte homeostasis. As a result, birds were severely underweight by d21. Therefore, repeated IP exposure of young birds to DEX can be a useful model in diseases and hormone mediated stress related studies to induce physiological changes in chicks where chronic weight loss and gut insult are the focus of interest.

Key Words: dexamethasone, broiler, intraperitoneal, intestinal permeability, FITC-dextran

460P Not Presented

461P Effect of *Bacillus*-DFM (Norum™) on leaky gut, bone mineralization, serum peptide YY concentration and ammonia excretion in turkey poults fed with a rye-based diet. Guillermo Tellez*¹, Jesus Maguey², Matias Michel³, Juan Latorre¹, Margarita Arreguin-Nava⁴, Christine Vuong¹, Billy Hargis¹, and Guillermo Tellez¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²UNAM, Mexico City, Mexico, ³Universidad Nacional del Nordeste, Corrientes, Argentina, ⁴Eco-Bio LLC, Fayetteville, Arkansas, United States.

Rye contains high concentrations of non-starch polysaccharides (NSP), leading to reduced digestibility. Since poultry have little or no intrinsic enzymes capable of hydrolyzing these NSP, exogenous carbohydrases as feed additives are used in an attempt to reduce the anti-nutritional effects of these polysaccharides. Previously, an in vitro study conducted in our laboratory showed that inclusion of certain *Bacillus* direct-fed microbial (DFM) candidates that produce exogenous phytase, lipases, proteases, cellulases and xylanases in high NSP diets significantly reduced both digesta viscosity and *Clostridium perfringens* proliferation. In the present study, rye-based turkey starter diets with or without *Bacillus*-DFM were administered *ad libitum* to day-of-hatch poults to evaluate the effect of this DFM on leaky gut, peptide YY, bone mineralization and ammonia excretion. Day-of-hatch turkey poults were randomly assigned to either a control diet (CON) or a DFM treated diet ($n = 25$ birds/group). At 10 d-of-age, poults in both groups were given an appropriate dose of FITC-d by oral gavage. One hour later, all poults were euthanized. Blood was collected to evaluate serum FITC-D and peptide YY concentrations. Liver samples were aseptically collected to evaluate bacterial translocation. Both tibias were removed for assessment of bone parameters. Poults fed the *Bacillus*-DFM candidate had increased ($P < 0.05$) tibia diameter, breaking strength, ash content, calcium content, and phosphorus content when compared with CON poults. Interestingly, poults fed the DFM showed a significant reduction in Peptide-YY. Furthermore, poults fed with a rye-based diet without DFM showed an increase in serum FITC-d and liver bacterial translocation, accompanied by higher ammonia concentrations in the manure. However, these adverse effects were prevented by the inclusion of selected DFM candidates in high NSP diets.

Key Words: *Bacillus*-DFM, bone mineralization, peptide YY, leaky gut, ammonia

462P Comparative effects of *in ovo* injection of oligosaccharides (xylotriase, xyloetraose, mannotriose, and mannotetraose) on growth performance and gut health parameters of broilers.

Amit Singh*, Utsav Tiwari, Birendra Mishra, and Rajesh Jha, *University of Hawaii at Manoa, Honolulu, Hawaii, United States.*

In ovo feeding of prebiotic is a novel strategy to enhance the gut health of broilers starting early post-hatch. To explore the beneficial role of prebiotic, 4 oligosaccharides (xylotriase, xyloetraose, mannotriose, and mannotetraose) were fed to the embryo in the egg of Cobb 500 broilers and the parameters of growth, cecal fermentation, histomorphometry, and immune-related genes were evaluated. A total of 192 fertilized eggs were divided into 6 treatment groups with 4 replicate trays containing 8 eggs per replicate. Group 1–4 was assigned to the respective oligosaccharides, group 5 to normal-saline (NS) control and group 6 to no-injection control. On 17.5 d of egg incubation, 3 mg of oligosaccharides (except for NS) dissolved in 0.5 mL of 0.85% NS was injected in the amniotic sac of eggs in respective treatments. The chicks were raised for 28 d post-hatch under standard husbandry practices and were fed commercial broilers diet *ad libitum*. The feed intake and body weight were recorded weekly to determine feed conversion ratio and growth performance. Ileal samples were collected on 1, 7 and 28 d for histomorphometric and gene expression analysis. Simultaneously, the cecal digesta were collected for DNA extraction and genomic sequencing, and VFA determination. The hatchability, growth performance, and relative organ weights of breast, drumstick, liver, proventriculus, and gizzard were not different across the treatments ($P > 0.05$). The xylotriase injection increased the total VFA production at 28 d compared with controls ($P < 0.05$). Villus height, crypt depth, and their ratio were not different across treatments at 7 d and 28 d post-hatch ($P > 0.05$). However, the villus height to crypt depth ratio was significantly higher in xyloetraose group compared with controls at 7 d post-hatch ($P < 0.01$). The innate immunity related pro-inflammatory and anti-inflammatory cytokines and adaptive immunity related T-cell and B-cell marker genes were not expressed differently across treatments at 7 d post-hatch. However, on the hatch day, the level of CD3 (a T-cell marker) was increased by xylotriase, while IL-10 (a marker of anti-inflammatory cytokine) was reduced by xylotriase ($P < 0.074$) and mannotetraose ($P < 0.05$) compared with the controls. The results of this study indicate that the effect of these oligosaccharides on ileal mucosa and immunity is transient, but the effect on fermentation is prolonged. Further research on the mechanism and potential of prebiotic products is warranted before their extensive use alone or in combination with other additives as a gut health promoter in poultry.

Key Words: broilers, gut health, immunity, *in ovo*, oligosaccharides

463P Effects of early feeding with resistant starch during post-hatch on growth performance and gut health parameters of broilers. Amit Singh*, Birendra Mishra, and Rajesh Jha, *University of Hawaii at Manoa, Honolulu, Hawaii, United States.*

Early feeding with special diets to chicks immediately after hatch is becoming a popular and effective strategy to influence the overall growth and health of broilers. Resistant starch (RS) refers to the type of starch that resists digestion in upper GIT of host and can be fermented by the lower gut microbiota to produce volatile fatty acids (VFA). This study investigated the effect of feeding RS type 2 (RS-2) and type 4 (RS-4) during post-hatch yolk dependent period on the parameters of growth, cecal fermentation, histomorphometry and immune parameters of Cobb 500 broilers. A total of 300 d-old chicks were randomly distributed to 30 cages, and 5 replicate cages were allocated to each of 6 treatments. The treatments included i) no feed (starved), ii) RS-2, iii) RS-4, iv) 50% commercial feed (CF) + 50% RS-2, v) 50% CF + 50% RS-4 (CF+RS),

and vi) CF (control). The treatment diets were fed for 2 d post-hatch (48 h. \pm 2 h.). Later, all birds were fed the same CF *ad libitum* for 21 d. The feed intake and body weight were recorded weekly to determine feed conversion ratio and growth performance. Ileal samples were collected on 7 and 21 d for histomorphometric and immune-related gene expression analysis. Simultaneously, the cecal digesta were collected for DNA extraction and genomic sequencing, and VFA determination. At 21 d, there was no difference in FCR among treatments ($P > 0.05$), but ADFI and ADG were higher in CF+RS2, CF+RS4 and CF groups compared with starved, RS2 and RS4 groups ($P < 0.01$). There was no difference in terms of ADFI and ADG at 21 d among CF+RS2, CF+RS4 and CF groups ($P > 0.05$). The treatments did not affect total cecal VFA production at 21 d ($P > 0.05$). The ileal histomorphometric analysis at 21 d did not show a significant effect of any treatment on villus height, crypt depth, and their ratio ($P > 0.05$). At 21 d, we did not find any differential expression in the level of innate and adaptive immunity-related genes that were measured for T- and B-lymphocytes, and pro- and anti-inflammatory cytokines ($P > 0.05$). Likewise, we did not find any difference in the ileal histomorphology among treatments at 7 d ($P > 0.05$). However, RS4+CF group had higher expression of CD3 (a T-cell marker) genes while the level of IL-4 was reduced in RS4 and RS4+CF groups compared with control ($P < 0.05$) at 7 d. The results indicate that the effect of early post-hatch feeding with RS on the immunity of chicks is transient. Further research on the use of RS4 as a potential prebiotic that can act as an immunostimulant in broilers is necessary for its effective use in nutritional strategy to modulate the gut health of broilers.

Key Words: broilers, gut health, histology, immunity, resistant starch

464P Not Presented

465P Intradermal injection of lipopolysaccharide and peptidoglycan in chickens results in divergent leukocyte infiltration profiles. Gisela Erf*, Marites Sales, and Chelsea Ellington, *University of Arkansas, Fayetteville, Arkansas, United States.*

Bacterial cell wall products such as lipopolysaccharide (LPS) and peptidoglycan (PGN) of Gram-negative and Gram-positive bacteria, respectively, are known stimulants of inflammatory responses. In chickens, like in mammals, LPS has been shown to activate heterophils and macrophages in culture, increase heterophil levels in the blood, and result in rapid heterophil accumulation at the site of injection. Heterophil infiltration is followed within hours by monocytes, whereas lymphocytes are a minor part of the LPS response. The response to LPS is transient, with heterophils and macrophages reaching peak levels by 6 h and 24 h, respectively, and returning to pre-injection levels by 48 h. Knowledge regarding leukocyte recruitment initiated by PGN is limited. We have developed the growing feather (GF), a skin derivative, as a minimally invasive platform to examine local cellular responses to test-materials injected into the dermis of the GF pulp. Simultaneous injection of multiple GFs of a chicken with test-material, and subsequent periodic collection of GFs for laboratory analysis, provides a window into local activities initiated by test-material, similar to the window into systemic activities provided by periodic blood sampling. Injection of LPS or PGN into the pulp of GFs resulted in leukocyte infiltration profiles in the dermis of PGN-injected GFs that differed ($P < 0.05$) from those observed in LPS-injected GFs. Specifically, GFs (20 per bird) were injected with 10 μ L of LPS or PGN (50 μ g/mL) or vehicle (endotoxin-free PBS) ($n = 6$ chickens per treatment) and GFs collected before (0 h), and at 0.25, 1, 2, 3, 5, and 7 d post GF-injection. At each

time point, pulp cell suspensions prepared from collected GFs were immunofluorescently stained and subjected to leukocyte population analysis by flow cytometry. While the leukocyte infiltration profiles to LPS were as described above, the leukocytes infiltration response to PGN was dominated by mononuclear leukocyte infiltration consisting primarily of T and B lymphocytes. Lymphocyte levels (% pulp cells) in the dermis of PGN injected GFs increased by 6 h, continued to increase to maximal levels by 1 d, and then remained at this level through 5 d. By 7 d post-injection, lymphocyte levels in the PGN injected pulps had dropped to levels still above baseline. This immediate and sustained lymphocyte recruitment to the site of PGN injection was consistently observed independent of the type of chicken used, and when LPS and PGN were injected into different GFs of the same chickens. Current research is focused on gene-expression profiles that may explain the qualitative differences in the types of leukocytes recruited and stimulated by PGN compared with LPS.

Key Words: innate immunity, lipopolysaccharide, peptidoglycan, inflammation, leukocytes

466P Effect of *Eimeria maxima* infection on intestinal permeability and mRNA expression of tight junction genes in meat-type chickens. Samuel Aggrey*, Marie Milfort, Gustavo Schneiders, James Foutz, Ufuoma Sorhue, Alberta Fuller, Ahmed Ghareeb, Jennifer Richter, and Romdhane Rekaya, *University of Georgia, Athens, Georgia, United States.*

Coccidiosis in poultry is caused by protozoa of the genus *Eimeria*. Chickens infected with *Eimeria spp* have impaired nutrient utilization, weight loss and poor feed efficiency. *Eimeria spp* invades the intestinal mucosa and compromises the intestinal barrier. However, the ontogeny of tight junction permeability during *Eimeria spp* infection requires detailed investigation. The objective of this study was to investigate the progression of tight junction permeability and gene expression of tight junction genes when broiler chickens are infected with a pure single oocyst isolate of *Eimeria (E.) maxima*. Two hundred and 20 14d old broilers (Ross708) were divided equally into 2 (treatment and control) groups. The treatment group was infected via gavage with 2×10^5 *E. maxima* sporulated oocysts suspended in water. The control group was infected with water. Five birds each from the treatment and control groups were sampled daily for 10 consecutive days, post infection (dpi). The sampled birds gavaged with 2.2 mg/mL of fluorescein isothiocyanate dextran for the assessment of intestinal permeability. Intestinal tissue samples, fecal oocyst shedding, lesion scores were also collected. We also assessed the mRNA expression of tight junction genes; claudin 1 (CLDN1), CLDN3 and occludin (OCLN) between the treatment and control groups using ileal tissue. The data was analyzed by with one-way ANOVA. Intestinal permeability was significantly increased at 4–6 dpi compared with the control group ($P < 0.0001$), while oocyst shedding assessed by oocyst per gram of feces were significantly increased at 6 (15753 ± 9083) and 7 dpi (64068 ± 38635) when compared with the controls ($P \leq 0.0006$). Macroscopic intestinal lesions were significantly higher from 4 to 7 dpi ($P \leq 0.016$), while microscopical lesions were significantly higher from 2 to 7 dpi ($P \leq 0.016$). The OCLDN1 gene was downregulated in the treatment group from 2 to 3 dpi, and upregulated from 4 to 7 dpi compared with the control group. CLDN3 was downregulated in the treatment group compared with the control group at 3 dpi, and upregulated from 6 to 7 dpi. The OCLN gene was downregulated in the treatment group from 3 to 7 dpi. The data show that opening of the intercellular tight junctions starts at 5 dpi before oocyst shedding and by 7 dpi, the tight junctions of the infected chickens have been restored to that of the controls. The tight junction gene expression changes suggest that the

gap junction repair processes probably begin at 4 dpi. The ontogeny of gap junction permeability during *E. maxima* infection is critical for gut health. Also, proper management especially 5–7 dpi is essential to limit other opportunistic infections leading to necrotic enteritis.

Key Words: gut permeability, *Eimeria maxima*, tight junction genes, oocyst shedding, gut health

467P Dietary spray-dried plasma influences growth performance in *Salmonella*-challenged broiler chicks. Yasin Jababu¹, Candice Blue¹, Devendra Shah², and Yewande Fasina*¹, ¹*North Carolina A&T State University, Greensboro, North Carolina, United States*, ²*Washington State University, Pullman, Washington, United States.*

Reducing intestinal *Salmonella* spp. colonization in live poultry to mitigate transmission to humans, continue to be a priority in the poultry industry. Alternative non-antibiotic strategies such as dietary incorporation of spray dried porcine plasma (SDPP) are constantly being explored. SDPP contain immunoglobulins and glycoproteins that possess antibacterial properties and improve animal growth. A 14-d experiment was conducted to investigate the effect of spray-dried porcine plasma (SDPP) on the growth performance of broiler chicks challenged with nalidixic-acid resistant *Salmonella* Enteritidis (SAL). A total of 180 d-old Ross 708 male chicks were obtained from a commercial hatchery, out of which 20 were confirmed free of our nalidixic-acid resistant SE (marker strain). Remaining chicks were weighed and randomly assigned to 4 dietary treatments in a completely randomized design (CRD). Treatment CX (fed corn-soybean meal (SBM) basal), Treatment SP (fed corn-SBM basal containing SDPP at 30 g/kg diet), Treatment SE (fed diet similar to CX and challenged with SAL at 10^8 cfu / mL at day-old), and Treatment PSE (fed diet similar to SP and challenged with SAL at 10^8 cfu / mL at day-old). Each treatment consisted of 4 replicate pens, each consisting of 10 chicks. Intestinal SAL infection was confirmed and monitored throughout the experiment by enumerating cecal SAL concentration. Body weight (BW), body weight gain (BWG), feed intake, and feed conversion ratio (FCR) were also recorded to assess growth performance. Results showed that compared with SE treatment (5.47 Log_{10} cfu / mL), chicks in PSE had lower (4.48 Log_{10} cfu / mL) cecal SAL concentrations by d 7 ($P < 0.05$), while chicks in CX and SP treatments had negligible levels. There were no differences in BW, BWG, and FI of chicks in all treatments ($P < 0.05$), but differences were observed for FCR. Specifically, FCR of chicks in the PSE treatment (0.85) was similar to those of CX (1.12) and SP (1.12; $P > 0.05$), but superior to that of SE treatment (1.36; $P < 0.05$). It was concluded that SDPP showed potential to reduce intestinal colonization by *Salmonella* Enteritidis and circumvent associated reduction in growth performance.

Key Words: spray-dried porcine plasma, broiler chicks, *Salmonella* Enteritidis, growth performance

468P Assessment of whole blood biochemical and gas parameters using two diagnostic systems in Pekin ducks. Jean-Marc Larivière*¹, Robert Charette², and Daniel Venne³, ¹*ITA La Pocatière, La Pocatière, Québec, Canada*, ²*Demeter Veterinary Services Inc-Brome Lake Ducks Ltd, Knowlton, Québec, Canada*, ³*Scott Hatchery, Scott, Québec, Canada.*

Biochemistry and blood gas has been well documented in chickens and laying hens but very little has been investigated in waterfowls. The purpose of this trial was to establish reference intervals in Peking ducks. A total of 16 Peking ducks (Star 53 medium, Grimaud & Frères) were

blood sampled from wing vein at 21 and 35 weeks of age, respectively. Measurements of blood chemistries and electrolytes, hematology and blood gases were obtained with the I-Stat CG-8+ cartridge (Abbott) and the VetScan© Avian Diagnostic Profile reagent rotor (Zoetis). In the first method, pH, partial pressure of carbon dioxide (pCO₂, mm Hg), partial pressure of oxygen dioxide (pO₂), base excess (BE) BE_{ecf}, HCO₃, total concentration of carbon dioxide (TCO₂), saturation of hemoglobin (sO₂%), sodium (Na, mmol/L), potassium (K⁺, mmol/L), ionized calcium (iCa, mmol/L), Glucose (Glu, mg/dL), hematocrit (Hct % packed cell volume [PCV]) and hemoglobin (Hb, g/dL) were analyzed. Second method established values of aspartate aminotransferase (AST), bile-acid (BA), creatine kinase (CK), uric acid (UA), total calcium (Ca⁺⁺), phosphorous (PHOS), total protein (TP), albumin (ALB), globulin (GLOB), hemolyse (HEM), lipemia (LIP), ictericia (ICT). In addition, birds were individually weighed, body temperature was assessed with an infrared thermography camera, length measurement of wing's feather (mm) and bladder to carcass weight ratio (%) were evaluated. Statistical analysis showed significant differences between some blood biochemistry/gas and age. Finally, information obtained will help to build reference values for optimizing performance and diagnosing disorders in Peking ducks.

Key Words: blood, biochemistry, ducks, hematology, diagnostic

469P Variations of total sulfur amino acid (TSAA) in antibiotic-free and supplemented diets changes gut microbial community and functions in broilers. Sanjay Kumar^{*1}, Pratima Adhikari^{1,2}, Brian Oakley³, and Woo Kim¹, ¹University of Georgia, Athens, Georgia, United States, ²Mississippi State University, Starkville, Mississippi, United States, ³Western University of Health Sciences, Pomona, California, United States.

Commercial broiler diets, based upon soybean meal as the primary protein source, are limited in essential total sulfur amino acids (TSAA). Additionally, there is an escalating consumer pressure to raise chickens without antibiotics (AB) in the US To address this hot-button issue, the poultry industry has gained interest in searching for alternatives to replace AB as feed additives and maintain optimal animal health. Since gut microbial communities play vital roles in broiler health and productivity and nutrient levels may influence gut microbiome in broilers, we investigated the effect of TSAA on cecal microbiome in broilers raised with antibiotic free or supplement diets. A total of 360, one-day old Cobb broiler chicks were randomly distributed to 6 dietary treatments in a 2 × 3 factorial design, with 2 levels of AB (0 and 0.05%) and 3 levels of TSAA either for starter (0.7, 0.8 and 0.9%) or finisher chicks (0.52, 0.62 and 0.72%). Cecal digesta from each replicate (n = 10) were sampled at d21 and 42, and DNA was extracted, amplified and sequenced. Bioinformatic analyses were performed using QIIME, and functional profiles of the inferred metagenome were analyzed using PICRUST. Statistical difference was determined by 2-way ANOVA and PERMANOVA using R-script. Clustering of cecal communities using PCoA showed clear separation of microbial communities based on age ($P < 0.05$) of birds and between low and medium/ high levels of TSAA. At d21, bacterial richness and diversity were higher than at d42 where *Clostridium* cluster XI and *Lactobacillus* were found most abundant. No variability in richness at the genus level was observed with AB and TSAA supplementation. Inter-bird variation for richness was greater at d42 compared with d21. The mean fold difference of richness was 1.5 times greater with low and high TSAA levels, suggesting interactive effects of AB and TSAA in the diet. KEGG function profiles suggest that the cecal microbiome increased glycolysis and energy generation correlated with increased TSAA supplementation at d42. This study enhanced our understanding of microbial dynamics and functions that

are relevant to host nutrition and may help us tailoring alternative strategies for raising birds under AB-free facilities.

Key Words: total sulfur amino acids, cecum, microbiome, broilers, antibiotic

470P Standardized ileal digestible methionine requirement of Ross 308 male broilers from 10 to 21 d of age. Su Hyun An^{*}, HyeIn O, and Changsu Kong, *Kyungpook National University, Sangju, Korea (the Republic of).*

The objective of the present study was to determine the standardized ileal digestible (SID) methionine requirement of male broiler chickens (Ross 308) from 10 to 21 d of age with using different statistical approaches. A total of 288 10-d-old birds (309.9 g ± 1.88) were allocated to 6 dietary treatments in a randomized complete block design with 8 replicate cages per treatment and 6 birds per cage. The 6 experimental diets were formulated based on corn, soybean meal, and synthetic amino acids to contain dietary SID methionine equally increased from 0.30 to 0.55% with consistent ratio of other indispensable amino acids to methionine. Data for body weight gain (BWG), feed intake, and gain to feed ratio (G:F) at 21 d of age were fitted to linear and quadratic response curves using the GLM procedure of SAS. The SID methionine requirements for both BWG and G:F were estimated by one-slope broken-line, 95% of the upper asymptote of the quadratic, and quadratic-broken-line models. There were linear and quadratic increases ($P < 0.05$) of BWG, feed intake, and G:F with increasing dietary SID methionine. The estimated SID methionine requirements based on BWG and G:F were 0.476 ($R^2 = 0.991$) and 0.472% ($R^2 = 0.980$) for one-slope broken-line model, and 0.523 ($R^2 = 0.993$) and 0.513% ($R^2 = 0.984$) for 95% of the upper asymptote of the quadratic model, respectively. For the quadratic-broken-line model, the SID methionine requirement based on BWG was not able to be estimated whereas the requirement for G:F was estimated to be 0.540% ($R^2 = 0.984$). In conclusion, the SID methionine requirements of Ross 308 male broiler chickens fed corn-soybean meal-based diets from 10 to 21 d of age ranged from 0.476 to 0.523% for BWG and from 0.472 to 0.540% for G:F, respectively.

Key Words: amino acids, broiler chickens, methionine, requirement, standardized ileal digestibility

471P L-Methionine decreases oxidative stress and increases egg production in laying hens subjected to heat stress. Sanjay Kumar^{*1}, Hyo-Young Kim², Fernanda Castro¹, Young-Gi Hong², and Woo Kim¹, ¹University of Georgia, Athens, Georgia, United States, ²CJ Corporation, Seoul, Korea (the Republic of).

Heat stress (HS) experienced by poultry birds is an economical and welfare issue for the poultry industry. HS leads to increased generation of reactive oxygen species, which eventually cause oxidative stress and injuries to the cell. High temperature cause HS in the laying hens and lead to decrease egg quality and alters gene expression. In this study, we investigated the effect of total sulfur amino-acids (TSAA) supplemented with 100% L- and D-methionine on egg production and gene expression in pullet hens subjected to chronic cyclic HS. For this experiment, a total of 72 Hy-Line W36 d-old-chicks were allocated in collective cages and distributed in a completely randomized design, with 2 treatments, 6 replications of 12 birds each. The birds were subject to 95°F/7h/d throughout this phase. The main factors were diets and rooms. The dietary treatments used were: T1: 100% TSAA+L-Met; T2: 100% TSAA+D-Met (Hy-Line W36 recommendation). At 6 and 18 weeks, liver and duodenum samples were collected, and total

RNA was extracted from each sample. Stranded sequence libraries for chicken was prepared from total RNA at the Georgia Genomics Facility and paired-end 75 bp reads (PE-75) generated on a single Illumina NextSeq run using a high output flow cell. Read quality of raw and trimmed RNA-Seq data was assessed using FastQC. Trimmomatic was used to remove adapters and quality trim raw read data. Trimmed reads were aligned to the *G. gallus*-5.0 genome using Tophat2. Cuffdiff 2.1.1 was used to detect differential expression (FDR adjusted $P < 0.05$). To characterize functional differences between L- and D-methionine treatments, Gene ontology and KEGG pathway enrichment analysis, implemented in DAVID database (<http://david.abcc.ncifcrf.gov/>) was performed. From liver samples, 260 and 453 genes were identified as differentially expressed ($P < 0.05$), whereas for duodenum samples the DEGs were 1207 and 373 at wk 6 and 18, respectively. The expression of *HSP90*, *STAT5B* and *IGBPF1* genes were increased in laying hens with L-Met after acute heat exposure. An increase in the expression of *HSP90* in the L-Met supplemented group, thus, implies that oxidative stress was more relieve in this group than D-Met supplemented treatment. In addition, upregulation of *STAT5B* and *IGBPF1* enhanced the functions of ovarian. Further, increase *HSP90*, *STAT5B* and *IGBPF1* expression may be related with activation on cell protection and growth to promote egg production under heat stress. Therefore, L-Met may be effective in relieving heat stress in laying hens.

Key Words: heat stress, laying hens, RNA-Seq, L-methionine, oxidative stress

472P Optimizing amino acid density for Ross 708 female broilers grown for a small bird debone market. Craig Maynard*¹, Chance Williams², and Michael Kidd¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²Wayne Farms LLC, Oakwood, Georgia, United States.

With an increase in rearing broilers sex separately to fulfill consumer demands for various products, research elucidating optimal amino acid density for female broilers is needed. Therefore, an experiment was conducted to determine the optimal amino acid density for Ross 708 female broilers reared for a small bird debone market using the blending technique. Four amino acid density diets were created by formulating summit and dilution diets for each phase of a 3 phase feeding program (0 to 18 d starter, 19 to 28 d grower, and 29 to 35 d finisher) that were subsequently batched and blended to create the 4 dietary treatments. Dietary treatments were based on changes in amino acid density where digestible amino acid ratios were maintained and consisted of: Diet 1 with a digestible Lys (dLys) of 1.128, 0.987, and 0.846; Diet 2 with a dLys of 1.164, 1.019, and 0.873; Diet 3 with a dLys of 1.200, 1.050, and 0.900; and Diet 4 with dLys of 1.236, 1.082, and 0.927% in the starter, grower, and finisher diets, respectively. These treatments resulted in feed intake corrected dLys values of 0.958, 0.989, 1.019, and 1.050% for Diets 1, 2, 3, and 4, respectively. Three hundred and 80 4 female broiler chicks were distributed across 32 pens (12 bird/pen) and were randomly assigned to 1 of the 4 treatments. Body weight gain, feed intake, and feed conversion ratio were determined for the entire growout period. At 35 d, all birds were processed for evaluation of carcass traits and to determine body weight CV. All data were analyzed using a one-way ANOVA and means were separated using a Tukey's test where appropriate. No differences were detected in body weight gain, feed intake, or body weight CV for the 0 to 35 d period. Feed conversion ratio was significantly lower ($P < 0.05$) for birds fed Diet 2 compared with birds fed Diet 1, while birds fed Diets 3 and 4 were intermediate ($P > 0.05$). No differences ($P > 0.05$) were detected for any carcass traits except for tender yield. Tender yields were highest ($P < 0.05$) for broilers fed

Diet 3, lowest ($P < 0.05$) for broilers fed Diet 1, and broilers fed Diets 2 and 4 were intermediate ($P > 0.05$). In conclusion, when rearing female broilers for a small debone market, increasing amino acid density may not be necessary to obtain optimal growth performance and satisfactory carcass composition.

Key Words: amino acid, small bird, broiler, performance, carcass traits

473P Optimum dietary level of balanced protein for broilers determined by economic approach. Jefferson Azevedo*¹, Nilva Sakomura¹, Matheus Reis¹, and Rob Gous², ¹Universidade Estadual Paulista—UNESP, Jaboticabal, São Paulo (SP), Brazil, ²University of KwaZulu-Natal, Pietermaritzburg, KZN, South Africa.

Either the excessive supply of dietary amino acids or unbalanced amino acid profile are associated with impairments in the efficiency of protein utilization affecting negatively the performance and economic return. Therefore, the ideal balanced protein (BP) is essential to formulate diets for broilers. The objective of this study was to estimate the optimum balanced protein level in diets for broilers based on economic analysis of the gross margin over feed cost. A dose-response trial was conducted to evaluate the response of male chicks Ross 308 to increase levels of BP, specially the effects on body weight (BW) and carcass weight (CW) at 42 and 56 d old. The BP was defined based on lys and ideal profile recommended by Brazilian Table (2017). The trial was conducted in 4 phases, evaluating 6 BP levels in each phase: starter (1 to 14 d) 14.5, 16.5, 19.4, 22.3, 25.1 and 28.0% of BP; grower (15 to 28 d) 12.3, 14.1, 16.8, 19.6, 22.3 and 25.0% of BP; finisher-I (29 to 42 d) 11.0, 12.6, 15.0, 17.4, 19.8 and 22.3% of BP; and finisher-II (43 to 56 d) 10.4, 11.9, 14.1, 16.4, 18.6 and 20.9% of BP. Diets were produced using the dilution technique, where a summit diet (high-protein) was graded diluted with a low-protein diet. The treatments were randomly assigned to 24 experimental units of 26 birds each, 4 replicates per treatment. The response variables, feed intake (FI), BW and CW were fitted quadratic, quadratic plateau and linear plateau models and the best model to describe the data was chosen by AIC criterion. Gross income (GI), feed costs (FC) and gross margin (GM) were calculated based in local costs to determine the optimum economic level of BP. The maximum profitability depends on how the broilers are marketed (live body weight or parts), considering the prices of 0.83 US\$/ kg of live body weight and 1.07 US\$/ kg of carcass. From 1 to 42 d the optimum BP to maximize the live BW was 1029.3g and 63.3g/lys while to maximize the GM considering the sale of the live bird was 973.9g/BP and 59.3g/lys, to maximize the CW the BP was 1048.1g and 64.7g/lys and 1016.5g/BP and 62.3g/lys to maximize the GM considering the sale of carcass. From 1 to 56 d, the of BP to maximize the live BW was 1697.2g and 97.7g/lys while BP to maximize the sale of the live bird was 1618.4g and 91.54g/lys, the BP to maximize the CW and GM of carcass sale was 1684.1g and 96.6g/lys and 1622.1g and 91.8g/lys, respectively. These results show that is important to consider the sale objective and economic aspects to define the amino acids level in broiler diets.

Key Words: amino acids, economic analysis, ideal protein, lysine, broilers

474P Branched-chain amino acids and α -keto acids blood concentrations in broilers fed different leucine and isoleucine dietary levels. Georg Dusel³, Aude Simongiovanni¹, William Lambert*¹, Jörg Bartelt², and Thomas Flécher¹, ¹Ajinomoto Animal Nutrition Europe, Paris, France, ²Kaesler Nutrition GmbH, Cuxhaven, Germany, ³University of Applied Sciences, Bingen, Germany.

Branched-chain amino acids (BCAA), like others amino acids, are catabolized if present in excess in the blood. BCAA share the same 2 first enzymatic steps: the first one is a reversible transamination reaction by the BCAA transaminase (BCAT) which produces branched-chain α -keto-acids (BCKA, α -ketoisovaleric acid (KIV), α -ketoisocaproic acid (KIC) and α -keto- β -methylvaleric acid (KMV) from valine (Val), leucine (Leu) and isoleucine (Ile) respectively). BCKA are further irreversibly decarboxylated by the branched-chain α -keto acid dehydrogenase (BCKDH) complex. KIC have been described as the regulator of the BCKDH complex activity. Other studies have shown that an oversupply of dietary Leu increases catabolism of the 3 BCAA, and thus decrease Val and Ile availability for protein deposition. Leu interaction with the 2 other BCAA has been extensively studied in pigs (e.g., Witalfsky et al., 2010 and Wessels et al., 2016), but less in broilers. The aim of this study was to investigate the effect of Leu dietary levels in combination with different Ile levels on the BCAA metabolism in broiler. A trial with 108 male Ross 308 broilers was conducted. From d-1 to d-21, the birds were fed a common commercial diet. At d-21, the chickens were randomly allocated to one of the 6 experimental diets in a 3x2 factorial design, including 2 levels of Leu (105 and 140% SD Leu:Lys) and 3 levels of Ile (56, 62, 68% SD Ile:lys). Feed and water were provided *ad libitum*. At d-35, birds were slaughtered and blood samples were taken to analyze BCAA and keto-acid profiles. All data were analyzed by a 2-ways ANOVA with Leu and Ile dietary levels as main factors using SPSS software. Means were separated using the Tukey post-hoc test. Increasing dietary Ile level resulted in an increase of Ile and KMV blood concentrations ($P < 0.05$) whatever the Leu level but did not affect the concentration of Val and Ile in the blood. Surprisingly, increasing SD Ile:Lys dietary level numerically increased KIC and significantly decreased KIV blood concentrations at 105% TD Leu:Lys, but had no effect at 140%. When dietary Leu level was increased, blood Leu concentration increased ($P < 0.05$), while blood Val and Ile concentrations decreased ($P < 0.05$), irrespective of the Ile dietary level. Excessive Leu content reduced also KMV and KIV concentrations especially for deficient Ile diets. A stable pool of KIC with high Leu diet might be explained by its degradation by the BCKDH complex. This study showed that Leu excess decreases Val and Ile availability and confirms the findings from piglet studies. Further research on BCAT and BCKDH complex activity might give more insights on the metabolic faith of BCAA and BCKA in broilers.

Key Words: broilers, branched-chain amino acids, interactions, α -keto acids, blood profile

475P Effect of dietary protein and lysine levels on performance characteristics of female ring-necked pheasant chicks from 0 to 8 weeks of age. Christopher Delfelder* and Richard Beyer, *Kansas State University, Manhattan, Kansas, United States.*

Ring-necked pheasants held for breeding stock or raised for release must be provided appropriate nutrition for survival out in the wild. A trial was conducted to evaluate the effect of graded protein and lysine levels in the starter phase on female pheasant chick performance from 0 to 4 weeks of age and then subsequently measured for compensatory growth during the 4 to 8-week growth period. Pens of pheasants were fed diets with 26% or 28% protein and 3 levels of dietary lysine (1.5, 1.6, 1.7%) for each of the protein levels for the first 4 weeks. At the end of the first 4-week period all birds were weighed and put on a commercial 24% CP chick starter. Hens and feed were weighed again at the end of the second 4-week feed period and any compensatory growth was measured. Birds were placed in Petersime batteries with 8 chicks per pen and 6 replications per treatment for the first 4-week feeding period.

For the following 4-week feeding period the pheasant hens were moved to open floor pens with an outside exercise pen. Throughout the trial birds were allowed *ad libitum* access to feed and water. Mortality, BWG, FI, and FCR were determined for both the 4-week and 8-week feeding periods. Treatments were arranged in a completely randomized design and data were analyzed using the GLIMMIX procedure of SAS (9.4). Statements of significance were compared using least squares means by contrast and considered significant at $P \leq 0.05$. During the 4-week and 8-week starter periods, no significant difference in FI, BWG, or mortality were observed. At 28d of age the 28% CP diet with 1.6% lysine had a significantly lower FCR than the 26% and 28% CP diets with 1.5% lysine levels. No significant difference in FCR were detected at 56d of age. At 28d a significant difference in total average BW was observed between the 26% CP (1.5% lysine) diet and the 28% CP diets with 1.6% and 1.7% lysine levels, as well as the 26% CP diet with 1.7% lysine levels. However, this difference in body weight was not observed in the second phase with the all pens of birds showing similar body weights by 56d of age. The results indicated that hen pheasant chick performance was affected based on starter ration protein and lysine levels during the first 4 weeks of growth but BW and FCR were not significantly affected during the second feed period. Pheasants raised commercially for release programs may be able to utilize compensatory growth patterns to decrease feed costs during long-term holding periods but more research is needed to determine if these growth patterns continue until the birds reach market age.

Key Words: ring-necked pheasant, reduced protein, lysine, compensatory growth, amino acids

476P Meta-analysis of the effect of lowering crude protein in broilers diet: Exploration of the meta-design to identify modulating factors. Angel Alfonso Avila*², William Lambert¹, and Marie-Pierre Létourneau-Montminy², ¹*Ajinomoto Animal Nutrition Europe, Paris, France*, ²*Laval University, Quebec, Canada.*

Reducing dietary crude protein (CP) in broilers is a nutritional strategy that allows to decrease nitrogen excretion. This approach involves environmental, economic, health and welfare issues, all of concern in poultry industry. In practice, the reduction of CP is performed by substituting part of soybean meal, the principal protein source, by crystal amino acids and corn. However, there is no consensus about the possibility to maintain growth performance when broilers receive low CP diets. It was thus hypothesized that other feed components are also modulated and could affect the success of the low CP strategy. Given the large number of publications on CP reduction in broilers, an initial database of 191 publications, reporting 364 trials and 1358 treatments was built. Then, the database has been restricted by keeping experiments where energy and digestible lysine were fixed when CP was reduced leading to 105 trials and 332 treatments. The aim of the present study was to characterize the design of meta-analytic data (meta-design) by studying the impact of reducing dietary CP on other feed components. Diet composition in all nutrients was recalculated based on feedstuff tables. Pearson correlation coefficients were then performed to explore CP associations with feed components. The results confirmed that the main strategy used to decrease CP was the substitution of soybean meal ($r = 0.57$, $P < 0.001$). As expected, low CP diets were negatively associated ($P < 0.001$) with energy:protein ratio ($r = -0.95$), starch content ($r = -0.79$) and corn-soybean meal ratio ($r = 0.31$). Regarding other dietary components, high positive correlations were found between sulfur ($r = 0.71$), dietary electrolyte balance ($\text{Na} + \text{K} - \text{Cl}$; $r = 0.63$), potassium ($r = 0.60$), magnesium ($r = 0.59$), oligosaccharides (sucrose + stachyose + raffinose; $r = 0.57$), sugars ($r = 0.56$), ash (0.49) and CP ($P < 0.001$).

Similarly, moderate positive correlations were observed between phosphorus (0.34), dietary cation-anion difference (Na + K - Cl - S; $r = 0.34$), crude fiber ($r = 0.32$), acid detergent fiber ($r = 0.30$), manganese ($r = 0.29$), and dietary CP ($P < 0.001$). In addition, a negative relationship was found between dietary chloride ($r = -0.25$) and low CP diets ($P < 0.001$). On the basis of meta-design, nutritional strategies that reduce dietary CP increase energy density by rising starch content. Further, macro-minerals and the resulting electrolyte dietary balance are altered as CP decreases. It is therefore important to take into account these aspects when formulating low CP diets.

Key Words: Low crude protein, meta-design, broilers

477P Supplementation of methionine, choline and betaine in Japanese quail diets in production. Almir da Silva, Carlos Rabello*, Bruna Santos, Marcos Santos, Rogerio Silva Junior, and Heraldo Oliveira, *Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brazil.*

The objective of the current study was to evaluate the performance, eggs quality and serum biochemistry profile of Japanese quails fed different Methionine, Choline and Betaine supplementations in diets. A total of 216 quails (63 d old) were distributed in a completely randomized design, 6 treatments, 6 replicates and 10 quails per cage. The treatments were: positive control (PC) – diet attend the nutritional requirements of quails; negative control (NC) – Methionine reduced in 20.5% without DL-Methionine, Choline and Betaine supplemented; NC+Choline – NC plus 1,000 mg/kg of Choline supplemented; NC+0.07% Betaine – 500 and 700 mg/kg of choline and Betaine supplemented, respectively; NC+0.10%Betaine – NC with 300 mg/kg of Choline plus 1,000 mg/kg of Betaine supplemented; NC+0.12%Betaine – NC with 1,200 mg/kg of Betaine supplemented. The performance - feed intake (FI), egg output (EO), egg mass (EM), feed conversion ratio (FCR); egg quality - yolk, albumen and shell, albumen height and shell thickness and serum biochemistry - uric acid, total proteins, albumin, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (AL) were evaluated. The variables were analyzed to test the assumptions of normality of errors and homoscedasticity of variance by the Cramer-von Mises tests. Afterward, the data obtained from the performance was submitted to univariate ANOVA. Data of Serum blood variables were standardized for mean 0 and standard deviation = 1, after that they were submitted to multivariate ANOVA. Means were compared by the Tukey test ($P < 0.05$). There was no difference in FI between treatments ($P > 0.05$). There was difference to EO ($P < 0.05$). NC+0.10%Betaine was higher than NC group and PC in 2% and 5%, respectively. Regarding EM the laying hens fed NC+0.07%Betaine did not differ from the PC group ($P > 0.05$) except for NC that produced 6% less than the others. The FCR (kg/kg) was statistically different ($P < 0.05$) with the lowest value for NC+0.07%Betaine and NC+0.12%Betaine groups not differing from PC. Regarding egg quality, highest yolk weight and the shell percentage ($P < 0.05$) was observed in NC+Choline differing from other treatments. Serum blood variables were not influenced by the treatments evaluated ($P > 0.05$). Betaine and Choline can be utilized in diets for quails in diets 20% deficient in methionine.

Key Words: performance, egg quality, betaine, coline, cistine

478P Update of lysine requirements for broilers. Matheus Reis*, Nilva Sakomura, Bernardo Nogueira, Carolina Nascimento, and Mirella Melaré, *Universidade Estadual Paulista—UNESP, Jaboticabal, São Paulo (SP), Brazil.*

To update the digestible lysine (dLys) requirements for broilers, 3 trials were conducted using male and female broiler chickens (Cobb 500), one for each phase, initial (1–14 d old), grower (15–28 d old), and finisher (29–42 d old). A total of 1280 birds were used, with treatments randomly distributed in 64 experimental units of 20 birds each, totaling 16 treatments of 4 replications each. The experimental diets were formulated based on the dilution technique by mixing 2 isoenergetic feeds, high protein (HP) and nitrogen free (NF), obtaining feeds with the same amino acid profile. Levels of dLys ranged from 0.87% to 1.60% for starter, from 0.78% to 1.75% for grower, and from 0.64 to 1.28% for finisher phase. To prepare counterproof and confirm that dLys were the first limiting nutrient in the feed, the diet with the lower level of dLys were replicated with the addition of L-lysine, to achieve the second level of dLys used. The mean value of dLys intake (g/kg) was considered as an independent variable and body weight gain (BWG) was the dependent variable. Broken-line (BL), Quadratic-Broken-Line (QBL) and Quadratic (QUAD) functions were adjusted to estimate the optimum intake of dLys, according to the response variable. The BL is described as follows: $Y = L + U * (R - X)$, where Y is the BWG (g/day), L is the maximum BWG response (g/dia); U is the slope and R is the ideal intake of dLys (%) for maximum BWG response. Same approach was adopted for QBL as follows: $Y = L + U * (R - X)^2$. The QUAD was adjusted as described: $Y = a + b * X + c * X^2$, where a is the intercept in the x-axis, b is the regression parameter for the linear component, and c is the regression parameter for the quadratic component. Akaike Information Criterion (AIC) were used to evaluate both models. The results of counter proof demonstrate that dLys was the first limiting nutrient in the feed ($P < 0.05$). The AIC indicate a better fit for QBL model, predicting levels of 0.49, 1.73 and 2.61 g of dLys / bird / day for male and 0.49, 1.50 and 1.83 g of dLys / bird /day for female to maximize body weight gain, respectively for starter, grower and finisher phases.

Key Words: dose response, amino acid

479P The effects of supplemental L-isoleucine on egg composition, hepatic attributes and blood parameters in laying hens fed a low crude protein, amino acid balanced diet from 19 to 48 weeks of age. Ilona Parenteau*¹, Marvin Stevenson², Anna-Kate Shoveller¹, John Cant¹, and Elijah Kiarie¹, ¹University of Guelph, Port Perry, Ontario, Canada, ²Halchemix Canada, Port Perry, Ontario, Canada.

Branched-chain amino acids (BCAA) have been implicated in metabolic diseases in humans and are considered novel therapeutic tools for the treatment of fatty liver and improving lean muscle mass. We previously reported that L-Ile supplementation to a low crude protein (LCP) diet fortified with AA (Met, Lys, Trp, Thr and Val) improved breast muscle composition, and that leaner birds had a higher egg mass output. Therefore, the aim of this study was to further investigate the effects of L-Ile supplementation to a LCP, AA-balanced diet on AA partitioning and hepatic health. One hundred and 80 White Shaver laying hens were housed in 30 battery cages (6 birds/cage, $n = 6$) for a period of 30 weeks, in a 2-phase (19–27 wks and 28–48 wks) feeding program. Diets were isocaloric and included a high protein control (HCP; 18 and 16% CP for phases 1 and 2, respectively), and 4 LCP diets (16 and 14% CP for phases 1 and 2, respectively) with graded levels of L-Ile (70, 80, 90, 100% digestible Ile:Lys). Birds had free access to feed and water throughout the experiment. At 29 and 48 wks of age, 2 birds/cage were bled for plasma AA, serum metabolites (uric acid, creatinine, glucose, cholesterol) and serum enzymes indicating liver and tissue damage (alkaline phosphatase [ALP], aspartate aminotransferase [AST], gamma-glutamyl transferase [GGT], glutamate dehydrogenase [GLDH]). Livers were subsequently collected from 1 bird/cage, weighed and analyzed

for fat content using Dual x-ray Absorptiometry (DEXA). Internal egg composition was additionally measured from 2 eggs/cage at the end of every 4-wk period. Data were subjected to 1-way ANOVA in SAS Studio, and orthogonal contrasts were performed to determine HCP vs LCP and linear and quadratic effects of Ile. Hens that were fed the LCP diet with no added Ile laid eggs with larger yolks compared with the HCP control ($P = 0.001$) and exhibited an 11% increase in liver fat ($P > 0.1$), with no differences in liver enzymes ($P > 0.1$). As Ile was added into the diet, the proportion of egg albumen relative to yolk increased ($P < 0.01$) with no significant effects on liver size or composition. At 29 wks, plasma BCAA and serum GGT decreased linearly ($P < 0.05$), AST increased linearly ($P = 0.004$), and ALP responded quadratically ($P = 0.08$) to Ile supplementation. No differences in blood BCAA or liver enzymes were observed at 48 wks, however Glu linearly increased with Ile supplementation, which may be indicative of increased BCAA catabolism. The results from this study suggest that dietary Ile has little effect on hepatic health and function, and likely redirects the utilization and diversion of dietary AA, although more research is needed.

Key Words: low crude protein, laying hens, isoleucine, hepatic health, egg quality

480P Leucine requirement for maintenance determined with rooster. Mirella Melaré*, Nilva Sakomura, Matheus Reis, and Bernardo Nogueira, *Unesp, Jaboticabal, São Paulo, Brazil.*

Leucine is an essential amino acid most related to protein biosynthesis, which reinforces the importance to supply the demand of this amino acid for growing animals. The use of a factorial approach to determine amino acid requirement is an efficient method to estimate the intake of amino acids for poultry. This technique is based on the necessity of amino acid for maintenance and growth. In this sense, an important step toward construction of factorial model is the determination of maintenance for each amino acid, however, there is a lack of information about Leucine in such area. The objective of this study was to determine the requirement of digestible Leucine (dLeu) for maintenance, using roosters in a nitrogen balance trial. A total of 6 treatments (6 levels of dLeu) were randomly distributed in 36 experimental units of one rooster each, performing 6 replications per treatment. Diets were formulated according to dilution technique, blending a high protein diet with a nitrogen-free diet, obtaining 6 crescent levels of dLeu (0.00%, 0.35%, 0.69%, 1.04%, 1.39%, and 1.74%). At the beginning of the trial, roosters were individually weighed and then fed during 5 d, using a standard precision fed (crop-intubation) technique, assuring the consume of 40 g of experimental feed daily. Excreta were collected in trays, weighed, homogenized, dried and ground and the nitrogen contents of excreta and diets were then determined. A linear regression between nitrogen retention (NR) and dLeu intake was performed using the PROC REG from SAS 9.3 software. The equations estimated was $NR = -41.9(\pm 4.7) + 0.6(\pm 0.02) \times dLeu$ (mg / kg of BW / d), using the intake of dLeu per kg of body weight, and $NR = -54.5(\pm 4.9) + 0.6(\pm 0.02) \times dLeu$ (mg / kg^{0.75} of BW / d), using the intake of dLeu per kg of metabolic body weight. The amount of dLeu required to maintain the body at zero NR was estimated as 70 mg/kg of body weight/d and 89 mg/kg^{0.75} of body weight/d.

Key Words: amino acid, nitrogen retention, nitrogen balance

481P Gompertz-Laird model prediction of optimum utilization of methionine and cysteine by French Guinea fowl broilers. Samuel Nahashon*, *Tennessee State University, Nashville, Tennessee, United States.*

This study was undertaken to assess the influence of dietary methionine and cysteine on growth parameters of the French Guinea fowl (FGF), a meat-type variety. In a 3×3 factorial arrangement, 405 1-d-old FGF keets were randomly assigned to experimental diets containing 3 concentrations of methionine (0.50, 0.45, and 0.40%) each in combination with 3 concentrations of cysteine (0.35, 0.40, 0.45%). The diets were isocaloric (3,100 Kcal ME/kg) and isonitrogenous (21% CP) and were fed from hatch to 8 weeks of age (WOA). Each dietary treatment was replicated 3 times with 15 birds per replicate, and both feed and water were provided *ad libitum*. A 23-h light regimen was provided to the birds for the 8 weeks. The experimental birds were evaluated for body weight (BW) and feed to gain (F:G) to determine optimal dietary methionine and cysteine. Using BW and F:G data from hatch to 8 WOA, the Gompertz-Laird growth model was employed to estimate growth patterns of the FGF. Treatment means were compared using the *t*-test. Although mean differences in exponential growth rate were not significant, average BW at inflection point and asymptotic BW were higher in birds fed 0.5–0.45% methionine and 0.45–0.4% cysteine (0.83 kg and 1.56 kg, respectively) than other treatment groups. The F:G was significantly lower ($P < 0.05$) in birds fed diets containing 0.5–0.45% methionine and 0.45–0.4% cysteine at 0–8 WOA. Therefore, based on the Gompertz-Laird growth model estimates, feeding 0.45% methionine and 0.4% cysteine at hatch to 8 WOA can be recommended as adequate and economical for optimal growth of FGF broilers.

Key Words: French guinea fowl, methionine, cysteine, Gompertz-Laird model

482P Characterization of Brazilian soybean quality and effect of the protease CIBENZA® DP100 on broiler digestibility using a commercial soybean meal. Raquel Araujo¹, Juan Ruiz¹, Sandra Rodrigues*¹, Everton Krabbe², Juxing Chen³, and Karen Wedekind³, *¹Novus do Brasil, Indaial, São Paulo, Brazil, ²Embrapa Suínos e Aves, Concórdia, Santa Catarina, Brazil, ³Novus International Inc., St. Charles, Missouri, United States.*

Urease activity (UA) and protein solubility in KOH (SOL) are the most commonly used in vitro quality measurements for soybean (SB) products used as feed ingredients for broilers in Brazil. The analyses of these parameters were reported to be correlated with SB processing parameters that theoretically indicate anti-nutritional factors (ANFs) deactivation such as trypsin inhibitor (TI). The objectives of the study were: 1) determine the levels of TI, UA and SOL in a total of 359 SB samples used by integrators from 4 Brazilian regions and analyzed by 2 laboratories, 2) validate whether UA and SOL are correlated with TI, and 3) evaluate a protease (CIBENZA® DP100, Novus International, Inc. (PRO)) effect on digestibility of a commercial solvent-extracted soybean meal (SBM) high in TI. Results were submitted to ANOVA and means compared by Tukey test. TI in SBM, heat-inactivated full-fat soybean (HIFFS) and full-fat-extruded soybean (FFES) varied from 1.2 to 5.5; 0.9–5.0 and 3.3–10.0 mg/g respectively. UA varied from 0.006 to 0.150 pH unit and solubility from 70.6 to 90.8% in SBM. By region, TI was lower in the SBM used by Southern companies than Northeastern and Southeastern considering laboratory B results ($P < 0.05$). As expected, raw soybean presented the highest TI followed by FFES, SBM and HIFFS ($P < 0.05$) according to both laboratories. TI was poorly correlated with UA ($R^2 = 0.09$, $n = 261$) and SOL ($R^2 = 0.19$, $n = 70$) in SBM. The highest correlation was obtained for TI and SOL in HIFFS ($R^2 = 0.39$, $n = 24$). In addition, a digestibility trial was conducted with 192 Cobb male broilers reared in cages. A common corn-SBM diet was fed to all chicks from d 1 to 21. Test diets (20% CP) containing 0.5% chromium oxide as digestibility marker were fed from

d 22 to 28 and ileal digesta was collected on d 28. Both treatments (with or without 500 g/MT PRO, 12 replicates each) used commercial SBM with TI 4.2 mg/g (solubility 90% and urease 0.06 or 0.01 analyzed by laboratory A or B, respectively) as the unique protein source. Data were submitted to ANOVA analyses. PRO improved apparent ileal amino acid digestibility of all AAs except for Lys ($P = 0.06$), His and Trp ($P > 0.05$). Digestibility was increased by 5.4% for Met, 13% for Cys, 5.1% for Thr and 5.5% for Val. Digestibility coefficient of gross energy increased ($P < 0.001$) from 74.3 to 79.9%. In summary, SOL and UA are inexpensive and easily analyzable to assess SB quality. However, these variables are not correlated to TI which is considered the best in vitro method to predict ANFs levels in SB products. Thus, additional TI analysis is advisable to determine SB quality. PRO was efficacious in ameliorating the negative TI effects of commercial SBM by improving digestibility of AA and energy.

Key Words: trypsin inhibitor, urease activity, protein solubility, amino acids, energy

483P Effect of lipase on the performance of broilers fed reduced energy, protein and amino acid diets. Fernanda Castro^{*1}, Junyong Gong², and Woo Kim¹, ¹University of Georgia, Athens, Georgia, United States, ²Shenzhen Leveking Animal Nutrition Co., Ltd., Shenzhen, China.

The aim of this study was to investigate the effect of lipase (provided by Shenzhen Leveking Animal Nutrition Corp.) on growth performance of broilers fed diets with different energy and protein levels. A total of 480-d-old male chicks (Cobb 500) were distributed randomly in a factorial arrangement 2 by 2, with 6 replicates of 20 birds, allocated to floor pens and grown for 42 d under a normal condition. Nutritional levels and inclusion of lipase were considered the main factors. The treatments consisted of positive control (PC: recommended metabolized energy and crude protein levels – 3010 kcal/kg and 21.33%, 3090 kcal/kg and 19.61%, and 3150 kcal/kg and 19.00% for starter, grower and finisher diets, respectively) and negative control (NC: reduction in 100 kcal/kg ME, 1% CP and 5% Lys, Met, Thr, Met+Cys levels) diets supplemented or not with 100g/T of lipase. At 14, 28 and 42 d, feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were measured. Two-way ANOVA was performed, and no significant interaction was found between the factors for any variable or phase. Thus, means of the main factors were compared by F-test ($P < 0.05$). From 1 to 14 d, BWG and FCR were both affected by nutritional levels, and birds fed PC diet showed the highest BWG and the lowest FCR ($P < 0.001$). Similar result was observed from 1 to 28 d ($P < 0.001$). From 1 to 42 d, FI and BWG were both affected by nutritional levels and the inclusion of lipase. Birds fed PC showed the lowest FI and highest BWG ($P < 0.03$), whereas birds fed lipase showed the highest FI and BWG ($P < 0.01$). The FCR was lower when birds were fed PC compared with NC ($P < 0.001$). In conclusion, the reduction in the nutritional levels led to poor growth performance throughout the experiment. Additionally, considering the overall period (1–42 d), the inclusion of 100g/T of lipase resulted in higher FI and BWG compared with the treatment without the enzyme.

Key Words: lipase, broilers, performance

484P Growth performance, breast yield and gastrointestinal weight in conventional and slow-growing strains of broiler chickens fed corn-soybean meal diet without or with multi-enzyme supplement. Mohsen Mohammadigheisar^{*}, Victoria Shouldice, Stephanie Torrey, Tina Widowski, and Elijah Kiarie, *University of Guelph, Guelph, Ontario, Canada.*

Emerging market differentiation for broiler meat production in terms of conventional (C) and slow-growing (SG) strains is necessitating comparative research on various production aspects of these strains. We investigated effects of dietary multi-enzyme supplement (MES) on growth performance, breast yield and gastrointestinal (GIT) weight in C and SG strains of broiler chickens. A total of 640 1 d-old chicks of 4 strains (C and 3 SG: F6, J10, and N14) were housed in metabolic cages (10 birds/cage). The strains differed by time they took to reach 2.2 kg BW corresponding to 37, 43, 47, and 50 d for C, F6, J10, and N14, respectively. An antibiotic-free corn soybean meal-basal diet was formulated for a 2-phase program (starter and grower), with 3040 and 3090 kcal/kg ME, respectively. Half the birds per strain received the basal diet with 0.04% MES containing phytase, protease, cellulase, β -glucanase at 4,000 FYT/g, up to 15,000 PRT/g, 213 U/g and 186.7 U/g, respectively. Diets were allocated by strain, creating a 4x2 factorial arrangement of treatments ($n = 8$). Birds were allocated equal amount of feed in each phase (starter, 476 g; grower, 1360 g) and had free access to water. Birds were sacrificed at the end of the trial for breast, gizzard, small intestine and ceca weights. Strains C and F6 took 28 d to finish feed allocation whereas strains J10 and N14 took 31 d. Hatch BW was used as covariate in growth data analyses. There was no interaction ($P > 0.10$) between strain and diet on growth, breast yield and GIT weight. There was strain effect ($P < 0.01$) on BW, BWG, and FCR throughout the experiment. Overall, C (1.45) and F6 (1.70) birds showed better FCR than J10 (1.93) and N14 (2.08), with J10 being better than N14. A diet effect was only observed in grower phase; birds fed MES had higher BWG (713 vs. 684 g/d, $P = 0.03$) than birds not fed without MES. Subsequently, birds fed MES had a tendency for higher final BW (1,104 vs. 1,072 g, $P = 0.08$) compared with the control. There was no ($P > 0.10$) MES effect on FCR. Strain C and F6 had higher ($P > 0.01$) breast yield compared with strains J10 and N14 which were in turn had similar ($P > 0.10$) breast yield. Gizzard and ceca (g/kg BW) weights were higher ($P \leq 0.02$) for strain N14 compared with strain C while strains J10 and F6 were intermediate. Birds fed MES had 8.8% ($P = 0.02$) heavier gizzard compared with birds not fed MES. The data indicated that SG strains (J10 and N14) gained less on allocated feed allowance but also exhibited poorer feed efficiency and breast yield linked to heavier GIT mass. The MES improved growth and gizzard weight independent of strain, indicating effects of feed enzymes are not influenced by genetic background of broiler chickens.

Key Words: conventional broilers, enzyme, growth performance, slow-growth broilers

485P Effect of graded levels of supplemental phytase in wheat-based diets on phosphorus retention and phytate degradation in young turkeys. Vasil Pirgozliev^{*1}, S. Rose¹, C. Brearley², and M. Bedford³, ¹Harper Adams University, Newport, Shropshire, United Kingdom, ²University of East Anglia, Norwich, Norfolk, United Kingdom, ³AB Vista, Marlborough, United Kingdom.

This experiment is focused on the effect of graded levels of dietary phytase on phosphorus retention (PR), and phytate degradation as determined by measurement of inositol-phosphate (IP) isomers and inositol (INS) in the excreta of female turkeys fed wheat-based mash diets from 71 to 77 d age. Dietary N-corrected apparent metabolizable energy (AMEn) was also determined. A basal diet, negative control (NC), with reduced available P (calculated to be 0.53%; determined 0.6%) and Ca (calculated to be 1.12%; determined 1.62%) compared with the breeders recommendations was prepared. The basal diet was then split in 5 parts and phytase enzyme (Quantum Blue 5G, ABVista, UK) was added at 0, 100, 1000, 10000 and 100000 FTU per kg diet respectively.

Female BUT Premium turkeys were obtained from a commercial hatchery (Faccenda Foods Ltd., Dalton, UK) at day old and were placed in a single floor pen and fed on a proprietary wheat–soybean turkey feed until 70 d of age. At 71 d age 2 birds were randomly allocated to one of 30 raised-floor pens with 0.36 m² floor area and given the experimental diets. Each diet was fed to 6 pens following randomization. Access to the feed and the water was *ad libitum*. The droppings were collected for the last 4 d of the study from 74 to 77 d age, freeze-dried, milled and analyzed for marker (titanium dioxide), gross energy, N, total P and IP concentration. There was a positive linear relationship between phytase activity and dietary PR and NR ($P < 0.05$), although the relationship with AMEn and INS was curvilinear ($P < 0.05$). There was a negative curvilinear relationship between phytase activity and excreta IP isomer concentrations ($P < 0.001$), as the levels increased up to a maximum and then decreased. These results indicate that phytase supplementation alters the degradation of dietary phytate, and that increasing activity increased the PR, NR and AMEn. Research including different phytase activities and/or a combining enzymes, may provide further information on phytate degradation.

Key Words: phytase, inositol phosphates, turkeys

486P Withdrawn

487P Effects of phytase supplementation on performance, plasma mineralization and intestinal phytase activity of broiler fed high rice bran and low available phosphorus. Yuwares Ruangpanit^{*1}, Attawoot Phaiboon¹, Pavatpong Noilert², Bongkot Topan², and Kwanticha Prommetta¹, ¹Kasetsart University, Kamphaengsaen, Nakhon Pathom, Thailand, ²Animal Supplement and Pharmaceutical Co., Ltd., Bangkok, Thailand.

Feeding high level of rice bran is considered a challenge since it contains high level of phytate. A study was conducted to evaluate growth response, plasma Ca and P and intestinal phytase activity of broilers fed high rice bran (11%) diets containing low avP supplemented with phytase (PhySnap[®], Animal Supplement and Pharmaceutical Co., Ltd., Thailand). One thousand 4 hundred and 40 male broilers (Ross 308) were divided into 4 dietary treatments using Completely Randomized Design. The diets were 1) PC; normal avP with no phytase, 2) NC; 0.18% avP reduction with no phytase, 3) NC + phytase 500 FTU/kg and 4) NC + phytase 1000 FTU/kg. Each treatment consisted of 12 replications with 30 birds each. All birds were raised in an evaporative cooling house for a period of 39 d. Water and feed in pellete form were provide *Ad libitum* throughout the experiment. Broiler fed diet containing low avP had a reduction in body weight gain, feed intake and carcass yield but not FCR. Phytase supplementation at both levels improved body weight gain, feed intake and carcass yield of broilers fed low avP. Feeding low avP diet increased Ca and decreased P concentrations in plasma ($P < 0.01$). However, these negative effects were eliminated by phytase supplementation. Phytase activity in duodenum, jejunum and ileum of PC and NC groups was not difference ($P > 0.05$). An increase of phytase level in the diets significantly increase intestinal phytase activity ($P < 0.01$). These results indicated that the supplemented phytase had capability to act in broiler gut which could led to an improvement in growth performance, carcass traits and plasma mineral content of broiler fed diet containing low avP and high level of rice bran

Key Words: broiler, phytase, plasma Ca and P, phytase activity, rice bran

488P Acid and alkaline protease combination on broiler performance. Thiago Ribeiro^{*1}, Andréia Massuquetto¹, Matias Appelt¹, Meghan Schwartz², Daniel Monteiro¹, and Sebastiao Borges¹, ¹Tec-tron Innovation and Technology, Toledo, Paraná, Brazil, ²Schwartz Consulting Services, Louisburg, North Carolina, United States.

This study investigated the effects of the supplementation of an acid (*Aspergillus niger*) and an alkaline (*Bacillus subtilis*) protease combination in pelleted diets on broilers performance. In total, 320 male broilers (Cobb 500) were housed in metabolic cages from 1 to 35 d of age. Birds were distributed according to a completely randomized design in a 2 × 2 factorial arrangement of treatments with 2 dietary crude protein (CP) and metabolizable energy (ME) levels, and presence or absence of a combination of acid and alkaline proteases (0 or 125 g/ton for 2,500 U/g), with 4 treatments and 8 replicates of 10 birds each. The treatments were (PC) a positive control basal diet containing corn and soybean meal, formulated to meet nutrient requirements of Cobb 700 broilers; (NC) a negative control formulated to a lower nutrient specification (5% reduction in CP and 20 kcal/lb in ME); PC + 125g/t of the proteases blend; and NC + 125g/t of the proteases blend. All birds and feed remaining in the feeders were weighed by repetition, to determine the average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). The data were submitted to ANOVA analysis and significant means were compared by Least Significant Difference ($P < 0.05$). There were essentially no differences that could be associated by interaction ($P > 0.05$) between the different nutritional levels and enzyme addition. There was no difference on ADG or ADFI of the broilers fed different nutritional levels nor protease supplementation ($P > 0.05$). There was noted a trend on FCR of the birds fed NC, that had higher FCR than PC, 1.58 and 1.54 respectively ($P < 0.08$). There were also a trend in the inclusion of protease blend that reduced the FCR in comparison to the NC, 1.54 and 1.58 respectively ($P < 0.06$). There are several numbers of potential modes of action that can explain the beneficial effects of proteases. Proteases may reduce the requirement of amino acids and energy, improve the digestibility of dietary protein, hydrolyze protein-based anti-nutrients etc. Some evidences in the literature can indicate that proteases may improve the performance of the birds receiving or not diets with low-crude protein and amino acids. Furthermore, the combination of proteases can amplify the protein utilization at different pH conditions along the gastrointestinal tract of broilers. In the conditions evaluated, the inclusion of 125g/t of the blend of acid and alkaline proteases improve the feed conversion of 35-d-old broilers.

Key Words: amino acid digestibility, exogenous enzymes, metabolizable energy, protein, poultry

489P Comparative study to evaluate different phytase enzyme sources on growth performance, meat quality and serum biochemistry of broilers fed with deficient phosphorus and calcium. Kyeong Seon Ryu^{*1}, Chun Ik Lim¹, Md. Masud Rana¹, and Kostas Stamatopoulos², ¹Chonbuk National University, Jeonju, Korea (the Republic of), ²DSM Singapore PTE LTD, Singapore, Singapore.

The aim of the study was to investigate the effects of 2 phytase products on growth performance, meat quality and serum biochemical indices of broilers fed diets with required or low available phosphorus (AP) and calcium (Ca). A total of 800 broiler chicks (Cobb 500) were randomly allotted to 8 experimental groups with 10 replicates and 10 birds in each replicate. Treatment consisted of a positive control (PC) diet and a negative control (NC) diet. The positive control (PC) diet was formulated to contain 0.97, 0.88 and 0.79% Ca; and 0.45, 0.40 and 0.35% AP in pre-starter (0–11 d), starter (12–25 d) and grower (26–42 d) diets, respectively. The negative control (NC) diet was formulated

with reduced by 0.17% Ca and 0.15% AP from the pre-starter, starter and grower diets of PC. The NC diet was sub-divided into 6 treatments and supplemented with 2 sources of phytase: at the inclusion levels of 600, 1200, 1800 g/ton feed (Sunphase, Sunhy Biology Co; Ltd.) and 100, 200, 300 g/ton feed (Ronozyme HiPhos GT, DSM Nutritional Products), respectively. Data were analyzed by one-way ANOVA using the general linear model of SAS (SAS 9.1, 2009). Body weight gain (BWG) was significantly decreased in birds fed NC diet during pre-starter ($P < 0.01$) and starter ($P < 0.05$) period. Supplementation of both phytase enzymes with different inclusion levels to the NC diet had significantly improved BWG which was similar to that of PC group. At the period of 26–42 d and 0–42 d, BWG ($P < 0.01$) and feed intake (FI, $P < 0.05$) were significantly affected among the treatments and depressed in NC diet without phytase group. The NC diet with different concentration of both phytase had ameliorated these negative effects. However, in the overall study, the BWG and FI were observed maximum in birds fed NC diet with phytase at 300 g/ton of feed group. No differences of dietary treatments were observed on breast muscle color, pH value, drip loss, cooking loss and shear force ($P > 0.05$). At 42 d of age, serum IL-2 ($P < 0.02$) and IL-6 ($P < 0.03$) were significantly lower in birds fed NC diet while inclusion levels of both phytase enzymes were added to the NC diet significantly influenced IL-2 and IL-6 concentration. Phosphorus ($P < 0.03$) and calcium ($P < 0.01$) concentration in serum was significantly enhanced by increasing both phytase inclusion levels with NC diet than birds fed unsupplemented groups, as well as plasma inositol was tended to be higher ($P < 0.09$) in phytase treated groups compared that of PC and NC treated group. It can be concluded that phytase supplementation can alleviate the negative impact of nutrient density (AP and Ca) and enhance broiler performance as well as increase plasma biochemical retention of broilers.

Key Words: phytase, calcium, available phosphorus, performance, broiler chickens

490P Effects of multi-enzyme supplement on growth performance in broiler chickens and turkey poults fed corn or wheat-based diets formulated with or without respective fibrous co-products. Juan Sanchez*¹, Rob Patterson², and Elijah Kiarie¹, ¹University of Guelph, Guelph, Ontario, Canada, ²Canadian Bio-Systems Inc., Calgary, Alberta, Canada.

The high content of anti-nutritive fibrous and recalcitrant protein fractions in co-products from the biofuel and milling industries can negatively impact bird growth and feed efficiency. Although feed enzymes can mitigate these negative effects, such enzymes should be able to hydrolyze large array of indigestible components that exists in feedstuffs. Moreover, there is dearth of comparative research on broilers and turkey fed fibrous feed feedstuffs and the impact of feed enzymes. The present study investigated interactive effects of grain (corn or wheat), fiber (low or high), and multi-enzyme supplement (MES) in broiler chicks (Exp. 1) and turkey poults (Exp. 2). High fiber diets were created by adding corn DDGS and wheat middlings at 10% in corn and wheat-based diets, respectively. The MES supplied 2,500 U xylanase, 300 U β -glucanase, 700 U invertase, 10,000 U protease, 1,200 U cellulase, 24,000 U amylase and 20 U mannanase per kg of feed. Diets were formulated to meet specifications for respective poultry species and prepared in pellet form. A total of 960-d old Ross x Ross 708 male chicks (Exp. 1) and 720-d old male hybrid toms (Exp. 2) were allocated to 8 diets ($n = 6$). Birds had free access to feed and water for 28 d. Body weight and feed intake were recorded on d 0 and 28. Mortality was recorded to calculate adjusted feed conversion ratio (FCR). The data were subjected to GLM procedures of SAS with grain, fiber, MES

and associated interactions as fixed factors. There were no ($P > 0.05$) treatment effects on feed intake and FCR in both experiments. In Exp. 1, an interaction ($P < 0.05$) between grain, fiber and MES, was such that adding fiber in corn diet reduced Final BW (FBW) and BW gain (BWG) but MES addition improved these parameters by ~3.5% to the level of corn diet without fiber. Broilers fed high fiber wheat diets with MES had a lower ($P < 0.05$) FBW and BWG compared with birds fed all other diets. Broilers fed corn had a higher ($P < 0.05$) FBW (1,462 vs. 1,424g) and BWG (1,416 vs. 1,378g) than birds fed wheat diets linked to a tendency ($P = 0.07$) for increased feed intake in broiler fed corn diets. In Exp. 2, there was no interaction ($P > 0.05$) between grain, fiber and MES or main effects of MES on FBW and BWG. Poults fed wheat diets had a higher ($P < 0.05$) FBW (1,441 vs. 1,148g) and BWG (1,376 vs. 1,343g) than poults fed corn diets. In conclusion, wheat-based diets increased growth in turkey poults but had opposite effect in broilers. Unlike broilers, the data indicated turkey could be fed up to 10% cereal co-products without negative effects on growth performance. Adding fiber in corn diets reduced growth in broilers which was improved to same level of corn diet without fiber by addition of MES.

Key Words: broiler, turkey, fiber, growth performance, multi-enzyme supplement

491P Effects of dietary protein levels and protease supplementation on growth performance and carcass traits of meat ducks fed a miscellaneous meal based diet. Qiudan Wang*, Ying Zhang, Ping Wang, Shi Bai, Mei Ding, and Feng Zeng, *Sichuan Agricultural University, Chengdu, Sichuan, China.*

The study investigated the effects of dietary crude protein levels and protease supplementation on the growth performance and carcass traits of Cherry Valley duck fed diets containing meals of cottonseeds, rapeseeds, and feather. A total of 960 14-d-old male ducks were weighed and randomly allotted to a 2×5 factorial arrangement of treatments with 6 replicated pens of 16 ducks each to 49 d of age. Experimental factors included 5 dietary protein levels at 13.5%, 14.5%, 15.5%, 16.5% and 17.5%, and without or with protease (200mg/kg). The results showed that BW (d 35 and d 49), BWG and feed intake (d 15–35, d 36–49 and d 15–49), breast meat yield (d 35 and d 49) linearly increased ($P < 0.05$), and feed to gain ratio (d 15–35 and d 15–49) and leg meat yield (d 35 and d 49) linearly decreased ($P < 0.05$) when dietary protein levels increased. Ducks fed diets with 16.5% or 17.5% crude protein (CP) had a better ($P > 0.05$) growth performance and carcass traits than fed diets with other levels of CP, and ducks fed diets containing 13.5% CP had the poorest ($P < 0.05$) growth performance and carcass traits among all experimental treatments. Protease supplementation had no effect ($P > 0.05$) on growth performance and carcass traits of ducks. However, adding protease to low protein diets (13.5% or 14.5%) significantly increased ($P < 0.05$) the breast meat yield of ducks at 49 d of age. In conclusion, a miscellaneous meal based diet containing 16.5% or 17.5% CP could meet the requirement of protein, and supplementation of protease could improve the deposition of breast meat in meat ducks aged from 15 to 49 d.

Key Words: meat duck, crude protein, protease, growth performance, carcass traits

492P Not Presented

493P Association of phytases and acids and alkaline proteases in laying hens diets. Thiago Ribeiro*¹, Andréia Massuquetto¹,

Sebastiao Borges¹, Edson Fontinelli¹, Matias Appelt¹, Ariane Nogueira², and Antonio Bertechini², ¹Tectron Technology and Innovation, Toledo, Paraná, Brazil, ²Federal University of Lavras, Lavras, Minas Gerais, Brazil.

This study investigated the effects of the supplementation of an acid (*Aspergillus niger*) and an alkaline (*Bacillus subtilis*) proteases in association with phytase on laying hens performance. In total, 280 laying hens (Lohmann Lite) were housed in cages from 30 weeks of age. Birds were distributed according to a completely randomized design in 4 treatments, with 7 replicates of 10 birds each. The treatments were (PC), a positive control basal diet containing corn and soybean meal, formulated to meet nutrient requirements of Lohmann Lite laying hens; (NC) a negative control formulated to a lower nutrient specification (0.635% reduction in Crude Protein [CP], 50 kcal/kg Metabolizable Energy [ME], 0.15% of Calcium [Ca] and Available Phosphorus [aP]); NC + 125g/t of the protease blend and 300 FTU/g of phytase; and NC + 125g/t of the protease blend and 600 FTU/g of phytase. The treatments were evaluated in 3 periods of 21 d each. Each period was considered as subplot. It was evaluated feed intake (FI), egg production (EP), feed conversion (FC) and albumen viscosity (VI). The data were submitted to ANOVA analysis and significant means were compared by Tukey Test ($P < 0.05$). The only parameter that were not noted differences was FI ($P = 0.256$). The birds that received diets of the NC had reduction in the EP, VI and worst FC than PC ($P < 0.05$). On another hand, all the birds that received supplementation of enzymes had EP, FCR and VI similar with PC ($P < 0.05$). The use of enzymes exogenous have been studied for many years in poultry nutrition. The results achieved in this study are similar with several others. One of the reasons for these results may be explained by increase of the nutrients digestibility, especially amino acids and minerals, principally because the synthetic enzymes used was protease and phytase, respectively. The best result of viscosity is usually attributed to the best digestibility of amino acids. Furthermore, the combination of proteases can amplify the protein utilization at different pH conditions along the gastrointestinal tract of laying hens. In the conditions evaluated, was possible reduce the levels of CP (0.635%), ME (50 Kcal), Ca (0.15%) and aP (0.15%) in case of supplementation of 125g/t of blend of proteases and 300 or 600 FTU of phytase, without compromising egg production and albumen viscosity of laying hens from 30 weeks of age.

Key Words: amino acid digestibility, egg production, environmental impact, enzymes exogenous, phosphorus

494P Effects of higher doses of microbial phytase on performance, bone ash, and incidence of woody breast in broilers fed non-phytate phosphorus markedly deficient corn-soybean meal based diets. Megharaja Manangi*, Steve Richter, Vivek Kuttappan, Juxing Chen, Deana Hancock, and Mercedes Vazquez-Anon, *Novus International Inc., St. Charles, Missouri, United States.*

A 44d floor pen experiment was conducted to evaluate the effects of supplementing higher doses (up to 4 times the standard dose of 500U) of phytase (CIBENZA® PHYTAVERSE®) in broiler chicks fed non-phytate phosphorus (npp) deficient corn-SBM based diets. A total of 1584 Ross-308 male broiler chicks were assigned to 6 treatments with 12 pens/treatment and 22 chicks/pen. Treatments consisted of T1 with reduced levels of npp and no added phytase (Negative Control, NC); T2 to T5 with 500, 1000, 1500 and 2000U of phytase/kg diet added to T1, respectively; T6 with industry levels of npp (Positive Control, PC). For starter, grower and finisher diets, the PC npp levels (analyzed in parenthesis) were maintained at 0.48 (0.48), 0.45 (0.39) and 0.42 (0.34)%, respectively, and PC Ca levels at 0.78, 0.86 and 0.80%, respectively.

The NC npp for all 3 phases was reduced by 0.24% from the PC. The NC Ca for the starter phase was maintained at the same level as the PC. For grower and finisher phases, the NC Ca levels were reduced by 0.15% from the PC. All treatments diets within each phase were isocaloric and isonitrogenous. The analyzed starter, grower, and finisher npp (Phytate P in parenthesis) values for T1-T5 (basal) were 0.25 (0.25), 0.15 (0.27), and 0.15 (0.25)%, respectively. The study was carried out as RCBD and the data were analyzed using 1-way ANOVA. Significance was tested at $P \leq 0.05$. Birds in NC were terminated at d 22 due to welfare (inability to reach feeders). At d 40, significant treatment effects were observed for cumulative gain, feed intake (FI), mortality, grams of ash/tibia and woody breast incidence (d 44). T2 had reduced ($P \leq 0.05$) gain (2.88 vs. 3.05 kg/bird), FI (4.28 vs. 4.5 kg/bird) and g of ash/tibia (3.02 vs. 3.47%) compared with T6. The responses for gain, FI and ash were similar ($P \geq 0.05$) for all other doses including PC, and higher ($P \leq 0.05$) compared with T2 suggesting 500U/kg diet could not compensate for the deficiency caused by the npp reduction. The mortality was higher for T2 (7.7%), T3 (8.0%) and T4 (6.8%) compared with T6 (2.7%) ($P \leq 0.05$), while T5 (4.2%) was not different ($P \geq 0.05$) from T6 or other treatments. On d 44, T5 had a 15 percentage point improvement in incidence of WB (score 0+1; less severe) compared with T6 (PC) ($P \leq 0.05$) while the response was not different for all other doses (T2 to T4) compared with T6 ($P \geq 0.05$). The response for gain, FI and ash plateaued after 1000U (Quadratic, $P \leq 0.05$). In conclusion, supplementing 1000 and 1500U/kg diet from d 0 alleviated the effects resulting from the 0.24% npp reduction as seen by improved performance and ash that were similar to the PC but supplementing 2000U further added value by reducing severity of WB incidences compared with the PC.

Key Words: phytase, broiler, woody breast

495P Effect of dietary non-phytate phosphorus and exogenous phytase on performance, egg quality and plasma biochemistry of Hy-line brown laying hens at older ages. Kyeong Seon Ryu¹, Md. Masud Rana^{*1}, Chun Ik Lim¹, Sang Ho Kim², and Hwan Ku Kang², ¹Chonbuk National University, Jeonju, Korea (the Republic of), ²National Institute of Animal Science, Pyeongchang, Korea (the Republic of).

A study was conducted to evaluate the effects of phytase enzyme in diets formulated with different non-phytate phosphorus (nPP) levels on performance, egg quality and plasma biochemical indices of laying hens from 73 to 80 weeks of age. In a completely randomized design, a total of 540 Hy-line brown laying hens were randomly assigned into 6 treatments with 5 replicates consisting of 18 hens each replicate, and were kept in cages (2 hens/cage). Three iso-nitrogenous and iso-caloric diets were formulated to contain 0.20, 0.25 and 0.30% nPP with constant level of 4.5% Ca, and with or without supplementation of phytase at the inclusion level of 1000 FYT/kg feed (Ronozyme HiPhos-L, 6-phytase produced by the strain of *Aspergillus oryzae*). Productive performance parameters were summarized for an 8 week (73 to 80 weeks of age). At the termination of the experiment, 30 eggs were randomly collected from each treatment to determine egg quality and blood samples were taken (2 hens per replicate) to analysis serum biochemical parameters. Data were analyzed by SAS software (version 9.1, 2009). The results showed that egg production was significantly ($P < 0.04$) affected among the treatments and hens fed diet containing 0.25% nPP with phytase had greater egg production. However, the egg production was increased by about 1.50, 1.64 and 0.97% on 0.20, 0.25 and 0.30% nPP with supplemented phytase respectively, when compared with the unsupplemented groups. On the other hand, other performance traits egg weight, egg mass, feed intake and feed conversion ratio were not affected ($P > 0.05$) by the

treatments. Egg shell thickness was significantly ($P < 0.04$) influenced due to addition of phytase in all nPP diets whereas the treatments did not affect ($P > 0.05$) yolk color, albumen height and Haugh unit. In addition, phytase supplementation to nPP diets significantly increased serum albumin ($P < 0.02$) and high-density lipoprotein ($P < 0.05$) level than unsupplemented groups while no differences ($P > 0.05$) in serum cholesterol, glucose, total protein and alanine aminotransferase were observed. It can be concluded that phytase supplementation of laying hens diet may improve persistency of lay, shell quality and also have positive impact on immune status.

Key Words: laying hens, phytase, performance, egg quality, plasma

496P Effect of dietary enzymes supplementation on cecal microbiota of broiler chickens. Franciele Navarini Giacobbo¹, Cinthia Eyng^{*1}, Levy Teixeira², Ricardo Nunes¹, Cleverson de Souza³, and Cristiano Bortoluzzi⁴, ¹Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon, Paraná, Brazil, ²DSM, São Paulo, Brazil, ³Universidade Tecnológica Federal do Paraná, Dois Vizinhos, Brazil, ⁴USDA, College Station, Texas, United States.

The intestinal microbiota can positively affect poultry performance and health by influencing the digestive process, development and physiological aspects of the gastrointestinal tract. Several nutritional strategies have been associated with gut microbiota modulation, including the dietary inclusion of exogenous enzymes. The present study aimed to evaluate the effects of feeding exogenous enzymes on the cecal microbiota composition in broilers at 21 d of age. A total of 2,160 one-day-old male broilers were allotted to a completely randomized design, in a $3 \times 2 \times 2$ factorial scheme (3 corn hybrids, 2 drying temperatures - 80 and 110°C, with and without top-dressed enzyme blend (15,000 PROT/kg protease, 80 KNU/kg amylase and 100 FXU/kg xylanase), totaling 12 treatments, 9 replicates of 20 birds per experimental unit. The birds were given a mash diet with a corn grain ground into sieves with a diameter of 2 mm to provide corn particles with a geometric mean diameter of 600 µm. All experimental diets had 1,000 FYT/kg of phytase. However, the statistical analysis showed no effect ($P > 0.05$) of corn hybrid and drying temperature on broiler performance; only an effect of enzyme inclusion was reported, which broilers fed diets containing the enzyme blend had better performance from 1 to 21 d. Therefore, for the analysis of intestinal microbiota at 21 d of age, the initial treatments were grouped into 2 groups (with or without the dietary inclusion of enzyme blend) with cecal content samples from 6 birds for each replicate ($n = 9$) of the group. Identification of the microbiota was performed by sequencing the V3/V4 regions of the 16S rRNA gene using the Illumina Miseq Platform, and the abundance of bacteria was analyzed through a non-parametric one-way ANOVA (Kruskal-Wallis test) in SAS. The taxonomic composition at the phylum level revealed that Firmicutes (94.98%) was the dominant phylum of the cecal community in broilers. The abundance of genus *Lactobacillus* increased ($P = 0.07$; 56.76% x 43.08%) while those of genus *Blautia* decreased ($P = 0.001$; 1.99% x 8.94%) in broilers fed diets containing enzymes compared with animals fed diets without the blend. Furthermore, the abundance of *Lactobacillus helveticus* was 93.3% higher ($P = 0.02$) in broilers fed diets with the top-dressed enzyme blend. Overall, the dietary inclusion of exogenous enzymes changed the composition of the cecal microbiota in broilers. However, these modifications do not explain the improvement observed in broiler performance.

Key Words: amylase, cecal microbiota, protease, xylanase

497P Effects of enzyme combinations on cecal microbiota of broiler chickens. Franciele Navarini Giacobbo^{*1}, Cinthia Eyng¹, Levy Teixeira², Ricardo Nunes¹, Cristiano Bortoluzzi³, and Cleison de Souza¹, ¹Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon, Paraná, Brazil, ²DSM, São Paulo, Brazil, ³USDA, College Station, Brazil.

The use of exogenous enzymes improves the utilization of dietary nutrients by poultry and may influence the composition of their intestinal microbiota. The intestinal microbiota plays an important role in broiler health and performance by acting on the digestive and immune systems. This study aimed to evaluate the effect of enzyme combinations on the cecal microbiota composition in broilers. A total of 1,320 one-day-old male broilers were allotted to a completely randomized design, in a 2×3 factorial scheme (2 drying temperatures for corn - 80 and 110°C and 3 enzyme combinations) and 2 control treatments: a positive control (meeting the nutritional requirements of broilers for each phase) and a negative control (reduction of 150 kcal in the apparent metabolizable energy of corn), totaling 10 treatments with 6 replicates of 22 birds per experimental unit. Enzymes were added to the negative control diet considering the following combinations: Amylase (80 KNU/kg); Amylase + Xylanase (100 FXU/kg); and Amylase + Xylanase + Protease (15,000 PROT/kg). All experimental diets had 1,000 FYT/kg of phytase. However, the statistical analysis showed no effect ($P > 0.05$) of drying temperature on performance traits; only an effect of enzyme combinations was reported, and the dietary enzymes were efficient in providing nutrients to broilers. Therefore, for the analysis of intestinal microbiota at 21 d of age, the initial treatments were grouped regardless of the effect of drying temperature, with cecal content samples from 4 broilers for each replicate of the group ($n = 6$). Identification of the microbiota was performed by sequencing the V3/V4 regions of the 16S rRNA gene using the Illumina Miseq Platform. The abundance of bacteria was analyzed through a non-parametric one-way ANOVA (Kruskal-Wallis test), and the means were compared by the Dunnett's test in SAS. Regardless of enzyme supplementation, the filo Firmicutes (88.87%) was the most abundant, followed by Bacteroidetes (9.40%) and Proteobacteria (1.57%). At the genus level, regardless of enzyme combinations, *Lactobacillus* prevailed, with *Lactobacillus helveticus* (22.43%) and *Lactobacillus salivarius* (22.32%) as the most frequent species. Furthermore, a high frequency of the species *Subdoligranulum variable* (23.91%) was reported. Even though the dietary inclusion of the enzyme combinations improved poultry performance, it was not possible to correlate this improvement with the abundance of the main bacterial groups in the cecal microbiota.

Key Words: amylase, cecal microbiota, enzyme combination, protease, xylanase

498P Effect of dietary phytate and microbial phytase on broilers diets at 21 days of age. Ricardo Nunes^{*1}, Jomara Broch¹, Vaneila Savaris¹, Cristine Kaufmann¹, Edinan Cirilo¹, Lucas Wachholz¹, Cinthia Eyng², Jarred Oxford³, and Gene Pesti³, ¹Western Paraná State University, Marechal Cândido Rondon, Paraná, Brazil, ²Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon, Paraná, Brazil, ³University of Georgia, Athens, Georgia, United States.

Broilers diets are usually composed of high amounts of phosphorus in the form of phytate which can form a complex with positively charged molecules in the diet. To maximize feed utilization by poultry, as well as the reduction of costs, the use of animal by-products in the diets has become common. This study was conducted to evaluate the effects of different levels of phytase on diets with different amounts of phytate

on live performance and bone characteristics of broiler chickens at 21 d. A total of 2,625 male, 1-d-old Cobb 500 broilers were allocated to 15 dietary treatments. Treatments consisted of a 3x5 factorial arrangement, with high (HP), medium (MP) and low (LP) phytate (2.45, 2.34, 2.23 g kg⁻¹ of phytate P, respectively) and a positive control (PC); negative control (NC) with a reduction of 0.15% of calcium (Ca) and phosphorus (P) and NC diet plus 0, 500, 1000 or 1500 FTU kg⁻¹ of phytase. Data were analyzed by SAS - Version 9.1. Polynomial regression between levels of inclusion of the enzyme was performed excluding the positive control treatment. The Dunnett's and Tukey's test were performed at 5% probability. No significant interaction ($P > 0.05$) was found on weight gain (WG) and feed conversion ratio (FCR). WG and FCR showed an improvement with increasing levels of phytase ($P < 0.05$). Regression equations had the best fit with quadratic adjustment and the levels that provided the maximum responses were estimated at 233 and 1180 FTU kg⁻¹, for FI and WG, respectively. A significant interaction was found on broiler's FI. Birds fed the NC and NC+500 FTU kg⁻¹ and diets with HP had 11% and 4.1%, higher FI when compared with birds on the LP diets. With 1000 FTU kg⁻¹ of inclusion there was no differentiation between FIs. A quadratic effect was observed between phytase supplementation and LP diet and the level of phytase that provided the maximum FI responses was estimated at 1051 FTU kg⁻¹. Bone parameters analysis showed that broilers fed NC diets without phytase supplementation, exhibited the lowest breaking strength (BS), dry matter (DM) and bone ash (BA) content, and a higher value for hypertrophic cartilage zone (A2), by Dunnett's Test. Most measurements had best fit with quadratic adjustments and the equations derived showed the greatest values for supplementation at 1140 (BS), 1008 (DM), 1304 (BA) FTU kg⁻¹; for A2, 1308 FTU kg⁻¹ may provide a tendency of tibial dyschondroplasia. However, for DM and BA there were a significant interaction. Phytase supplementation improved the performance and bones of birds. The use of 1101 FTU kg⁻¹ resulted in better bone characteristics when fed with the lowest phytate level, this level does not negatively affect the other parameters evaluated.

Key Words: poultry nutrition, phytic acid, diets, enzyme

499P Effects of xylanase supplementation on growth performance in broilers fed reduced-energy corn-soybean meal diets.

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The objective of this study was to evaluate the effects of xylanase inclusion on growth performance of male broilers. Ross 708 male broilers (n = 1,290) were randomly assigned to 3 treatment groups with 10 replicate pens of 43 birds. The experimental design included a positive control (PC) representing an industry style corn-soybean meal based diet with corn DDGS, a reduced energy (-110kcal/kg) negative control (NC) diet, and an additional treatment consisting of the NC diet supplemented with xylanase at 40,000 U/kg (NC+X). The dietary program consisted of 4 feeding phases (starter 0-12d, grower 13-28d, finisher 29-36d, and withdrawal 37-43d). Average body weight (BW), feed consumption (FC), and mortality adjusted feed conversion ratio (FCR) were determined for each phase. Data were analyzed using one-way ANOVA, and significantly different means were separated using Duncan's Multiple Range Test ($P \leq 0.05$). Starter FCR was significantly ($P < 0.05$) greater when broilers were fed the reduced energy NC when compared with the PC. Cumulative FCR from 1 to 28d was also greater ($P < 0.05$) for the NC as compared with the PC, while inclusion of xylanase in the NC improved ($P < 0.05$) FCR to levels similar to the PC. A similar improvement was

observed with xylanase inclusion on cumulative FCR from 1 to 36d. Cumulative FC from 1 to 36d was greater ($P < 0.05$) in the reduced-energy NC as compared with the PC and NC+X. Inclusion of xylanase in the NC increased ($P < 0.05$) FC compared with the PC during the withdrawal phase. The increases in FC along with numerical differences in BW of the NC+X led to reductions ($P < 0.05$) in withdrawal FCR to levels comparable to the PC. Cumulatively from 1 to 43d, FC of NC-fed birds was greater ($P < 0.05$) than the PC, while xylanase inclusion in the NC resulted in an intermediate FC compared with the control diets. At the conclusion of the trial, the reduction in energy increased ($P < 0.05$) FCR in the NC compared with the PC, with the inclusion of xylanase in the NC yielding intermediate results. No significant differences were observed in BW or mortality throughout the duration of the trial. These data demonstrate the growth performance benefits of including xylanase in reduced-energy corn-soybean meal diets.

Key Words: broiler, performance, xylanase, low-energy

500P Relative pH range and IP6 degradation rate: An *in vitro* efficacy examination of two commercially available phytases.

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Traditionally, phytase assays are ran at pH 5.5 and using Na-phytate as substrate. But early and rapid removal of phytate to minimize anti-nutritional effects of phytate means a phytase should have high activity at gastric pH (e.g., 3.5). Plus phytase should be able to accommodate a variety of phytate/protein complexes. The goal of this *in vitro* project was to determine: 1) the activity of each tested phytase in a pH range of 1.5 to 6.5, relative to pH 5.5 activity, and 2) the efficacy of each toward degradation of phytate in a corn/SBM diet or a phytate-lysozyme protein complex at pH 3.5. Two phytases were tested: a *Buttiauxella* sp. phytase (phytase B, expressed in *Trichoderma reesei*) and a *Hafnia* sp phytase (phytase H, a hybrid phytase consisting of *Hafnia* sp., *Yersinia* sp and *Buttiauxella* sp, expressed in *Aspergillus niger*), with normalized activity at pH 5.5 for both phytases. In test 1, each phytase was reacted with Na-phytate in a multibuffer solution (pH 1.5-6.5) at 37°C for 60 min. In test 2, each of the phytases, at levels corresponding to 250, 500, 750 and 1000 FTU/kg, were incubated in a corn/SBM feed slurries in a pH 3.5 buffer preparation for 10 min at 37°C. Phytate degradation was measured by anion exchange HPLC as relative to no phytase. In addition, the rate of lysozyme protein-phytate hydrolysis was evaluated as a decrease in turbidity of the complexed substrate at pH 3.5. Equimolar aliquots (60µL) of lysozyme and phytate (1%) were reacted with phytase for a final concentration of 0.14 FTU/ml. Test 1 demonstrated that at pH 3.5, the activity relative to pH 5.5 was 188 and 89% for phytase B and H respectively. With lysozyme protein-phytate complex in test 2, phytase B and H, respectively, degraded phytate by 95 and 19% after 4 min. In the corn/SBM feed slurry, phytase B and H, respectively, hydrolysed phytate by 65 and 17% at 500 FTU/kg, and by 85 and 36% at 1000 FTU/kg after 10 min. In conclusion, these tests reveal large differences between tested phytases in relative activity as well as in degradation of complexed phytate/protein structures at pH 3.5 wherein phytase B shows earlier and more rapid phytate destruction than phytase H.

Key Words: phytase, pH, phytate degradation, phytate complexes

501P Phytase activity at pH 1.5 to 6.5 and IP6 degradation rate: An *in vitro* study. Janet Remus^{*1}, Trine Christensen², Rie Mejl-dal², and Yueming Dersjant-Li³, ¹Danisco US Inc., DuPont Animal Nutrition, Cedar Rapids, Iowa, United States, ²DuPont Nutrition Biosciences ApS, DuPont Industrial Biosciences, Brabrand, Denmark, ³Genencor International, B.V., Danisco Animal Nutrition, Leiden, Netherlands.

Standard phytase activity is measured at pH 5.5. However, to reduce phytate's anti-nutritional effect early in the GI tract, a phytase should be highly active at stomach pH (e.g., 3.5), thus allowing for early, efficient removal of phytate given varying complexes it may be in. This *in vitro* study determined: 1) the activity of different phytases in a pH range of 1.5 to 6.5, relative to pH 5.5, and 2) the efficacy in rdegrading phytate in a corn/SBM diet or a phytate-lysozyme protein complex at pH 3.5. Four phytases were tested: a *Buttiauxella* sp. phytase (phytase B); 2 *E. coli* phytases (phytase E1 and E2) and a *Citrobacter braakii* phytase (phytase C), with normalized activity at pH 5.5 for all phytases. In test 1, each phytase was reacted with Na-phytate in a multibuffer solution (pH 1.5–6.5) at 37°C for 60 min. In test 2, each phytase, at a level of 250, 500, 750 and 1000 FTU/kg, was incubated in a corn/SBM feed slurry in a pH 3.5 buffer preparation for 10 min at 37°C. Phytate degradation was measured by anion exchange HPLC as relative to no phytase. In addition, the rate of lysozyme protein-phytate hydrolysis was evaluated as a decrease in turbidity of the complexed substrate at pH 3.5. Equimolar aliquots (60µL) of lysozyme and phytate (1%) were reacted with phytase for a final concentration of 0.14FTU/ml. Test 1 demonstrated that at pH 3.5, the activity relative to pH 5.5 was 188, 97, 101, and 118% for phytase B, C, E1 and E2 respectively. In test 2, the lysozyme protein-phytate complex measurements for phytase B, C, E1 and E2 revealed phytate degradation by 95, 15, 33 and 9%, respectively, after 4 min. For the corn/SBM feed slurry, phytase B, C, E1 and E2, respectively, hydrolyzed phytate by 65, 48, 39 and 12% at 500 FTU/kg, and by 85, 75, 69 and 24% at 1000 FTU/kg after 10 min. These results indicate that *in vitro* measurements can be helpful in identifying potential efficacy differences between phytases. In conclusion, clear differences measured in pH activity as well as the ability to degrade natural substrates like phytate/protein complexes and cereal phytate in corn/soy feed between the tested phytases reveal phytase B as the phytase favored to produce early and rapid phytate degradation at gastric pH.

Key Words: phytase, pH, phytate degradation, phytate complexes

502P Effect of phytase and myo-inositol supplementation on the expression of inositol transporters in the small intestine of laying hens, and blood and yolk inositol concentrations. Eugenia Herwig^{*1}, Karen Schwean-Lardner¹, Carrie Walk², Andrew Van Kessel¹, Michael Bedford², and Henry Classen¹, ¹University of Saskatchewan, Saskatoon, Saskatchewan, Canada, ²AB Vista, Marlborough, United Kingdom.

Myo-inositol (inositol), is an important second messenger, and its release is believed to be one of the positive effects of phytase action. An experiment was designed to assess the effects of inositol in pure form (0.16%) or derived from phytase action (3000 FTU/kg) on inositol absorption in laying hens when fed amino acid sufficient and deficient diets. To assess this, 5 treatment diets were chosen: high balanced protein (HBP – 19% CP), HBP with inositol (HBP-I), reduced balanced protein (RBP – 16% CP), RBP with phytase (RBP-P), and RBP with inositol (RBP-I). Each treatment was fed *ad libitum* to 48 Lohmann LSL lite hens housed in conventional cages (4 replicates per treatment) from 19 to 39 wk of age. At 39 wk of age, all eggs were collected per replicate to determine egg yolk inositol content. The following day, 4 birds per

replicate were euthanized and contents from the gizzard and distal ileum were collected to determine inositol and phytate esters concentrations. In addition, 3 birds per replicate were bled (to determine blood inositol levels), euthanized and samples from mid duodenum and jejunum were collected for gene expression of MUC2 (mucin 2), ALPI (alkaline phosphatase), SLC2A13 (HMIT, H⁺/inositol transporter), SLC5A3 (SMIT1, Na⁺/inositol co-transporter) and SLC5A11 (SMIT2, Na⁺/glucose or Na⁺/inositol co-transporter). Data were analyzed using the MIXED MODEL one-way ANOVA procedure of SAS 9.4. Four orthogonal contrasts were also performed to compare RBP-P vs. RBP-I, HBP vs. HBP-I, and the effects of protein level and inositol. All differences were considered significant at $P \leq 0.05$. Phytase decreased, or tended to decrease, IP6 ($P = 0.004$ and $P < 0.001$) and IP5 ($P = 0.095$ and $P < 0.001$) in gizzard and distal ileum digesta, respectively. Inositol levels in the gizzard and distal ileum digesta were highest in HBP-I, RBP-P and RBP-I treatments ($P < 0.001$ both). Gene expression analyses showed a trend for inositol to upregulate MUC2 ($P = 0.066$) and HMIT ($P = 0.090$) in the duodenum, and no differences were found between phytase and inositol treatments. In the jejunum, phytase, but not inositol, upregulated ALPI ($P = 0.033$) and SMIT1 ($P = 0.033$). A trend was found for dietary inositol to increase blood inositol concentration ($P = 0.065$) and these values were equal to the levels resulting from feeding the phytase diet. Similarly, feeding inositol or using dietary phytase increased egg-yolk inositol concentration ($P = 0.037$). In conclusion, inositol supplementation via enzymatic hydrolysis of phytate or pure inositol results in equal concentrations of inositol in egg yolk and blood. However, source, phytate or free inositol, affects absorption location along the small intestine.

Key Words: inositol, phytase, alkaline phosphatase, inositol transporters, mucin

503P Exogenous enzyme supplementation can overcome amino acid deficient diets. Victoria Ayres^{*}, Timothy Boltz, Angela Lamp, and Joseph Moritz, West Virginia University, Morgantown, West Virginia, United States.

The current study hypothesized that exogenous enzyme supplementation can overcome a corn and soybean meal based diet, with a 15% reduction in crude protein and amino acids. The objective of the study was to determine broiler performance of diets that vary in amino acid concentration and enzyme inclusion. Dietary treatments included a positive control (PC) (100% amino acid recommendations), negative control (NC) (85% amino acid recommendations), and 6 additional diets containing commercially available enzyme supplements (Single Dose Phytase, Super Dose Phytase, Single Dose NSPase, Super Dose Phytase + Single Dose NSPase, Protease 1, and Protease 2) added to the NC based on manufacturers' recommendations. Each diet was conditioned at 70°C and fed as crumbles to 12 replications of 10 male Hubbard x Ross 708 broiler chicks for 21d. Treatments were arranged in a randomized complete block design. The 15% calculated reduction in crude protein and amino acids produced expected 7, 14 and 21d broiler live weight gain (LWG) differences between the PC and NC ($P < 0.05$). Dietary treatments including phytase, at either level or when combined with the NSPase, had similar d7, 14, and 21 LWG compared with the PC ($P > 0.05$). These data suggest that with the inclusion of the particular phytase, regardless of dose or when combined with NSPase, improved broiler LWG beyond the NSPase alone or either tested protease without detriment to feed conversion ratio relative to the PC.

Key Words: phytase, NSPase, protease, amino acid, deficiency

504P Effect of grain particle size, dietary nutrient levels, and phytase on the performance of broiler chicks to 3 weeks of age.

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Commercial broiler diets are pelleted to assist with feed handling, improve ease of consumption, and decrease wastage. These pellets disintegrate rapidly after consumption when moisture is contacted in the crop. The wet mash enters the gizzard where additional grinding of the feed particles occurs; however, because the cereal grain was previously ground for pellet manufacturing, the gizzard is less active in grinding particles. For this reason, broiler gizzards are often reduced in mass and function. Because the physical impact on grain particles in an acidic environment could affect the dynamics of nutrient bioavailability due to mixing and surface preparation, a study was conducted to test the effect of combinations of particle size and phytase enzyme on broiler chick performance. Corn was ground using a lab scale hammer mill to particle sizes (PS) of 450 nm (small) and 800 nm (large). Corn-soy rations were formulated to meet the recommended nutrient requirements of Cobb male chicks (Positive control, PC) with rations made using either small or large corn particles and were fed in mash form. Each PC diet was fed with or without 1500 FYT of phytase (Ronozyme HiPhos 2700). A second set of negative control rations (NC) were formulated to within 3% restricted energy and protein and with available P reduced to 0.4%. Each NC diet was manufactured with small or large PS corn and fed with or without 1500 FYT of phytase similar to the PC. The Petersime battery trials were conducted with 6 chicks per pen and 6 replications of each of the 8 feed rations. Performance data and gizzard weights were recorded at 18d. Data were analyzed as a 2x2x2 factorial arrangement using main effects of diet (NC vs PC), PS (small vs large) and phytase level (no vs yes) using SAS (9.4) with significance levels set at $P \leq 0.05$. As expected, rations with reduced nutrient levels and small PS had significantly reduce BWG and FC compared with the PC birds fed any PS or phytase supplement. When the NC and PC diets were supplemented with phytase, both BWG and FC were significantly improved ($P \leq 0.05$). There was a significant improvement ($P \leq 0.05$) of large PS at either nutrient level without added enzyme. Phytase addition also decreased mortality when added to NC and PC rations. Gizzard weight increased as particle size increased. The data indicate that there was significant ($P \leq 0.05$) PS by phytase interaction with BWG and FC improved in chicks fed the larger PS in the presence of phytase. NC but not PC fed chicks had increased gizzard weight due to the addition of phytase to the ration. The results indicate that PS and enzyme combinations may affect broiler chicks differently when the cereal grain fraction is ground to different sizes.

Key Words: particle size performance, phytase, feed milling, broilers, performance

505P Effects of dietary lysine and exogenous protease inclusion on apparent ileal amino acid digestibility in turkeys.

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Previous research has shown that exogenous proteases increase protein digestion in poultry diets. Thus, a 42-d experiment was conducted to evaluate the effects of dietary lysine level and exogenous protease inclusion on apparent ileal amino acid digestibility (AIAAD) in turkeys. Female poults (Hybrid Genetics, Ag Forte LLC, Aurora, MO; n = 780) were weighed and randomly assigned to 1 of 32 floor pens with 24 or 25 poults per pen. Poults were maintained on a 24-h lighting schedule

in an environmentally controlled room with *ad libitum* access to feed and water. Poults were randomly assigned to 1 of 4 treatments within location block for a total of 8 replications per treatment. Treatments were arranged in a 2 x 2 factorial of digestible lysine and commercial protease. Poults were fed a starter diet from 0 to 28 d and a grower diet from 29 to 42 d. Starter diets were formulated to provide 1.52% and 1.62% digestible lysine for the low and adequate digestible lysine treatments, respectively; grower diets were formulated to provide 1.39% and 1.49% digestible lysine, respectively. Exogenous protease was added to the protease diets at 125 g/tonne, and the same inclusion of sand was added to the diets without protease. Diets were balanced by energy and had equal amino acid ratios. Titanium dioxide was included in the grower diets at 0.5% as an indigestible marker for determination of AIAAD. Diet samples were collected from each pen during the grower phase and composited by treatment for analysis of titanium dioxide. On d 42, ileal contents from 2 poults per pen were collected, composited, and immediately frozen. Frozen ileal samples were subsequently freeze-dried and ground for analysis of titanium dioxide. Data were analyzed using the GLIMMIX procedure in SAS 9.4 with pen as the experimental unit and pen location as the blocking factor. There was a lysine level x protease interaction ($P = 0.01$) for AIAAD of Trp. There was no evidence of difference in AIAAD of Trp in the low lysine diets with or without protease (89.8 vs. 89.3%, respectively). However, in the adequate lysine diets, AIAAD of Trp was greater in poults not consuming protease compared with those consuming protease (91.7 vs. 88.6%, respectively). There was no evidence of difference ($P > 0.29$) in AIAAD of Lys among treatments (90.5, 90.2, 91.3, and 90.3%, respectively). Similarly, there was no evidence of difference ($P > 0.60$) in AIAAD of Arg, Met, Cys, Thr, Ile, Leu, or Val due to dietary lysine level or protease inclusion. Overall, there was no benefit of increased dietary lysine level or protease inclusion on AIAAD in poults at 42 d of age.

Key Words: amino acid, digestibility, lysine, protease, turkeys

506P Enzyme complex in broiler diets in barley or rye based diets with reduced metabolizable energy. Marcia Sakamoto*, Paulo Pozza, Caio Souza, Alceu Hirata, and Alice Murakami, *Universidade Estadual de Maringá—UEM, Maringá, Paraná, Brazil.*

Two experiments were conducted to evaluate performance, digestive, and blood parameters when an enzyme complex (EC) was used in barley and rye based broiler diets with or without dietary energy level reduction. The EC (Allzyme SSF® - Alltech Inc.) had pectinase, protease, phytase, β -glucanase, xylanase, cellulose, and amylase. In Exp I, 108 17-d old Cobb male broilers were distributed in a completely randomized design into a 2x2x2 + 1 factorial arrangement of 2 base feeds (20% barley or 20% rye), 2 EC levels (0 and 0.02%), and 2 energy levels (3,025 and 3,125 kcal AME/kg), plus a control treatment based on corn and soybean meal, totaling 9 treatments with 6 replicates (2 broilers/replicate). Total collected excreta methodology was applied with diets adaptation (5 d), followed by total excreta collection during 5 d. In Exp II, the same 2x2x2+1 factorial treatment with 1,080 1-d old Cobb male broilers were used. The dietary energy levels were modified to be 2,875 and 2,975 kcal AME/kg from 1 to 21 d and 3,025 and 3,125 kcal AME/kg from 21 to 42 d. and 2 levels of the EC (0 and 0.02%), plus a control treatment based on corn and soybean meal, totaling 9 treatments with 6 replicates (20 birds each). Performance, carcass, cut yields, intestinal viscosity, intestinal morphometry and blood parameters were evaluated. Data were analyzed by one-way ANOVA using SAS software (2009) at a significance level of 5%. Whenever a difference was found, the means of the factors were compared using Student's *t*-test. No interactions ($P > 0.05$) were observed among factors for any of the

analyzed variables (Exp I and II). In Exp I, the addition of EC provided higher metabolizability coefficient of dry matter ($P < 0.0001$), crude protein ($P = 0.0045$), neutral detergent fiber ($P < 0.0001$), crude fat ($P < 0.0001$), and apparent metabolizability coefficient of gross energy ($P = 0.0432$) in diets. In Exp II, the EC improved performance of broilers from 1 to 21 d and from 1 to 42 d of age, providing greater weight gain ($P < 0.05$) and better feed conversion rate ($P = 0.05$). Carcass ($P = 0.0007$) and breast ($P = 0.0018$) yields were greater for broilers fed diets containing EC. Intestinal viscosity at 21 d ($P < 0.0001$) and 42 d ($P < 0.0001$) of age was reduced with addition of the EC. Villus: crypt ratio of duodenum ($P < 0.0001$) and jejunum ($P < 0.0001$) were higher with the enzyme addition. Blood glucose ($P = 0.0009$), total cholesterol ($P = 0.0137$), and triglyceride ($P = 0.0060$) concentrations were higher with the addition of EC. Therefore, the use of the enzyme complex reduced intestinal viscosity caused by barley or rye intake, improving nutrient digestibility, performance and carcass yield.

Key Words: arabinoxylans, exogenous enzymes, performance, viscosity, B-glucans

507P Enzyme complex in broiler diets with barley or rye and energy level reduction from 21 to 42 days. Caio Souza, Marcia Sakamoto*, Ivan Camilo OspinaRojas, Paulo Pozza, and Alice Murakami, *Universidade Estadual de Maringá—UEM, Maringá, Paraná, Brazil.*

The experiment was carried out to evaluate the performance, carcass yield, and relative weight of digestive organs of broilers receiving diets containing an enzyme complex (EC) and based on barley and rye, with or without metabolizable energy reduction. The EC (Allzyme SSF[®]; Alltech Inc.) has the following enzymes: pectinase, protease, phytase, β -glucanase, xylanase, cellulose, and amylase. A total of 975 21-d-old Cobb male broilers were randomly distributed into a 2x3x2+1 factorial arrangement, consisting of 2 feedstuffs (18% barley or 18% rye), 3 EC levels (0, 0.02, and 0.04%) and 2 energy levels (3,050 or 3,150 kcal AME/kg), plus a control treatment based on corn and soybean meal, totalizing 13 treatments with 5 replicates (15 broilers/replicate). Data were analyzed by one-way ANOVA using SAS software (2009) at a significance level of 5% and means were compared using Tukey test (CE levels) and Student t- test (feedstuff and energy levels). No interactions ($P > 0.05$) were observed among the studied factors (feedstuff x enzyme complex x energy) for any of the evaluated parameters. The EC provided greater weight gain ($P < 0.0001$) and improved feed conversion rate ($P < 0.0001$) independent of the supplementing level. There was no effect of feedstuff ($P > 0.05$) or energy level ($P > 0.05$) on carcass and cut yields. Carcass ($P = 0.0202$) and breast ($P = 0.0184$) yields were greater for broilers fed diets containing EC independent of the supplementing level. Addition of 0.04% EC reduced ($P = 0.0087$) the small intestine length. Therefore, under the conditions of this experiment, broilers fed diets containing Allzyme[®] SSF showed improved performance, resulting in greater carcass and breast yields without difference among the 2 levels of enzyme complex used.

Key Words: carcass yield, exogenous enzymes, non-starch polysaccharides, weight gain

508P Economical and practical evaluation of phytase supplementation in broiler diets. Rony Riveros Lizana*¹, Ruth Sumarriva Loayza², and Carlos Vilchez², ¹Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil, ²Universidad Nacional Agraria La Molina, Lima, Lima, Peru.

The objectives of the study were to evaluate a practical model for performance response prediction and economic evaluation, and a practical non-parametric evaluation the response of broilers fed commercial diets supplemented or not with phytase. Four-hundred-50 one day-old Cobb 500 male chicks were randomly distributed in 18 groups with 25 birds each. The experimental dietary treatments were: PC, Positive Control with adequate Ca and avP levels; NC, Negative Control with reduction of 0.16% Ca and 0.17% avP with respect with to PC and NC+1000, NC with supplementation of 1000 FTU/kg of phytase. Water and feed (pellet form) were offered *ad libitum*. Body weight (BW) and feed intake (FI) were recorded weekly and then body weight gain (BWG) and feed conversion ratio (FCR) were calculated. The economical evaluation was performed as Profitability (\$/bird) = (Broiler Price – Feed Cost). At d 21 and 42, 2 birds per pen were randomly picked-up for an individual evaluation of walking ability with Gate Score System (GS), *Varus-Valgus* leg deformities, and the Femoral Head Separation (FHS). Data of BW, FI, BWG and FCR were submitted to one-way ANOVA and the means were compared by Tukey test ($P < 0.05$). The GS, *Varus-Valgus* and FHS data were analyzed by Kruskal-Wallis nonparametric test. For each treatment, a quadratic equation (QE) were fitted in accordance to $FI = f_{(Age)}$ response, and a cubic equation (CE) were adjusted for $BW = f_{(FI)}$. The adjusted equations of $f_{(Age)}$ and $f_{(FI)}$ from PC were considered as the Standard Curve to compare the economic analyses of Feed Save and Time Save between treatments. At d 42, the NC+1000 showed a significant effect on BW and BWG ($P < 0.05$) compared with PC and NC. The NC+1000 showed similar feed efficiency with respect to PC and it was higher ($P = 0.0238$) than that obtained with NC. The nonparametric evaluation showed that at d 21 more prevalence of GS = 3 ($P = 0.097$) and significant expression for *Varus* deformities ($P = 0.049$) for the NC; similar results were observed for PC and NC+1000. The R^2_{aj} CE and QE values were over 99% for all treatments. The Feed Save with phytase was 6.2% vs without enzyme earning 9.3% less and the Time Save to reach BW of the standard curve was 2 d with enzyme and 4 d and more without enzyme. The results represents a profit of 0.457 and a loss of 0.422 \$/bird, with or without phytase enzyme supplementation, respectively. In conclusion, the decision to either supplement or not with an enzyme are directly related with costs and that the feed efficiency and the time of production can be modeled with linear functions and used by broiler industry to increase its profit with the advantage of reducing leg disorders in growing birds

Key Words: modelling, economical, nonparametric, broiler, phytase

509P Phytase and carbohydrases in broiler feed: Formulation adopting metabolizable energy savings on nutrient digestibility and carcass production cost. Caroline Bavaresco², Thais Stefanello*¹, Edenilse Gopinger³, Ari Sandi⁴, Everton Luis Krabbe⁴, Victor Fernando Roll², Bruno Wernick⁵, and Fernando Martinez⁵, ¹Federal University of Rio Grande do Sul, Porto Alegre, Brazil, ²Federal University of Pelotas, Pelotas, Brazil, ³Federal Institute of Education Science and Technology of Rio Grande do Sul, Sertão, Brazil, ⁴Embrapa Swine and Poultry, Concórdia, Brazil, ⁵BASF S.A, Maringá, Brazil.

The aim was evaluate the use of hybrid phytase or its association with carbohydrases in diet with nutritional reduction of metabolizable energy (ME), Ca and available P (aP) on ileal nutrient digestibility of ME and the impact on cost of one kg produced broiler carcass. Broilers male Cobb 500 (1875 animals), distributed in 5 treatments with 15 replicates and 25 birds each, were fed diets formulated with nutritional reduction of metabolizable energy (70 and 100 kcal/kg), 0.16% calcium and 0.15% aP, supplemented or not with hybrid phytase (500FTU/kg),

xylanase (560TXU/kg) and glucanase (250 TGU/kg). The treatments are described as follows: T1-basal corn soybean meal diet (BD) meeting nutritional requirements; T2 – BD reduction of 70kcal/kg, Ca and aP; T3- BD reduction of 100kcal/kg, Ca and aP; T4- T2 + 500 FTU/kg hybrid phytase; T5- T3 + 500 FTU/kg hybrid phytase + xylanase+ glucanase. At 23 d age, birds received the respective diets with an indigestible marker (CrO) for 5 d, after this period (28 d) 3 birds/experimental unit (body weight \pm 5%) were euthanized for ileal content collection (dry matter, crude protein and metabolizable energy). The remaining birds were feed until 42 d and the end of period 2 birds/experimental unit (body weight \pm 5%) were selected for carcass yield evaluation (CY) through the formula: CY = cold carcass weight X 100 / body weight. The carcass cost calculation (\$/kg cold carcass) followed EMBAPA's methodology. The results were submitted to ANOVA in statistical software R, and the means were compared by Tukey's test ($P < 0.05$). The results of metabolizable energy ($P < 0.0001$) confirm that the enzyme supplementation with hybrid phytase (T4 – 3273ab), as well as the association with specific carbohydrases (xylanase + glucanase) – T5 (3216ab) improved feed efficiency. The ME of control treatment (T1) was 3368a. By treatments with nutritional reduction without enzyme supplementation decrease of ME was observed (T2 – 3203b and T3 – 3192b). Poultry industry is constantly seeking to increase production efficiency at the lowest cost. This study showed that the treatment with nutritional reduction of the 100 kcal/kg, 0.16% Ca and 0.15% aP associated with 500 FTU/kg hybrid phytase + 560 TXU/kg xylanase + 250 TGU/kg glucanase (T5), resulted in carcass cost of 0,752b (USD/kg cold carcass) ($P = 0.05$). Carcass cost for control treatment was 0,782a. Other treatments (T2 and T3) showed carcass costs of 0,769ab and 0,758ab, respectively. The use of the studied enzyme and nutritional matrix adoption improved a carcass cost reduction of USD 0.03/kg.

Key Words: energy, enzymes, nutrient reduction, price, poultry

510P Faba bean as a feedstuff for broilers: Effects on growth performance, carcass traits, saleable cuts and feed-associated carbon intensity of chicken meat production. Matt Oryschak¹, Daniella Batres¹, Emmanuel Opoku-Yeboah¹, and Eduardo Beltranena*^{1,2}, ¹Alberta Agriculture and Forestry, Edmonton, Alberta, Canada, ²University of Alberta, Edmonton, Alberta, Canada.

Feed accounts for approximately 35% of the total carbon footprint of chicken meat production in Western Canada. Fava bean (*Vicia faba*) is a high-yielding pulse crop that contains levels of starch (~40%) and digestible protein (~25%) that make it suitable as a feedstuff for modern broiler chickens. The crop's ability to fix atmospheric N results in a lower carbon footprint compared with other cereal or oilseed feedstuffs. The present experiment, therefore, evaluated the effect of increasing the dietary inclusion of fava bean grain on broiler growth, carcass traits, saleable cuts and feed-associated carbon intensity. In a randomized complete block design with 7 replicate pens of 10 broilers per treatment, 360 male Ross 708 were housed in bedded floor pens and fed a common starter phase diet (0–10d). Pens of broilers were then assigned to one of 4 dietary regimens consisting of grower (11–25d) and finisher (25–41d) phase diets including 0, 10, 20, or 30% fava bean var. Snowbird. Test diets were fed in coarse mash form and were formulated to meet or exceed recommended nutrient density in proportion to target AME. Estimates of carbon intensity for each ingredient derived from ECO-ALIM feedstuff database (INRA, France) were incorporated into the formulation matrix. Individual weight of broilers, pen feed added and orts were weighed for each growth phase to calculate ADFI, ADG and gain-to-feed. Antemortem wt, chilled carcass wt and wt of saleable carcass cuts were measured on d 42. Data were analyzed as generalized

linear mixed models using the GLIMMIX procedure of SAS (v9.4) that tested the main effect of fava bean dietary level. Models also included block as a random effect and tested for linear effects of dietary fava bean inclusion. Whereas dietary fava bean inclusion level had no effect on overall ADFI, broilers fed diets containing 30% fava bean exhibited reduced ($P < 0.01$) overall ADG (63 vs. 68 g/d) and gain-to-feed (0.577 vs. 0.657) compared with those fed the wheat/SBM-based control diet. Differences ($P < 0.05$) were observed between 10% and 30% fava bean regimens for antemortem wt (2877 vs. 2681 g), carcass wt (2174 vs. 1965 g) and dressing percent (74.7 vs. 73.5%) due to increased dietary fiber. There was no effect of fava bean inclusion on proportional yield of saleable cuts. Feed-associated carbon intensity per bird marketed linearly declined ($P < 0.001$) by 2.5% per each 10% increase in dietary fava bean inclusion. These results not only confirmed that fava bean grain can be fed at relatively high levels of inclusion to modern broilers, but also demonstrated its potential for reducing the carbon intensity of chicken meat production.

Key Words: broiler chickens, carbon footprint, faba bean, growth performance, saleable cuts

511P Canola seed as a feedstuff for broilers: Effects on growth performance, carcass traits, saleable cuts and feed-associated carbon intensity of chicken meat production. Matt Oryschak¹, Emmanuel Opoku-Yeboah¹, Daniella Batres¹, and Eduardo Beltranena*^{1,2}, ¹Alberta Agriculture and Forestry, Edmonton, Alberta, Canada, ²University of Alberta, Edmonton, Alberta, Canada.

The tonnage of canola (*Brassica napus*) oilseed produced in Canada has nearly doubled since 2007. Consequently, the supply of non-food grade canola seed (CS) has increased proportionally. Non-food grade CS has potential as a source of both energy (>40% oil) and protein (>20%) in cereal-based rations for poultry. Because unprocessed CS has approximately 50% of the carbon intensity of the corresponding amount of commercially-produced oil and solvent-extracted meal, the inclusion of CS in broiler rations may reduce the carbon intensity of feed. The present experiment therefore evaluated the effect of feeding increasing dietary inclusions of CS on broiler growth, carcass traits, saleable cuts and feed-associated carbon intensity. Male broilers (Ross 708; n = 336) housed in bedded floor pens were fed a common starter diet (0–10d) and then assigned to one of 4 dietary regimens for the grower (11–25d) and finisher (25–41d) phases of a 41-d trial in a randomized complete block design with 7 replicates per regimen. Dietary regimens consisted of phase-specific diets containing 0, 10, 20, or 30% rolled CS formulated to meet or exceed recommended nutrient density in proportion to target AME. Estimates of carbon intensity for each ingredient were derived from ECO-ALIM feedstuff database (INRA, France). For each phase, individual BW (initial and final) ADFI, ADG and gain-to-feed were determined. On d 42, broilers were slaughtered and antemortem wt, chilled carcass wt, and wt of saleable carcass cuts were measured. Data were analyzed as generalized linear mixed models using the GLIMMIX procedure of SAS (v9.4). Models included the main effect of CS inclusion, block as a random effect and a contrast statement to test for linear effects of CS inclusion. Birds fed diets including 30% CS had lower BW ($P < 0.01$) at d 25 and 41, and tended to have lower overall ADG ($P < 0.06$) compared with other regimens. Birds fed diets containing 10, 20 and 30% CS consumed 8.6 g/d more feed ($P < 0.04$) compared with the 0% CS controls, which linearly reduced ($P < 0.01$) gain-to-feed by approximately 0.4 g/g for each 10% increase in CS inclusion. Birds fed 30% CS had reduced antemortem wt ($P < 0.01$), carcass wt ($P < 0.03$) and reduced dressing % ($P < 0.07$) compared with birds fed 0, 10 or 20% CS. There was no dietary regimen effect

on feed-associated carbon intensity per kg gain or per bird marketed. The present experiment demonstrated that dietary inclusion of CS up to 20% has little adverse effect on broiler growth, carcass traits, and yield of saleable cuts, but had limited influence on feed-associated carbon intensity. Further investigations into CS digestible nutrient content are needed to optimize its inclusion in broiler diets.

Key Words: broiler chickens, canola seed, carbon intensity, carcass traits, growth performance

512P Effect of high dietary inclusion of canola or camelina expeller-pressed cake or solvent-extracted meal on hen performance and egg quality. Matt Oryschak² and Eduardo Beltranena^{*1,2}, ¹University of Alberta, Edmonton, Alberta, Canada, ²Alberta Agriculture and Forestry, Edmonton, Alberta, Canada.

Oilseed co-products are good dietary sources of amino acids for poultry, and those with remaining oil content can also supply a portion of the energy requirements. Anti-nutritional factors, however, may adversely affect productivity or product quality when poultry are fed high dietary inclusions of co-products. The objective of the present study therefore was to compare the productivity of and egg quality from hens fed high dietary levels of canola (*Brassica napus*) and camelina (*Camelina sativa*) co-products. In a randomized complete block design with 6 replicates per treatment, hens (Lohmann LSL Lite; n = 120) housed 4/cage in a conventional layer battery were fed a wheat-soybean meal based control diet, or one of 4 test diets for the duration of a 12 wk study (40–52 wk of age). Test diets met or exceeded recommended nutrient requirements in proportion to AME, and included 25% solvent-extracted canola (SECAN; 36.7% CP, 3.3% Cf_{at}, 11.2% CFiber) or camelina (SECAM; 37.9% CP, > 0.5% Cf_{at}, 10.5% CFiber,) meal, or 30% expeller-pressed canola (EPCAN; 23.7% CP, 12.1% Cf_{at}, 6.9% CFiber) or camelina (EPCAM; 33.8% CP, 11.0% Cf_{at}, 9.4% CFiber) cake. Individual hen BW, cage feed disappearance (ADFD), egg mass-to-feed (EF) ratio, egg wt, lay percentage, and objective egg quality were determined at 3-wk intervals during the study. Data were analyzed as generalized linear mixed models using the GLIMMIX procedure of SAS (v9.4). Models included the main effect of diet and block as a random term. Overall hen ADFD was not different among dietary treatments and the control diet other than EPCAM, which was lower (97 vs. 121 g/d; $P < 0.01$) than controls. There was no difference in EF among treatments, suggesting that observed differences in lay percent ($P < 0.01$) and egg wt ($P < 0.01$) were attributable to different feed intake patterns rather than a nutrient imbalance. Feeding hens both SECAM and EPCAM yielded eggs with greater ($P < 0.01$) proportional wt of albumen and lower proportional wt of yolk compared with controls. Hen fed all test diets produced eggs with equivalent or superior Haugh and eggshell thickness measurements compared with those from controls. The present study demonstrated that SECAN, SECAM and EPCAN can be fed at high inclusion levels in practical laying hen diets with no adverse effect on productivity or egg quality. Further characterization of EPCAM may identify anti-nutritional factors responsible for the reduction in ADFD observed in the present study.

Key Words: camelina, canola, laying hen, egg quality, hen productivity

513P Effect of high (30%) dietary inclusion of lentil and chickpea in layer diets on hen performance and egg quality. Matt Oryschak¹ and Eduardo Beltranena^{*1,2}, ¹Alberta Agriculture and Forestry, Edmonton, Alberta, Canada, ²University of Alberta, Edmonton, Alberta, Canada.

Western Canada is one of the world's largest exporting regions of pulse crops, including lentil (*Lens culinaris*) and chickpea (*Cicer arietinum*). A small proportion of the annual tonnage produced (<10%) that does not achieve export grade is occasionally available for use as a feedstuff. However there is limited empirical evidence that might indicate any practical limitations to dietary inclusion for laying hens. The objective of the present study therefore was to compare the productivity of and egg quality from hens fed diets containing a high dietary inclusion levels of lentil and chickpea. In a randomized complete block design with 6 replicates per treatment, hens (Lohmann LSL Lite; n = 120) housed 4/cage in a conventional layer battery were fed a wheat-soybean meal based control diet, or one of 4 test diets for the duration of a 12 wk study (40–52 wk of age). Test diets met or exceeded recommended nutrient requirements in proportion to AME, and contained 30% red (RL) or green lentil (GL), or 'Desi'- (DC) or 'Kabuli'-type chickpea (KC). Hen BW, feed disappearance (ADFD), egg mass-to-feed (EF) ratio, egg wt, lay percentage, and objective egg quality were determined at 3-wk intervals during the study. Data were analyzed as generalized linear mixed models using the GLIMMIX procedure of SAS (v9.4). Models included the main effect of diet and block as a random term. There was no effect of treatment on overall ADFD, EF or lay percentage. The wt of eggs from hens fed GL was less than those from DC or KC, whereas eggs from the RL treatment and controls were intermediate ($P < 0.01$). Egg mass from hens fed KC was greater than that of hens fed RL ($P < 0.01$), but no treatment egg mass production was different from controls. Other than a difference in proportional yolk weight, which was slightly reduced in comparison to controls by feeding DC, GL and RL ($P < 0.01$), there was no effect of pulse treatment on any objective measure of egg quality. The present study demonstrated that lentil and chickpea can be fed at 30% of laying hen diets with little adverse effect on hen productivity or objective egg quality.

Key Words: chickpea, egg quality, hen productivity, laying hen, lentil

514P Dietary approaches to reducing the carbon intensity of table egg production: Capping dietary carbon intensity. Matt Oryschak¹, Daniella Batres², Emmanuel Opoku-Yeboah², and Eduardo Beltranena^{*1,2}, ¹Alberta Agriculture and Forestry, Edmonton, Alberta, Canada, ²University of Alberta, Edmonton, Alberta, Canada.

Approximately 65% of the carbon footprint of table egg production in Western Canada is directly attributable to the carbon intensity (CI) of feed. Life-cycle analyses (LCA) that have been conducted for several common feed ingredients demonstrate considerable variability in their relative CI based, due primarily to crop inputs and the extent of feed processing. It may be possible therefore to reduce the feed associated CI of egg production through including ingredient CI as an additional parameter in feed formulation matrices and consideration of lower CI ingredients. The present study therefore sought to demonstrate a conservative approach to the application of LCA-based estimates of ingredient CI to feed formulation with the aim of reducing the CI of laying hen diets. Treatments consisted of a least cost control diet and least-cost formulations that capped LCA-based CI to 5, 10 or 15% below that of the control. Estimates of CI for feed ingredients were obtained from the ECOALIM database (INRA, France). Hens (Lohmann LSL-Lite; n = 128) housed in conventional battery cages (4 hens/cage) were fed one of the 4 dietary regimens for a 16 wk experimental period in a randomized complete block design with 8 replicate cages per treatment. Hen BW, ADFI, lay percent, egg mass production, and objective egg quality were measured at 4 wk intervals. Data were analyzed as generalized linear mixed models using the GLIMMIX procedure of SAS (v9.4). Models included the main effect of CI reduction, block as a random term and a

contrast to test for linear effects of CI reduction. Reducing dietary CI did not affect hen BW, feed disappearance or lay percentage. Reducing dietary CI did, however, linearly increase both egg mass production ($P < 0.01$) and egg-to-feed ratio ($P < 0.02$). The feed-associated CI of egg production (in g CO₂ eq./kg egg mass production) was linearly reduced ($P < 0.01$) by 15 g for each 1-% reduction in dietary CI intensity. There were no adverse effects of treatment on any objective measure of egg quality, feed costs, income or profitability per hen-day. The results of the present study confirmed that by incorporating LCA-derived estimates of CI for feed ingredients and the imposition of conservative CI caps as a formulation constraint, the CI of egg production can be reduced without adversely affecting hen productivity, egg quality or relative profitability.

Key Words: carbon intensity, egg quality, egg production, feed formulation, layers

515P Dietary approaches to reducing the carbon intensity of egg production: Optimization of dietary AME density and formulation objective.

Matt Oryschak¹ and Eduardo Beltranena^{*1,2}, ¹Alberta Agriculture and Forestry, Edmonton, Alberta, Canada, ²University of Alberta, Edmonton, Alberta, Canada.

Life-cycle analyses (LCA) have revealed that approximately 65% of the carbon footprint of egg production is attributable to the carbon intensity (CI) of the feed, and that considerable variability exists in the relative CI of common feed ingredients. Additionally, feed efficiency or dietary AME density could be positively or inversely related to the environmental impact of table egg production depending on the situation. The objective of the present study therefore was to elucidate the relationship between feed-associated CI of egg production and dietary AME density in regimens formulated to minimize either cost or CI. In a randomized complete block design with 8 replicates per treatment, layers (Lohmann LSL-Lite; $n = 192$) housed in conventional battery cages (4 hens/cage) were fed one of 6 dietary regimens for a 16-wk experimental period. Dietary regimens consisted of laying hen diets formulated to either minimize cost (LC) or minimize CI (LI), and to meet 2.65, 2.8 or 2.95 Mcal/kg AME in a 2×3 factorial arrangement. All diets were fed in mash form and met or exceeded recommended nutrient density in proportion to AME. Estimates of CI for feed ingredients were derived from the ECOALIM database (INRA, France). Hen BW, ADFI, lay percent, egg mass production, and objective egg quality were measured at 4 wk intervals. Data were analyzed as generalized linear mixed models using the GLIMMIX procedure of SAS (v9.4). Models included the main effect of formulation objective, energy density and the 2-way interaction with block included as a random term. Hen BW and egg-to-feed ratio both increased with increasing AME density ($P < 0.01$). Except for eggshell thickness, formulation objective (LC, LI) did not affect any hen productivity or egg quality parameter independently of AME density. Eggshell thickness was 3% greater for 2.65 compared with 2.80 and 2.95 Mcal/kg diets ($P < 0.02$). Profitability declined by 3% as AME density increased from 2.65 to 2.95 Mcal/kg. Feed disappearance declined as AME increased in LI diets, but not in LC diets ($P < 0.05$). Avg egg wt and egg mass production was not different among AME densities for LI diets, but was greater for 2.95 compared with 2.65 and 2.8 Mcal/kg in LC diets ($P < 0.01$). Feed-associated CI followed different patterns within LC and LI categories, but the greatest difference (21%) was observed between LC x 2.80 Mcal/kg and LI x 2.65 Mcal/kg diets (1124 vs 880 g CO₂ eq/kg egg mass). The present study confirmed that feed-associated CI of egg production can be measurably reduced, with minimal adverse effect on hen performance or egg quality by incorporating ingredient CI into feed formulation matrices and optimizing dietary AME density.

Key Words: carbon intensity, egg quality, egg production, feed formulation, layers

516P A comparison of solvent-extracted and expeller-pressed Camelina sativa co-products as feedstuff for laying hens. Matt Oryschak¹ and Eduardo Beltranena^{*1,2}, ¹Alberta Agriculture and Forestry, Edmonton, Alberta, Canada, ²University of Alberta, Edmonton, Alberta, Canada.

Camelina (*Camelina sativa*) is an ancient oilseed that has received renewed interest as a potential bio-industrial crop for the production of bio-based jet fuel. Oil pressing from the seed results in co-product cake with remaining oil content, which after solvent-extraction results in meal with slightly greater protein content; both cake and meal have potential as feedstuff for poultry. The objective of the present study therefore was to compare the effect of increasing dietary inclusion of either expeller-pressed (EPCAM) cake or solvent-extracted meal (SECAM) on hen productivity and egg quality parameters. In a randomized complete block design with 6 replicates per treatment, laying hens (H & N Brown Nick; $n = 264$) housed in a conventional battery were fed one of 11 dietary regimens for a 36-wk laying cycle. Dietary regimens consisted of early and late lay phase diets containing no camelina co-products (control), or phase-specific diets containing 5, 10, 15, 20 or 25% of EPCAM or SECAM for a 36-wk laying cycle. Hen BW, feed disappearance (ADFD), egg mass-to-feed (EF), avg egg wt, lay percentage, and basic measures of egg quality were determined at 4-wk intervals during the study. Data were analyzed as generalized linear mixed models using the GLIMMIX procedure of SAS (v9.4). Models included main effect of co-product inclusion level (0, 5, 10, 15, 20, 25%), co-product (EPCAM vs. SECAM), the 2-way interaction and block as a random term. The ADFD and EF of all co-product treatment regimens were not different from those of the control. Co-product interacted with inclusion level ($P < 0.01$) to affect avg egg wt, lay percent, egg mass production and proportional wt of albumen, shell and yolk. The avg egg wt within EPCAM regimens was greater or equal for all inclusion levels compared with the control, whereas increasing dietary inclusion of SECAM linearly reduced avg egg wt ($P < 0.05$). Patterns in lay percent and egg mass production differed between EPCAM- and SECAM-containing diets; however, neither metric ($P > 0.17$) differed between 0% and 25% dietary inclusion regimens for either co-product. The proportional wt of albumen increased at the expense of proportional yolk wt with increasing dietary inclusion of either co-product ($P < 0.01$). The results of the present study indicate that laying hens can be fed diets containing up to 25% of either EPCAM or SECAM without adversely affecting hen performance or basic egg quality. Further research is warranted to determine possible differential effects of oil extraction method on the nutrient digestibility in the resulting co-products.

Key Words: camelina, egg quality, egg production, laying hen, oilseed coproducts

517P Replacement of soybean meal with canola meal at two feed energy levels on production performance and egg attributes of laying hens. Matt Oryschak¹, Miranda Smit¹, and Eduardo Beltranena^{*1,2}, ¹Alberta Agriculture and Forestry, Edmonton, Alberta, Canada, ²University of Alberta, Edmonton, Alberta, Canada.

Layers may sustain egg production consuming a lower cost feed if they are able to increase consumption of reduced energy diets including alternative protein sources. Feed intake is also typically greater in larger Brown- compared with lighter White-shelled egg layers, so genotype may offer additional opportunities to reduce feed energy concentration

replacing common, standard protein sources with lower-cost alternative ones according to layer strain. Therefore, the objective of this experiment was to evaluate the effect of replacing soybean meal (SBM) with canola meal (CM) at 2 dietary energy levels on laying performance, measures of egg components and quality and cost vs. benefit. Brown- and White-shelled egg layers ($n = 512$) housed 8 per paired cages (a replicate) in a 3-tier battery were used to evaluate replacing SBM with CM either at 2.6 or 2.9 Mcal AME/kg at 4-wk intervals from 37 to 53 wk of age (8 replicates per treatment x hen strain). Diets were formulated to meet or exceed 105% of the recommended apparent ileal digestible amino acid content in proportion to AME. Production and egg quality data were analyzed using PROC MIXED (SAS v 9.4). The main effects in the $2 \times 2 \times 2$ factorial model were hen strain, energy level, supplemental protein ingredient and interactions. Block was random and 4-wk period was the repeated term. There were no hen strain effects or interactions. Energy level x protein meal effects were evident for periods 1, 2 and entire trial. Overall, hens fed the 2.9 Mcal CM diet consumed 5.7 g/d less feed than those fed other diets (120.4 g/d). Hens fed the 2.9 Mcal SBM diet produced 3.4 g/d more egg mass than those fed other diets. In contrast, independent energy level and protein meal effects were more evident for periods 3 and 4. Hens fed the 2.9 Mcal diets averaged 53 g heavier BW, consumed 6.4 g less feed and produced 3.7 g more egg mass than those fed the 2.6 Mcal diets for periods 3 and 4. Hens fed SBM diets averaged 72 g heavier BW, produced 2.8 g/d greater egg mass and had 1 g heavier egg yolk than those fed CM diets for periods 3 and 4. Despite lower FE, CM can profitably replace SBM feeding reduced energy diets to laying hens because the 2.6 Mcal CM diet had the lowest cost per calorie.

Key Words: canola meal, soybean meal, dietary energy, laying hen, egg production and quality

518P Dietary approaches to reducing the carbon intensity of chicken meat production: Optimization of dietary AME density and formulation objective. Matt Oryschak¹ and Eduardo Beltranena^{*1,2}, ¹Alberta Agriculture and Forestry, Edmonton, Alberta, Canada, ²University of Alberta, Edmonton, Alberta, Canada.

Life-cycle analyses (LCA) have revealed considerable variability in the relative carbon intensity (CI) of common feed ingredients. Feed efficiency or dietary AME density could either be positively or inversely related to the environmental impact of chicken meat production. The objective of the present study was to elucidate the relationship between feed-associated CI of chicken meat production and dietary AME density in feeding regimens formulated to minimize either cost or CI. In a randomized complete block design with 7 replicates per treatment, broilers (Ross 708; $n = 504$) housed in floor pens were fed a common starter phase diet from d0 to 10 of age. Beginning on d11, pens of broilers were assigned to one of 6 dietary regimens in a 2×3 factorial consisting of grower (d11–24) and finisher (d25–41) diets formulated to either minimize cost (LC) or minimize CI (LI) and to meet 100% (REC), 95% (RD5) or 90% (RD10) of recommended AME density. Diets were fed in mash form and met or exceeded recommended nutrient density in proportion to AME. Estimates of CI for feed ingredients were derived from ECOALIM database (INRA, France). Growth performance variables included ADFI, ADG and gain-to-feed. On d 42, broilers were slaughtered and antemortem wt, chilled carcass wt and wt of carcass cuts was recorded. Data were analyzed as generalized linear mixed models using GLIMMIX procedure of SAS (v9.4). Models included the main effects of formulation objective, energy density and 2-way interaction with block included as a random term. Broiler BW on d41 ($P < 0.01$), overall ADG ($P < 0.02$) and gain-to-feed ($P < 0.01$) differed between

LC and LI regimens only for REC. The ADFI of broilers fed LC diets was lower than that of LI ($P < 0.03$). Antemortem wt for LC x REC broilers was greater than that of broilers fed all other dietary regimens ($P < 0.02$). Dressing percent and yield of carcass cuts other than breast were slightly greater (i.e., 1%-point) for broilers fed LC compared with LI regimens ($P < 0.01$). Net income per kg of gain was unaffected by dietary AME density in broilers fed LC regimens, but increased in LI regimens as AME density decreased ($P < 0.01$). The feed-associated CI per bird marketed was 12% lower for LI compared with LC regimens ($P < 0.01$) and 10% lower for both RD5 and RD10 regimens compared with REC. The maximum reduction in feed associated CI with reference to LC x REC regimen was achieved feeding the LI x RD10 regimen (21%, $P < 0.05$). The present study confirmed that feed-associated CI of chicken meat production can be measurably reduced with minimal adverse effect on broiler growth, carcass traits or saleable cuts by incorporating ingredient CI into ingredient formulation matrices and reducing dietary AME density.

Key Words: broiler chickens, carbon intensity, carcass traits, growth performance, saleable cuts

519P Dietary approaches to reducing the carbon intensity of chicken meat production: Capping dietary carbon intensity. Matt Oryschak¹ and Eduardo Beltranena^{*1,2}, ¹Alberta Agriculture and Forestry, Edmonton, Alberta, Canada, ²University of Alberta, Edmonton, Alberta, Canada.

Approximately one-third of the carbon footprint of chicken meat production in Western Canada is attributable to the carbon intensity (CI) of feed. Life-cycle analyses (LCA) that have been conducted for several common feed ingredients demonstrated considerable variability in their relative CI due primarily to crop inputs and extent of feed processing. It may be possible therefore to reduce the feed associated CI of chicken meat production through including ingredient CI as an additional parameter in feed formulation matrices and consideration of lower CI ingredients. The present study therefore sought to demonstrate a conservative approach to the application of LCA-based estimates of ingredient CI to feed formulation with the aim of reducing the CI of broiler diets. In a randomized complete block design with 7 replicates per treatment, broilers (Ross 708; $n = 336$) housed in floor pens bedded with wood shavings were fed a common, least-cost starter phase diet from d0 to 10 of age. Beginning on d11, pens of broilers were assigned to one of 4 dietary regimens consisting of least-cost grower (d11–24) and finisher (d25–41) diets or phase-specific least-cost formulations that capped LCA-based CI to 5, 10 or 15% below that of the control regimen. All diets were fed in mash form and met or exceeded nutrient density recommendations. Feed formulation utilized LCA-based estimates of CI for feed ingredients obtained from the ECOALIM database (INRA, France). Broilers, feed added and orts remaining were weighed at the beginning and end of each production phase to calculate ADFI, ADG and gain-to-feed. On d 42, broilers were slaughtered and antemortem wt, carcass wt and wt of carcass cuts was recorded. Data were analyzed as generalized linear mixed models using the GLIMMIX procedure of SAS (v9.4). Models included the main effect of CI reduction, block as a random term and a contrast to test for linear effects of CI reduction. Reducing dietary CI did not affect broiler BW on d 41, overall ADFI or ADG. Reducing dietary CI did, however, linearly ($P < 0.01$) decrease overall gain-to-feed. Reducing dietary CI did not affect antemortem wt, carcass wt, dressing %, or the yield of saleable carcass cut as proportion of chilled carcass wt. Reducing dietary CI linearly ($P < 0.01$) decreased the feed-associated CI (g CO₂ per bird marketed) by approximately 22 g for each 1-% reduction in feed formulated CI. The results of the

present study confirmed that by incorporating LCA-derived estimates of CI for individual ingredients and the imposition of conservative CI caps as a formulation constraint, the CI of chicken meat production can be reduced without adversely affecting growth performance, carcass traits or yield of saleable cuts.

Key Words: broiler chickens, carbon intensity, carcass traits, growth performance, saleable cuts

520P Impact of dietary calcium level, non-phytate phosphorus level and feeding time on phosphorus digestibility of SBM in broilers. Qian Zhang*, Frances Yan, Ashley Provin, Mercedes Vazquez-Anon, and Deana Hancock, *Novus International, St. Charles, Missouri, United States.*

In addition to dietary calcium (Ca) level, dietary non-phytate phosphorus (nPP) level and feeding duration may be important factors to impact the phosphorus (P) digestibility of ingredients, as birds will likely adapt to P deficiency over time. Therefore, a battery study was conducted to evaluate the dietary Ca level, nPP level and feeding duration on P digestibility of SBM in broilers. A total of 512 birds were allocated to 8 dietary treatments in a $2 \times 2 \times 2$ factorial arrangement (0.59% or 1.02% dietary Ca; SBM with 0.17% nPP or SBM-dicalcium phosphate blend with 0.30% nPP; 5 d or 40 h feeding). The 5 d or 40 h feeding was from d 16 to d 21 or from d 19 to d 21. Each diet was provided to 8 replicate pens of 8 birds in mash form. On d 21, all birds were sacrificed to collect ileal digesta for nutrient digestibility. Data were analyzed by 3-way ANOVA to evaluate the main effect of dietary Ca level, nPP level, feeding duration and their interactions on P digestibility of SBM. Statistical difference or a tendency were declared at $P \leq 0.05$ or $0.05 < P \leq 0.10$. The results showed a tendency ($P = 0.09$) to have a significant interaction between dietary Ca level and feeding duration on P digestibility of SBM. At 40 h feeding, high Ca significantly decreased the P digestibility of SBM from 40.2% to 29.4%. However, at 5 d feeding, the P digestibility of SBM was not impacted by dietary Ca level; the values were 45.5% and 42.1% with low and high Ca, respectively. The results at 5 d feeding were not able to show the influence of dietary Ca on P digestibility of SBM, as the values at 5 d feeding were most likely overestimated. This may be due to the birds have already adapted to P deficiency at 5 d feeding. Although the interaction among the 3 factors was not detected by the statistical model, the data showed dietary nPP level impacted the P digestibility of SBM differently at the 2 feeding durations and dietary Ca level combinations. At 5 d feeding with high Ca, the P digestibility of SBM in 0.17% nPP group was 9.5% higher than that in 0.30% nPP group; while at 5 d feeding with low Ca, the difference of P digestibility of SBM between 2 nPP groups was 0.1%. At 40 h feeding with low or high Ca, the difference of digestibility between 2 nPP groups was 0.0% or 1.5%. The results suggested that, at 5 d feeding with high Ca, the P digestibility of SBM was decreased with incremental nPP level, but the digestibility was consistent with altered nPP levels at 40 h feeding with both low and high Ca. Taken together, 40 h feeding duration is recommended for the evaluation of P digestibility of SBM, which was observed to be 40.2% and 29.4% at 0.59% and 1.02% dietary Ca inclusion rates, respectively.

Key Words: phosphorus digestibility, feeding duration, calcium, non-phytate phosphorus, SBM

521P The effect of pelleting diets composed of large particle corn using standard conditioning and more thermally aggressive conditioning utilizing a hygienizer. Kari Harding*, Angela Lamp,

Timothy Boltz, Victoria Ayres, and Joseph Moritz, *West Virginia University, Elkview, West Virginia, United States.*

The use of large particle corn in broiler diets may decrease energy costs associated with feed grinding and improve nutrient retention and digestion in the gastrointestinal tract. However, large particle corn has been suggested to decrease pellet quality, that may negatively affect broiler performance. This study hypothesized that more thermally aggressive conditioning utilizing a hygienizer will attenuate negative effects of incorporating large particle corn into diet formulations. The objectives were to pellet broiler grower diet formulations containing either 980, 1,250, or 1,600 μm roller milled corn using standard conditioning and more thermally aggressive conditioning utilizing a hygienizer and evaluate pellet mill efficiency and pellet quality. Diets were corn and soybean meal based and contained 5% DDGS and 4.3% mixer added soybean oil. Pellet mill throughput was 30% above equipment manufacturer recommendations to assimilate broiler integrator pellet production practices. Standard conditioning was defined as 70°C conditioning for 15 s and more aggressive conditioning was defined as 80°C conditioning for 60 s and 45 s retention within a hygienizer. Hygienizers incorporate jacketed heat that enables feed to maintain a desired temperature for an extended time. All conditioned feed was extruded through a 4.8 \times 38 mm pellet die. Treatments were replicated 3 times across 3 d of feed manufacture. Treatments were arranged in a 3 (particle size) \times 2 (level of conditioning) factorial with more thermally aggressive conditioning following standard conditioning each day and corn particle size treatments being alternated in a 3 \times 3 Latin square. Corn particle size did not affect pellet mill motor load, hot pellet temperature, or pellet quality ($P > 0.05$). Perhaps the diet formulation and high production rate contributed to factors that could not be exacerbated by increasing corn particle size beyond the large particle size of 980 μm . More thermally aggressive conditioning utilizing a hygienizer decreased pellet mill motor load and increased hot pellet temperature and pellet quality ($P < 0.05$). These observations were likely the result of increased lubrication due to increased steam volume and increased starch gelatinization and protein gelation due to greater heat, moisture, and retention. Diets containing high levels of mixer added fat, DDGS, and large particle corn may produce pellets of improved quality if manufactured using more thermally aggressive conditioning with a hygienizer.

Key Words: particle size, hygienizer, pellet quality, pelleting, conditioning

522P ω 3 fatty acid profile of broiler chickens fed different levels of flaxseed during finisher period. Ali Daneshmand*, Hassan Kermanshahi, and Ali Javadmanesh, *Ferdowsi University of Mashhad, Mashhad, Khorasan Razavi, Iran (the Islamic Republic of).*

The purpose of this study was to find the optimum level of ground flaxseed to fortify broiler meat with ω 3 fatty acids (FA). A total of 360 d-old male Ross 308 chicks were obtained from a local hatchery, and reared till d 24 based on Ross guideline. On d 25, chickens were weighted, transferred into floor pens which assigned to 4 dietary treatments containing 6 replicates of 15 birds in each replicate. Treatments were: corn-soybean meal based diet as control (Ctrl); Ctrl replaced in part by 3% ground flaxseed (Flax3); Ctrl replaced in part by 6% ground flaxseed (Flax6); Ctrl replaced in part by 9% ground flaxseed (Flax9). On d 34 and 45, 2 birds from each replicate (12 birds/treatment) were randomly selected and euthanized, the viscera was excised, and the relative weight of the carcass (with skin), skin, muscles (breast and thigh, without skin), internal organs (liver and heart), and abdominal fat pad (AFP) were recorded and stored at -20°C for fatty acid analysis. All data were analyzed in a completely randomized design using GLM

procedure of SAS software and means were compared by Tukey's test ($P < 0.05$). Birds fed with Flax6 had higher ($P < 0.05$) polyunsaturated fatty acids (PUFA) and $\omega 3$ FA in breast and thigh muscles and lowered ($P < 0.05$) the relative weight of AFP compared with control group. Eicosapentaenoic acid (EPA) both in 34 and 45 d of age in skin and AFP increased ($P < 0.05$) and $\omega 6/\omega 3$ ratio decreased ($P < 0.05$) at flax6 when compared with those of other treatments ($P < 0.05$). Total $\omega 3$ FA was also increased ($P < 0.05$) at skin of chickens at 45 d by flax6. In conclusion, feeding flax6 to chickens at finisher period (d 25 to 45) may improve $\omega 3$ FA content and $\omega 6/\omega 3$ ratio of meat products, and reduce fat depots in AFP.

Key Words: flaxseed, $\omega 3$ fatty acids, broiler chickens, meat fortification

523P Conditioning time and pellet binder levels affect pellet quality and pellet mill efficacy in a poultry feed plant. Ali Daneshmand*, Hassan Kermanshahi, Abolghasem Golian, and Amir Attar, *Ferdowsi University of Mashhad, Mashhad, Khorasan Razavi, Iran (the Islamic Republic of).*

The present study was conducted to evaluate the effects of conditioning times and processed sodium bentonite (PSB)-based pellet binder (G. Bind, PAYA FARAYAND H.N. Co., Mashhad, Iran) levels on pellet quality and pellet mill efficacy in a local poultry feed plant. In a completely randomized design, diets were assigned to a 2×3 factorial arrangement including 2 conditioning times (2 and 4 min) and 3 levels of PSB (0, 7.5 and 15 g/kg) with 6 replicates. All diets were formulated to meet or exceed the minimum requirements of Ross 308 male chickens for the grower period (11–24 d). All diets were sampled after production to test for pellet durability index (PDI) and hardness. PDI was measured using a Holmen Pellet Tester. The pellet hardness was assessed using a hardness tester machine. For pellet mill efficacy, the used amperages were recorded. The amperages of the pellet mill motor were recorded when various experimental diets passed through the pellet mill machine to calculate relative electrical energy usage (REEU). Data were analyzed using 2-way ANOVA of the General Linear Model (GLM) procedure of SAS software to determine the main effects and 2-way interactions and means were compared using Tukey's test ($P < 0.05$). The results showed that the diet with a 2 min conditioning time and containing 15 g/kg PSB had the highest ($P < 0.05$) PDI and hardness. Diets contained 15 g/kg PSB and conditioned for 2 min had the highest ($P < 0.05$) PDI and hardness at different time intervals and had the lowest ($P < 0.05$) REEU when compared with other diets. It was concluded that a corn-soybean meal diet conditioned for 2 min and containing 15 g/kg PSB may improve pellet quality and pellet mill efficacy when prepared for broiler chickens during the growing period.

Key Words: conditioning time, pellet quality, pellet mill, electricity usage, broiler chickens

524P Effect of feeding diets containing high amounts of fat on productive performance and egg quality, and economics of laying hens from 18 to 70 weeks of age. Gi Ppeum Han*¹, Jong Hyuk Kim¹, Jomari Delos Reyes¹, Hyeon Seok Choi¹, Geun Hyeon Park¹, Hwan Ku Kang², and Dong Yong Kil¹, ¹*Chung-Ang University, Anseong-Si, Korea (the Republic of),* ²*Rural Development Administration National Institute of Animal Science, Pyeongchang, Korea (the Republic of).*

Dietary fat for laying hen has been reported to improve performance and egg quality of laying hens. However, the use of high amounts of fat in layer diets has not been practiced widely because of increasing

feed costs. Consequently, there is a lack of researches regarding the effect of feeding diets containing high amounts of fat on laying hens during the entire laying cycle (from 18 to 70 weeks of age). Therefore, the objective of this experiment was to investigate the effect of feeding diets containing high amounts of fat on productive performance, egg quality, and economics of laying hens from 18 to 70 weeks of age. A total of 384 18-week-old Hy-line Brown laying hens were allotted to 1 of 3 dietary treatments with 8 replicates. Each replicate had 16 hens. A basal diet was prepared without additional fat addition. Other 2 diets were prepared by adding 1.5 or 3.0% tallow to the basal diet. However, AME_n and other nutrient concentrations were equalized among 3 dietary treatments. Productive performance was recorded every 4 weeks during 52 weeks of experiments. Egg quality was measured with 12 eggs per replicate every 4 weeks. The data were analyzed by ANOVA in completely randomized design using the PROC MIXED procedure. During 52 weeks of the experiment, feeding diets containing 1.5% or 3.0% tallow decreased FCR ($P < 0.05$), but feeding diets containing 3.0% tallow only resulted in decreased FI and increased egg weight than feeding the basal diets ($P < 0.05$). There were no differences in other productive performance (i.e., egg production rate, egg mass, and broken egg production rate) among dietary treatments. For egg quality, increasing amounts of tallow in diets decreased egg yolk color ($P < 0.05$), but feeding diets containing 3.0% tallow only induced decreased eggshell thickness ($P < 0.05$). Other egg quality including eggshell color and Haugh unit was not affected by dietary treatments. In the economic analysis, increasing inclusion of tallow in diets increased total feed costs for a 52-week laying period. However, total income minus total feed costs did not differ among dietary treatments possibly due to increasing egg mass and decreasing FI. In conclusion, the use of 1.5% tallow in layer diets during the entire laying period has a benefit on productive performance without negative effects on egg quality and the final income of egg production. It is likely that the use of 3.0% tallow in layer diets impairs eggshell thickness despite other possible benefit on productive performance of laying hens.

Key Words: dietary fat, economics, egg quality, laying hen, productive performance

525P ISA brown and Shaver white egg-type pullet breeders fed algae or flaxseed products as sources of n-3 polyunsaturated fatty acids: Embryonic uptake of fatty acids. Reza Akbari Moghadam Kakhki*¹, David W. L. Ma¹, Kayla Price², Janna Moats³, Niel Karrow¹, Tina Widowski¹, and Elijah Kiarie¹, ¹*University of Guelph, Guelph, Ontario, Canada,* ²*Alltech Canada, Guelph, Ontario, Canada,* ³*O & T Farms, Saskatoon, Saskatchewan, Canada.*

Enriching egg products destined for human use with n-3 polyunsaturated fatty acids (PUFA) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) is widely practiced. However, there is limited information on the impact of feeding egg-type pullet breeders PUFA on dynamics of embryonic fatty acids (FA) uptake. The objective of this study was to investigate the effect of feeding 2 sources of PUFA to egg-type pullet breeders on FA composition of hatching eggs and uptake by the embryo during incubation. Twenty-six wk old ISA brown and Shaver white breeders were fed one of 3 diets: 1) Control (CON), 2) CON + 1% of a dried micro-alga (*Aurantiochytrium limacinum*) fermentation product (DMA), as a source of DHA, and 3) CON + 2.48% of LinPRO, a dry extruded full-fat flaxseed (FFF) product, as a source of α -linolenic acid (ALA). Test diets (DMA and FFF) had equal amounts of n-3 and n-6: n-3 ratio. Breeders were housed in 3 group pens of 27♀ and 4♂ per strain and had free access to diets and water for 30 d upon which 660 fertile eggs were collected for incubation. Additional eggs (16 per

diet) were analyzed for baseline concentration of FA in the phospholipid fraction (PL) of the yolk. Residual yolk (RY) samples (10 per treatments) were taken at hatch for FA analyses in PL. Fatty acids consumption was calculated by subtracting concentration of FA in RY from the baseline concentration. Data were analyzed as completely randomized design with a factorial arrangement (strain, diet and interaction) using GLIMMIX procedures of SAS. There was an interaction between strains and diets ($P < 0.05$) on ALA and EPA of yolk PL; both n-3 sources decreased ALA and increased EPA to a greater extent in Shaver white compared with ISA brown. Shaver white also deposited 29.4% more DHA in yolk PL than ISA brown ($P < 0.001$). The interactive effect of strains and diets ($P = 0.019$) on consumed ALA was such that DMA and FFF reduced ALA uptake to a greater extent in Shaver white compared with ISA brown. There was no interaction effect between strains and diets on consumed EPA and DHA ($P > 0.05$). While embryos of DMA and FFF consumed identical EPA ($P = 0.950$), less ALA ($P = 0.007$) and total PL ($P < 0.001$), they preferentially consumed more DHA (DMA: 13.69 and FFF: 12.53 mg) compared with CON (10.43 mg). Shaver white embryos consumed more ($P < 0.05$) ALA (6.9%), EPA (90.9%), and DHA (37.4%) compared with ISA brown. In conclusion, our results suggest that feeding egg-type breeders n-3 PUFA can modify the pattern of FA uptake by embryos. The preferential consumption of DHA might be attributed to higher demand for embryonic development and warrant further investigations to characterized potential benefits on the progeny.

Key Words: egg-type breeders feeding, omega-three fatty acids, embryonic period

526P Crude protein digestibility of soybean meals from beans produced in USA, Brazil, and Argentina: An in vitro study. Lewis Aguirre*¹, O. Chandi¹, L. Cámara¹, A. Smith², J. J. Arroyo³, and Gonzalo Mateos¹, ¹UPM, Madrid, Madrid, Spain, ²DSM Nutritional Products (UK) Ltd., Heanor, Derbyshire, United Kingdom, ³DSM Nutritional Products Iberia S.A., Alcalá de Henares, Madrid, Spain.

An assay was conducted to determine the in vitro CP digestibility of soybean meals (SBM) from beans of 3 origins. Samples ($n = 25$ by origin) from USA, Brazil (BRA), and Argentina (ARG) were collected from European crushing plants or at the arrival of the vessels to European ports. The samples were analyzed for key nutrient [CP, amino acids, crude fiber, neutral detergent fiber (NDF), sucrose, oligosaccharides, and minerals] and for protein quality variables [trypsin inhibitor activity (TIA), KOH solubility (KOH), protein dispersibility index (PDI), and heat damage indicator (HDI)]. On 88% DM bases, the BRA SBM had more CP (47.0%) and NDF (9.3%) and less sucrose (5.7%) and stachyose (4.3%) than the USA and the ARG meals (46.3 and 46.4%, 8.6 and 8.8%, 7.4 and 6.8%, and 5.1% and 4.5%, respectively) TIA, KOH, and PDI values were higher but HDI values was lower ($P < 0.01$) for the USA SBM than for the BRA and the ARG SBM. The in vitro test used in the study (2 steps) simulated the processes occurring in the gastrointestinal tract of the chicken (pepsin and pancreatic digestibilities). Briefly, 0.4 g of the sample was ground using a 0.75 mm screen and mixed with 5 mL of distilled water in falcon tubes. The samples were incubated with 10 mL of pepsin solution (containing 2,500 U/mg, Sigma) and 0.12 N of HCl solution at pH 1.9 at 39°C and 190 rpm for 45 min. Then, 0.18 mL of NaOH and 13.5 mL of the pancreatin solution (8 × USP, Sigma) was added to the sample and incubated during 1 or 3 h at pH 7.8 and 39°C. After incubation, the samples were placed on ice and 5 mL of trichloroacetic acid were added. The tubes were equilibrated by weight, centrifuged (8,700 rpm), and filtered. Crude protein was determined by the Kjeldahl method. Data were analyzed as completely randomized design with origin of the SBM as main effect. The Tukey test was used

to separate treatments means. Origin of the SBM affected the in vitro digestibility of the protein fraction, with USA meals presenting higher digestibility than the BRA and the ARG meals (78.4, 76.1, and 75.8% at 1 h of incubation and 82.1, 80.7, and 80.5%, at 3 h of incubation, respectively; ($P < 0.001$)). The data suggest that country of origin of the beans of these 3 origins affects the digestibility of the CP of the SBM. The data on CP digestibility obtained in vitro, favor the use of SBM from USA over the use of South American SBM.

Key Words: crude protein digestibility, in vitro test, soybean origin

527P Influence of energy concentration and standardized ileal digestible lysine content of the diet on performance and egg quality of brown-egg laying hens from 19 to 59 weeks of age.

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An experiment was conducted to determine the effects of the AMEn concentration and standardized ileal digestible lysine (SID Lys) content of the diet on performance and egg quality of brown Classic Lohmann hens from 19 to 59 wk of age. There were 8 treatments organized as a 2×4 factorial with 2 AMEn concentrations (2,750 and 2,650 Kcal/Kg) and 4 levels of SID Lys (0.68, 0.72, 0.76, and 0.80%) in the diet. All other indispensable amino acids were formulated according to the ideal ileal protein concept. Each treatment was replicated 9 times and the experimental unit was a cage with 9 hens. The experiment lasted for 10 periods of 4 wk each. All birds had free access to feed and water throughout the trial. Production data (egg rate, feed intake, egg weight, egg mass, FCR, BW gain, and mortality) were collected by period. Egg quality traits (egg weight, Haugh units, and egg shell resistance to breakage) were measured in 8 eggs randomly select from each replicate the last 2d of each of the 10 experimental periods. Data were analyzed as a completely randomized design using the mixed procedure of SAS with energy concentration, level of SID Lys and the interaction as main effects. In addition, treatment sum of squares of the level of SID Lys, was partitioned into lineal (L) and quadratic (Q) effects. All differences were considered significant at $P < 0.05$. No interactions between AMEn and SID Lys were found for any trait and therefore, only main effects are presented. An increase in the energy content of the diet, improved egg weight ($P < 0.001$) and FCR ($P < 0.05$) but did not affect any of the other variables studied. The diet with the higher energy content had more added fat than the diet with the lower energy content (3.5% vs. 1.3%). Consequently, the higher level of added fat might have resulted in the improvement in egg size observed. An increase in the level of SID Lys from 0.68% to 0.80% improved linearly egg weight ($P < 0.05$) and egg mass production ($P < 0.05$) but did not affect ADFI, FCR, BW gain, or hen mortality. The level of SID Lys of the diet did not affect egg shell resistance to breakage or albumen quality. In conclusion, an increase in the energy content of the diet from 2,650 to 2,750 Kcal AMEn did not affect the number of eggs produced but increased egg weight. Hens require no more of 0.68% digestible Lys in the diet (736 to 752 mg SID Lys per day for the low and high energy diet respectively) to optimize egg production. However, if the objective is to maximize egg weight or egg mass production, higher levels of SID Lys might be required.

Key Words: egg weight, egg production, energy, laying hens, standardized ileal digestible lysine

528P Not Presented

530P Effect of encapsulated citral and cinnamaldehyde on growth performance, intestinal morphology and meat quality of broiler chickens. Chongwu Yang^{*1,2}, Janghan Choi³, Marion Mogire¹, Argenis Rodas-Gonzalez¹, Moussa Diarra², Qi Wang³, Joshua Gong², and Chengbo Yang¹, ¹University of Manitoba, Winnipeg, Manitoba, Canada, ²Guelph Research and Development Centre, Agriculture Agri-Food Canada, Guelph, Ontario, Canada, ³University of Manitoba Animal Science, Winnipeg, Manitoba, Canada.

This study investigated the effects of encapsulated citral (CIT) and cinnamaldehyde (CIN) on growth performance, intestinal morphology and meat quality in broilers. A total of 320 0-d-age male broilers (Cobb 500) were allocated 32 pens with 8 pens per treatment. The treatments included: 1) Corn-wheat-SBM basal diet (negative control); 2) basal diet with 30 ppm avilamycin premix (positive control); 3) basal diet with 50 ppm encapsulated CIT and CIN (CCL); 4) basal diet with 100 ppm encapsulated CIT and CIN (CCH). Statistical analyses were conducted according to a complete randomized design using the GLM procedure (SAS 9.4) with treatment groups as sources of variation and the individual pen as experimental units (8 pens/treatment group). There were no significant differences between treatments in growth performance ($P > 0.05$) at each feeding stage. Additionally, birds had similar relative organ weights of heart, liver, spleen and bursa ($P > 0.05$). The higher ratios of villus height to crypt depth (VCR; $P < 0.05$) were detected in broilers fed either CCL or CCH, with an average being 14.67 and 15.13 in the duodenum, and 15.13 and 13.58 in the jejunum, respectively. Regarding meat quality, a higher pH value (pH = 6.21) in the breast was observed in 24-h post mortem chickens fed CCL ($P < 0.05$). No significant differences of pH were detected in the breast in 96-h post mortem birds. The breast color (redness, yellowness, and lightness) in either 24-h or 96-h post mortem chickens was not significantly different among treatments ($P > 0.05$). No change in purge loss (%) was observed in the breast after 48 h. The severity of white striping (WS) or woody meat (WB) was higher in the positive control (WS = 0.58; WB = 0.15) than in other treatments ($P < 0.05$). In conclusion, dietary treatment with encapsulated CIT and CIN improved gut health and meat quality of broilers by increasing VCR in the small intestine and reducing the severity of WS/WB in the breast, respectively, without affecting growth performance.

Key Words: broiler, encapsulated essential oils, gut health, meat quality, intestinal morphology

531P Effects of source and level of available phosphorus in the diet on performance and egg quality of brown laying hens from 33 to 45 weeks of age. A. Huerta¹, A. F. de Juan^{*1}, J. García^{1,2}, R. Scappaticcio^{1,2}, L. Cámara¹, and Gonzalo Mateos¹, ¹UPM, Madrid, Madrid, Spain, ²Camagroalimentaria S.L., Toledo, Spain.

The objective of the experiment was to study the influence of the source and level of available phosphorus (AP) in the diet on performance and egg quality of brown Classic Lohmann hens from 33 to 45 wk of age. The experimental diets were based on corn and soybean meal and contained 2,710 kcal AMEn/kg, 0.78% SID Lys, and 3.9% Ca. A commercial phytase, at the recommended dose, was added to all diets. The experimental design was completely randomized with 8 treatments arranged as a 2 × 4 factorial with 2 sources of phosphorus [calcined bone phosphate (CBP) and monocalcium phosphate (MCP)] and 4 levels of AP (0.26, 0.30, 0.34, or 0.38%) as main effects. Each treatment was replicated 13 times and the experimental unit was an enriched cage with 9 hens. The

experiment lasted for 12 wk (3 periods of 4 wk). Egg production, hen mortality, and the incidence of dirty, broken, and shell-less eggs were recorded daily. Feed intake was measured by period and cumulatively. All the hens were weighed the end of each of the periods. Egg weight was determined by period in all eggs produced the last day of each wk. From these data, egg mass, ADFI, feed conversion ratio (FCR), and BW gain were calculated by period and cumulatively. Haugh units and eggshell quality were determined in 8 eggs chosen at random from each replicate the last 2 d of each of the 4 wk-periods. In addition, data were analyzed as a completely randomized design with source of P and level of AP as main effects. Furthermore, the effect of the level of AP on all the variables studied was partitioned into the linear (L) and quadratic (Q) components. No interaction between P source and AP level in the diet was observed for any of the performance or eggshell quality traits studied and therefore, only main effects are presented. Neither source of P nor AP content of the diet affected any of the performance traits studied. Hens fed the diets containing MCP had higher ($P < 0.05$) incidence of dirty eggs. An increase in the level of AP of the diet increased (L, $P < 0.05$) the incidence of broken eggs and tended (L, $P = 0.098$) to reduce eggshell resistance to breakage. In summary, neither source of P nor level of AP of the diet affected egg production in hens from 33 to 45 wk of age. The incidence of dirty eggs increased when MCP was used as a phosphorus source. The incidence of broken eggs increased as the level of AP in the diet increased from 0.26 to 0.38%, with most of the negative effects observed in the last period of the trial. From 33 to 45 wk of age laying hens require no more than 0.26% AP in the diet.

Key Words: available phosphorus, calcined bone phosphate, egg quality, laying hen, monocalcium phosphate

532P Influence of the length of the post-hatch holding time on growth performance and the development of the proximal part of the gastrointestinal tract of broilers from 1 to 21 d of age. G. Fondevila^{*}, L. Cámara, J. Archs, A. F. de Juan, Lewis Aguirre, and Gonzalo Mateos, UPM, Madrid, Madrid, Spain.

We studied the influence of the length of the post-hatch holding time (HT) on the development of the organs of the proximal part of the gastrointestinal tract and growth performance of broilers from 1 to 21 d of age. In experiment 1, 120 birds were pulled from the hatchery 24 or 48 h after the first egg hatched, vaccinated against major diseases, and prepared for shipping (3 h). Then, chicks were transported (6 h) to the experimental farm. The final length of the HT was 33 and 57 h, respectively. Neither water nor feed were supplied during this period. Each treatment was replicated 6 times and the experimental unit was a cage with 10 birds. The broilers were fed a common low-density diet (EMAn = 2,820 kcal/kg; digestible Lys = 0.92%) in mash form throughout the experiment. Feed consumption, BW, and FCR were determined by replicate at 0, 7, 14, and 21 d of age. At 7 and 21 d of age, 2 birds per cage chosen at random were weighed individually and used to measure the weight (full and empty) and the pH of the crop and the gizzard. In addition, the DM content of the crop digesta was determined. The experimental design was completely randomized. The effects of HT on growth performance and organ traits and its interaction with age were analyzed using the MIXED procedure of SAS. An increase in HT length from 33 to 57 h reduced the BW of the broilers at the arrival to the experimental farm (40.5 vs. 35.6 g; $P < 0.001$) and at 21 d of age ($P < 0.001$). From 1 to 21 d of age, bird mortality increased ($P < 0.05$) as the length of the HT period increased. From 1 to 7 d of age, the longer HT period impaired ($P < 0.05$) FCR but the effect disappeared after this age. In experiment 2, chick management and experimental design were similar to those described for experiment 1 but a high-density diet (EMAn = 2,970

kcal/kg; digestible Lys = 1.31%) was used. An increase in the length of the HT period reduced the BW of the broilers at the arrival to the experimental farm (40.7 vs. 36.3 g; $P < 0.001$) and at 21 d of age ($P < 0.05$). Also, the longer HT period impaired FCR from 1 to 7 d of age ($P < 0.05$) but not thereafter. Length of the fasting period after hatch did not affect any of the organ traits studied in any of the 2 experiments at any age. Regardless of the energy and amino acid density of the diet, long fasting periods after hatch reduced growth performance and reduced the feed efficiency of the birds during the first week after hatch. The data indicate that food and water deprivation after hatch delays the start of the growth of the chicks without affecting the development of the proximal part of the gastrointestinal tract.

Key Words: crop, gizzard, broiler growth performance, post-hatch holding time

533P Not Presented

534P Efficacy of *Saccharomyces cerevisiae* cell walls (SCIW) in reducing the toxic effects of aflatoxin B₁ in broilers fed dietary treatments from hatch to 21-day of age. Madainey Humphrey^{*1,2}, David Ledoux¹, Colwayne Morris¹, and Patsy Ann Francis², ¹University of Missouri–Columbia, Columbia, Missouri, United States, ²University of Guyana, Georgetown, Guyana.

The aim of this study was to evaluate the efficacy of *Saccharomyces cerevisiae* cell walls (SCIW) at dietary concentrations of 0.1%, and 0.15% in ameliorating the deleterious effects of 2 mg aflatoxin B₁ (AFB₁) /kg of feed in broilers fed dietary treatments from hatch to 21d. The SCIW was obtained from a yeast byproduct manufacturing company in Brazil which utilized the yeast extracts from local sugarcane processing. Cobb 500 broilers (n = 125) were sourced from a commercial hatchery, wing-banded, weighed, and randomly placed into chick batteries in a temperature and humidity-controlled room, and allowed *ad libitum* access to feed and water. A Completely Randomized Design (CRD) was used, comprising 5 dietary treatments, 5 replicates per treatment and 5 birds per replicate. Dietary treatments were assigned as follows: T1) Basal Diet (BD) – containing no SCIW or AFB₁, which was the positive control (PC); T2) BD plus 0.15% SCIW and 0 AFB₁; T3) BD plus 2 mg AFB₁ /kg of feed and 0% SICW, which was the negative control (NC); T4) BD plus 0.1% SCIW and 2 mg AFB₁ /kg of feed; and T5) BD plus 0.15% SCIW and 2 mg AFB₁ /kg of feed. There were no significant ($P > 0.05$) differences among treatments for body weight gain, feed intake or feed conversion. However, compared with the PC birds, birds fed the NC diet gained 6% less weight. There were no significant ($P > 0.05$) differences in percent toe ash among treatments. Aspartate aminotransferase increased ($P < 0.05$) in birds fed 0.1% SCIW + AFB₁, and decreased from elevated values ($P < 0.05$) in birds fed 0.15% SCIW + AFB₁ back to values observed for the PC diet. Relative liver weight was increased ($P < 0.05$) in birds fed 0.15% SCIW + AFB₁ when compared with the birds fed the PC diet but was not different from birds fed the NC diet. Compared with the PC birds (score 0.3) and birds fed SCIW alone (score 0.2), higher liver lesion scores were observed in birds fed the NC diet (score 1.0), and those fed AFB₁ in combination with SCIW (0.10%, score 1.3, and 0.15% score 1.5). Mild bile duct proliferation was observed in livers of all birds fed AFB₁ and the combinations of SCIW and AFB₁. In conclusion, results indicate that SCIW may have been beneficial with respect to growth performance but not effective with respect to reducing or preventing liver lesion scores.

Key Words: broilers, aflatoxin B₁, *Saccharomyces cerevisiae*

535P Responses of different dietary energy levels on carcass traits and abdominal fat accumulation in Pekin duck from hatch to 21 days. Jun Seon Hong*, Hyun Min Cho, Shemil Macelline, Samiru Wickramasuriya, and Jung Min Heo, *Chungnam National University, Daejeon, Korea (the Republic of).*

An experiment was conducted to study the responses of 6 different dietary apparent metabolizable energy levels (AME: from 2,700 to 3,200 kcal/kg, 100 kcal/kg disparity) on carcass traits in Pekin duck from 1 to 21 d of age. A total of 432 one-day-old male white Pekin ducklings were randomly allocated in to one of 6 dietary treatments to give 6 replicates (12 birds per pen). The diets were formulated based on corn-soybean meal to meet or exceed NRC (1994) nutrient recommendations for Pekin duck. The carcass traits and abdominal fat were evaluated on d 21 using a randomly selected duck from each cage. Breast-, leg-meat weight and empty body weight were measured and calculated breast and leg meat yield in proportionate to empty body weight. Our data was statistically analyzed using one-way ANOVA in GLM procedure of SPSS 24.0 (Statistical Package for the Social Sciences, 2016). Our results in the current study indicated that ducks fed higher dietary energy (over 2900 kcal/kg) diets increased ($P < 0.05$) the proportion of abdominal fat. Nevertheless, proportion of breast meat weight did not show any differences ($P > 0.05$) among dietary treatments. However, proportion of leg meat gradually decreased ($P < 0.05$) while dietary energy level increased. In conclusion, higher dietary energy level did not affect breast meat yield, nonetheless abdominal fat increased when dietary apparent metabolizable energy was beyond 2900 kcal/kg.

Key Words: carcass, duck, energy, leg meat, breast meat

536P Relative metabolizable energy values for oxidized fats and oils determined using a slope-ratio precision-fed rooster assay. Patrick von Schaumburg^{*1}, Pamela Utterback¹, Brian Kerr², Rob Shirley³, Jason Lee⁴, and Carl Parsons¹, ¹University of Illinois-Urbana-Champaign, Urbana, Illinois, United States, ²USDA-ARS-National Laboratory for Agriculture and the Environment, Ames, Iowa, United States, ³Adisseo USA Inc., Alpharetta, Georgia, United States, ⁴Texas A&M University, College Station, Texas, United States.

Over time, lipids exposed to certain elements (e.g., UV light, high temperatures, oxygen) can become oxidized, thereby affecting their taste and odor, as well as decreasing their metabolizable energy content. Two slope-ratio precision-fed rooster assays were conducted with several fats and oils fed at different dietary levels to determine their relative metabolizable energy value (RME). The fat and oil sources evaluated in Experiment 1 were refined corn oil (RCO), canola oil (CO), poultry fat (PF), oxidized canola oil (OCO), and oxidized poultry fat (OPF). Both CO and PF were oxidized by heating each oil for 60 h at 90°C being cycled between 8 h hot and 16 h cold. Ambient air was percolated through the lipids at a rate of 12 L/min. For OCO and OPF there was an increase in the peroxide and p-anisidine values and a decrease in iodine values post-oxidation. In Experiment 1, birds were precision-fed 0, 5, and 10% of the fats as part of a ground corn diet. Data were analyzed using multiple regression of TMEn regressed on dietary fat level and then slope-ratio analysis was used to estimate RME, where the regression coefficient of the test lipid was divided by the regression coefficient of the reference RCO fat source (RME value set at 100%). The results of the slope-ratio analysis indicated that the RME values for the CO, PF, OCO, and OPF were not significantly different ($P > 0.05$) from 100% (R^2 value of 0.87 for the multiple regression), although the RME values for the CO and PF were numerically decreased by 10–11 percentage units by oxidation. In Experiment 2, soybean oil (SO) was used and was thermally processed at different temperatures and different lengths of

time with air infused at 30 L/min. The oils are classified as 22.5, 135, 180, and 225 which corresponded to the temperature (°C) at which the SO was thermally processed, with processing lengths of 0, 42, 21, and 1.5 h, respectively. Peroxide and p-anisidine values for the SO increased as processing temperature increased. Similar to Experiment 1, the oils for Experiment 2 were precision-fed at 2 inclusion levels, 7.5% and 15%, added at the expense of the ground corn diet. Multiple regression slope-ratio analysis of TMEn regressed on dietary fat level was used to estimate RME with the 22.5 SO being used as the reference standard (RME set at 100%). The RME values for the 135 and 225 SO were significantly lower ($P < 0.05$) than 100% with an R^2 value of 0.91 for the multiple regression. The results of this study indicated that the RME of fats and oils may be decreased by thermal processing and that the magnitude of the decrease in RME is influenced by the temperature, length of thermal processing, and inclusion of air during thermal processing.

Key Words: oxidized fats, metabolizable energy, oils, precision-fed rooster, slope-ratio

537P Not Presented

538P The effect of corrugated roller mill processing on the nutrient digestibility of full-fat camelina and flax seed fed for laying hens. Matt Oryschak¹ and Eduardo Beltranena^{*1,2}, ¹Alberta Agriculture and Forestry, Edmonton, Alberta, Canada, ²University of Alberta, Edmonton, Alberta, Canada.

The dietary inclusion of full fat oilseeds such as flax (*Linum usitatissimum*) or camelina (*Camelina sativa*) can be used to enrich table eggs with n-3 fatty acids. Because of a hard hull surrounds the seed that would limit digestion, mechanically breaking the seed hull by processing may enhance nutrient and lipid digestibility. The objective of the present study was therefore to compare the effect of corrugated roller milling on energy and nutrient digestibility of flax and camelina seed fed to laying hens. In a randomized complete block design with 6 replicates per treatment, laying hens (H & N Nick Chick; $n = 180$) were fed a basal diet, or one of 4 test diets containing 70% of the basal diet and 30% of either whole or rolled camelina or flax during a 14-d digestibility study. All diets contained titanium dioxide as an indigestible marker and were offered to birds ad libitum. Excreta were collected from each cage over the 48-h period preceding digesta collection to yield a single pooled sample of excreta for each cage. On d 14, hens were humanely euthanized by cervical dislocation and the last third of the ileum was excised from each bird. The digesta therein was gently expressed to yield a single pooled specimen for each cage. Diets, ingredients, digesta and excreta were analyzed for nutrient and titanium content and GE value. The ATTD of DM, GE and the AID of amino acids and CP were calculated first for diets. Subsequently the digestibility of test ingredients was calculated using the difference method assuming additivity between the basal and test ingredient portions of diets. Data were analyzed as mixed models using the MIXED procedure of SAS (v9.4). Statistical models included the main effects of oilseed (flax vs. camelina), processing (whole seed vs. rolled) and the 2-way interaction, with block included as a random effect. There was no interaction between seed type and processing for ATTD of DM, GE or the AID of essential AA ($P > 0.07$), indicating a similar effect of roller milling for both oilseeds. The AID of CP (55.3 vs 45.2%; $P < 0.01$), MET (70.4 vs. 64.6%; $P < 0.04$), THR (48.2 vs. 34.2%; $P < 0.01$), HIS (57.1 vs. 40.1%; $P < 0.01$) and total AA (59.8 vs. 54.5%; $P < 0.05$) were all greater for camelina compared with flax. Other than ARG ($P < 0.02$), roller milling did not increase the AID of any essential AA. Roller milling did however increase the ATTD of both

DM (52.7 vs 44.0%; $P < 0.05$) and GE (44.6 vs. 34.6%; $P < 0.01$) for both oilseeds. The results of the present study indicate that corrugated roller milling substantively enhanced the AME value, but not the digestible AA content of flax and camelina seed for laying hens.

Key Words: camelina, flax, laying hen, nutrient digestibility, roller milling

539P Omega-3 fatty acids sources fed to broiler breeders and/or their progeny: Impact on growth performance and breast yield in 42-day old broiler chickens. Aizwarya Thanabalan^{*1}, Janna Moats², Kayla Price³, and Elijah Kiarie¹, ¹University of Guelph, Guelph, Ontario, Canada, ²O&T Farms Ltd., Saskatoon, Saskatchewan, Canada, ³Alltech, Guelph, Ontario, Canada.

Lipids are essential for perinatal growth and organ development. Enriching broiler breeder (BB) diets with functional omega-3 fatty acids (n3FA) such as α -linoleic acid (ALA) and docosahexaenoic acid (DHA) may improve chick quality and growth performance. The objective of this study was to determine the transgenerational impact of 2 sources of dietary n3FA on growth performance and breast yield in broiler chickens. A total of 588 pullets (Ross 708) and 60 cockerels d-old (Ross) were placed in separate rearing pens (28♀ and 20♂/pen) and fed 3 diets: corn and soybean meal control (CON) or CON + 1% of dried micro-algae (*Aurantiochytrium limacinum*) fermentation product, as a DHA source or ~2.5% flaxseed-based product as a ALA source. DHA and ALA diets were formulated to have same total n3FA and n6FA: n3FA ratio. Birds were fed (♀ pens; $n = 9$ for CON & $n = 6$ for DHA/ALA) to sexual maturity (wk 22), moved to laying house and grouped based on rearing diets (60♀ and 10♂/pen) and fed 7 diets. Birds reared on CON were split into 3 groups: CON-CON; fed CON, CON-DHA; fed DHA and CON-ALA; fed ALA. Birds reared on DHA and ALA either continued these diets (i.e., DHA-DHA and ALA-ALA) or switched to CON (i.e., DHA-CON and ALA-CON) diet. Fertile eggs (210/diet) were collected at wk 34, incubated, sexed and hatchlings placed in cages (8 chicks/cage) based on rearing-laying diet, sex and BW and allocated 15 diets. The diets were: CON-CON-CON, CON-DHA-DHA, CON-DHA-CON, CON-CON-DHA, CON-CON-ALA, CON-ALA-CON, CON-ALA-ALA, DHA-DHA-DHA, DHA-DHA-CON, DHA-CON-DHA, DHA-CON-CON, ALA-ALA-ALA, ALA-CON-CON, ALA-CON-ALA and ALA-ALA-CON. Body weight and feed intake (FI) were taken on d 10, 15 and 42. On d 10, all birds were orally challenged with *Eimeria* culture. Birds were sacrificed on d 42 for breast yield. Data were analyzed in a factorial arrangement of diet, sex and interaction. There was no ($P > 0.05$) interaction between sex and diet. On d 10, chicks from BB fed DHA during laying and rearing and maintained on DHA post-hatch (DHA-DHA-DHA) had higher BW gain (BWG, 228 vs. 204 g, $P = 0.001$) than CON-CON-CON while BWG of chicks fed other diets were intermediate. Chicks on ALA-CON-CON and DHA-DHA-CON ate more feed and had poor FCR ($P < 0.05$) relative to other chicks on d 10. There was no ($P > 0.05$) diet effects on growth, FI and FCR on d 15 and 42. However, birds on CON-ALA-ALA had higher breast yield (23.6 vs. 19.6%, $P = 0.005$) than CON-CON-CON birds. Generally, males grew faster ($P < 0.05$) than female however, female had higher ($P = 0.005$) breast yield relative to males. In conclusions, feeding DHA to both BB and their progeny improved growth in the first 10 d post-hatch while ALA improved d 42 breast yield.

Key Words: omega-3 fatty acids, broiler breeders, growth performance

540P Heat-induced protein oxidation of soybean meal impairs growth performance and antioxidant capacity of broilers. Peng

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This study investigated the effects of soybean meal (SBM) modified by heating on the growth performance and antioxidant capacity of broilers. A total of 400 one-day-old Arbor Acres Plus chicks were randomly divided into 5 treatment groups with 8 replicates (10 birds per replicate). Birds in the control group were fed diet containing non-heated SBM, and those in the treatment groups were fed SBM heated at 100°C for 1, 2, 4, and 8 h, respectively. Heated SBMs (HSBMs) showed a relatively higher protein carbonyl (PC) level in vitro ($P < 0.05$). The increase in PC level indicated that protein of SBM was oxidized by heating since PC is a common used marker of protein oxidation. The average daily gain decreased linearly, whereas the feed/gain ratio increased linearly ($P < 0.05$) in broilers fed HSBMs at each feeding stage. The average daily feed intake decreased linearly ($P < 0.05$) only during d 22 to 42. The results demonstrated that diets containing oxidized protein could decrease growth performance. Reactive oxygen species, malondialdehyde, and PC levels increased linearly ($P < 0.05$) in the plasma, liver, and jejunum of broilers fed HSBMs. Furthermore, the total antioxidant capacity in the plasma and jejunum, and the total superoxide dismutase and glutathione peroxidase activities in the plasma, liver, and jejunum decreased linearly ($P < 0.05$) in broilers when feeding HSBMs. The increase of oxidized products and the decrease of antioxidant enzyme activity suggested that the antioxidant capacity of broiler chickens was impaired by feeding HSBMs. In conclusion, protein oxidation of SBM could be induced by heating, and heat-induced protein oxidation of SBM reduced the growth performance and antioxidant capacity of broilers, and this negative effect was exacerbated with increasing heating time.

Key Words: antioxidant capacity, broiler, heat, oxidation, soybean meal

541P Determining the relationship among average particle size consumed and gastrointestinal characteristics of two broiler strains in the starter phase. Andrew Brown*¹, Mark Lemons¹, Chris McDaniel¹, Joseph Moritz², and Kelley Wamsley¹, ¹Mississippi State University, Mississippi State, Mississippi, United States, ²West Virginia University, Morgantown, West Virginia, United States.

During the starter phase, a companion study investigated the impact of 2 feed forms and 3 feed qualities on 2 genetic strains (Fast Growing (FG) or High Yielding (HY)) to determine the effect of, not only these treatments on broiler performance, but also the relationship among performance characteristics and average particle size consumed (APSC). Data from D 0–6 and 0–18 demonstrated that APSC was dependent upon strain and feed form. Additionally, there was a positive relationship between APSC and enhanced performance characteristics throughout the aforementioned time periods. Literature detailing the relationship between APSC and gastrointestinal characteristics has focused mainly on APSC of specific ingredients and less on APSC of complete feed. Therefore, the present study was conducted to determine the relationship between APSC and gastrointestinal characteristics in an attempt to help explain the performance observed in the companion study. The current study utilized a sub-sample of pens from the companion study, where 1 d old chicks (480/S; 0.093 m²/bird) were randomly allocated by strain to 48 pens. For each day of the starter phase, APS placed was determined for each treatment by hand sieving 2x the breeder's recommendations for FI/bird/day, using a stack of 8 sieves (over a collection pan) for 2 min. The sieved feed was then weighed for determination of particle size, and then recombined before being fed on the corresponding day. Each day, the previous day's feed was sieved using the same

manner to determine feed remaining on each sieve. On D 3, 5, 7, 10, 14, and 18, one bird/pen was randomly selected for the determination of yolk sac, crop fill (dry matter basis), and gizzard weights as well as small intestinal segment weights and lengths. Birds were weighed to calculate relative organ weight (ROW) according to BW. For the HY strain, from D5 to 18, data demonstrated negative correlations of APSC with gizzard, duodenum, jejunum and ileum ROW, in which as APSC increased, ROW decreased ($P < 0.045$, $r = -0.423$ to $r = -0.68$). However, APSC for the HY strain was positively correlated with yolk sac weight ($P = 0.018$, $r = 0.498$). Also at D 18, there was a positive correlation of APSC with ileum length ($P = 0.044$; $r = 0.423$). For the FG strain, on D10 there was a negative correlation between APSC and ileum ROW ($P = 0.026$; $r = -0.463$), and on D18 a positive correlation of APSC with crop fill ($P = 0.0031$, $r = 0.602$). In conclusion, as shown in the companion study, strain influences APSC; but, as revealed in the present study, APSC impacts gastrointestinal characteristics, ultimately, altering performance. These findings may be due to birds proportioning energy positively to influence growth performance.

Key Words: average particle size, feed form, feed quality, gastrointestinal, strain

542P The interactive influence of dietary feed form and energy density on nutrient digestibility and broiler performance. Thais Stefanello*¹, Everton Luis Krabbe², Valdir Avila², Edenilse Gopinger³, Andréa Ribeiro⁵, and Eduardo Xavier⁴, ¹Federal University of Rio Grande do Sul, Porto Alegre, Brazil, ²Embrapa Swine and Poultry, Concórdia, Brazil, ³Federal Institute of Education Science and Technology of Rio Grande do Sul, Sertão, Brazil, ⁴Federal University of Pelotas, Pelotas, Brazil, ⁵UFRGS, Porto Alegre, Brazil.

In animal nutrition, the physical form of diets is an important factor associated with productive performance and nutrient utilization. Pelleted and crumble diets provided benefits such as improved feed intake and conversion, decreased segregation of ingredients and wastage, increased density and overall palatability of feed. This study aimed to compare the influence of processing and the energy level of diets on nutrient digestibility and productive performance in broiler. The diets were formulated based on corn and soybean meal, then mixed and pelleted under normal feed mill conditions (80 ± 2°C and 9 s conditioning) using a die with 4,75 mm hole diameter and 50 mm length. Two different feed form, mash and crumble, with 2 metabolizable energy levels, 2,900 and 3,000 kcal/kg AME were produced, resulting 4 different diets/treatments. All other nutrients were set to meet animal requirements. The pelleted diets were ground to eliminate the physical form factor but maintaining the effect of conditioning and processing. The mean geometric diameter (DGM) of the diets mashed and crumbled were 640µm and 1236µm, respectively. In total, 480-dayold broiler were used, Cobb 500, male, housed in metabolic cages, distributed in completely randomized block design with 12 replicates and 10 birds per cage. The experimental performance (average weight-AW, feed intake-FI, body weight gain-BWG, feed conversion ratio-FCR) period was from 1 to 28 d and the nutrient digestibility (dry matter-DM, nitrogen-N, ether extract-EE) was evaluated from 24 to 28 d age, consisting of the total excreta collection method. The data were submitted to ANOVA (ANOVA) and the presence of interaction between the factors were evaluated with Tukey test ($P < 0.05$). An interaction ($P < 0.05$) between physical form and energy was observed for all performance variables at 1–28 d. The animals receiving the crumbled form of the diet significantly increased the AW, FI, BWG compared with those who received the mashed form, for both 2,900 and 3,000 kcal/kg AME diets. However, the crumbled form with 3,000 kcal/kg showed a better FCR. For nutrient digestibility,

an interaction ($P < 0.05$) was observed for EE from 24 to 28 d of age. The animals that received a diet with energy density of 3000 kcal/kg obtained a higher EE digestibility in both diets (mash and crumble); however, the effects of energy were more effective when associated with the crumbled form. In conclusion, the crumbled physical form of the diet provides improvements of broiler performance in both energy densities, however, for nutrient digestibility, these improvements were not clearly observed.

Key Words: energy, nutrition, pelleting

543P Extraction of cell wall material (CWM) of SBM and profiling of non-cellulosic polysaccharides content in CWM. Pramir Maharjan*, Katie Hilton, Jordan Weil, Antonio Beitia, Nawin Suesutajit, and Chris Coon, *University of Arkansas, Fayetteville, Arkansas, United States.*

Commodity soybean meal (SBM) (~47% CP) was obtained from University of Arkansas feed mill. The SBM was the freeze-dried and ground to a fine powder (particle size <1mm). Protein extraction was carried out by treating with sodium dodecylsulphate (1.5% wt/vol). The residue was then subjected for starch gelatinization for 1 h at 85°C. The residue obtained after centrifugation (3000g, 30 min) was suspended in buffer solution (pH 6.5) containing 10 mM maleic acid, 10 mM NaCl, 1mM CaCl₂ and 0.05% NaN₃. Porcine α amylase was added (0.2 mg for 10 g residue) and incubated at 30°C for 19 h. The pellet/residue after centrifugation was washed with DI water, and centrifuged before freeze-drying. The residue after soluble/starch CHO extraction was the cell wall material (CWM). The yield of CWM was then calculated for defatted SBM. The dried CWM was further subjected for non-cellulosic polysaccharides (NCP) analysis as described by Englyst et al., 1984. Briefly, CWM was acid hydrolyzed and individual monosaccharides obtained were derivatized by acetylation before they were analyzed with gas chromatography-flame-ionization detector (GC-FID). The CWM was handled in 6 replicates for analysis. Predominant monosaccharides obtained (in % DM basis) in CWM of SBM were galactose 12.69 (± 0.04) followed by arabinose 5.70 (± 0.34), glucose 3.40 (± 0.47); xylose 2.66 (± 0.07), rhamnose 1.12 (± 0.12), fucose 0.98 (± 0.12) and mannose 0.55 (± 0.15). The yield obtained for CWM from defatted SBM was 59.99%. Results indicated that NCP content in CWM of SBM was as high as ~27%, the nutrient portion that could be potentially exploited using the appropriate carbohydrases in feed.

Key Words: cell wall material, soybean meal, extraction, non-cellulosic polysaccharides

544P Effects of grinding corn with different moisture concentrations on subsequent particle size and flowability characteristics. Michaela Braun*, *Kansas State University, Manhattan, Kansas, United States.*

The objective of this study was to determine the effects of whole corn moisture and hammermill screen size on subsequent ground corn particle size and flowability. Whole yellow dent #2 corn was used for this experiment. Treatments were arranged as a 2 \times 2 factorial design with 2 moisture levels (12%-dry and 16.5%-high) and ground using 2 hammermill screen sizes (3 mm and 6 mm). Corn was ground using a Bliss Hammermill (Model 6K630B) at 3 separate time points to create 3 replications per treatment. Increasing initial whole corn moisture was accomplished by adding 5% water and heated at 55°C for 3 h in sealed glass jars using a Fisherbrand Isotemp Oven (Model 15-103-051). Ground corn flowability was calculated using angle of repose (AOR), percent

compressibility, and critical orifice diameter (COD) measurements to determine the composite flow index (CFI). The average analyzed mean moisture contents were 11.7, 16.4, 11.9, and 16.6% for the 3 mm dry, 3 mm high, 6 mm dry, and 6 mm high, respectively. There was a tendency for a screen size \times moisture interaction ($P < 0.064$) for geometric mean particle size and mean standard deviation. The observed interaction was due to decreased mean particle size and increased standard deviation of high moisture corn compared with dry corn when ground using a 6 mm screen. However, there were no differences between dry and high moisture corn when ground with a 3 mm screen. The average analyzed mean particle sizes were 348, 401, 438, and 563 μ m and standard deviations were 2.49, 2.39, 2.75, and 2.41 for the 3 mm dry, 3 mm high, 6 mm dry, and 6 mm high, respectively. There were no screen size \times moisture interactions for AOR, COD, compressibility, or CFI. There was an increase ($P < 0.03$) in percent compressibility as screen size increased from 3 to 6 mm. A marginally significant decrease ($P < 0.056$) was seen in AOR from corn ground using a 6 mm screen compared with a 3 mm screen. Moisture content of corn produced a marginally significant decrease ($P < 0.056$) in AOR and COD. Additionally, the CFI value decreased ($P < 0.038$) as corn moisture increased. The AOR were 30.0, 26.0, 27.3, and 23.3°, COD were 30.0, 26.0, 27.3, and 23.3 mm, compressibility's were 26.8, 26.1, 25.4, and 23.0%, and CFI were 38.5, 46.6, 45.6, and 52.4 for the 3 mm dry, 3 mm high, 6 mm dry, and 6 mm high, respectively. In conclusion, decreasing screen size reduced particle size and had poorer flowability as measured by percent compressibility and AOR. Moisture level also influenced corn particle size when ground using a 6 mm screen but not when using a 3 mm screen. Increased moisture in ground corn increased CFI.

Key Words: corn, grind, moisture, flowability, particle size

545P Effect of nutrition plan of one and two stages and environment temperature on Japanese quail growth. João Souza Silva, Jose Jordão Filho, Claudiana Souza, Mario Lima, and José Vilar Da Silva*, *Universidade Federal da Paraíba, Solânea, Paraíba, Brazil.*

The objective of this study was to estimate the body growth of Japanese quail raised in 2 thermal environments and fed with 3 nutritional plans (NPs) from 1 to 39 d of age. The NP evaluated were: NP₁ = 2 diets (1 to 15 and 16 to 39 d); the NP₂ = 2 diets (1 to 10 and 11 to 39 d); and NP₃ = single diet (1 to 39 d). The diets of NP₁ and NP₃ were recommended by the Brazilian Tables for Japanese and European Quail (Silva & Costa, 2009) and NP₂ diets followed the suggestions of the Brazilian Poultry and Pork Tables (Rostagno et al., 2017). A total of 312 birds were housed in an environment of 25°C, considered to be of thermal comfort, and another 312 birds were housed at a continuous temperature of 35°C, considered as temperature of heat stress. The experimental design was completely randomized in a 3 \times 2 factorial scheme resulting in 6 treatments with 13 replicates of 8 birds. The weighings of the birds were carried out every 3 d until the 30 - ninth day of life of the quail to obtain body weight. To estimate the growth of the quail, the Gompertz model fit to the weight-for-age series was used. The estimates of weight at maturity (W_m), maturity rate (k) and age at maturity (t) were estimated from the derivative of the Gompertz model and the means were compared by the Tukey test ($P \leq 0.05$). The W_m of the quail fed with NP₂ was higher in the comfort environment compared with the quail housed in the heat environment (235.2^a vs. 213.50^b g). NPs did not affect any parameter of the Gompertz model in the thermal comfort environment, but affected in the warm environment, where NP₂ reduced the W_m compared with NP₁ and NP₃ (230.70^a, 224.35^b and 233.60^a g). The parameter k fell from the comfort environment to the thermal discomfort environment of, respectively, 0.0579^a to 0.0522^b g/day in the NP₁; 0.0578^a to 0.0552^b

g/day in NP₂; and 0.0576^a to 0.0531^b g/day in NP₃. The NP₁ delayed maturity age at 1.3 d for quail in the warm environment (20.21^b vs. 21.55^a days). Heat reduces the maturity rate and shows that growing Japanese quail do not tolerate an environment with a temperature of 35°C. The low weight at maturity of the NP₂ quail housed at a temperature of 35°C was probably a result of the higher concentration of amino acids in the diets and, thus, greater catabolism and heat production increasing the thermal discomfort of birds. The NP₁ with 2 diet and NP₃ with a single diet are more suitable for quail from 1 to 39 d of age.

Key Words: body weight, growth rate, weight at maturity

546P Additivity of available amino acids in various hatchery by-products for broiler chickens. Hyeon Seok Choi^{*1}, Gi Ppeum Han¹, Geun Hyeon Park¹, Jong Hyuk Kim¹, Sang Yun Ji², and Dong Yong Kil¹, ¹Chung-Ang University, Anseong-Si, Korea (the Republic of), ²Rural Development Administration, Wanju, Korea (the Republic of).

Korean poultry industry produces more than 4,000 tons of hatchery by-products (HBPs) every year. The HBPs contain high amounts of crude protein (22–56%), and therefore, there is an increasing interest in the use of HBPs as a feed ingredient in the Korean feed industry. In a diet formulation, the fundamental assumption is that the total supply of available amino acids (AAs) in a mixed diet is equal to the sum of available AAs in individual feed ingredients. This assumption is referred to the additivity of available AAs in a diet formulation. However, no previous studies were performed to validate the additivity of available AAs in various HBPs although HBPs are produced generally as a mixture of individual HBP with different inclusion levels. Thus, the objective of the current experiment was to determine the additivity of available AAs of individual HBP in broiler diets. We collected 4 different HBPs including infertile eggs (IFE), unhatched eggs (UHE), low grade and dead chicks (LDC), and a mixture (MIX) containing 55% IFE, 10% UHE, 10% LDC, and 25% hatched eggshells from a commercial hatchery. A new mixture of HBPs was artificially created by mixing 12.5% IFE, 12.0% UHE, 11.0% LDC, and 12.5% MIX to test an additivity of available AAs in various HBPs. A total of 4-hundred-20 21-d-old broiler chickens were randomly allotted to 1 of 5 dietary treatments with 7 replicates per treatment. Each diet contained 55.6% IFE, 48.3% UHE, 34.2% LDC, 60.6% MIX, and 48% mixture of 4 HBPs as the sole source of AAs. Additional 125 birds were used to estimate ileal endogenous losses of AAs. The apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of AAs in 5 HBPs were determined. The additivity of AAs in HBPs was tested by comparing measured values for the AID and SID of a mixture of 4 HBPs and its predicated values calculated from measured AID and SID of individual HBP. The differences between measured and predicted values were analyzed using the confidence interval. If the differences between measured and predicted values are not different from zero, the additivity is assumed to be validated. Results indicated that average differences for essential AAs and non-essential AAs were 0.31 and 0.26 percentage units, respectively, for both AID and SID. The AID and SID of the most AAs (13 AAs out of 18 AAs) in individual HBP were additive in the mixture of 4 HBPs; however, we failed to validate the additivity of His, Ile, Trp, Val, and Glu. In conclusion, the amounts of available AAs in individual HBP as determined in the current experiment may be applied to a diet formulation if varying inclusion levels of individual HBP or mixture of HBPs are used in broiler diets.

Key Words: additivity, amino acid, broiler chicken, diet formulation, hatchery by-product

547P Calcium digestibility of limestone in broiler chickens as influenced by age and dietary crude protein content. Laura David^{*1}, Reza Abdollahi¹, Carrie Walk², and Ravi Ravindran¹, ¹Massey University, New Zealand, Palmerston North, Manawattu, New Zealand, ²AB Vista, Marlborough, Wiltshire SN8 4AN, United Kingdom, Marlborough, United Kingdom.

The objective of the present study was to investigate the effects of age and dietary crude protein content on the calcium (Ca) digestibility of limestone for broiler chickens. Six treatment groups of different ages, namely d 1–7, 8–14, 15–21, 22–28, 29–35 and 36–42, were utilized. A corn-based diet (crude protein, 79 g/kg) containing limestone as the sole Ca source and, supplying 9.0 g/kg Ca, was fed to 6 replicate cages of broilers during these 6 periods. The birds were fed a commercial broiler diet until the introduction of experimental diet, except the d 7 age group. Ileal digesta were collected on d 7 (12 birds per cage), 14 (10 birds per cage), 21 (8 birds per cage), 28 (6 birds per cage), 35 (6 birds per cage) or 42 (6 birds per cage). An additional corn-based diet containing a crude protein content of 153 g/kg was developed with the objective to examine the effect of dietary protein on the Ca digestibility on d 21 and fed to 6 replicate cages (8 birds per cage) from d 15 to 21 post-hatch. The birds belonging to the age group d 15–21 and fed the 79 g/kg crude protein diet served as the control treatment to determine the effect of dietary crude protein effect. Titanium dioxide (5 g/kg) was included in both diets as an indigestible marker for apparent ileal digestibility measurements. Total-tract Ca retention and pH of gizzard digesta were also measured at each age. Data were analyzed by ANOVA and orthogonal comparisons were performed to determine the linear and quadratic effects of broiler age. The effects of dietary protein content were determined by Student's *t*-test. Ileal Ca digestibility coefficients declined linearly ($P < 0.001$) with advancing age, from 0.51 at d 7 to 0.27 at d 42. The reductions in digestibility with age were closely associated with increased feed intake (linear effect, $P < 0.001$; quadratic effect, $P < 0.05$). A quadratic response ($P < 0.01$) was observed for Ca retention. The retention declined till d 21 and then plateaued. Age quadratically ($P < 0.01$) affected the gizzard pH, with the lowest and highest values observed at d 21 and 42, respectively. Increasing the dietary protein content had no effect ($P > 0.05$) on the ileal digestibility and retention of Ca. In conclusion, the present results showed that the Ca digestibility of limestone in broilers reduces with advancing age when fed corn-based diet with the limestone supplying 9 g/kg Ca.

Key Words: age, broiler, calcium, crude protein, digestibility

548P Effects of the *in ovo* injection of vitamin D₃ and 25-hydroxyvitamin D₃ on the performance and meat yield of broilers challenged with coccidiosis. Saman Fatemi¹, Katie Elliott¹, Abdulmohsen Alqhtani¹, Abiodun Bello², and Edgar Peebles^{*1}, ¹Mississippi State University, Strakville, Mississippi, United States, ²University of Alberta, Edmonton, Alberta, Canada.

Broiler performance has been shown to be improved by the use of supplemental 25-hydroxyvitamin D₃ (25OHD₃) during a coccidiosis challenge. Therefore, the objectives of this study were to determine effects of the *in ovo* administration of vitamin D₃ (D₃) and its metabolite, 25OHD₃, on broiler performance and breast meat yield after a coccidiosis challenge. Live embryonated Ross 708 broiler hatching eggs were randomly assigned to one of the following 5 *in ovo* injection treatments at 18 d of incubation (doi): 1) non-injected; 2) diluent; diluent containing either 3) 2.4 µg D₃, 4) 2.4 µg 25OHD₃, or 5) 2.4 µg D₃ + 2.4 µg 25OHD₃. A 50 µL solution volume was injected into each egg using an Inovoject multi-egg injector. At hatch, 4 male chicks were randomly assigned to each of 80 battery cages in each of 2 rooms with 8 replicates per treat-

ment group. Only *in ovo*-injected treatments were challenged with a 20x live coccidial vaccine at 14 d of age (doa). At 14 and 28 doa, 8 birds per treatment group were sampled before and 2 wk after challenge (14 and 28 doa, respectively). Weights of bursa, liver, spleen, duodenum, jejunum, and ileum and lengths of duodenum, jejunum, and ileum were determined. Mean BW, average daily BW gain, and feed intake, as well as feed conversion ratio (FCR) were determined from 0 to 14, 15 to 28, and 29 to 41 doa. At 42 doa, birds were processed for determination of carcass weight, and the absolute and relative (% of carcass weight) weights of carcass parts. All data were analyzed as a one-way ANOVA using SAS 9.4. No significant differences were observed for performance or for processing data and the weights and lengths of organs before coccidiosis challenge. However, duodenum ($P = 0.001$) and jejunum ($P = 0.027$) weight decreased in unchallenged birds as compared with challenged birds. Spleen weight ($P = 0.081$) tended to increase in response to injection of 25OHD₃ alone relative to the non-injected and diluent-injected treatments. Also, a severe decline occurred for BW ($P = 0.001$) from 14 to 41 doa and for FCR ($P = 0.001$) from 14 to 28 doa in challenged birds as compared with unchallenged birds. At 41 doa, birds in the 25OHD₃ alone had an increased BW as compared with diluent-injected birds. These results indicate that the *in ovo* injection of either vitamin D source did not affect broiler performance during a coccidiosis challenge, but 25OHD₃ at 2.4 µg improved broiler performance as compared with D₃ at the same level of injection. This effect may be due to an improvement in small intestine morphology.

Key Words: In ovo injection, 25-hydroxyvitamin D₃, coccidiosis, broiler performance, meat yield

549P Effects of different mineral premixes on the storage stability of vitamins in broiler diet premixes. Tuoying Ao*, Marquisha Paul, Anthony Pescatore, Lizza Macalintal, Mike Ford, and Karl Dawson, *Alltech-University of Kentucky Nutrition Research Alliance, Lexington, Kentucky, United States.*

To investigate the effect of different mineral premixes on the storage stability of vitamins in broiler diet premixes, 2 mineral premixes were added to marginal level of vitamin premix (30% commercial level) separately to get MV1 and MV2 and stored in room temperature for 90 d. MV1 was using inorganic mineral salts for Zn, Cu, Mn, Fe and Se at commercial level. MV2 was using organic mineral proteinates (Bioplex[®] mineral, Alltech Inc. Nicholasville, KY) for Zn, Cu, Mn & Fe at recommended level by the company (about 40% of the commercial level) and organic Se (Sel-Plex[®], Alltech Inc., Nicholasville, KY) at the same level as sodium selenite. Four-week chicken trial with 12 replicate pens of 22 chicks indicated that chickens fed diet with MV1 had lower ($P < 0.01$) weight gain and feed intake than those fed diet with MV2. Later, the activity of vitamins in both premixes was analyzed (Eurofins Scientific Inc., Des Moines, IA). Compared with the calculated activity of vitamins at the beginning, all vitamins except pantothenic acid, niacin and biotin in MV1 and thiamin, riboflavin, pantothenic acid, niacin and biotin in MV2 lost a significant portion (>60%) of activity at the end of storage. The vitamins in MV1 lost more activity than those in MV2. The activity of vitamin A and vitamin K in MV1 was only 10.36 and 6.11%, respectively, the activity in MV2. The activity of thiamin and folic acid in MV1 was only 23.77% and 33.27%, respectively the activity in MV2. The activity of vitamin D3 lost almost 50% when inorganic mineral premix was used compared with organic mineral premix. The activity of riboflavin and pyridoxine lost 35% when inorganic mineral premix was used compared with organic mineral premix. The results from this study further confirmed that replacement of inorganic trace minerals

with organic minerals can increase the storage stability of vitamins in broiler diet premix containing both vitamins and trace minerals.

Key Words: organic trace minerals, inorganic mineral premix, vitamin activity, mineral proteinate, storage stability

550P The source of selenium hydroxy-selenomethionine is used by intestinal Caco-2 cells and protects against oxidative stress.

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Since selenium (Se) supplementation is required for optimal growth, its incorporation in animal diets is of great importance. Se performs biological functions through its incorporation into selenoproteins in the form of selenocysteine. In the intestine, inadequate dietary Se is considered a risk factor for several chronic diseases associated with oxidative stress and inflammation. The present study investigates the role of the hydroxy-selenomethionine (2-hydroxy-(4-methylseleno)butanoic acid: HMSeBA), a novel organic form of Se, in supporting selenoprotein synthesis and protecting against oxidative stress, in comparison with sodium selenite. The protein and gene expression and activity of different selenoproteins were studied in Se-deprived and Se-supplemented models of Caco-2 cells. GPx activity together with both protein and gene expression of SelP in the Se-deprived model was significantly decreased; while GPx2 gene expression and ROS production significantly increased. In the Se-supplemented model, GPx activity and SelP protein expression were significantly increased irrespective of the selenium source. In contrast, gene expression of SelP and GPx2 were only significantly increased for the organic source. Similarly, decreases in ROS, lipid peroxidation and protein carbonylation after H₂O₂ treatment were only observed for the organic source HMSeBA. Thus, the difference observed between the effects of the Se sources on GPx2 and SelP gene expression may be of importance in terms of protection against oxidative stress. In conclusion, our results indicate that the organic form HMSeBA is used as a Se source by Caco-2 cells and contributes to maintaining intestinal epithelial homeostasis by protecting against oxidative stress.

Key Words: selenium, oxidative stress, cell model

551P Impact of a protected vitamin and mineral premix enriched with metabolite extracts when reducing the inclusion rate of these nutrients in broiler diets.

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Feed cost is significant expense animal production. Because vitamins can be affected by oxidation from environmental conditions and interaction with trace mineral, it is a normal practice to use them in high levels in the diet. However, this increases the cost and environmental impact in the production system. The protection of the vitamin and minerals in a coating matrix can prevent this loss and help to reduce the inclusion levels in the diets. Two experiments were conducted to investigate the effects of using a vitamin and mineral premix protected in fat matrix (Jefo Matrix Technology) associated to microorganism metabolite extract (ME) to lower the inclusion levels of nutrients in diet. In the first experiment, 500 ROSS 308 male chicks were randomly distributed in 2 treatments with 10 replicates per treatment. Feed was corn-soybean based in 3 phases and produced without additional vitamins to add them

at different inclusion rate. Treatment 1 (T1) contain a commercial free vitamins premix at 100% recommended dose (0.5 kg/T), treatment 2 (T2) contain a protected vitamin premix with 60% reduction of vitamins levels of T1 plus ME (0.2 kg/T). In the second experiment, 112 ROSS 308 male chicks were divided in 2 treatments with 14 replicates per treatment. Feed was wheat based in 2 phases and produced without additional minerals and vitamins to add them at different inclusion rate. Treatment 1 (T1) contain a commercial free minerals and vitamins premix at 100% of recommended dose (1.5 kg/T), and treatment 2 (T2) contain a protected vitamin and mineral premix with 50% reduction of vitamins and mineral levels of T1 plus ME (0.7 kg/T). Each week, feed intake, body weight and feed conversion rate were measured in all trials. At the end, data was analyzed using one way ANOVA, followed by post hoc Fischer LSD test. No statistical difference ($P > 0.05$) for feed intake, body weight gain and feed conversion ratio were observed between control and reduced treatments. These results demonstrate that the use of protected vitamins and mineral premix enriched with ME can be supplemented at 60% reduction of the vitamins and 50% of vitamins and minerals commercial levels in broiler diets without affect the animal performance. In conclusion, protected vitamins and mineral enriched with ME can reduce the feed cost, while obtaining equivalent results to the commercial dose. This will have a positive effect on the economic and environmental impact of animal production.

Key Words: vitamins reduction, coated matrix protection, microorganism metabolite, broiler

552P Effect of copper proteinate as growth promoter in broiler diets challenged with *Salmonella* Heidelberg. Maryelle de Oliveira¹, Heloisa Helena Mello¹, Jose Stringhini^{1,2}, Marcos Cafe¹, Helder de Oliveira¹, Patricia de Araujo^{1,2}, and Hyorrara Oliveira^{1,2}, ¹Universidade Federal de Goias, Goiania, Goias, Brazil, ²CNPq, Brasilia, Distrito Federal, Brazil.

Copper is recognized as having an antimicrobial effect when used at high levels in diets. Thus, it may be possible to use of copper to support performance in place of antibiotics used for growth promotion. An experiment was conducted to evaluate the effect of copper proteinate in broiler diets challenged with *Salmonella* Heidelberg. A total of 192 1-d old male Cobb500® broiler chicks were randomly housed into 24 pens where 4 treatments were provided resulting into 6 replicates with 8 birds each. The treatments were: (1) basal diet containing antibiotic (zinc bacitracin, 0.013%), (2) basal diet without antibiotic + 25 ppm of copper proteinate (Bioplex Cu, Alltech Inc.), (3) basal diet without antibiotic + 150 ppm of copper sulfate + 25ppm of copper proteinate, (4) basal diet without antibiotic + 150 ppm of copper sulfate. All chicks were orally inoculated at 1-d old at housing with 0,5 mL of saline solution containing *Salmonella* Heidelberg. After 24 h of inoculation, excreta samples were collected to analyze bacteria recovery. The dietary program consisted into 2 phases including the pre-starter from d 0 to d 7 and starter from d 7 to d 28. The animals received the same amount of nutrients and energy and had free access to feed and water. The performance of the broilers was evaluated at 7, 21, and 28 d of age. At 10 and 29 d of age, one bird/pen totaling 24 birds, were euthanized by cervical dislocation for *Salmonella* Heidelberg presence testing in liver and ceca samples. The results were submitted to ANOVA and Scott-Knott test at 5% probability. After 24 h of inoculation *Salmonella* Heidelberg was detected in all excreta samples. At both time points *Salmonella* Heidelberg was not detected in any treatment. At 7 d old, birds fed a diet containing zinc bacitracin showed lower feed intake ($P = 0.012$) and better feed conversion ratio ($P = 0.001$) compared with birds fed 25 ppm of copper proteinate but similar feed intake and feed

conversion ratio to birds fed 25 ppm of copper proteinate + 150 ppm of copper sulfate and those that received diets containing 150 ppm of copper sulfate. At 21 and 28 d of age, the performance of broilers did not differ among treatments ($P > 0.05$). Under the conditions of this experiment, we conclude that in antibiotic free diets, copper proteinate at 25 ppm did not negatively impact growth performance in chickens challenged with *Salmonella* Heidelberg.

Key Words: antibiotic-free diets, bacterial challenge, mineral, nutrition, performance

553P Evaluation of three sources of selenium on performance, drip loss, selenium content in feathers, antioxidant capacity and lipid oxidation in breast (*Pectoralis major*) of broilers. Alex Espinoza*¹ and Carlos Vilchez², ¹Redondos S.A., Lima, Peru, ²Universidad Nacional Agraria La Molina, Lima, Peru.

The objective of the study was to determine the effect of 3 sources of selenium on performance, drip loss, selenium content in feathers, antioxidant capacity (AC) and lipid oxidation (LO) in the breast (*Pectoralis major*) of broilers. Nine hundred and 60 one-day-old Ross AP95 line male chicks were randomly placed in 24 experimental units (EU) with 40 birds each. Eight (8) EU received, during 42 d, one of the following treatments: T₁, diet with sodium selenite; T₂, diet with hydroxy-selenomethionine, and T₃, diet with selenium protein. The amount of supplemental selenium was 0.300 ppm, regardless of the source. The diets were formulated to meet the nutritional requirements of the line, pelleted and offered, together with water, *ad libitum*. Live body weight, feed intake, feed conversion and mortality were recorded weekly. At 42 d, a total of 84 birds (28 per treatment) were slaughtered and the breast of each bird was collected and identified. Breasts of 30 birds of the same treatment were separated into 3 groups of 10 for drip loss determination at 96 and 192 h post-slaughter. The 54 remaining breasts (18 per treatment) were separated into groups of 6 to determine the AC (Trolox Equivalent Antioxidant Capacity, TEAC) and LO (Malondialdehyde, MDA) at 24, 96 and 192 h post-slaughter. In addition, amounts of feathers from 6 birds per treatment were collected for determination of selenium content. The recorded data were submitted to ANOVA under the corresponding statistical design. The differences between treatments were separated through Tukey's test. The final liveweight of birds was not influenced ($P > 0.05$) by dietary treatments; however, birds that received the T₃ showed higher values ($P < 0.05$) feed intake and feed conversion compared with those which received the T₁ and T₂, and these were not different ($P > 0.05$) between them. The drip loss of the breasts, both at 92 and 192 h post-slaughtered, were not affected ($P > 0.05$) by dietary treatments. Selenium content of feathers from the experimental birds were also not influenced ($P > 0.05$) by dietary treatments. Lower oxidative capacity and increased lipid oxidation ($P < 0.05$) were obtained in breasts from birds that received the diet supplemented with sodium selenite (T₁) when comparison with those from the other 2 treatments (T₂ and T₃) which were not different between them ($P > 0.05$). In conclusion, based on the results, sources of organic selenium, in comparison to the inorganic source, improve animal response, particularly the oxidizing capacity and lipid oxidation of broiler breasts.

Key Words: selenium methionine, broilers, inorganic selenium, antioxidant capacity

554P Trans-generational effect of metal amino acid complex nutrition on bone health from turkey breeders to their progeny. Colwayne Morris*¹, Leonardo Linares², Marco Rebollo², Duarte

Neves², and Jeffrey Firman¹, ¹University of Missouri -Columbia, Columbia, Missouri, United States, ²Zinpro Corporation, Eden Prairie, Minnesota, United States.

Efficacy of dietary amino acid complexed (AAC) trace mineral (TM) supplementation on bone health of 0 to 16-week-old male turkey poults, of breeders fed inorganic minerals (IM) or Availa[®]ZMC (AvZMC) was studied. The objective was to chronicle histological profiles and skeletal pathology severity. Day-old Hybrid Converter male poults (n = 1,248) were randomized in a factorial design, with 2 breeder × 4 progeny treatments, 12 replicate pens, and 13 birds/rep. Birds were fed corn-SBM based diets *ad libitum*: Starter (S) 0–21d; Grower (G)1 22–49d; G2 50–70d; Finisher (F)1 71–91d; F2 92–112d. Progeny S and G1 diets: T1, 125ppm Zn, 125ppm Mn, 7ppm Cu from sulfates (SULF); T2, 60ppm Zn from Av[®]Zn+65ppm Zn, 125ppm Mn, 7ppm Cu from SULF; T3, 40ppm Zn, 40ppm Mn from Av[®]Z/M+85ppm Zn, 85ppm Mn, 7ppm Cu from SULF; T4, 40ppm Zn, 40ppm Mn, 7ppm Cu from Av[®]ZMC+85ppm Zn, 85ppm Mn from SULF. G2 diets had 25ppm less Zn and Mn from SULF for all S diet treatments; F1 and F2 diets had 50ppm less Zn and Mn from SULF for all S diet treatments. Data were analyzed by ANOVA, with statistical significance for performance characteristics considered at $P < 0.10$. Progeny effect ($P = 0.07$) was noted from 0 to 13-weeks, where SULF had the highest adjFCR. There was no difference in tibia weight, ash, or cross-sectional measures among breeder or progeny treatments. Breeder progeny fed AvZMC had higher ($P < 0.10$) Mn tibia ash concentration. Histological analysis showed increased growth plate proliferative zone (PZ) length; PZ was shorter in turkeys fed AvZn of breeders fed AvZMC ($P < 0.05$). Greater severity and occurrence, of microfractures and fibrous cortical defects (FCD) were seen at d25 in progeny fed SULF, and in those fed AvZ/M of breeders fed AvZMC, FCD differences were significant ($P = 0.03$). Histomorphometric measures of tibia growth plate (40X) revealed different degrees of rickets, possibly due to Ca or P imbalances, shown as widened chondrocyte proliferative or maturation zones. Severity was elevated in progeny fed SULF over those of breeders fed AvZMC ($P = 0.01$). Miscellaneous bone alterations improved for progeny fed AvZMC, of breeders fed AvZMC ($P < 0.05$). Tibia PZ of progeny fed SULF was wider over those fed AvZn ($P = 0.04$). No evidence of microfractures occurring in association with FCD was observed at 112 d-old, in contrast to 25 d-old. In conclusion: bone undergoes varied abnormalities during growth that tends to correct over time; feeding supplemental AAC TMs to turkey breeders statistically and numerically impacted progeny bone characteristics and performance, when compared with progeny of breeders fed IM.

Key Words: copper, manganese, rickets, turkey, zinc

555P Effect of iron source (organic vs inorganic) on broiler performance under a pathogen exposure. Jack Garrett^{*1} and James McNaughton², ¹QualiTech Inc., Longmont, Colorado, United States, ²AHPharma, Hebron, Maryland, United States.

Iron has been shown to influence broiler performance when exposed to normal or higher than normal pathogen levels. This study was conducted to validate the hypothesis that an organic form of iron (SQM Iron[®], QualiTech, Inc.) could limit the availability of iron to such pathogens and reduce their impact on broiler performance as was observed in a previous study. A total of 2496 mixed sex broiler chickens (Ross 708) were randomly assigned to one of 48 pens (12 pens/replicate). Diets (corn-soy based) were formulated to meet industry standards for a Prestarter (0–7d), Grower (8–35d) and Finisher (36–42d) program (Crumbles 1–21 d; pellets 22–42 d). Treatments included 1) iron sulfate as the sole supplemental iron source, 2) SQM Iron instead of iron

sulfate, 3) Trt 1 receiving a pathogen exposure on d 7, and 4) Trt 2 receiving a pathogen exposure on d 7. Broilers received Cocci-Vac d 0. Sub-clinical challenge was provided by obtaining used litter containing *E. acervulina* (>50,000 oocysts/bird), *C. perfringens* (>104/bird) and *E. coli* (>106/bird). Performance was measured every 7 d; lesion scoring, and pathogen counts were done on d 21 and 42. Data was analyzed as a 2x2 factorial design ANOVA using NCSS (2011). Broiler growth rate was significantly ($P < 0.001$) improved by organic iron and impaired ($P < 0.02$) by the pathogen exposure every week of the study. Iron source (organic) and pathogen exposure had a significant ($P < 0.05$) improvement on weight and efficiency through wk 5 and at wk 6 when adjusted to a common body weight. Bird mortality showed a significant ($P < 0.03$) interaction between source and exposure with those bird receiving the organic source reducing mortality under the exposure conditions. Lesion scores were significantly ($P < 0.01$) reduced by feeding the organic iron source (exposed or unexposed). Feeding the organic iron source significantly ($P < 0.01$) reduced *E. coli*, *C. perfringens* and cocci counts under pathogen exposure, typically bringing level close to unexposed control levels. Results showed that feeding SQM Iron as an organic iron source reduced the growth rate and influence of pathogens. This supported the previous research showing similar results and supports the reduction in growth rate of pathogens due to limiting the accessibility of iron.

Key Words: iron, broiler, pathogen exposure, organic, SQM

556P Not Presented

557P Effects of 25-OH-D₃ on performance, egg quality and serum characteristics of laying hens with high stocking density.

LingYun Qiu^{*}, Ying Zhang, Feng Zeng, Mei Ding, Shi Bai, Huanwei Peng, and Ping Wang, Sichuan Agricultural University, Chengdu, Sichuan, China.

The present study was conducted to evaluate the effect of 25-OH-D₃ on the performance, egg quality and serum characteristics of laying hens with high stocking density. 800 45-week-old Roman powder-shell laying hens were selected in this experiment. Two 25-OH-D₃ levels (0 ug/kg, 69 ug/kg) and 2 feeding densities (506 cm²/chicken, 338 cm²/chicken) were designed by 2*2 factor experiment. There were 4 treatments, 10 replicates per treatment and 1 replicate per 4 continuous cages. The experiment was conducted with corn-soybean meal diet for 16 weeks. Layers were fed and drank freely during the experiment. All data were analyzed by 2-way ANOVA. High stocking density significantly reduced the egg production rate, feed intake and egg weight of laying hens during 5–8wk, 9–12wk, and 1–16wk ($P < 0.01$) but had no significant effect on feed conversion ratio ($P > 0.05$). In the fourth week, high stocking density significantly increased yolk color ($P < 0.05$), 25-OH-D₃ decreased L* value ($P < 0.05$), increased a* value and thickness of egg shell ($P < 0.05$). While yolk color ($P < 0.01$) was also reduced by high stocking density. 25-OH-D₃ decreased significantly ($P < 0.05$) and increased the b* value of eggshell ($P < 0.01$). High stocking density significantly increased the levels of follicular estrogen (FSH) ($P < 0.05$), testosterone (T) ($P < 0.01$), corticosterone (CORT) ($P < 0.05$), lipopolysaccharide (LPS) ($P < 0.05$) in serum of laying hens, and significantly decreased the levels of anti-Muller hormone (AMH) and adrenocorticotrophic hormone (ACTH) ($P < 0.01$). 25-OH-D₃ significantly increased the levels of follicular estrogen (FSH) ($P < 0.01$), carbonic anhydrase (CA) ($P < 0.01$) and diamine oxidase (DAO) ($P < 0.05$), and significantly decreased the levels of anti-Muller hormone (AMH) ($P < 0.01$), corticosterone (CORT) ($P < 0.01$) and lipopolysaccharide (LPS) ($P < 0.05$). The results showed that high stocking density significantly reduced the

production performance, eggshell quality and increased serum stress-related hormone levels. 25-OH-D₃ can improve the eggshell color and alleviate stress-related hormone levels increased.

Key Words: density, 25-OH-D₃, performance, eggshell quality

558P Effects of 25-OH-D₃ on intestinal microbiota of laying hens with high stocking density. Ru Jia*, LingYun Qiu, Ying Zhang, Feng Zeng, Mei Ding, Shi Bai, Huanwei Peng, and Ping Wang, *Sichuan Agricultural University, Chengdu, Sichuan, China.*

The present study was conducted to research the effect of 25-OH-D₃ on the intestinal microbiota of laying hens with high stocking density. 800 45-week-old Roman powder-shell laying hens were selected in this experiment. Two 25-OH-D₃ levels (0 ug/kg, 69 ug/kg) and 2 feeding densities (506 cm²/chicken, 338 cm²/chicken) were designed by 2*2 factor experiment. There were 4 treatments, 10 replicates per treatment and 1 replicate per 4 continuous cages. The experiment was conducted with corn-soybean meal diet for 16 weeks. Layers were fed and drank freely during the experiment. All data were analyzed by bioinformatic analysis. There was no significant difference in Alpha diversity index of high stocking density ($P > 0.05$). 25-OH-D₃ significantly decreased observed species, Chao1 and ACE index of cecum ($P < 0.05$). In the composition of the flora, the dominant bacteria in the cecum are bacteroidetes and firmicutes, which account for 40.50%~60.28% and 32.26%~51.21%, respectively. High stocking density significantly reduced the ratio of bacteroidetes ($P < 0.05$) and spirulina ($P < 0.01$), and also significantly increased the proportion of firmicutes ($P < 0.05$). Density and 25-OH-D₃ interacted in the proportion of bacteroidetes and firmicutes. At low stocking density, 25-OH-D₃ increased the proportion of bacteroidetes and decreased the firmicutes ($P < 0.05$). 25-OH-D₃ reduced the proportion of bacteroidetes at high stocking density, and increased the proportion of firmicutes. The dominant genus of the cecal is bacteroides, accounting for 16.68%~26.61%, followed by lactobacillus, fecalibacterium, and desulfovibrio. The density interacted with 25-OH-D₃ in the proportion of bacteroides. At low stocking density, 25-OH-D₃ increased the proportion of bacteroides, and in the case of high stocking density 25-OH-D₃ reduces the proportion of bacteroides ($P < 0.05$). The results showed that the change of density altered the structure of microflora, and 25-OH-D₃ may regulate intestinal function by changing the proportion of intestinal microflora.

Key Words: density, 25-OH-D₃, intestinal microbiota

559P Evaluation of egg quality and performance in late-lay hens fed different combinations of manganese, zinc and copper complexed with sulfate or amino acid ion. Jill Nelson*¹, Gabrielle House¹, Eric Sobotik¹, Marco Rebollo², and Greg Archer¹, ¹Texas A&M University, College Station, Texas, United States, ²Zinpro Corp., Eden Prairie, Minnesota, United States.

Research has shown improvement in egg shell quality as a result of changes in membrane structure with dietary inclusion of inorganic sources of copper, magnesium, and zinc. Availa-Mins (Zinpro Corporation, Eden Prairie, MN) contain trace minerals bound to amino acid ions rather than sulfates. In this study, healthy 60-week old white Hy-Line W-36 laying hens (n = 378) were assigned to one of 7 treatments with 18 replicate cages each in a randomized complete block design. Hens were housed in groups of 3 within conventional cages and fed diets containing different combinations of trace minerals from sulfates or Availa-Mins for 15 weeks to assess egg production and quality. Treatments included: sulfates (T1; control), Availa-Cu (T2), Availa-Mn (T3),

Availa-Zn (T4), Availa-Cu/Mn (T5), Availa-Zn/Mn (T6), and Availa-Cu/Zn (T7). Trace minerals were added to a layer diet according to treatment combination to achieve target concentrations of 20ppm Cu, 40ppm Mn and 40ppm Zn. Egg weight was recorded daily. At wk 5, 10 and 15 egg performance was assessed via shell weight, thickness, breaking strength and deformation; specific gravity; vitelline membrane strength and Haugh unit. At wk 10 and 15 mineral content (Ca, P, Cu, Zn, and Mn) of eggshells, homogenized egg content and excreta was assessed and used to determine phosphorus retention (%). Data was analyzed in Minitab 17.1.0 using GLM, and mean separation was performed using the LSD post hoc procedure. A significant difference was defined as $P < 0.05$. Egg weight only differed during wk5-10 ($P = 0.02$): T6 had the highest egg weight, and T2 and T3 were intermediate. No treatment differences ($P > 0.05$) were observed for hen day production, breaking strength, specific gravity, Haugh unit, vitelline membrane strength, and shell deformation, thickness or weight during any time period. Excreta calcium content was significant ($P = 0.003$) at wk10: T2 and T5 had the lowest whereas T7 had the highest. Excreta phosphorus was significant ($P = 0.02$) at wk10, where T3 was lower than all other treatments. Excreta mineral content was significant for zinc at wk10 ($P < 0.001$) and wk15 ($P = 0.001$); for copper at wk10 ($P < 0.001$) and wk15 ($P < 0.001$); and for manganese at wk10 ($P < 0.001$) and wk15 ($P < 0.001$). Mineral content of egg shells and contents did not differ ($P > 0.05$) at any time point. Availa-Cu or Mn alone, and Availa-Zn/Mn combination increase egg weight in 65- to 70-week old layers with no effect on egg quality. Mineral source did not affect egg mineral deposition and egg quality measures. Some Availa-Mins enhanced zinc, calcium, and manganese retention, however Availa-Cu increased copper excretion. Only Availa-Mn enhanced phosphorus retention.

Key Words: layer, egg production, egg quality, minerals

560P An evaluation of five different sources of trace minerals on manganese, zinc and copper retention in the tibia of 21-day old broilers. Mathias Corteel*¹ and Sunday Adedokun², ¹BASF Corporation, Florham Park, New Jersey, United States, ²University of Kentucky, Lexington, Kentucky, United States.

Various types of trace minerals are used in broiler nutrition. Based on the disparity in product chemistry, differences in efficacy are to be expected, but it remains unclear which products result in the best mineral uptake and retention. Hence, the current study was conducted to evaluate the effect of different sources of 3 key trace minerals (TM; manganese, zinc and copper) from 5 distinct product categories: a glycinate, an amino acid complex, a hydroxychloride, a proteinate, and a sulfate. Cobb 500 male broiler chicks were fed a corn-SBM-casein-based broiler starter diet that was moderately deficient in Mn, Zn and Cu from d 0 to 14 (50% below NRC 1994). On d 14, birds were individually weighed and 486 birds were allotted to 6 dietary treatments in a completely randomized block design (CRBD) with 9 replicate cages consisting of 9 birds per replicate cage. The 6 experimental diets consisted of a negative control (NC) diet that was similar to the starter diet fed from d 0 to 14. To the NC diet, TM from different commercial sources (A: glycinate, B: amino acid complexes, C: hydroxychlorides, D: proteinates and E: monohydrate sulfates) were added at the expense of ground corn to meet the birds' requirements for Mn, Zn and Cu (NRC, 1994). On d 21, 7 d after start of supplementation, the 3 biggest birds within each cage were euthanized after which both tibias were removed (6 tibia/cage). Birds were sampled 7 d into the experiment to be able to pick up any difference in TM deposition in bones before when the bones would have been completely mineralized. Bones were analyzed for breakings strength, ash content and TM (Mn, Zn and Cu) contents. Data

were analyzed using Proc GLM procedure of SAS that is appropriate for a CRBD. The effect of the different dietary treatments on growth performance was not significantly different. Diet containing source A resulted in higher ($P < 0.05$) bone breaking strength. Manganese in the bones of birds on source D was higher ($P < 0.05$) than for birds on TM sources B and C. Tibia Cu content was not influenced by the source of TM. Overall, TM source D showed a trend for better tibia Mn and Zn retention resulting in 3.5 to 10% higher TM levels in bones compared with sulfates while TM source A resulted in between 3 and 6% higher TM retention in tibia compared with the sulfates.

Key Words: broiler, copper, manganese, trace mineral, zinc

561P Efficacy between chelated vs organic and inorganic trace minerals in broilers. Karen Wedekind*, Juxing Chen, Mercedes Vazquez-Anon, Steve Richter, and Mark Minier, *Novus International Inc., St. Charles, Missouri, United States.*

The primary objective of this trial was to compare efficacy of chelated trace minerals (combination of Zn-Cu-Mn methionine hydroxy analog chelate (MMHAC, MINTREX®, Novus International, Inc.; 32–8–32 mg/kg diet; Zn, Cu, Mn, respectively) vs other organic trace minerals (OTM; proteinate, glycinate) at equivalent Zn-Cu-Mn concentrations; 32–8–32, respectively, as well as 2 inorganic trace mineral treatments (ITM provided as sulfates at 32–8–32 mg/kg and at commercial levels of ITM; 100–125–90 mg/kg Zn, Cu, Mn, respectively) and lastly a deficient control diet w/o added Zn-Cu-Mn. A total of 1800 Ross 308 d-old male broilers were randomly allotted to one of 6 treatments (12 reps/trt) to floor pens (25 birds/pen). All experimental diets (starter and grower phase) were semi-purified in nature (corn-corn grits-potato protein concentrate-soy isolate basal) and fed for 21 d. The control starter diet was analyzed to contain 11, 11 and 25 mg/kg Zn, Cu, Mn, respectively and all 6 treatments were iso-methionine. To simulate more commercial-type conditions, the following were utilized: 1) used wet litter, 2) high stocking density, and 3) unplanned; high humidity and a spike in temperature during the course of the trial. Multiple pair-wise comparison procedures (LSD) were used to compare treatment means using a one-way ANOVA analyses. With the exception of cumulative feed intake, significant differences between mineral sources were observed for d21 body weight (BW), cumulative feed conversion rate (cFCR), cumulative mortality (cMortality), cumulative performance index (cPerfdix), and for bone Mn and Zn concentrations ($P < 0.05$). Collectively, among the OTM sources evaluated, rankings based on cPerfdix were: MMHAC > Glycinate > Proteinate corresponding to 171.9, 160.4, and 155.0, respectively ($P < 0.05$) which were higher than the commercial and low ITM treatments (147.7 and 150.6, respectively). Similar rankings were observed for cFCR and BW ($P < 0.05$). Chicks fed the deficient control had the highest cMortality (3.0%) and only MMHAC was significantly lower than control (0.0%; $P < 0.05$). Likewise, chicks fed the control diet has the lowest bone Mn and Zn concentrations; all trace mineral sources increased bone Zn ($P < 0.05$), but only chicks fed ITM at commercial levels and MMHAC significantly increased bone Mn ($P < 0.05$). Under antagonistic conditions, higher addition of minerals may not improve performance. MMHAC performed better than proteinate, glycinate and ITM sources.

Key Words: chelate, zinc, copper, manganese, broiler

562P Updated amino acid, choline, betaine, folic acid, and vitamin B₁₂ concentrations in soybean meal and corn. Kari Estes*¹, Barbara Barton¹, Ryan Dilger², and Thomas Powell¹, ¹*Balchem*

Corporation, New Hampton, New York, United States, ²*University of Illinois, Urbana, Illinois, United States.*

Choline and water-soluble vitamins are essential nutrients for monogastric species. Choline specifically acts as a biochemical building block involved in cell structure and function making it an influential nutrient in cognitive, cardiovascular and liver health. Additionally, choline along with methionine (Met), betaine, folic acid and vitamin B₁₂ are considered methyl donors and play important roles in DNA methylation. Choline deficiency symptoms can include fatty liver, neuromuscular issues, poor immune function and poor growth rates which can affect animal production profitability. While the Poultry NRC (1994) has set forth dietary requirements for poultry, there is a lack of current information related to how the concentrations of water soluble vitamins/methyl-containing compounds differ in varying ingredients. Improvements in analytical testing procedures, changes to the genetic makeup of crops and/or current processing methods may collectively influenced nutrient concentrations in feedstuffs. The purpose of this project was to sample and analyze numerous soybean meal (SBM) and corn samples collected in 2018 for choline and other methyl-containing compound concentrations to build an updated library. Twenty-nine SBM samples and 22 corn samples were collected from 2 international countries and 10 states within the United States. Samples were then analyzed at Eurofins Scientific (Des Moines, IA) for amino acids, choline, betaine, folic acid, and vitamin B₁₂ concentrations. In general, corn exhibited lower analyzed methyl-containing nutrient concentrations and was more variable when compared with SBM. Choline, folic acid, betaine and Met concentrations in corn were roughly 25%, 13%, 67%, and 21% of those in SBM. Vitamin B₁₂ concentrations in both SBM and corn were low and below detection limits (<4.40 and < 5.96 µg/kg, respectively). Of these nutrients, the Poultry NRC has published only choline and Met values for SBM and only choline, folic acid, and Met values for corn. Analyzed choline levels were 24% lower for SBM and 9% lower for corn than those reported by the Poultry NRC. Met content of SBM and folic acid content of corn were numerically similar to those reported by the NRC, however, Met content of corn was determined to be 22% lower than that reported by the NRC. This has important implications as formulating diets based on nutrient concentrations from the Poultry NRC over predicts dietary choline concentrations. The lack of data on other methyl-donor compound concentrations in these 2 feedstuffs would suggest there are likely inaccuracies in formulating rations to meet the requirements for optimal growth and health of poultry.

Key Words: choline, methyl donor, vitamin, soybean meal, corn

563P Expression of genes involved in medullary bone resorption and accretion during the inactive and active phases of eggshell formation in laying hens. Audrey Gloux*¹, Nathalie Le Roy¹, Jacky Ezagal¹, Nathalie Mème¹, Christelle Hennequet-Antier¹, Marie-Liesse Piketty², Prié Dominique², Gaëlle Benzoni³, Joel Gautron¹, Yves Nys¹, Agnès Narcy¹, and Michel Duclos¹, ¹*BOA, INRA, Université de Tours, Nouzilly, Centre Val de Loire, France,* ²*INSERM U11513, Paris, Ile de France, France,* ³*Neovia, Saint-Nolff, France.*

In laying hens, eggshell formation is a discontinuous process requesting a high rate of Ca exportation during the dark period, when the hens have no access to the feed, and thus implies a daily cycle of bone resorption and accretion. To limit bone losses, laying hen diets commonly contain coarse particles of calcium carbonate (CaCO₃) to extend the provision of soluble Ca in the digestive tract. The objective of this study was to describe the expression of candidate genes involved in bone remodeling in laying hens fed fine or coarse Ca particles. Laying hens at the peak of lay were accommodated in individual cages to record the oviposition

time, and subjected to a 14h L: 10h D lighting program. They were fed a diet supplemented with fine (AP1) or a mix of fine (30%) and coarse (70%) CaCO₃ (AP2). Laying hens were selected at 0–1, 9–10 and 18–19 h post oviposition (PO ; n = 6 to 7 per group). Blood samples were collected before euthanasia, dissection and collection of bone samples. Total medullary bone RNA were extracted and subsequently reverse transcribed into cDNA. Plasma inorganic P (iP) and 1,25(OH)₂D₃ were measured. The mRNA levels of 10 candidate genes, selected on their involvement in bone resorption (ACP5, CA2, CTSK), bone accretion (COL1A1, ITGB1 and MMP2) or their regulation (RANK, RANKL, OPG and VDR) were measured by high-throughput microfluidic RT-qPCR, using validated primers. All data were analyzed with a linear model by robust regression (rlm function in R), including the main effects of the stage PO, of the diet and their interaction. No interaction between the stage PO and the diet was observed for any parameter. Plasma iP and 1,25(OH)₂D₃ rose with the time PO ($P < 0.01$). The levels of 1,25(OH)₂D₃ were significantly higher with AP1 than with AP2 ($P < 0.05$). At 18–19 h PO, 4 h after dark, the higher gene expression of RANK, the higher level of plasma iP and the elevated level of 1,25(OH)₂D₃ ($P < 0.05$) indicated that bone resorption was initiated. The high expression of ACP5 and CTSK ($P < 0.05$) also suggested that resorption was enhanced in hens fed AP1. The 3 candidate genes implicated in bone accretion and the inhibitor of bone resorption OPG were also overexpressed in hens fed AP1 compared with hens fed AP2 ($P < 0.05$). These data further demonstrate that bone turnover was reduced by the provision of coarse Ca particles in the diet. The recorded plasma parameters and mRNA levels of candidate genes could provide markers for the evaluation of new dietary sources of Ca allowing progressive and extended diffusion of soluble Ca during the dark period. This is of particular interest regarding the current challenge of extending the laying period up to 100 weeks.

Key Words: calcium, bone remodeling, eggshell formation, laying hens, gene expression

564P Amino acid chelated trace minerals reduces the expression of stress-and inflammatory-genes and severity of woody breast in heat-stressed broilers. Elizabeth Greene*, Mikayla Baxter, Juan Latorre, Guillermo Tellez, Barbara Mallmann, and Sami Dridi, *University of Arkansas, Fayetteville, Arkansas, United States.*

Heat stress (HS) causes a financial and physiological burden in poultry production and mitigating the negative effects of HS is essential. The purpose of this experiment was to determine if trace minerals supplementation would reduce the adverse effects of HS. On day of hatch, 480 chicks were allocated into 12 environmental chambers in 2x2 factorial design. Each chamber was divided into 2 pens containing separate feeders and water jugs. Amino acid chelated trace mineral (Tracer Minerals, Cimarron, KS) was supplemented in the water (0.5 oz per gallon) in one pen from each chamber. On d 28, temperature was increased to 35°C to induce HS in 6 chambers and maintained until d 42, while the remaining 6 chambers were maintained at 25°C. Feed and water intake were recorded daily, body weight and breast muscle palpation for woody breast was measured weekly. Blood and tissues, including the gut, were collected 2 h post HS (acute) and at the end of the experiment (chronic) to measure blood gasses and electrolyte levels via iSTAT portable analyzer and gene expression of stress and inflammatory markers. At the end of the experiment, the chickens were processed to determine carcass weight, composition, and woody breast scores. Data were analyzed by 2-way ANOVA with temperature and mineral supplementation as the main effects. When ANOVA revealed significant effects, means were compared by Tukey's multiple comparison test and considered

significant at $P < 0.05$. Chronic HS caused an increase in core body temperature and water intake and decreased feed intake, body weight, and feed efficiency. HS reduced carcass weight, causing a decrease in the weight and yield of all parts. Acute HS increased plasma corticosterone (CORT) and blood oxygen saturation (sO₂). Additionally, there was a significant interaction between HS and mineral supplementation during chronic HS on the blood total CO₂. HS increased the expression of stress markers (HSP70), inflammasome (NLRP3) and proinflammatory cytokines (IL-6, IL-1 β) in both circulation and gut. Although amino acid-chelated trace minerals had no statistical effect on growth performance, supplementation significantly reduced the expression of stress- and inflammation-related genes under HS but not under thermo-neutral conditions. Interestingly, chickens treated with the minerals had a significant reduction in the severity of woody breast syndrome by at least 5%, regardless of heat treatment. Together, these data indicate the potential for amino acid chelated trace minerals to improve inflammatory status and woody breast in heat stressed broilers.

Key Words: amino acid chelated minerals, heat stress, woody breast, gene expression

565P Impact of varying calcium and phosphorus concentrations on bone mineralization and performance. Dalton Dennehy*, Tri Duong, Brooke Bodle, and Jason Lee, *Texas A&M University, College Station, Texas, United States.*

Calcium and phosphorus are essential nutrients that are important in bone development and mineralization and critically important for broiler chickens to reach their maximum genetic potential. In this study, we investigated the effect of feeding varying calcium and phosphorus concentrations during the grower and finisher phases of production. Male Cobb 500 broilers (n = 1020) were allocated to 3 experimental treatment groups with 4 replicate pens of 85 birds arranged as a randomized complete block design. The treatment groups consisted of a low, medium, and high mineral diet with Ca and aP maintained at a 2:1 ratio. Broilers were fed a common ration (0.45% aP) during the starter phase (d 0–14); rations with 0.40, 0.425, or 0.45% aP during the grower phase (d 14–28); and rations 0.35, 0.40, or 0.45% aP during the finisher phase (d 28–42). Average body weight (BW), feed consumption (FC), mortality corrected feed conversion ratio (FCR), and mortality were evaluated at d 14, 28 and 42, and tibia bone ash was evaluated at d 7, 14, 21, 28, 35 and 42. Results were analyzed using ANOVA, and significantly different means were separated using Duncan's multiple range test ($P \leq 0.05$). Significant increases in BW were observed with each incremental increase in mineral concentration on d 28 ($P < 0.01$); while, on d 42, BW of the low mineral treatment was significantly lower than the other treatments ($P < 0.05$). Cumulative FC of the high and medium mineral fed broilers was significantly greater compared with the low mineral fed broilers ($P < 0.01$). FCR of broilers fed the high mineral diet was lower than the low mineral diet during the grower phase ($P < 0.05$); while cumulative FCR of the medium and high mineral treatments was lower when compared with the low mineral diet. Bone ash % was lowest when broilers were fed the low mineral diet on d 21 ($P < 0.01$) and d 28 ($P < 0.05$) when compared with the medium and high mineral diets. Although the differences were not observed to be statistically significant, this trend continued on d 35 and d 42. The results observed for bone ash weight were similar, with the bone ash weight of the low diet being significantly lower than the medium and high mineral diets at d 21 ($P < 0.01$). At d 35, the high mineral diet significantly increased ($P < 0.05$) bone ash weight when compared with the low mineral diet with the medium diet being intermediate. On d 42, the high mineral diet significantly increased bone ash weight when compared with the

low and medium diets. These data confirm the importance of calcium and phosphorus inclusion in broiler diets and demonstrate the negative impacts of an aggressive reduction in calcium and phosphorus on growth performance and bone mineralization.

Key Words: broiler, calcium, phosphorus, performance, mineralization

566P Exploring the capacity of the chicken to catch-up in growth and bone mineral deficit depending on P and Ca levels.

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Broiler production sustainability implies the formulation of diet optimizing growth performance and bone mineralization while matching P excretion with plant's needs. A study was conducted to evaluate broilers response to dietary Ca and P supply during grower phase and the capacity to recover or not bone mineralization with high or normal Ca and P supply. Six dietary treatments differing in calcium (Ca) (0.55, 0.70 and 0.85%) and phytase (0 or 1000FTU/kg; AXTRA® PHY) with a basal low level of available phosphorus (aP) (0.2%) were given to 2268 broilers divided in 50 pens of 45 birds from 10 to 20 d of age. At 20 d, 6 birds per pen were slaughtered and scanned using Dual-X-Ray (DXA) considering the entire body or femur or tibia. The experimental design was a completely randomized designed with 2x3 factorial arrangement. Contrast has been used to test Ca x Phytase and Ca linear and quadratic effects using proc mixed. From 20 to 30 d, broilers received a negative control diet (C-) with 0.60% of Ca, 0.09% of aP (no phosphate addition) and 1000FTU/kg of phytase, or a positive control (C+) which corresponds to grower treatment 0.70% of Ca, 0.2% of aP and 1000FTU/kg of phytase that was maintained in the finisher period and should maximize bone mineralization. Data were compared with C+ with Dunnett test. During the grower phase, phytase addition improve final body weight (fBW), Average daily gain (ADG), Average daily feed intake (ADFI), feed conversion ratio (FCR), and bone mineral content (BMC) of the whole body and the femur. Its effect was higher when increasing dietary Ca for tibia BMC (CaxPhytase; $P = 0.04$). Dietary Ca affected FCR in a quadratic manner with the medium Ca level having the lowest FCR. Thus, it seems that no more than 0.55% of Ca in 1000 FTU/kg phytase is enough for bone mineralization with 0.2% aP. During the finisher phase, there was no difference in ADG, ADFI and FCR between C+ and C-. Thus, birds having received a diet without phytase during the grower period remains with lower fBW ($P = 0.002$) compared with C+ while those having received phytase were similar to C+. There was no difference in tibia and whole body BMC while femur BMC was lower in birds fed low and moderate Ca diet without phytase ($P = 0.02$). This shows that birds have the capacity to catch-up bone mineral deficit depending of previous Ca supply and bone area, but they cannot cope with deficit in growth performance induced by P deficiency. In conclusion, these results help to fine-tune Ca and aP levels in phytase supplemented diets during the grower phase and showed that birds can be raised without phosphate in the finisher phase and have similar growth performance and bone mineralization with 60% less phosphate.

Key Words: broiler, phosphorus, calcium, phytase

567P Amino acid complex minerals in diets for laying hens improves intestinal histomorphology.

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An experiment was designed to evaluate the intestinal histomorphology in layer hens during the production period, as affected by the trace mineral nutrition of initial, growth and production period. From 1st to 30th weeks-of-age pullets were raised in cages according to the manual guidelines in 2 groups fed with inorganic minerals sources (IM) or with amino acid complex minerals (AACM) partially replacing by IM. Minerals partially replaced were: Zn, Mn, Cu and Fe, as following: IM, 70 ppm Zn + 70 ppm Mn + 8 ppm Cu as ZnO, MnO, and CuSO₄H₂O, respectively; or ZMC-AACM 40 ppm Zn + 40 ppm Mn + 2.75 ppm Cu (from IM sources) and 30 ppm Zn + 30 ppm Mn + 5.25 ppm Cu (from AACM sources). From 30 to 50th weeks-of-age, 800 laying hens (Lohmann Brown Lite) originated from the 2 initial groups of treatments were distributed in complete randomized design, under a 2 × 4 factorial arrangement of treatments. Each treatment had 10 replicates, with 10 birds per cage. The 2 initial diet groups were divided into 8 treatments (4 groups per initial diet): IM; AACM-ZMC; AACM-ZMCFE (AACM-ZMC + 10 ppm Fe from FeSO₄ and 40 ppm Fe as Fe-AACM); AACM-Fe (IM + 10 ppm Fe from FeSO₄ and 40 ppm Fe-AACM). In the 50th experimental week, one bird of each replicate was euthanized by cervical dislocation. Samples of 3 cm of the middle duodenum and jejunum were collected from 3 birds per treatment immersed in formalin, dehydrated in series of alcohols, xylol and embedded in paraffin. The slides were assembled with 5 µm sections and stained hematoxylin-eosin. The villus height was measured from its apex to the basal region, and the depth of the crypt was measured from the base of the crypt to the base of the villi. The villi: crypt ratio (V:C) was calculated. In each segment, 18 measurements were performed, totaling 54 measures per group. Data were submitted to multivariate ANOVA and Tukey test ($P < 0.05$). Intestinal morphometry was improved ($P < 0.05$) when birds were fed with AACM supplemented diets both on growth and production periods. In the growth period, there was an increase in crypt depth and reduction in the V:C ratio for 2 segments evaluated for birds fed only with IM ($P < 0.05$). During the production period, there was increased in villus height in the duodenum for birds that received any AACM treatment ($P < 0.05$). There was interaction for crypt depth for the group that received AACM during the growth and production period ($P < 0.05$). There was deeper crypt for the group of birds supplemented only with IM and lower crypt deep when they received AACM in any period of life ($P < 0.05$). Overall, the supplementation of laying hens diets with AACM during initial and production periods improves intestinal morphology.

Key Words: gut health, intestinal integrity, mineral source, trace mineral

568P Dietary supplementation of amino acid complexed minerals for pullets.

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This study aimed to evaluate the effect of partial supplementation of inorganic sources of zinc, manganese, and copper by metal-amino acid complex sources in the diets of pullets. Performance parameters, development of organs of the lymphoid system, hepatic, digestive and bone characteristics were evaluated. Lohmann Brown Lite hens (n = 1120) from 35 to 98 d of age, were housed in cages and randomly allotted to 2 treatments, 20 replicates, and 28 birds per experimental unit. The treatments consisted of a diet supplemented with 70, 70 and 8 mg/kg of zinc, manganese, and copper, respectively, from inorganic sources, and a diet supplemented with 40, 40 and 2.75 mg/kg of zinc, manganese, and copper, respectively, from inorganic sources and additional 30, 30 and 5.25 mg/kg of zinc, manganese and copper, respectively, from metal-

amino acid complex sources. Water was supplied *ad libitum* and the feed adjusted according to the strain guideline. The experimental period comprised 9 weeks, from the 42 to the 98 d old. Data was submitted to ANOVA and the means were compared by Student *t*-test ($P < 0.05$) using software SAS. The supplementation of zinc, copper and manganese in the form of metal-amino acid complex did not influence ($P > 0.05$) the performance (weight gain, feed intake, feed conversion and uniformity) and bone characteristics (tibial weight and length, bone resistance and Seedor index) of replacement pullets at 98 d of age. However, higher concentrations of calcium and phosphorus were found in the tibia of birds fed minerals complexed to amino acids ($P < 0.05$). No significant differences ($P > 0.05$) were observed in the weight of the pancreas and length of the cecum. However, the weight of the small and cecum and the length of the small intestine were higher for the birds that consumed the diet supplemented with metal-amino acid complex ($P < 0.05$). This suggests a better development of the gastrointestinal tract, a greater area of nutrient absorption and intestinal integrity. In conclusion, mineral supplementation with metal-amino acid complex improves the development of the gastrointestinal tract, as well as the deposition of calcium and phosphorus in the bones of the replacement pullets.

Key Words: bioavailability, bone characteristics, complexed micro-minerals, mineral composition

569P Response of broilers chickens to gelatin inclusion in diets and coccidiosis vaccination. Catiane Orso¹, Carolina Haubert Franceschi*¹, Bruna Cony¹, Thais Stefanello¹, Danlei Nogueira¹, Julio Furtado¹, Ines Andretta¹, Pedro Ferzola¹, and Andréa Ribeiro², ¹Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, ²UFRGS, Porto Alegre, Brazil.

The objective of this study was to evaluate the effects of the inclusion of gelatin in broiler diets on mitigating the negative impact of vaccination for coccidiosis on performance. A total of 280 Cobb 500 male broiler chicks were randomly assigned to 28 pens at d 1 of age and housed on used litter up to 42 d. Half of the chicks were vaccinated through the water with live oocysts on the first day of age and received non-medicated feed, whereas the other half received coccidiostat-medicated (salinomycin) feed up to 6 wk of age. The standard diet was composed of corn and soybean meal and, when added, gelatin was included at 2% in the formulation. Four treatments were applied: T1 – Unvaccinated; T2 – Unvaccinated + Gelatin; T3 – Vaccinated, and T4 – Vaccinated + Gelatin. Data obtained were analyzed using the general linear models procedures of SAS 9.4. Polynomial contrast was applied to determine the effects of treatments and probabilities lower than 0.10 are considered. Adding gelatin to the diets of the unvaccinated chicks improved body weight, weight gain and feed conversion rate throughout the period of trial (d 1 to 21) ($P < 0.05$). Vaccinated chick had decreased performance when compared with unvaccinated chicks ($P < 0.10$). Adding gelatin to the diets fed to vaccinated chicks did not prevent the poorer performance when compared with the unvaccinated chicks. However, adding gelatin improves weight gain and feed conversion rate from 22 to 42 d of vaccinated chicks ($P < 0.05$). The coccidiosis vaccine affects the performance of broilers. Unvaccinated chick that received gelatine improved performance over unsupplemented chick. In the same way, vaccinated chick were better with the inclusion of gelatine in comparison to those that did not receive the additive in the diet.

Key Words: *Eimeria*, performance, poultry, salinomycin, vaccine

570P Performance of broilers submitted to coccidiosis vaccination and vitamin E supplementation. Catiane Orso¹, Thais

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We aimed to evaluate if the inclusion of vitamin E, a non-steroidal anti-inflammatory agent, could reduce post-vaccination pro-inflammatory effects that may affect broiler performance. A total of 280 Cobb 500 male broiler chicks were randomly assigned to 28 pens at d 1 of age, housed on used litter as bedding up to 42 d. They were orally vaccinated with a live attenuated vaccine or were provided with in-feed salinomycin for the first 6 weeks. The basal inclusion of vitamin E (VitE) in the standard diet (SD) was 30 UI, while the supplemented diet received 65 UI of VitE. Four treatments were applied: T1– Unvaccinated; T2–Unvaccinated + VitE; T3 – Vaccinated and T4 –Vaccinated + VitE. Data obtained were analyzed using the general linear models procedures of SAS 9.4. Polynomial contrast was applied to determine the effects of treatment. The VitE supplementation for unvaccinated chicks improved body weight (BW) and weight gain (WG) of 01–21d ($P < 0.10$). Vaccinated birds presented lower BW at 21d and 42d, and lower WG of 1–21 d ($P < 0.10$). Despite receiving VitE, vaccinated chicks had lower performance than non-vaccinated ($P < 0.10$). In conclusion, the addition of vitE to unvaccinated chicks improves the performance of 01–21d. Vaccination for coccidiosis causes loss of performance and the addition of VitE is not sufficient to reverse the damage caused by the vaccine.

Key Words: *Eimeria*, non-esteroidal, poultry, inflammation, salinomycin

571P Reduction of *Campylobacter* on poultry thighs using sequential treatments of antimicrobials. Melissa Landrum*¹, Nelson Cox², Jeanna Wilson¹, Mark Berrang², Mark Harrison³, Brian Fairchild¹, Woo Kim¹, and Arthur Hinton², ¹University of Georgia, Crawford, Georgia, United States, ²United States Department of Agriculture, Athens, Georgia, United States, ³University of Georgia, Athens, Georgia, United States.

Campylobacter is a major concern for poultry processors. The objective of this study was to evaluate CMS PoultrypHresh, a low pH processing aid, and peracetic acid using consecutive and sequential dip treatments to reduce *Campylobacter* numbers on chicken thighs. Thighs (n = 3/treatment group) were inoculated with a *C. coli* gentamicin resistant marker strain (10⁸). Each inoculated thigh was dipped into bags containing 1 L of treatment one for 6 s. Thighs were allowed 5 s to drip, placed onto foil for 60 s, and dipped into treatment 2 for 6 s. After 5 s drip time, each thigh was placed in a bag with 150 mL buffered peptone water and hand shaken for 60 s; controls followed the same procedure with no treatment. Rinsates were serially diluted, plated onto Campy Cefex agar with 200 ppm gentamicin and incubated microaerobically for 48 h at 42°C. Procedures were replicated 5 times. Statistical differences were evaluated using a General Linear Model and means separated by a Tukey Multiple Comparison test. Significant reductions ($P < 0.05$) compared with untreated control samples were found when using consecutive dips of PoultrypHresh and PAA were 98.2% and 99.3%, respectively. Treatments of PoultrypHresh then peracetic acid reduced *Campylobacter* 99.2% from untreated thighs. Peracetic acid then PoultrypHresh showed significant reductions compared with all other treatments (99.9% from untreated). These data suggest that treatment with an oxidizing agent (PAA) following by an acidic dip maximizes *Campylobacter* reduction. Treating with this sequence may allow processors to meet strict performance standards relative to *Campylobacter* on broiler parts.

Key Words: *Campylobacter*, CMS PoultrypHresh, broiler, carcass rinse, immersion

572P Particle size-dependent bioactivity of novel eggshell membrane preparations. Garima Kulshreshtha*, Tamer Ahmed, Ling Wu, Fiona Dong, and Maxwell Hincke, *University of Ottawa, Ottawa, Ontario, Canada.*

The chicken egg is a valuable nutritious food, and large markets exist for processed / purified egg yolk and egg white. However, more research is necessary to develop value-added applications for waste eggshell and eggshell membranes. The eggshell membranes (ESM) are a fibrous meshwork of highly cross-linked proteins which show significant promise to be utilized as a biomedical resource. Proteomic analysis of egg shell membrane has shown the presence of antimicrobial proteins including lysozyme C, ovotransferrin, ovocalyxin-32 and peptides such as avian β -defensins-9, -10 and -11 required for defense responses against bacteria. However, one of the biggest challenges for the use of ESM bioactive substrate is its poor solubility and bioavailability. These limitations could be resolved by reducing the size of the ESM particles to enhance the solubility and increasing the bioavailability of bioactive components. The goal of this study is to produce novel ESM preparations with reduced particle size to improve its bioactivity against microbial infection and inflammatory disease condition, with an enhanced emphasis on skin health application. White eggs from Lohmann Leghorn hens (mid-lay) were used as a source to produce manually peeled eggshell membranes with minimum chemical processing. Top-down approaches, including manual grinding, Emuslifix (Avestin Inc.) and cryomilling using Bead Ruptor 96 (OMNI International) and Retsch cryomill (ATS) were used to reduce ESM particle sizes. ESM particles in different preparations were characterized by scanning electron microscopy (SEM), and antimicrobial activity in various size ranges was evaluated. A combination of top-down approaches resulted in size reduction of ESM particles down to the nano scale, approaching 200nm. Amino acid composition of ESM particles in various preparations was similar to the literature values of intact ESM material, indicating little effect of processing on biomaterial composition. Lysozyme activity was significantly higher ($P < 0.05$, $n = 3$) in $< 53 \mu\text{m}$ ESM preparation ($328.1 \pm 16.1 \text{ U/mg}$) compared with bulk ESM material ($288.9 \pm 9.3 \text{ U/mg}$). ESM particles exhibited a size-dependent antimicrobial activity against Gram-positive *Staphylococcus aureus* (*S. aureus*) and Gram-negative *Pseudomonas aeruginosa*. In addition, a bacteriostatic mechanism of action was determined against *S. aureus*. The results from our research will lead to the development of new high-value cosmetic biomaterials from ESM by-products. (Supported by Egg Farmers of Canada and NSERC).

Key Words: eggshell membrane, biomaterial, antimicrobial, skin-health

573P Dietary supplementation of Woodsout™ on wooden breast syndrome of broilers: Incidence, blood chemistry, gene expression and tissue morphology. Rebecca Delles*¹, Cody Durham¹, Katie Eastridge¹, Rijin Xiao¹, Mike Ford², Karl Dawson¹, Anthony Pescatore², Ronan Power¹, and Tuoying Ao¹, ¹Alltech Inc., Nicholasville, Kentucky, United States, ²University of Kentucky, Lexington, Kentucky, United States.

Quality defects in fresh meat products of broilers lead to downgrades or condemnations, resulting in economic losses. Wooden Breast Syndrome (WB) of broilers is a myopathy that alters the chemical composition, textural parameters and functional properties of the *pectoralis major*. The objective of this study was to investigate the occurrence and severity of WB, gene expression patterns, blood chemistry and histological attributes of breast muscle from birds fed Woodsout, a proprietary formulation containing plant extracts and antioxidants (Alltech Inc., KY). One-day old Ross 708 broiler chicks were randomly placed in 20 pens

with 24 chicks per pen. Pens were randomly assigned to either one of 2 dietary treatments, a corn-soy control diet or control diet supplemented with 1 kg/MT of Woodsout. On d 46 and 49, 2 birds per pen were randomly selected, tagged, and blood was collected from the brachial vein, 100 μL blood was dispensed directly into the well of an Element POC blood analyzer test card (Heska, CO). Subsequently, tagged birds were processed to evaluate occurrence and severity of WB myopathy in the *pectoralis major*. On d 50, 6 additional birds per treatment were randomly sampled for gene expression assay and histology. Blood chemistry data were subjected to least square differences (LSD) in Statistix 9.0 to determine the significance of dietary treatments. Differentially regulated genes were subjected to functional and association analysis (Genespring and Ingenuity Pathway Analysis). Under the conditions of this study dietary supplementation with Woodsout reduced the severity of WB by 31.25%, compared with control. Birds fed Woodsout tended ($P = 0.0963$) to have higher blood gas oxygen levels on d 46 and 49 compared with birds fed control diets. Microarray results of the Woodsout treatment group revealed an increased expression ($P = 0.0068$) of carbonic anhydrase 5B gene (CA5B) and potassium channel gene ($P < 0.05$), KCNK2, compared with control group. CA5B is a gene that controls the production of mitochondrial carbonic anhydrase that plays an important role in acid-base balance and may serve as an antioxidizing agent. KCNK2 is a gene associated with resting membrane potential and is linked to the regulation of cellular response to hypoxia. Ultrastructural examination of myofibril cross-sections highlighted expansive fibrosis and lipodosis with subsequent myofibril degeneration in control birds. The results of the current study indicate that dietary supplementation with Woodsout has the potential to reduce the severity of WB, increase blood oxygen levels, upregulate CA5B, and maintain healthy muscle tissue morphology.

Key Words: wooden breast syndrome, gene expression, blood gas, dietary supplementation

574P A study on sensory attributes of meat from three Sri Lankan indigenous chicken strains reared under semi-intensive systems. Madushika Ranasinghe* and D. D. Jayasena, *Uva Wellassa University, Badulla, Sri Lanka.*

The aim of the present study was to assess and compare sensory attributes of meat from 3 Sri Lankan indigenous chicken strains namely; Village/Non-descriptive Chicken (VC), Long Legged (LL), Naked Neck (NN). A total of 54 birds at similar age and live weight from both sex were purchased and sacrificed separately according to the commercial guidelines. Both breast and leg meat samples were prepared by boiling in water (1:2 wt/vol) while attaining to an internal temperature of 85°C for 30 min. Meat samples of 2.0 \times 3.0 \times 1.5 cm sized, were presented on coded white dishes and served with drinking water to 35 un-trained panelists. Samples were assessed for color, flavor, odor, tenderness, chewiness, juiciness, and overall acceptability attributes using a 9-point hedonic scale (1 = unlike extremely, 5 = like moderately, 9 = like extremely). Breast and leg meat from commercially available broiler chicken (Cobb®) strain, served as the control. Data obtained through the sensory evaluation were analyzed using Friedmann non-parametric test in Minitab 17®. Tenderness, chewiness, and juiciness of both meat portions in each strain showed significance difference ($P < 0.05$) while color, odour, flavor, and overall acceptability did not show significant difference ($P < 0.05$) either in strain or type of meat portion. Among strains, the highest preference for the color (5.06), flavor (5.13), and overall acceptability (5.00) obtained by the breast meat of VC strain. Similarly, among 3 strains, the highest preference was recorded for tenderness (5.13), chewiness (5.31), and juiciness (5.13) by the breast meat

of VC strain while the least acceptance for tenderness (4.13), chewiness (4.44), and juiciness (4.38) was recorded from the leg meat of the NN chicken strain. In conclusion, the breast meat of Village/Non-descriptive Chicken strain is the highly preferred indigenous chicken meat reared under semi-intensive systems.

Key Words: indigenous chicken, non-descriptive chicken, sensory, chewiness, juiciness

575P Effects of rice protein coatings combined or not with propolis on shelf life of eggs. Paula Gabriela Pires*, Aline Kummer Souza, Carolina Haubert Franceschi, Bruna Cristina Kuhn Gomes, Aline Fernanda Rodrigues Leuven, Katia Maria Cardinal, and Ines Andretta, *Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.*

Although eggs are an excellent protein source, they are a perishable product. Many methods exist to extend shelf life of food and one of them is the use of protein coatings that may be combined with antimicrobial substances, as propolis. The effectiveness of rice protein coatings plus propolis on maintaining the interior quality and the eggshell breaking strength of fresh eggs was evaluated during storage at 20 Celsius degrees for 6 weeks. Egg quality was assessed by weight loss, Haugh unit (HU), albumen pH, yolk index (YI), shell strength and scanning electron microscopy in uncoated eggs (control treatment) and eggs coated with rice protein concentrate alone or combined with propolis at 5 or 10%. Statistical analysis was performed using the SAS PROC GLM method ($P < 0.05$). Weight loss increased ($P < 0.001$) during long-term storage. Uncoated eggs showed the highest weight loss (5.39%), while rice protein (4.27%) and rice protein plus propolis at 5% (4.11%) and 10% (4.40%) solutions were effective in preventing weight lost ($P < 0.001$). Uncoated eggs had the worst ($P < 0.001$) HU (58.47), albumen pH (9.48), and YI (0.33) after 6 weeks of storage. The eggs coated with rice protein and rice protein plus propolis presented similar results for similar internal quality during the entire storage period. Scanning electron microscopy demonstrated a lower surface porosity in coated eggshells, indicating that the use of the coating may provide a protective barrier against the transfer of gases and moisture. In conclusion, rice protein and propolis treatments help maintaining the egg quality for a longer time compared with uncoated eggs and could be a viable alternative for improving the shelf-life of fresh eggs in room temperature.

Key Words: egg quality, eggshell, protein coating, storage time

576P Application of segmental bioelectrical impedance analysis to detect woody and normal sections on raw broiler breast fillets. Laura Garner*, C. Herron, and Amit Morey, *Auburn University, Auburn, Alabama, United States.*

Currently, few technologies exist for the detection of wooden breast (WB) in the processing facility. Bioelectrical impedance (BIA) has been successfully used for many applications in the food industry and has been shown to provide a way to separate wooden breast (WB) fillets from the normal breast (NB) fillets in the processing facility. WB can vary in severity throughout the fillet, therefore, different areas of the fillet may have different bioelectrical impedance values (reactance and resistance). In each of 3 replications, boneless skinless fillets were randomly collected just after deboning from a local processing facility and transported back (approximately 1 h) to the laboratory. Fillets were stored at 4 C for 18 h before analysis. Fillets were categorized into WB severity using a scoring system (0 = normal fillet, 1 = mild WB, 2 = moderate WB, 3 = severe WB) and hand palpation. Bioelectrical

impedance (resistance and reactance) was evaluated on the ventral side of whole fillets by applying a specialized Certified Quality Reader (CQR) for BIA. The cranial, medial and caudal segments of each fillet were analyzed for the presence or absence of WB by hand palpation and BIA was measured using the CQR. Data was analyzed using one-way ANOVA and Tukey HSD to separate means with a significant difference $P \leq 0.05$. BIA was successful in separating fillets with differing WB scores, resistance and reactance values were higher in the whole fillets with a WB score of 3 compared with fillets with a WB score of 0–2 ($P \leq 0.05$). For the BIA data collected on segmental portions of the breast fillets, segments that expressed WB also had higher resistance and reactance values when compared with segments that did not express and WB characteristics ($P \leq 0.05$). Differences in the resistance and reactance values of whole breast fillets may be used to identify fillets with the most severe expression of WB. However, when the expression of WB is not even throughout the breast fillet, using a smaller distance in probe placement in individual segments of the fillet can be successful in identifying the expression of WB in each segment of the breast fillets. BIA is a quick, portable analysis of the breast fillets and offers a quantitative method in identifying WB fillets in a processing facility.

Key Words: bioelectrical impedance, meat quality, wooden breast, resistance, reactance

577P Effect of freezing and phosphates on the quality characteristics of ground woody breast meat compared to normal meat. Laura Garner*, Lasheda Brooks, Lindsey Spencer, John Rehm, and Amit Morey, *Auburn University, Auburn, Alabama, United States.*

Woody breast myopathy (WB) affected meat has a tough texture, higher cook loss and decreased water holding capacity (WHC) and thus lower consumer acceptability. The WB meat can be ground and the fresh meat can be converted into further processed products or alternatively, it can be frozen, stored, and shipped to further processors. Freezing and thawing of ground WB meat may further affect the quality of WB meat products. Hence, research is required to determine the effect of phosphates on quality of ground WB meat as well as its cryoprotective effect during frozen storage. The objective of this experiment was to investigate the effect of phosphate levels (0, 0.25, and 0.5%) on meat quality (MQ) in WB and normal breast fillets (NBF) before and after freezing. Normal breast fillets and severely affected WB fillets were procured from a local commercial processor. The meat was separated into various treatment groups according to the sodium tripolyphosphate inclusion levels (0, 0.25 and 0.5% wt/wt). The meat was ground in a mechanical grinder with respective phosphate treatments and subdivided into 22 vacuum-sealed bags (1 kg each). Half of the bags ($n = 11$) from each treatment were taken for meat quality analysis while the other bags were placed in a walk-in freezer (-18 C) for freezing and storage for 6 d. Fresh samples were analyzed within 6–8 h while the frozen samples were thawed for 24–36 h at 4 C before analysis. Samples ($n = 10$) were analyzed for gel strength (TAXT Plus), pH, color ($L^* a^* b^*$) and randomly selected samples ($n = 5$) were analyzed for aerobic plate count (APC) and proximate composition. Experiments were repeated in 2 separate trials and the data was analyzed using the general linear model procedure in SPSS (v. 22.2) with Tukey's HSD at $P \leq 0.05$. The gel strength (g) of the fresh normal ground breast meat (888.98 g) and woody meat (721.84 g) with 0% phosphate were significantly different ($P \leq 0.05$). Addition of phosphate (0.25 and 0.5%) significantly increased the gel strength of fresh woody meat but it was significantly lower than normal meat added with 0.25 and 0.5% phosphate treatment. After freezing, normal breast meat samples with 0.5 and 0.25% phosphate had significantly higher gel strength compared with the other

treatments. Addition of 0.5% phosphate increased the pH of meat from approximately 6.17 to 6.40 ($P \leq 0.05$). Treatments did not significantly impact APC ($P \leq 0.05$). Addition of phosphate increases the functionality of ground WB meat.

Key Words: woody breast, phosphate, gel strength, meat quality, freezing

578P Bigheaded Carp-based fishmeal as a potential methionine source for organic poultry: Effect on meat quality characteristics in market age broilers. Indu Upadhyaya*², Komala Arsi¹, Basanta Wagle¹, Sandip Shrestha¹, Abhinav Upadhyay³, Dan Donoghue¹, Chris Coon¹, Casey Owens¹, Barbara Mallmann¹, Juan Caldas-Cueva¹, Anne Fanatico⁴, and Annie Donoghue⁵, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²Tennessee Tech University, Cookeville, Tennessee, United States, ³University of Connecticut, Storrs, Connecticut, United States, ⁴Appalachian State University, Boone, North Carolina, United States, ⁵USDA-ARS, Fayetteville, Arkansas, United States.

Synthetic methionine is the only synthetic amino acid allowed in organic poultry feed and it has been a challenge to find a natural, cost effective source for producers. Fishmeal is an excellent source of methionine and an allowable natural substance under the National Organic Program livestock rule. However, there are critical issues regarding cost and sustainable acquisition without depleting ocean stocks. Bigheaded carp is an invasive fish species affecting the water quality and aquatic ecosystem balance in Mississippi river basin. In this study, we investigated the potential of fishmeal prepared from Bigheaded Carp (through dry extrusion) as a natural/sustainable source of methionine in poultry diets and studied the effects on broiler meat quality. The effect of extruded Bigheaded carp meal (CM) on broiler performance was previously reported. The objective of this study was to evaluate the characteristics of the meat quality of these birds. Day-old male broiler chicks ($n = 480$) were randomly divided into 2 treatments with 8 replicates/treatment and 30 birds/replicate and fed with control diet or dry extruded CM diet for 42 d. At the end of trial period, birds were processed on a commercial-style in-line system followed by evaluation of carcass yield, and meat quality (pH, color, cook loss, texture, fatty acid profile, proximate analysis). Sensory analysis of breast and thigh meat was also conducted with 75 subjects. Meat quality characteristics and consumer sensory test results were analyzed using paired *t*-test with Fisher LSD corrections on GraphPad Prism Ver 6.0. Carcass analysis revealed that birds maintained on CM diet did not differ in live weight, carcass weight, breast weight, ready-to-cook yield, wing yield, tenders and thigh yields. However, frame yield was higher in controls ($P < 0.05$). No difference in meat color was observed between treatments. The pH of breast fillet meat differed (5.9 in carp fed birds vs 5.7 in controls) by treatment, but was in the acceptable pH range for broiler meat. No difference in cook loss and MORS force for texture was observed. Proximate composition analysis showed no difference between the treatments except for differences in protein content (73.4% CM diet vs 69.9% in controls) and fat % (25% in controls vs. 19.5% CM diet). Consumer analysis revealed no differences in overall impression and purchase intent between thigh meat treatments. However, a lower purchase intent for the breast from birds fed with CM was observed. The results suggest that CM has the potential of replacing synthetic methionine in commercial poultry diet with limited impact on post-harvest meat quality. Funded by USDA-NIFA 2015–51106–23966.

Key Words: organic poultry, methionine, Bigheaded Carp, meat quality, post-harvest

579P Evaluating the efficacy of whole cooked enriched-eggs in modulating the health-beneficial biological activity. Emerson Nolasco*, Sheila Purdum, and Kaustav Majumder, *University of Nebraska-Lincoln, Lincoln, Nebraska, United States.*

Diets, genetic varieties, and cooking methods can modulate the synergistic bioactivity of different egg compounds and may play a critical role in modulating health beneficial biological activities. Thus, the objectives were to determine the effect of diet and genetic variety on digestibility of cooked egg and to evaluate the bioactivity of cooked whole egg hydrolysate in reducing oxidative stress through in vitro methods. Two hen lines (White Leghorn (WLH) and Rhode Island Red (RIR)) were fed with 2 diets (regular diet and flaxseed/marigold enriched diet). Eggs were collected after 3 mo and prepared by boiling and frying. Thus, a total of 8 different types of cooked egg samples were prepared for this study. The simulated gastrointestinal (GI) digestion of regular and enriched cooked eggs was performed with sequential digestion of α -amylase, pepsin, and pancreatin. Each sample degree of hydrolysis (DH) was calculated via the pH-STAT method. Pierce fluorometric assay was used to quantify the peptide concentrations in the hydrolysate. Hydrophilic and hydrophobic compounds were extracted from hydrolysates to measure the Oxygen Radical Antioxidant Capacity (ORAC). The amino acid analysis was performed through gas chromatography. A Two-way ANOVA was performed to determine the effect of genetic variety, hen diet, and the cooking methods in modulating the antioxidant capacity of the whole egg hydrolysates. No significant difference was observed in the simulated GI digestion of the 8 egg hydrolysates. The DH of the cooked samples was ranging from 25 to 28%. A higher peptide concentration was found for enriched RIR boiled hydrolysate (56%), compared with the enriched RIR fried (35%) ($P < 0.05$). The ORAC of boiled RIR regular eggs (309 Trolox Equivalent Antioxidant Capacity (TEAC)/g) proved to be higher than WLH regular eggs (271 TEAC/g) ($P < 0.05$), with no difference for enriched eggs. ORAC value of fried WLH regular and enriched eggs (325 and 274 TEAC/g) was higher compared with fried RIR (259 and 225 TEAC/g) ($P < 0.01$ and $P < 0.05$, respectively). Nonetheless, the antioxidant capacity of the fried WLH samples significantly decreased in enriched eggs compare with the regular eggs ($P < 0.05$). Additionally, the high ORAC was correlated with a low amount of leucine amino acid with 9 nM/mg and 25 nM/mg for regular WLH fried and enriched RIR boiled hydrolysates. Finally, factors such as diet, genetic variety, and cooking methods showed no influence on the egg digestibility. However, they may modulate the antioxidant capacity of the whole egg. These results are helpful to elucidate new strategies for egg production while educating consumers about the potential health benefits of the egg for human consumption.

Key Words: White Leghorn, Rhode Island Red, egg hydrolysate, antioxidant activity, biological activity

580P Phenotypic correlations between meat quality traits in turkeys (*Meleagris gallopavo*). Michelle Yahiro*¹, Ryley Vanderhout³, Ben Wood^{2,3}, Christine Baes³, Jeff Mohr², and Shai Barbut¹, ¹University of Guelph, Georgetown, Ontario, Canada, ²Hybrid Turkeys, Kitchener, Ontario, Canada, ³University of Guelph, Guelph, Ontario, Canada.

With the rise in unfavorable meat characteristics, there is increasing economic importance being placed on meat quality. The purpose of this study is to improve the selection process of live birds and provide a higher quality product to the consumer by estimating the phenotypic correlations among various meat quality traits as well as describing the relation among these variables. At 20–22 weeks of age, whole turkey carcasses from an industrial setting were processed and underwent

full cut-up. A total of 895 samples of turkey breast (*pectoralis major*) were assessed for quality through color analysis (lightness, redness, yellowness, chroma, hue), pH (at 45-min and 24-h post mortem), drip loss, cooking loss, and shear force. Phenotypic correlation coefficients between all traits were computed using R and meaningful traits were further analyzed using regression. Moderately positive correlations were observed between redness and drip loss ($r = 0.21, P \leq 0.05$) and between chroma and drip loss ($r = 0.22, P \leq 0.05$). Moderately negative correlations were also observed between pH 24-h and lightness ($r = -0.29, P \leq 0.05$), as well as pH 24-h and cook loss ($r = -0.24, P \leq 0.05$). These correlations indicate the effect of pH decline over 24-h post mortem had on protein denaturation; i.e., decreasing the water-holding capacity. The water retention within the meat is directly affected by the protein-water interactions, impacting the juiciness and yield of the product. This

supports known relationships between the faster development of rigor mortis on the rapid reduction of pH, increasing the likelihood of a paler colored product. This mechanism plays a role in pale, soft, exudative (PSE) meat development, and thus results in reduced meat quality. Other quality parameters displayed no significant correlations; however, routine collection of phenotypes is on-going. With the data set increasing by approximately 100 samples per week; more definitive conclusions can be made in the near future. In summary, the results from the study indicate that the pH 24-h post mortem, lightness, redness, and chroma are correlated to liquid loss. These traits have the potential to be early indicators of quality, allowing for the production of juicier and higher yielding products in the industrial turkey line.

Key Words: phenotypic, correlations, meat, quality, turkey

Poster Session—Tuesday

581P Gene expression differs at hatch and day 14 in cellular fractions of adipose tissue of chicks from lines predisposed to be lean or have excess fat. Yang Xiao*, Paul Siegel, Mark Cline, and Elizabeth Gilbert, *Virginia Polytechnic Institute and State University, Blacksburg, Virginia, United States.*

The Virginia lines of low body weight-selected (LWS) chickens are lean and resistant to accumulating fat and the high body weight-selected (HWS) counterparts accumulate excess adipose tissue. There are differences between lines with respect to energy utilization within the subcutaneous depot immediately after hatch, and accumulation within the abdominal depot during later growth. Due to the cellular heterogeneity of adipose tissue, it is unknown whether the functionality of adipocyte precursor cells and/or adipocytes contributes to the differences in adipose tissue development. Hence, we compared the mRNA abundance of genes encoding proliferation, adipogenesis, and apoptosis-related factors in the stromal-vascular fraction (SVF) and adipocytes in the subcutaneous depot at hatch and abdominal depot on d 14. Meanwhile, depot-specific glycerol-3-phosphate dehydrogenase specific activity and plasma nonesterified fatty acids (NEFAs) were determined on both ages. Data were analyzed by ANOVA and depending on the variable, the model included effects of genetic line and/or cellular fraction. Progenitor cell marker CD71 and proliferation marker Krüppel-like factor 1 mRNAs were greater ($P < 0.05$) in LWS than HWS at both ages in the SVF, while preadipocyte marker adipocyte enhancer binding protein 1 was greater ($P = 0.01$) in HWS than LWS at hatch. Delta like non-canonical notch ligand 1 was greater ($P = 0.002$) in LWS than HWS on d 14. HWS chicks expressed more ($P = 0.04$) lipoprotein lipase than LWS in adipocytes at hatch and the LWS chicks expressed more ($P = 0.01$) carnitine palmitoyltransferase 1A than HWS on d 14. With respect to apoptotic factors, caspase 3 mRNA was greater ($P = 0.003$) in the SVF than in adipocytes on d 14 whereas granzyme A was greater ($P < 0.0001$) in adipocytes than the SVF. Granzyme A was greater ($P = 0.03$) in HWS than LWS at hatch, but less ($P < 0.0001$) at d 14. NEFAs were lower ($P = 0.0002$) in HWS than LWS while G3PDH activity was greater ($P = 0.006$) in HWS than LWS on d 14. Results indicate that lipolysis and apoptosis both contribute to the negligible deposition of abdominal adipose tissue in the LWS chicks. Moreover, granzyme A-mediated apoptosis may contribute to differences in adipose tissue mass between chicks predisposed to have differences in feeding behavior and body composition.

Key Words: adipocytes, apoptosis, chicks, development, stromal-vascular fraction

582P Estimating genetic diversity and population structure of Asian chicken breeds in 8 country using 20 microsatellite markers. Hee-Jong Roh*¹, Kwan-Woo Kim¹, Jinwook Lee¹, Dayeon Jeon¹, Dong-Kyo Kim¹, Seung-Chang Kim¹, Yeoung-Gyu Ko¹, Chang-Yeon Cho¹, Solongo Batsaikhan², Triana Susanti³, Sergey Hegay⁴, Siton Kongvongxay⁵, Neena Amatya Gorkhali⁶, Lan Anh Nguyen Thi⁷, and Trinh Thi Thu Thao⁷, ¹National Institute of Animal Science, RDA, Namwon, Jeonbuk, Korea (the Republic of), ²National Centre for Livestock Genebank, Ulaanbaatar, Mongolia, ³Indonesia Research Institute for Animal Production, Bogor, Indonesia, ⁴Biotechnology Institute, Bishkek, Kyrgyzstan, ⁵Livestock Research Centre, Vientiane, Lao People's Democratic Republic, ⁶Nepal Agricultural Research Council, Kathmandu, Nepal, ⁷Institute of Animal Sciences for Southern Vietnam, Ho Chi Min, Viet Nam.

Estimating genetic diversity and population structure is an important factor toward the identification of unique and animal valuable genetic resources. There are many breeds of chicken in Asia. So, this study was conducted to assess genetic diversity and relationship among Asian chicken breeds in 8 Country based on allelic frequencies for 20 microsatellite marker. We used a total of 480 genomic DNAs from 22 breeds (Korea: 6, Laos: 4, Indonesia: 3, Vietnam: 2, Mongolia: 1, Kyrgyzstan: 1, Nepal: 1, Sri Lanka: 1 and White Leghorn, Cornish, Rhode Island Red). Total 194 alleles was observed and the number of alleles ranged from 3(MCW0103) to 20(LEI0192) with an average value of 9.7. As a result of genetic diversity within 22 chicken breeds, NPS(Nepal) had highest H_{exp} (0.718 ± 0.027) and PIC (0.663 ± 0.030) value. H_{obs} was highest (0.690 ± 0.039) in LCH(Laos). But White Leghorn(WLG) showed the lowest H_{exp} (0.372 ± 0.055), H_{obs} (0.384 ± 0.019), PIC (0.325 ± 0.049). In general, Korean native breeds showed low diversity compared with the other Asian breeds. That's because Korean native breeds may have a stronger selection than the other Asian breeds. The DA distance revealed the closest (0.089) between 2 Vietnam breeds (VYN, VNH). On the other hand, Farthest genetic distance (0.497) revealed between WLG and LCH(Laos). Compared with imported breeds (WLG, CON, RIR), Most Asian chicken breeds had close genetic distance with CON or RIR. But MGN(Mongolia) was closer with WLG than CON and RIR. PCoA analysis also showed similar results of phylogenetic analysis. The analysis in STRUCTURE revealed that 22 chicken breeds should be divided in 15 clusters based on the highest ΔK value (10.36). The results of this study may also be useful as an initial guide in defining objectives for designing future investigations of genetic variation and developing conservation strategies regarding Asian chicken breeds.

Key Words: genetic diversity, genetic relationship, Asian chicken breeds, microsatellite

583P Genetic analysis of production, physiologic, and egg quality traits in heat-challenged commercial white egg-laying hens using 600k SNP array data. Kaylee Rowland*^{1,2}, Chris Ashwell³, Michael Persia⁴, Max Rothschild¹, Carl Schmidt⁵, and Susan Lamont¹, ¹Iowa State University, Ames, Iowa, United States, ²Hy-Line International, Dallas Center, Iowa, United States, ³North Carolina State University, Raleigh, North Carolina, United States, ⁴Virginia Tech, Blacksburg, Virginia, United States, ⁵University of Delaware, Newark, Delaware, United States.

Background: Heat stress negatively affects the welfare and production of chickens. High ambient temperature is considered one of the most ubiquitous abiotic environmental challenges to laying hens around the world. In this study we recorded production traits, feed intake, body weight, digestibility, and egg quality of commercial white egg-laying hens before and during a 4 week heat exposure. For those phenotypes where heritabilities were estimated (using 600k data) to be greater than zero, SNP associations were tested using the same 600k genotype data. Results: Seventeen phenotypes had heritability estimates greater than zero including measurements at various times for feed intake, feed efficiency, body weight, albumen weight, Haugh units, egg mass, and also change in egg mass pre heat to post heat. QTL were identified for 10 of the 17 phenotypes with measureable heritability. Some phenotypes shared QTL including Haugh units pre exposure and after 4 weeks of exposure. Conclusions: The existence of measureable heritability indicates the existence of genetic control and, therefore, the potential for changing these 17 traits through selective breeding. The association

of different QTL across time points indicates a relative lack of shared genomic control pre and post heat exposure. This study contributes to the knowledge of genomic control of response to heat stress in laying hens. Keywords: GWAS, laying hens, chronic heat stress, acute heat stress, heritability, egg quality, production.

Key Words: GWAS, laying hens, chronic heat stress, acute heat stress, egg quality

584P Effects of monochromatic lighting and In ovo vaccination on the splenic transcriptome profiles of chicken. Mohamed Ibrahim^{*1,2}, Jill Nelson¹, Greg Archer¹, and Giri Athrey¹, ¹Texas A&M University, College Station, Texas, United States, ²Cairo University, Giza, Egypt.

The objective of this study was to investigate the effect of providing a green spectrum with or without In ovo vaccination on the profile of total mRNA genes expression of chicken eggs at d 18 of incubation and d 7 post-hatch to evaluate whether lighting treatment will affect the transcriptome profile after In ovo vaccination or not. A total of 240 Ross 708 broiler eggs were kept under 2 light exposure treatments during incubation: continuous dark and 12h of LED light with high levels of green spectrum daily to the end of the incubation period. In ovo vaccination was carried out at the time eggs were transferred from incubator to hatcher. From there on, the eggs were divided into a total of 4 groups: 2 dark treatment (vaccinated and unvaccinated) and 2 Light treatment (vaccinated and Unvaccinated). After hatch, the light treatments followed industry lighting recommendation. Spleen samples were collected on d 18 of incubation and d 7 post-hatch from all the treatments. Total RNA was isolated from each sample and subsequently prepared for RNA sequencing using the Lexogen 3' library preparation kit. Individual libraries were barcoded and sequenced on an Illumina NextSeq platform, generating 75bp single end reads. The comparison of RNASeq data between dark incubation treatment and regular lighting in vaccinated and unvaccinated treatment after hatch resulted in 2684 and 2159 upregulated gene respectively. Green spectra lighting treatment during incubation and after hatch in the vaccinated and unvaccinated group resulted in 3319 and 2794 upregulated gene respectively. The DEGs analysis between lighting and dark treatment during incubation showed 2 upregulated genes in dark treatment involve in molecular function regulation. Furthermore, the DEGs between regular lighting and green spectra lighting after hatching in the unvaccinated group resulted in 20 upregulated and 9 downregulated genes, wherein the vaccinated group did not show any differential expression. The analysis of differentially expressed genes (DEGs, FDR < 0.05). Our results indicated that the majority pattern of DEGs is due to the sampling time/age with most differences found between the pre-hatch and post-hatch groups. whereas there were fewer DEGs between light treatment and dark treatments. Gene ontology and pathway analysis indicated that those genes involved in a biological process like cellular component organization or biogenesis, rhythmic process, developmental process, response to a stimulus and immune system processes were explained by the DEG. Additional investigation and parameter are needed to be included in future work to make sure of developing circadian rhythm and stimulate the immune response.

Key Words: green light spectra, RNASeq, DEGs, chicken egg incubation

585P Development of a genomic selection strategy to include meat quality traits in turkeys (*Meleagris gallopavo*): Data collection update. Ryley Vanderhout^{*1}, Ben Wood^{1,2}, Shai Barbut¹,

Michelle Yahiro¹, Jeff Mohr², and Christine Baes^{1,3}, ¹University of Guelph, Guelph, Ontario, Canada, ²Hybrid Turkeys, Kitchener, Ontario, Canada, ³University of Bern, Bern, Switzerland.

Meat quality in the poultry industry is increasing in economic importance. The difficulty with selection for improved meat quality is the inability to measure certain traits antemortem. Post-mortem information on many related individuals and genomic strategies can be used to accurately estimate breeding values of selection candidates. The objective of this study was to collect several traits on full and half siblings of selection candidates to estimate accurate genetic parameters in 3 lines of turkeys (A, B, and C). Live weights at 18 and 20 weeks of age, walking ability scores, *pectoralis major* meat quality trait measurements (pH at 45-min and at 24 h post-mortem, color, drip loss, cooking loss, and shear force), carcass component weights (*p. major*, *p. minor*, thighs, drums, wings, breast skin, scapula, and rack), and body conformation photos were collected on over 1300 turkeys. All birds were genotyped with a 65K SNP array and over 20 generations of pedigree records are available on all birds. Preliminary trends show line C birds to be largest, averaging 5.80 kg larger than line A at 20 weeks of age, and line B birds to be smallest, averaging 3.26 kg smaller than line A at 20 weeks of age. Line C birds have a larger difference between pH at 45-min and 24 h post-mortem, as well as a larger percent cooking loss. These results suggest possible connections between the size of the bird, pH, and cook loss. Average pH measurements between lines A and B did not significantly differ. Lightness, redness, yellowness, and percent drip loss were highest in line A, suggesting a possible link between *p. major* meat color and water holding capacity. With this data set increasing by 75–100 birds per week, accurate genetic parameters for all 3 lines will be estimated in the near future. Upon completion of this activity, genome-wide association studies and functional analyses will be conducted on all meat quality traits. The results of this research will provide the turkey industry with the groundwork needed to implement genomic selection for meat quality into future breeding strategies.

Key Words: meat quality, genetic parameters, turkey

586P Longitudinal analysis of egg production traits in turkeys (*Meleagris gallopavo*). Hakimeh Emamgholi Begli^{*1}, Ben Wood^{1,2}, Emhimad Abdallah¹, Ryley Vanderhout¹, Owen Willems², Flavio Schenkel¹, and Christine Baes^{1,3}, ¹University of Guelph, Guelph, Ontario, Canada, ²Hybrid Turkeys, A Hendrix Genetics Company, Kitchener, Ontario, Canada, ³Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland.

The objective of this study was to estimate covariance functions using Legendre polynomial random regression models for egg production traits in turkeys. Egg production was recorded on 5,547 birds for 5 consecutive months. Variance components were estimated by the restricted maximum likelihood procedure, fitting a fixed effect of hatch-week, an overall fixed polynomial regression, and random polynomial regressions for animal additive genetic and permanent environment effects in the model. Heritability estimates along the production trajectory ranged from 0.18 to 0.45 and were higher at the early ages. Genetic correlations between egg production at different ages were moderate to high, varying from 0.30 to 0.98, with the highest correlations between adjacent months in the middle of the production period. Phenotypic correlations between different months varied from 0.07 to 0.38, which were consistently lower than the corresponding genetic correlations. As expected, both genetic and phenotypic correlations consistently decreased as the time interval increased between months. In general, the findings of this study showed that there is substantial genetic variation in the egg production curve of individual turkeys and that egg production within different laying

months may show only moderate genetic correlation, especially at the beginning and end of the production period.

Key Words: turkeys, egg production, random regression model

587P Different sources and levels of methionine modify the expression of the genes of the sulfurous amino acid metabolism in the pectoral muscle of chickens. David Pereira Da Silva, Patrícia Naves Givisiez, Danila Campos, Alexandre Moreira Filho, Silvana Lima dos Santos, and José Vilar Da Silva*, *Universidade Federal da Paraíba, Solânea, Paraíba, Brazil.*

The frequency with which the gene network is expressed in different tissues can be altered by the nutritional composition of the broiler diet. DL-Methionine (DL-Met) and DL-Methionine Hydroxy- Analog Free Acid (DL-MHAFA), the main industrial sources of Met in poultry diets, have chemical structures and are absorbed by different transport systems. Met sources can activate genes encoding for protein synthesis regulatory enzymes in mTORC1 in *P. major* cells. However, there is no information on the effects of levels and sources on the expression of sulfur amino acid metabolism genes in *P. major* of chickens. A total of 30 chicks were weighed and distributed in a completely randomized design in a 2x3 factorial scheme (2 sources and 3 levels of Met) totaling 6 diets with 6 replicates of 6 broilers. The chicks were fed with low, normal and excess of digestible Met levels and 2 supplementary sources of Met (DL-Met and DL-MHAFA). The levels of digestible Met were: 0.63, 0.88 and 1.13% from 8 to 21 d, 0.58, 0.83 and 1.08 from 22 to 33 d, 0.52, 0.77 and 1.02% from 34 to 49 d of age. The chickens were slaughtered at 49 d and samples of muscle tissue were obtained from the breast. The data were analyzed by the Tukey test ($P \leq 0.05$). Using RT-qPCR analysis, the expression of the MTRR (5-Methyltetrahydrofolate-homocyst methyltransferase reductase), CBS (Cystationina β -synthase) and GSS (Glutathione synthase) genes were not affected by the interaction ($P > 0.05$). There was interaction for expression of the BHMT gene, in which the normal level of MHA-FA increased BHMT gene expression ($P < 0.05$). Met deficiency in the diet increased the expression of the MTRR, CBS and GSS genes ($P \leq 0.05$). The opposite occurred with BHMT expression, where the highest expression was observed at the normal Met level. This gene encodes the enzyme that catalyzes the conversion of Betaine + Hcy to Dimethylglycine and Met. The highest MTRR, CBS and GSS gene expression in the chickens fed 1 to 49 d of age deficient in Met diet suggest a compensatory effect of activation of Met metabolism genes still unknown in chicken breast muscle. The deficiency of Met in the broiler diet increases the gene expression of metabolism in the breast muscle. MHA-FA enhances the expression of BHMT in the breast of broiler chickens.

Key Words: sulfur amino acids, metabolism, gene expression

588P Characterization of heritage and commercial turkeys using melanocortin 1 receptor (MC1R) haplotypes. Edward Smith*², A. Adikari¹, and Jun Xu², ¹Rajarata University of Sri Lanka, Blacksburg, Sri Lanka, ²Virginia Tech, Blacksburg, United States.

Future genomic selection approaches in the turkey, *Meleagris gallopavo*, require understanding differences among turkey strains to identify which can be used for introgression and the level of admixture/hybridization. A total of 261 birds were used in the resequencing of the MC1R gene. The birds were from 6 distinct populations/strains including commercial (Nicholas, 40), Broad Breasted Bronze (BBB, 48)Blue Slate (BS, 40), Midget White (MW, 40), Royal Palm (RP, 45), and Spanish Black (SB, 48). Using data from 181 birds/genotypes, a total of 4 haplogroups,

based on variation in 6 SNPs, were identified. The haplogroups ranged in frequency from 13 (23 birds) to 50% (91 birds). When commercial birds were included in the haplotype analyses, the number of haplogroups and the frequencies did not significantly change. Though the highest percentage of commercial birds shared the broad breasted bronze haplogroup, a few commercial birds shared haplogroups with the MW, BS, and SB. In both the analyses, that with and without the commercial birds, the RP remained the most unique with a well-defined haplogroup containing all but 3 that appear to share the SB haplotypes. We believe that the data show, in addition to earlier investigations, that the RP is unique from other turkey strains and should be regarded as a breed. Further, we show that the commercial bird is a hybrid of many heritage turkeys and that the MC1R locus could be used to monitor introgression involving diverse strains.

Key Words: turkeys, heritage, MC1R, haplogroups

589P Developmental regulation of elongase enzyme expression in broiler chick adipose tissue. Robert Mihelic, Kamille Piacquadio*, Suchita Das, Kurt Lamour, and Brynn Voy, *University of Tennessee, Knoxville, Tennessee, United States.*

Enriching adipose tissue of broiler chicks with docosahexaenoic acid (DHA; 22:6 n-3) could have a dual benefit for the broiler industry by both attenuating accretion of excess fat (Beckford et al., 2017) and by increasing nutritional value of the market product for consumers. The **Objective** of this study was to characterize the patterns of very long chain fatty acid elongase (ELOVL) gene expression during adipose development and to assess the relative roles of ELOVL2 and ELOVL7 in endogenous DHA synthesis in broilers. **Experimental design:** Adipose tissue was collected from broiler chicks (n = 5–7/age) at embryonic d 13, 15 and 17 (subcutaneous (SQ)) and at 7 and 14 d post-hatch (SQ, abdominal (AB) and neck) and snap-frozen. **Materials and methods:** Total RNA was isolated from each depot, quantified, quality-checked, and used to synthesize cDNA. A targeted RNaseq approach was used to profile expression of a panel of adipose- and lipid-related genes including 6 elongase genes (ELOVLs 2, 3, 4, 5, 6, 7). Primers for the panel were designed using Primer3Plus (primer3plus.com) to span exon boundaries, using the RefSeq for each gene as template. Raw sequencing reads were aligned to the chicken genome (Galgal 5.0) and reads were counted using CLC Genomics Workbench (Qiagenbioinformatics.com). **Statistical analysis:** RNaseq data were analyzed for effects of age and depot in SAS (V 9.4) using ANOVA and least squares means for contrasts ($\alpha = 0.05$). **Results:** Expression levels of 5 of the 6 ELOVL genes (ELOVL2, 3, 4, 5 and 7) varied significantly with age in SQ adipose ($P < 0.05$). Expression of ELOVL2 was relatively low in embryonic adipose increased sharply at d 7, and returned to lower levels by 14d, with 7d levels significantly higher than at all other ages ($P < 0.05$). In contrast, ELOVL7 was expressed at relatively high levels in the embryo, with significantly lower levels at 7d (~2.5-fold vs, E17; $P < 0.05$). Unlike each of the other ELOVL genes, expression of ELOVL7 significantly increased from d 7 to 14 (~5-fold, $P < 0.05$), and it was the most abundantly expressed elongase in SQ, AB and neck fat after hatch. In contrast, expression of ELOVL2 significantly decreased from 7 to 14 d in SQ and AB fat ($P < 0.05$). **Conclusions:** Based on gene expression, these results suggest that the novel elongase ELOVL7, which has not been characterized in chickens, may play the predominant role in synthesizing DHA in chick adipose tissue, both during embryonic development and after hatch. Further investigation of this enzyme and its regulation may reveal ways to enhance endogenous synthesis of DHA in broilers.

Key Words: adipose, elongase, broiler, fatty acid, gene expression

590P Levels and sources of methionine affect the expression of sulfur amino acid metabolism genes in the jejunum of broiler chickens. Claudiana Souza, Silvana Lima dos Santos, João Souza Silva, Patrícia Naves Givisiez, Danila Campos, Alexandre Moreira Filho, and José Vilar Da Silva*, *Universidade Federal da Paraíba, Solânea, Paraíba, Brazil.*

Studies have shown that the intake of DL-MHAFA stimulates rapid conversion of this source to Taurine and GSH in enterocytes. As DL-MHAFA is more absorbed by passive transport, this conversion mechanism maintains a gradient favorable to the passive absorption of DL-MHAFA in the intestinal epithelium. The objective of this study was to evaluate the effect of the level and source of methionine (Met) on the expression of sulfur amino acid metabolism (AAs) genes in chicken jejunum. A total of 540 male Cobb-500 male chicks from the 8-d-old randomly distributed in a 3×2 factorial arrangement (3 levels of Met + Cys and 2 sources: DL-Met, and DL-MHAFA) were used, resulting in 6 treatments with 6 replicates of 15 birds. Feeding was provided at will and Met + Cys levels (deficient, requirement and excess) following nutritional recommendations for the following breeding phases: 8 to 21 d (0.63, 0.88 and 1.13%), 22 to 33 d (0.58, 0.83 and 1.08%) and from 33 to 49 d of age (0.52, 0.77 and 1.02%). The chickens were slaughtered at 49 d and immediately, jejunum samples were collected from each bird, then frozen in liquid N. Total RNA was extracted with Trizol (Invitrogen) and purified with the RNeasy Mini Kit (Qiagen, 74.104) as determined by the manufacturers. Then, 2 μ g of the RNA were transcribed with the High-Capacity cDNA RT kit (Thermo Fischer), following standard protocol. 1.0 μ l of cDNA was taken as template in a 20 μ l qPCR mixture containing 1.0 μ l of the primers (primer and reverse) starting at 10 μ M and 2x Fast SYBR Green Master Mix (Agilent Technologies). The RT-qPCR analyzes were performed on 4 samples, one / animal, in triplicates. The data were analyzed by the $2^{-\Delta\Delta Ct}$ method and were normalized by β -actin expression. Expressions of the genes evaluated were: MAT 1 (Met adenosyltransferase 1), CBS (Cystationine β -synthase), MTR, BHMT, MTRR and GSS (Glutathione Synthase). Expressions of the MAT 1, CBS, MTR, BHMT and GSS genes were not altered by the Met sources and the levels evaluated ($P > 0.05$), however, MHA increased ($P < 0.05$) the expression of the MTRR gene on the deficiency and reduced its expression in the excess of Met in the diet, which indicates that the conversion of Met from Hys is a process of controlling DL-MHAFA concentration in jejunal enterocytes to maintain a concentration gradient favorable for passive absorption of DL-MHAFA by intestinal cells. Therefore, supplementation of diets with MHA with deficiency or excess of Met affects the synthesis of Met from Hys.

Key Words: molecular biology, homocysteine, sulfur amino acids

591P Not Presented

592P Effects of color, irradiation period, and intensity of LED illumination on production performance of broiler chickens. Ken Tatsuda*, *Hyogo Pref. Agri. Inst., Kasai, Hyogo, Japan.*

The objective of this study was to evaluate the effect of color, irradiation period, and intensity of LED illumination on production performance of broiler chickens. Two hundred and 40 d-old chunky chicks were divided into 6 groups (A, B, C, D, E, and F) of 40 birds each. The birds were housed in a windowless hen house until 49 d of age. During the experimental period, feed and water were provided ad libitum. The birds of groups A, B, C, D, and E were reared under LED illumination, and the birds of group F (control) were reared under incandescent bulb illumination. Birds were fed a starter feed for broilers until 21 d, a

grower feed for broilers until 42 d, and finally a finisher feed for broilers. Treatments of each group (illumination color, irradiation period, and intensity) were as follows: A group: yellow until 20 d, yellow and blue until 30 d, and then blue; B group: yellow until 16 d, yellow and blue until 24 d, and then blue; C group: green until 16 d, green and blue until 24 d, and then blue; D group: high intensity green until 3 d, green until 20 d, green and blue until 30 d, and then blue; E group: green until 20 d, green and blue until 30 d, and then blue; and F group: incandescent color until 21 d and then low incandescent color. Health status, body weight, feed intake, meat yield, meat quality (levels of amino acid and fatty acid composition in thigh meat), and economy (feed cost and budget estimates) were evaluated. Statistical analysis was performed using SAS GLM procedure. The health status of all groups was good. There were no problems in growth performance in any group. Average body weight for the B group (3,671 g) was significantly higher than that of the F group (3,548 g) ($P < 0.05$). Feed conversion rate and production score tended to increase more for the B, C and E group than for the F group. Meat yield (%) did not differ among the groups. Feed cost per 100 g of meat was lower for the B, C and E group than for the F group. It was estimated that the annual benefit of the C group is 750,000 yen higher than that of F group, for a 10,000-birds scale operation, except for electricity charges. The color switching of green to blue during the former period of fattening was suggested to improve production performance of broiler chickens.

Key Words: broiler, LED, illumination color

593P Plateau regression index between breeder age and productive parameters of broilers. Deibity Cordeiro¹, Natiele de Oliveira¹, Alisson Gouvea¹, Roberto Jardim³, Emmanuel Arnhold¹, and Jose Stringhini^{*1,2}, ¹*Universidade Federal de Goias, Goiania, Goias, Brazil*, ²*CNPq, Brasilia, Distrito Federal, Brazil*, ³*Sao Salvador Alimentos, Itaberaí, Goias, Brazil*.

Breeder age directly influence progeny development, and impacts livability of day-old chicks and performance throughout the production cycle. Literature brings data about breeder age and the progeny performance, reporting significant large intervals that are mostly not conclusive. Based on this, the study aimed to evaluate through the quadratic polynomial model with plateau response when the breeder age implies greater influence on the productive characteristics of broilers. Data from broiler breeders aging from 24 to 69 weeks of age from a total of 18,492 lots of broilers were used, taking into account some variables like: day-old chick weight, absolute feed conversion and proportional weight gain from one to 42 d of age, calculated as a reason of final and day-old weight. The polynomial regression analysis was performed in a quadratic model with plateau response and a residual outlier's analysis was also performed. To identify the significance of the equations ($P < 0.05$), we performed the ANOVA and test F. The model used to evaluate the response in plateau was the following $Y = a + bx + cx^2 + \xi$, if $x < x_0$ (quadratic); $Y = p + \xi$, if $x \geq x_0$ (plateau), in which: Y is the analyzed response for age and X the age of the breeders in weeks. Thus, for values of x smaller than X_0 , the model that describes the response Y is a quadratic function, and for values of X greater than or equal to X_0 , the equation is a constant or plateau. Evaluating the breeder age in relation to the weight of the day-old chick, it was observed a quadratic behavior in the weight of the chicks for breeders whose age is less than 60.58 weeks. From this, there is a plateau response with weight of the chicks of 48.11 g remained constant until the 69 weeks of age. In the analysis of the absolute feed conversion (1 to 42 d), the response is different, it is observed that the progeny of breeders with ages greater than 32.77 weeks do not show changes in the conversion, making it clear that

broilers of breeders aging less than 32.77 weeks tend to convert food better into weight gain. The proportional weight gain falls quadratically as the breeder ages and stabilizes when the hen reaches 51.19 weeks of age. The adjusted equations ($P < 0,001$) were: day-old chick weight - $Y = 15,9076 + 1,0639x - 0,0088x^2$ $r^2 = 0,9685$; Feed conversion - $Y = 1,4566 + 0,02x - 0,0003x^2$ $r^2 = 0,5601$; proportional weight gain - $Y = 124,5988 - 2,6178x + 0,0256x^2$ $r^2 = 0,8583$. It is possible to conclude that the breeder age is a preponderant factor to obtain satisfactory productive indexes, however, it was observed that from the evaluated parameters the breeder age presents different responses.

Key Words: response plateau, breeder age, polynomial regression, productivity, performance

594P Effect of sanitizing with bleach solutions on the amount of residual cuticle on broiler breeder eggs. Tia O'Brien*, Kayla Davis, Beryl Khakina, Corey Farmer, and Tomohiro Hamaoka, *Calpis America Inc., Peachtree City, Georgia, United States.*

Although the eggshell cuticle is known to serve as a primary defense against bacterial penetration, little is known about how sanitizers affect cuticle quality. Two trials were conducted to evaluate methods of sanitation for effect on residual cuticle (T1) and to investigate the effect of bleach concentrations on the cuticle of broiler breeder eggs (T2). In both trials, half of each egg (side A) was utilized as control and the other half (side B) was used as treatment. Residual cuticle was determined by measuring % reflectance with a spectrophotometer (Konica Minolta, CM-700d) at 650 nm before and after staining with MST cuticle blue dye. In T1, 7 materials were selected to test effect on cuticle thickness based on communication with several US hatcheries, such as rubbing with 320 grit sandpaper (Miady), 3% Hydrogen Peroxide (Kroger), 70% isopropanol (VWR), 4.25% chlorine solution (Clorox®), disinfecting wipes (Clorox®), 0.34 M EDTA (VWR) and egg albumen. Each test material, except the sandpaper treatment, was applied to side B of the test eggs (15 commercial broiler breeder eggs per treatment) using a cotton ball soaked in the solution. In T2, a total of 160 broiler breeder (47 wk, Ross 708) eggs without any defects were selected and randomly assigned to 8 treatment groups (20 eggs each). The treatments applied were 5.75% chlorine solution (Clorox® bleach) diluted to 10, 20, 40, 60, 80, 100, 1000, and 10 000 mg/L of chlorine. In T2, treatments were applied by soaking side B in the designated solution for 5 min. Results were expressed as % remaining cuticle (treatment side/control side). Data were analyzed by SAS Studio 3.71 with PROC TTEST for T1 and T2, PROC REG and PROC CORR with \log_{10} transformed treatment concentrations for T2. In T1, significantly lower remaining cuticle ($P < 0.0001$) was observed for the sand paper treatment and chlorine treatment. In T2, 10 mg/L did not show a significant difference, whereas all other concentrations showed significant reduction ($P < 0.0001$) of the residual cuticle compared with the control side. The amount of cuticle remaining decreased linearly ($r = -0.92$, $P < 0.0001$, $R^2 = 0.85$), such as 99.65% (10 mg/L), 89.35% (20 mg/L), 82.71% (40 mg/L), 77.75% (60 mg/L), 71.99% (80 mg/L), 59.78% (100 mg/L), 24.74% (1000 mg/L), and 13.07% (10 000 mg/L). The increase in chlorine concentration has a strong negative correlation ($r = -0.92$) with the amount of cuticle that can be observed on the surface of the shell post-treatment. Sanitizing eggs with a bleach solution could cause damage to the eggshell cuticle, a natural protective defense against contamination.

Key Words: broiler breeders, cuticle, bleach, egg washing, sanitation

595P Effect of age and weight at the beginning of mating on productive, reproductive performance and egg quality of

Japanese quails (*Coturnix coturnix japonica*). Lincoln Tafur, Otto Zea, Marcial Cumpa, Pedro Ciriaco, and Carlos Vilchez*, *Universidad Nacional Agraria La Molina, Lima, Peru.*

This study aimed to evaluate the effect of age and weight at the beginning of mating on productive, reproductive performance and egg quality of Japanese breeding quails. A total of 162 females and 54 males were randomly assigned into 27 experimental units (EU) of 8 birds each, maintaining a 3:1 female:male ratio. Three EU conformed one of the 9 treatments that resulted from the combination of 3 ages (45, 52 and 59 d) and 3 weights (130, 140 and 150 g) of the birds. All the experimental animals received a common breeding corn-SBM based during a 10-wk evaluation period. Feed (mash form) and water were offered *ad libitum*. Egg production (EP), egg weight (EW), egg mass (EM), embryo mortality (M), hatchability (H) and hatchling weight (HW) were recorded on a weekly bases. A total of 540 eggs were used to evaluate egg quality (Haugh Units, HU; Shell thickness, ST). The registered data was submitted to ANOVA under a Completely Randomized Design with a 3×3 factorial arrangement and means were separated by Duncan Test. The results showed that the productive performance of the animals were significantly influenced ($P < 0.05$) by the age and weight of the animals, particularly, for the groups of the 52 and 59 d of age and weighting 150 g. The reproductive performance variables were also significantly influenced ($P < 0.05$) both by the age and weight of the birds. Breeding quails of 59 d of age and weighting 140 g showed better ($P < 0.05$) hatchability, hatchling physical quality, and lower early mortality than the other experimental groups. Birds of 45 d of age and 130 g of weight showed the poorest reproductive performance. Egg quality variables were also influenced by the age and weight of the animals. Birds of 52 d of age and 140 g of weight produced eggs with higher HU while those of 59 d and 150 g of weight showed eggs with higher ST than those of the other groups. It is concluded that both the age and weight of the breeding female quails influence the productive, reproductive performance and egg quality.

Key Words: Japanese quail, mating age, performance, egg quality, reproduction

596P Black soldier fly larvae for ecological poultry feed: On-farm production. Anne Fanatico*, *Appalachian State University, Boone, North Carolina, United States.*

Black soldier fly larvae (*Hermetia illucens*; BSFL) have the potential to provide a high-protein feed for poultry, especially in ecological and organic production. High-quality protein feeds are limited in organic poultry production due to restrictions on synthetic amino acids and slaughter by-products. Black soldier fly larvae can bio-convert residues and waste into protein feed. Insects like these have an important role in sustainability because they can recycle nutrients and recover embedded energy in crop residues, by-products, and local food waste. BSFL are high in protein and methionine, as well as fat. BSFL production has been tested in the past on poultry farms using manure as a feedstock to reduce the volume of manure for poultry waste management; however, a recent definition by AAFCO requires that feedstock for BSFL be feed-grade if the larvae will be used for poultry feed. Our objectives were to develop an on-farm larval production-harvest-dehydration system using feed-grade feedstock and to conduct a preliminary poultry feeding trial, with the goal of using BSFL as a protein feedstuff for chicks in an alternative poultry production system. We hatched commercially-available BSF eggs and raised the larvae at 15 C on feed-grade, de-hulled sunflower seeds. We harvested larvae at 6 weeks before they pupated. We sifted the BSFL from their substrate and terminated them by freezing. We dehydrated the BSFL at 52 C for 36 h and then ground them. We also

developed an adult colony production system to produce our own BSFL eggs during warm months. In ecological and organic poultry production, alternative feeding methods may be used to incorporate innovative, local feedstuffs. As such, we mixed a protein supplement, using soybean meal (81%), BSFL (9%), oyster shell (3%), vitamin/mineral premix (7%). The Control protein supplement utilized fishmeal (FM) instead of BSFL. Our supplements were designed to be offered with energy feed crops grown on the farm. Groups of 25 d-old chicks were randomly assigned to one of the 2 treatments: BSFL-based protein supplement and FM-based protein supplement (Control; n = 1). Protein supplements and corn were offered in separate feeders. Chicks were raised for 28 d in floor pens in a small range brooder. Weight gains and FCR were similar between BSFL and FM chicks (these data are not reported due to preliminary status). Our findings indicate that on-farm BSFL production at a feed-grade level shows potential for alternative poultry production and warrants further testing for poultry feed. In addition, we will raise BSFL at a higher temperature for faster larval growth.

Key Words: black soldier fly, *Hermetia illucens*, organic, feed, chicken

597P Corn and finished feed multi-mycotoxin survey 2018.

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Introduction – Description of the problem Mycotoxins are a large family of toxic fungal metabolites which occur worldwide in various cereals and other feed commodities. During the whole chain from field to feeding, mycotoxins can be produced by molds in plant material. There are numerous mycotoxins which are very toxic to different poultry species and may cause different diseases. Material and Methods To test the occurrence of multiple mycotoxin metabolites, a method based on Liquid Chromatography coupled with tandem Mass Spectrometry was used (LC-MS/MS method, Spectrum 380®). As the sensitivity of this test method has improved, it can test many more mycotoxins (e.g., masked mycotoxins) in addition to the main mycotoxins aflatoxin (Afla), zearalenon (ZEN), deoxynivalenol (DON), fumonisins (FUM), T-2 toxin (T-2) and ochratoxin A (OTA). In 2018, a total number of 106 corn and 273 finished feed samples were analyzed from over 30 countries all over the world. Results Among the most common mycotoxin metabolites in finished feed samples found are zearalenone (92%) and the fusarium metabolite moniliformin (85%), detected in nearly all samples. Also Type B trichothecenes were detected in a high amount, deoxynivalenol occurred in 89% of all the samples with an average of 245 ppb. In total 83% of all finished feed samples contained between 30 and 60 mycotoxins and their metabolites. Considering corn samples, the Fusarium metabolites moniliformin (97% occurrence), aurofusarin (89%) and culmorin (88%) represent the most common toxins. Fumonisin B1 and B2 were detected in 84% and 75% of all corn samples at an average of 1991 ppb and 560 ppb, respectively. Two thirds of all corn samples contained between 20 and 40 different metabolites. Conclusion The survey results indicate that mycotoxins remain a serious concern in agricultural production. Several studies have highlighted the significance of interactions between these mycotoxins and intestinal diseases such as clostridial necrotic enteritis and coccidiosis. Results of these studies showed that co-occurrence of these mycotoxins often results in greater immunosuppression, reduced feed intake, decreased weight gain and nutrient utilization, as a consequence of cytotoxicity and inhibition of protein synthesis or other key metabolic pathways that are vital for cells. An effective mycotoxin risk management program should be applied to protect poultry species from negative effects of mycotoxins.

Key Words: mycotoxin, spectrum, corn, finished feed

598P Providing a photoperiod with different colors of LED light during incubation on growth performance and body temperature of broiler chickens during the first week of life. Xujie Li*, Janessa Henry, Kayla Graham, Janice MacIsaac, and Bruce Rathgeber, *Dalhousie University, Truro, Nova Scotia, Canada*.

Providing photoperiod during incubation has been shown to improve post-hatch development and reduce stress during the grow-out phase. This study was conducted to evaluate the effects of providing light of different colors during incubation on growth performance and body temperature of broiler chickens early in life. The experiment was conducted with 4 repeated trials with 2 incubators per treatment per trial. For each trial, 1200 Ross 308 broiler hatching eggs were randomly distributed into 4 lighting treatments. The incubation lighting treatments included: incubated under dark as control, illuminated with white, red or blue lights for 12 h per day. After hatch, broilers were randomly distributed into 48 pens by the lighting treatment and gender. Treatment allocation was rotated among trials to minimize potential undesired environmental differences. Three pens of chicks hatched from the same incubator and with the same gender were considered as an experimental unit. The body weight and feed consumption were measured at 6 h (7 p.m.) post placement and every 24 h (at 1 p.m.) during the first week. The vent temperature was measured at 24 h (8 a.m.) and 36 h (8 p.m.) post hatch in 5 chicks/pen/trial. Data were analyzed using the Mixed procedure of SAS. Chicks hatched under white and blue lights had heavier ($P < 0.05$) body weight and higher ($P < 0.05$) feed consumption than the control group during the first 6 h post placement. Performance data were analyzed as repeated measures using the Proc Mixed procedure of SAS. The chicks hatched under white and blue lights had heavier ($P < 0.05$) average body weight over week one than those hatched under dark. No difference in vent temperature was found among treatments at 24 h post hatch (Dark: 39.9°C; White: 40.0°C; Red: 39.9°C; Blue: 40.0°C). However, the vent temperature at 36 h post hatch was lower ($P < 0.05$) in chicks hatched under dark (39.7°C) than those hatched under white, red or blue light (39.9°C for all lights). Results indicated that providing photoperiodic blue and white light during incubation improved the production parameters of broilers during the first week of age which was accompanied by an increase in body temperature at 36 h post-hatch. It is not clear why body temperature was higher for chicks hatched from photoperiodic lighting treatments but this may be related to the advanced body weight gain and feed consumption.

Key Words: incubation lighting, light color, broiler, vent temperature, performance

599P The effects of different wavelengths on broiler production. Bruna Maria Remonato Franco*¹, Tory Shynkaruk¹, Bryan Fancher², Nick French², Scott Gillingham², and Karen Schwean-Lardner¹, ¹*University of Saskatchewan, Saskatoon, Saskatchewan, Canada*, ²*Aviagen Group, Huntsville, Alabama, United States*.

Light emitting diodes (LED) offer the ability to use monochromatic colors for broiler production. However, the productivity research published to date is inconsistent. The objective of this study was to determine the impact of 3 light treatments (L) (blue (BL, 455nm), green (GL, 510nm) and white (WL)) on welfare and production traits of broilers; this abstract covers production traits. Two genders (G) and strains (S) were tested from 0 to 35d of age (Ross EPM-708 and YPM-708) in 2 blocked trials (n = 14,256 birds). Nine environmentally independent and identical rooms (3 replicates per L) were subdivided in 12 pens (2 × 2.3m) for distribution of G and S. Birds were fed commercial diets based on Aviagen recommendations. The lighting program started at 23L:1D for d1, decreasing 1 h daily until reaching 16L:8D by d5,

(dawn-dusk transitions daily, 15 min). Weekly data collection included body weight, consumption and feed efficiency ratios. Daily mortality and culls were necropsied for cause of death. Data were analyzed as a 3x2x2 factorial design with L nested within room (Proc Mixed - SAS 9.4). Differences were considered significant when $P < 0.05$. Body weights at d1, 21, 28 and 35d were unaffected by L. At d7, WL birds were heavier ($P = 0.004$) than BL and GL birds (0.167, 0.164, 0.164kg respectively) while at d14 they remained heavier ($P = 0.03$) than GL birds only (0.489, 0.480kg respectively). Feed intake differed during wk1, as WL birds ate more than BL or GL birds (0.133, 0.130, 0.130 kg/bird respectively; $P = 0.006$). WL mortality (1.76%) was higher than BL birds (1.08%) from d28–35 ($P = 0.03$) but was not different overall. An interaction between L and S was noted in feed intake at d1–35 ($P = 0.03$), where BL, WL and GL YPM-708 birds consumed more feed than BL and GL EPM-708 birds. An interaction between L and G was noted for mortality at d1–35 ($P = 0.01$), where WL males had higher mortality than BL and WL females. No impact of lighting treatment was found on feed efficiency and mortality causes. Males were heavier, ate more, had better feed efficiency corrected for mortality and higher mortality. YPM-708 birds were heavier at d21, 28 and 35, higher feed intake after d14 and better mortality corrected feed efficiency. At d1–7, YPM-708 birds had higher mortality than EPM-708 ($P = 0.001$), but at d28–35, mortality levels of YPM-708 birds were lower ($P = 0.02$). An interaction between S and G was noted in feed intake and feed to gain at d14–21 and d1–35, as YPM-708 male birds ate more and had better feed efficiency. In conclusion, while gender and strains reveal some differences in productivity, using varying light spectrums to improve productivity resulted in minor impacts in production parameters.

Key Words: wavelength, LED, broiler, production

600P Effects of simulated transport conditions on meat quality and core body temperature of brown strain end-of-cycle hens.

Kailyn Beaulac*, Trever Crowe, and Karen Schwean-Lardner, *University of Saskatchewan, Saskatoon, Saskatchewan, Canada.*

Transportation conditions can influence core body temperature (CBT) and meat quality of poultry. This study evaluated 5 temperature (T) and relative humidity (RH) combinations (-10°C uncontrolled RH (-10), $+21^{\circ}\text{C}$ 30% RH (21/30), $+21^{\circ}\text{C}$ 80%RH (21/80), $+30^{\circ}\text{C}$ 30%RH (30/30), and $+30^{\circ}\text{C}$ 80%RH (30/80)), 3 durations (D) (4, 8, and 12 h), and 2 feather covers (FC) (well (WF) and poor (PF)), in a factorial arrangement. Brown feathered hens came from 3 commercial farms (90 WF and 90 PF/farm) at the end of their production cycle (66–72 wk) and were housed in floor pens for an adaptation period (3–5 d). Hens were feed restricted (6 h), weighed, orally administered an iButton, transported to environmental chambers, crated (target density of 54.5 kg/m²) and exposed to the treatment combinations above. Post-exposure body weight was recorded (live shrink calculated); hens were slaughtered via electrical stunning. The iButton was retrieved (Δ) CBT calculated; breast and thigh meat quality analysis included initial pH upon evisceration, final pH, drip loss and color (lightness (L*), redness (a*), yellowness (b*)) 30h post-slaughter. Breast meat was assessed for thaw loss and cook loss. Data were analyzed as a randomized complete block design (farm of origin as block) via ANOVA (Proc Mixed of SAS 9.4), differences were significant when $P \leq 0.05$. The Δ CBT in the last h of exposure was lowest for -10 , 12h, PF hens compared with hens in all other treatment combinations ($P = 0.01$). Live shrink was higher for hens exposed to 30/30 and 30/80 compared with hens exposed to 21/30 and 21/80 ($P < 0.01$), and increased with increasing D (1.33, 2.56, and 4.05% for 4, 8, and 12 h, respectively; $P < 0.01$). Breast initial pH was higher in hens exposed to 21/30 12 h compared with hens exposed to

21/30 4 h and 30/80 8 h ($P = 0.03$). Thigh L* was higher for hens exposed to 30/80 4 h compared with hens exposed to -10 all D ($P = 0.04$). Final breast pH ($P < 0.01$) and final thigh pH ($P < 0.01$) was highest in hens exposed to -10 compared with all other T/RH. Thigh b* was lowest for -10 compared with all other T/RH ($P < 0.01$). Thigh a* was higher for 30/30 compared with 21/30 and 21/80 ($P < 0.01$). Initial thigh pH was lower for -10 compared with 21/30, 30/30, and 30/80 ($P < 0.01$). Breast thaw loss was higher for hens exposed for 4 h compared with 12 h ($P < 0.01$) and breast L* ($P < 0.01$) was highest for hens in the 4 h compared with 8 and 12 h D. Breast a* was highest for 12 h compared with 4 h ($P < 0.01$) and thigh a* was highest for 12 h compared with both 4 and 8 h ($P < 0.01$). There was no effect of FC on meat quality. These results suggest that extreme thermal conditions during transport influence the hen's ability to maintain CBT and increase stress, which impacts meat quality.

Key Words: live shrink, drip loss, cook loss, meat color, meat pH

601P Interaction of yeast fermentation product supplementation and indicators of intestinal dysfunction in heat-stressed Pekin ducks.

Jill Nelson* and Greg Archer, *Texas A&M University, College Station, Texas, United States.*

Fear and stress levels differ based on genetic strain. To determine differences in fear and stress levels 4 strains of cage-free laying hens were raised to 32 weeks of age in 3.05 m by 4.57 m floor pens. 60 birds were allotted to each pen with 1 strain per pen. The stocking density of each pen was 0.232 m² per bird. Three white strains were used, W-36, W-80, and LSL, and one brown strain was used, Hyline Brown (HB). Fear and stress tests were conducted on each strain at 3, 16, and 30 weeks of age with additional testing done at 31 weeks where a 30 min period of confinement was administered to induce stress; after which blood was sampled to measure plasma corticosterone concentration (CORT) At 3 weeks of age Isolation testing (ISO, $n = 60$) and emergence testing (EMER, $n = 60$) was conducted. At 16- and 30-weeks tonic immobility (TI, $n = 60$), physical asymmetry of bilateral traits (metatarsal length, metatarsal width, middle toe length) (ASYM, $n = 60$), CORT ($n = 20$), and heterophil to lymphocyte ratios (HL, $n = 20$) were measured. Data was analyzed using GLM and fisher pairwise comparisons: a significant difference was considered $P < 0.05$. In ISO W-80 (59.0 ± 7.9 vocal/3 min) had the most vocalizations among all other strains ($P < 0.001$) with LSL and HB (34.3 ± 5.5 vocal/3 min) at an intermediate level and W-36 the lowest (12.3 ± 3.4 vocal/3 min). During emergence testing W-36 and LSL (39.9 ± 7.1 s.) took longer ($P < 0.001$) to leave a 18.5 L bucket than W-80 and HB (14.5 ± 3.3 s.). Righting time did not differ ($P > 0.05$) between strains in TI, however, time for each strain to alert showed a difference in first head movement ($P < 0.001$). HB (5.1 ± 2.3 s.) was quickest to alert at when compared with other strains. W-36 and W-80 (23.7 ± 7.1 s.) alerted at an intermediate level while LSL (58.475 ± 9.4 s.) was the slowest to alert. ASSYM score is a composite score calculated by taking the difference in left metatarsal length and right metatarsal length During ASYM scoring HB (1.9 ± 0.29 mm) had the greatest score ($P = 0.048$) with W-36 (1.5 ± 0.17 mm) having an intermediate score and W-80 and LSL (1.4 ± 0.13) having the lowest score. HB (0.52 ± 0.08 , 20424.3 \pm 1570.2 pg/ml) had higher HL ($P < 0.001$) and CORT ($P < 0.001$) than the other strains. The other strains (0.18 ± 0.03 , 14431.1 \pm 1581.0 pg/ml) did not differ from each other. After the period of stress LSL (21358.0 \pm 1367.0 pg/ml) showed a trend to have higher CORT than the other strains ($P = 0.057$). HB (19059.0 \pm 1225.8 pg/ml) had an intermediate level and W-36 and W-80 (16380.0 \pm 16183.1 pg/ml) did not differ from each other. Results of this study suggest that fear and stress vary depending on the strain of bird used.

Key Words: duck, intestinal health, heat stress, goblet cells, yeast fermentation product

602P Supplementation of a water-soluble zinc amino acid complex to reduce stress in broilers, pullets and layers. Jill Nelson*¹, Eric Sobotik¹, Marco Rebollo², and Greg Archer¹, ¹Texas A&M University, College Station, Texas, United States, ²Zinpro Corp., Eden Prairie, Minnesota, United States.

The water-soluble zinc-amino acid complex Zinpro-LQ (Zinpro Corporation, Eden Prairie, MN) has been shown to affect immune response and to mitigate reduced growth in heat-stressed pigs. This study consisted of 3 experiments to test the effects of administering Zinpro-LQ for different time intervals over the course of 35 d in stressed broilers, pullets, and layers. There were 6 treatments: non-stressed control (T1); stressed control (T2); stressed and supplemented Zinpro-LQ for 14d before stressor (T3), 10d before stressor (T4), 5d before stressor (T5), and 7d before and 7d during stressor (T6). In all experiments, birds in stressed treatments were vaccinated against Newcastle Disease Virus (NDV) on d28 of the experiment and exposed to random feed and water withdrawal d28–35 for 6h/d. Experiment 1 included Cobb 500 broilers (n = 360). Experiment 2 included 7-week old white Leghorn pullets (n = 120); additionally, pullets were beak-trimmed on d28 of the experiment. Experiment 3 included 50-week old white Leghorn layers (n = 120); additionally, layers were heat-stressed d28–35. Blood samples were collected at the end of each experiment to determine plasma chemistry, corticosterone (CORT), anti-NDV antibody titer, and heterophil/lymphocyte (H/L) ratio. Mortality, feed and water consumption and body weight were also recorded. All data was analyzed using GLM in Minitab 17.1.0 and means were separated using the LSD post-hoc procedure. A significant difference was considered $P < 0.05$. In Experiment 1, T2 had the lowest feed conversion ratio (1.41 ± 0.01) due to lack of feed withdrawal, and the lowest plasma chloride (109.2 ± 0.9). All supplemented treatments had higher glucose (256.5 ± 3.9) than SC (239.9 ± 3.9) and NC (234.8 ± 3.9); CORT and H/L ratio were highest in T2 (222.5 ± 37.5 ; 0.51 ± 0.03) than all other treatments (93.12 ± 37.5 ; 0.37 ± 0.03). In Experiment 2 cholesterol was higher in T3 (131.1 ± 2.2) than all other treatments (110.16 ± 2.2); T5 had lower CORT (2100 ± 261) and higher NDV titer (12011 ± 651) than T2 (4179 ± 261 ; 6938 ± 651). Treatments also differed in uric acid and H/L ratio ($P < 0.05$). In Experiment 3, T3 and T6 had higher glucose (119.8 ± 4.9) than all other treatments (76.48 ± 4.9), and chloride was lower in T4 (117.7 ± 0.5) than T2 (122.9 ± 0.5). Administration of Zinpro-LQ for 14d before a stressful event helps maintain circulating glucose and cholesterol in broilers, pullets and layers. Administration for either 5d or 14d before an anticipated stressor helps reduce stress and improve humoral immune response in pullets and layers.

Key Words: stress, zinc, broiler, pullet, layer

603P Comparing impact depth and pressure of various captive bolt devices. Austin Stiewert* and Greg Archer, Texas A&M University, College Station, Texas, United States.

Euthanasia has universally been a necessary action used commercially to remove injured or sick fowl from a flock, however the methods of euthanasia have differed greatly. The objective is to determine how captive bolts vary and how they impact the surface. It is believed that captive bolts will have different impact characteristics. To test this 2 parameters were determined for various devices. Parameter one tested the overall impact depth of the devices into modeling clay: treatments (T) were applied by device type. T1 consisted of Jarvis (set to 130

Psi), (T2) K.E.D, Dick stunner (T3), Zephyr E (T4) (set to 120 Psi), Zephyr EXL (T5) (set to 120 Psi), and (T6) T.E.D (set to 120 Psi). Each device was dry fired 10 times followed by a shot into level clay and was replicated 10 times for a total of 100 dry fires, 10 shots into clay. Each indentation was recorded in (cm) and compared. Parameter 2 tested the overall average Psi, peak Psi and minimum Psi of each device by using scanned FujiFilm pressure paper: (T1) Jarvis (set to 130 Psi), (T2) K.E.D, Dick stunner (T3), Zephyr E (T4) (set to 120 Psi), Zephyr EXL (T5) (set to 120 Psi), and (T6) T.E.D (set to 120 Psi). Each device was dry fired 10 times followed by a shot onto the FujiFilm for a total of 100 dry fires and 10 shots on film. Each impact was recorded and compared. Data was analyzed using Kruskal-Wallis followed by the Dwass, Steel, Critchlow and Fligner method for means separation ($P < 0.05$). The clay depth has shown to have significant differences ($P < 0.001$) between device and depth of impact. With T6 having the deepest impact with crossbow having the least. In comparison of Psi impact, differences were observed ($P < 0.001$) between all devices. This includes radius impact, minimum, maximum and average Psi of each T. In conclusion each device varies between impact size and pressure which could alter the efficacy of euthanasia and damage to the birds skull.

Key Words: euthanasia, device, psi, penetration depth

604P Investigating the effect of CO₂ concentration on the efficacy of gaseous euthanasia of broiler chicks on day of hatch..

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Immersion into a pre-filled chamber at 100% CO₂ is efficacious for euthanizing cull broiler chicks; however, 100% CO₂ may not be achievable in commercial hatcheries. To understand how varying CO₂ concentrations within the chamber affects the efficacy of CO₂ immersion euthanasia, broiler chicks (n = 64) on day of hatch were euthanized by immersion into 70, 80, 90 or 100% CO₂. Chicks were placed in pairs into a 42.5L Euthanex chamber (chamber as replicate unit; 8 reps/tx) that was pre-filled to the required CO₂ concentration. Live focal sampling by 2 observers and video recording measured behavior responses. The latency to headshaking (HS) and gasping (GS), as well as the duration and frequency of their occurrence, were measured as indicators of distress, latency to loss of posture (LOP) was used as an indicator of insensibility, and latency to cessation of rhythmic breathing (CRB) and movement (COM) were indicators of death. Behavior data were pooled for both birds in the chamber and (log+1) transformed before analyses. One-way ANOVA for main effect of induction treatment, with block (testing of one replicate per treatment) as random effect and chamber as experimental unit was performed (PROC MIXED, SAS 9.4). Tukey-Kramer post hoc test was used for means separation and differences were considered significant when $P \leq 0.05$. No difference was found between CO₂ concentrations for latency to distress behaviors (HS, GS). There was a difference between CO₂ concentrations for duration of GS ($P < 0.01$), with duration being significantly longer with 70% compared with 80% and 100% CO₂ (8.7, 6.6, 8.0 and 6.2s, for 70, 80, 90 and 100%, respectively). Time to LOP was longer ($P = 0.02$) with 70% compared with 100% CO₂ (14.2 and 10.7s, respectively). For indicators of death, latency to CRB ($P < 0.01$) and COM ($P < 0.01$) was longest for 70% (CRB: 532.5s; COM: 536.5s), intermediate for 80% (CRB: 159.3s; COM: 163.3s), and shortest for 90% and 100% CO₂ (CRB: 40.6 and 31.2s; COM: 48.6 and 43.9s, respectively). Overall, immersion into 100% CO₂ led to the shortest latency to insensibility and death, and

the shortest duration of GS; and 90% was almost equal to 100% CO₂ in regard to inducing rapid insensibility and death. Immersion into 70% CO₂ led to the longest latency to insensibility and death, as well as the longest duration of distress behaviors. To conclude, immersion of hatched broiler chicks into 90 or 100% CO₂ rapidly induces insensibility and death, with the shortest duration of distress.

Key Words: disposal, neonate, CO₂ euthanasia, insensibility, distress

605P Evaluating the impact of age on the efficacy of CO₂ euthanasia for broilers in early life. Bethany Baker^{*1}, Stephanie Torrey², Pat Turner², Tina Widowski², Tennille Knezacek¹, Jenny Nicholds³, and Karen Schwean-Lardner¹, ¹University of Saskatchewan, Saskatoon, Saskatchewan, Canada, ²University of Guelph, Guelph, Ontario, Canada, ³University of Georgia, Athens, Georgia, United States.

Neonatal poultry have a higher tolerance to CO₂ and hypercapnia than older birds, thus they require longer exposure time and higher CO₂ concentrations during gaseous euthanasia. To evaluate how the efficacy of gaseous euthanasia changes with age in early life, broiler chicks (n = 192) at either 0, 3 or 6 d of age were euthanized by gaseous euthanasia with CO₂. At each age, 2 chicks per replicate (8 reps/tx) were immersed into either 70, 80, 90 or 100% CO₂ and behavioral response was measured. Live focal sampling by 2 observers and video recordings measured latency to performance of headshaking (HS) and gasping (GS) as indicators of distress; loss of posture (LOP) indicative of insensibility; and cessation of rhythmic breathing (CRB) and movement (COM) indicative of death. Duration and frequency of HS and GS were also measured. Behavior data was averaged for both birds in chamber and (log+1) transformed before analyses for main effect of age and CO₂ concentration. Data was in a 4 (CO₂ concentration) * 3 (age) factorial arrangement and analyzed using PROC MIXED (SAS 9.4) with block of each 4 treatments as random effect and chamber as experimental unit. Tukey-Kramer tests were used for means separation. Both GS ($P = 0.05$) and LOP ($P < 0.01$) showed an age effect, with the longest latency seen for chicks at 6 d of age (GS: 2.1, 1.9 and 2.2s (SEM = 0.07); LOP: 12.4, 13.9 and 17.8s, for 0, 3 and 6 d, respectively). Age and CO₂ concentration interacted for latency to CRB ($P < 0.01$) and COM ($P < 0.01$), with 0-d old chicks immersed into 70% CO₂ having a longer latency for CRB and COM than all other ages and concentrations. The impact of CO₂ concentration on time to CRB and COM appeared to be greater at 0 d compared with 3 or 6 d. At 0 d of age, chicks displayed a longer duration of HS ($P < 0.01$) and GS ($P < 0.01$), and frequency of HS ($P < 0.01$) than at 3 and 6 d of age (Duration for HS: 7.1, 4.4 and 4.8s; GS: 7.4, 6.3 and 5.1s; Frequency for HS: 4.1, 2.9 and 3.1, in respective order of 0, 3 and 6 d). As age increased the latency to death decreased, indicating that at 0 d of age chicks are more tolerant to CO₂. This increased tolerance was not seen for GS or LOP. To conclude, the increased CO₂ tolerance found in 0-d old chicks means higher CO₂ concentrations and longer exposure time compared with 3 and 6 d old broiler chicks are required for efficacious gaseous euthanasia.

Key Words: day old chicks, gaseous euthanasia, immersion, pre-fill, time to death

606P The impacts of simulated transportation on meat quality of white-feathered end-of-cycle hens. Carley Frerichs^{*}, Kailyn Beaulac, Trever Crowe, and Karen Schwean-Lardner, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

The objective of this study was to assess the effect of temperature (T)/RH, duration (D), and feather cover (FC) on meat quality of white-feathered end-of-cycle hens (630; 65–70 wk of age, obtained from commercial farms) in a 5 (T/RH) x 3 (D) x 2 (FC) factorial arrangement (randomized complete block design). Three replicates of 210 hens (105 well feathered (WF), 105 poorly feathered (PF)) were exposed to either –10°C with uncontrolled RH (–10), 30°C with either 30 (30/30) or 80%RH (30/80), or 21°C with either 30 (21/30) or 80% RH (21/80) for either 4, 8, or 12h. Three transport crates per duration were divided in half (7 WF or PF/side) then placed in climate-controlled chambers. Before chamber entry, hens were feed withdrawn (6 h) then weighed. Hens were orally administered a miniature data logger to record internal core body temperature (CBT), and baseline measures were collected for 5–20 min. Post chamber exposure, hens were weighed (live shrink calculated), slaughtered, eviscerated, and carcasses were cut up (5 hens/treatment combination). Breast and thigh pH and weight (drip loss calculated) was measured immediately after evisceration and 30 h post-mortem. Breast and thigh color (lightness (L*), redness (a*), and yellowness (b*)) was measured 30 h post mortem. Breast muscles were stored at –30°C then analyzed for thaw and cook loss 5 wk post-slaughter. Data were analyzed using ANOVA (PROC MIXED (SAS 9.4)) with farm of origin as block. Significance was declared at $P \leq 0.05$. Live shrink (%) was larger for WF hens exposed to 30/30 compared with PF in 30/30, 21/30 and WF hens in 21/30, 21/80. Change in (Δ) CBT was larger ($P = 0.05$) for hens exposed for 12 h at 21/30 WF and PF, 21/80 WF and PF, 30/80 WF and PF, and 30/30 PF compared with –10 12 h PF. Δ breast pH was larger ($P = 0.04$) for hens exposed to 30/80 for 8 h compared with –10 8 and 12 h, and 30/30 for 8 h. Breast thaw loss (%) was smaller ($P = 0.03$) in hens exposed to –10 for 4 h compared with 21/30 for 8 h, 21/80 8 h, and 30/30 12 h. Cook loss (%) was larger ($P < 0.01$) for hens exposed to –10 WF compared with –10 PF but smaller than 21/80 for WF and PF. Breast L* was larger, ($P = 0.05$) for hens exposed to 21/80 WF and PF and 30/30 PF than –10 WF and PF. Thigh L* was smaller in WF and PF hens exposed to –10 compared with all other T/RH and FC treatments; PF hens exposed to 21/80 were larger than WF 30/80 and WF and PF hens exposed to –10. There were no treatment interactions for drip loss, a*, b*, and Δ thigh pH. The results suggest that extreme temperatures influence the hen's ability to regulate CBT and colder temperatures result in more transport stress which impacts meat quality parameters.

Key Words: layer, meat physiology, transport, hot and cold stress, welfare

607P Not Presented

608P The effects of simulated transport conditions on pullet meat characteristics. Samantha Lalonde^{*}, Kailyn Beaulac, Trever Crowe, and Karen Schwean-Lardner, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Thermal stress during transportation is a significant stressor for poultry. The objective of this study was to evaluate the effects of various temperature/RH combinations and exposure durations on the meat characteristics of white-feathered layer pullets (sourced from commercial farms) during simulated transport while crated at a density of 45.5 kg/m². In a 5x2 factorial arrangement of temperature (T) and RH, and duration (D) the experiment consisted of 3 replications of 8 birds per T/RH and D (n = 240 pullets). Pullets were randomly exposed to 1 of 5 T/RH combinations including 21°C30%RH (21/30), 21°C80%RH (21/80), 30°C30%RH (30/30), 30°C80%RH (30/80), and –15°C (–15)

for either a 4 or 8 h D. Before exposure, each bird was weighed and then placed in 1 of 2 environmental chambers. After exposure, final BW were taken for calculations of live shrink, and then all birds were slaughtered using a small-scale facility. After slaughter, all carcasses were eviscerated and an initial pH was taken from the right breast and thigh muscles. Breast and thigh muscles were removed from carcasses 6 h post-slaughter. Final pH and color values (lightness (L*), yellowness (b*), and redness (a*)) from the right breast and thigh muscles were obtained 30 h post-slaughter. Left breast muscles were analyzed for thaw loss and cook loss 4 wk post-slaughter. Data were analyzed as a randomized complete block design via a one-way ANOVA (Proc Mixed (SAS 9.4)), with farm of origin as the block. Differences were considered significant at $P \leq 0.05$. Live shrink (kg) was higher ($P = 0.035$) for 30/30 and 30/80 compared with 21/80, but when converted to a percentage of body weight, differences were no longer significant ($P = 0.066$). Breast muscle a* was higher ($P = 0.024$) for pullets exposed to 30/30 compared with pullets exposed to 21/30. Thigh muscle a* was lower ($P = 0.019$) for 21/30 compared with 30/30, and thigh muscle b* was higher ($P = 0.048$) for 21/80 compared with -15. With respect to D, live shrink was higher ($P = 0.004$ for kg, 0.011 for %) for pullets exposed for 8 h D compared with 4 h D. Breast thaw loss was lower ($P = 0.019$ for kg, 0.010 for %) in the 4 h D compared with the 8 h D. Breast b* was higher ($P = 0.038$) for pullets exposed for 8 h D compared with 4 h D. Breast pH and cook loss were not affected by T/RH or D. There were no effects of T/RH or D on thigh pH. These data indicate that due to heat exposure, and due to increased exposure D, pullets mobilized more energy stores, which resulted in a greater live shrink. Heat exposure caused a higher breast and thigh muscle a*, while cold exposure, and longer exposure D, caused a lower thigh b*, all of which negatively affected the muscle tissue.

Key Words: live shrink, thermal stress, muscle pH, muscle colour, cook loss

609P Keel bone deformities in commercial brown laying hens fed different zinc sources. Lauren Nolan^{*1}, Anthony Pescatore¹, Tuoying Ao², Lizza Macalintal¹, Marquisha Paul¹, and Mike Ford¹, ¹University of Kentucky, Lexington, Kentucky, United States, ²Alltech Inc, Nicholasville, Kentucky, United States.

Egg production can be effected by many factors, one of which being keel bone damage and deformation; making keel bone damage a major concern of the layer industry. This study was conducted to determine the effect of dietary inorganic Zn (ZnO) versus Zn proteinate (Bioplex® Zn, Alltech Inc.) on keel bone damage. One hundred and 60 commercial brown egg layers were divided among 5 diets. Diet 1 was a commercial corn-soy diet with no additional supplementation of Zn; diets 2 and 3 were a corn-soy diet supplemented with 80 or 30 mg Zn/kg diet (respectively) as ZnO; diets 4 and 5 were corn-soy diet supplemented with 80 or 30 mg Zn/kg diet (respectively) as Bioplex® Zn. The hens were housed 2 birds per cage (25 × 41 cm) and photo-stimulated with 16L: 8D. Feed and water were provided *ad libitum*. Keel bones were scored by 3 judges every 4 weeks for 36 weeks, starting at 29 weeks of age. Scoring was conducted using palpation, outlined by Casey-Trott et al., 2015. At the end of the 36-week period, 12 birds per diet were randomly selected. Breast muscles were removed and final cleaning was accomplished with exposure to Dermestidae beetles for 36 h. Digital pictures of all bones collected were taken, as well as imaged using the Volscan Profiler. ImageJ was used to measure length of keel, deviation of keel, as well as type of deviation. Keel bones were divided into 4 quadrants, and deviations from a straight keel were recorded. Keel bone scores were analyzed using the PROC FREQ function in

SAS 9.4; deviations and type of deviation were analyzed using PROC GLM. Keel bone scores increased from a score of 1 to a score of 3 over the 36-week trial, regardless of treatment. Birds without any deviation from normal were given a score of 1, any deviation from “normal” was given a 2; while a deviation resembling the shape of an ‘S’ was given a 3. At the start of the experiment a score of 1 was given to 27% birds, a 2 was given to 70%, and a 3 to 3%. At the end of the 36 weeks, a score of 1 was given to 8% of birds, a score of 2 to 55%, and a score of 3 to 37%. An ‘S’ shape had a significantly higher deviation of 42.40 mm ($P < 0.05$), compared with 27.02 mm of a ‘U’ shaped keel bone. Total deviation by diet, regardless of shape, demonstrated that birds on diet 3 had significantly greater deviations (41.08 mm; $P < 0.05$) compared with keel bones from diets 1, 2, and 4. From this data set, it can be concluded that a greater total deviation, indicates an ‘S’ shape curve and greater keel bone deformation. Hens receiving the lowest level of Zn as ZnO had greater incidence of keel bone deformity, which was not seen with Bioplex® Zn.

Key Words: keel bone, commercial brown layers, Bioplex® Zn

610P Effect of dietary omega-3 fatty acids and vitamin D₃ on production and welfare parameters in brown laying hens housed in multi-tier aviary system. Prafulla Regmi^{*1}, Cara Robison², Woo Kim³, and Darrin Karcher⁴, ¹North Carolina State University, Raleigh, North Carolina, United States, ²Michigan State University, East Lansing, Michigan, United States, ³University of Georgia, Athens, Georgia, United States, ⁴Purdue University, West Lafayette, Indiana, United States.

Eighteen percent of the total laying hens in US are currently housed in cage-free. Cage-free egg production is expected to increase further in the coming years. Nutrition and management practices for conventional cages are well established but information regarding cage-free systems is limited. Complex housing systems like the multi-tier aviary (AV) system pose unique challenges to hen well-being and management. The objective of this study was to examine the production and welfare effects of long-term inclusion of omega-3 fatty acids and vitamin D in laying hen diets housed in AV system. Lohmann brown birds were floor reared and then transferred to AV system at 17 wk. The dietary treatments started at 12 wk and consisted of basal (C), FL (Flaxseed oil), FS (Fish oil), and Vit (25-hydroxy Vitamin D₃; 2760 IU/kg). The omega-6 (n6) to omega-3 (n3) ratio was maintained at 0.5 for FL and FS whereas n6/n3 was 6.75 for C and Vit groups. Birds were randomly distributed to 320 birds per pen with 2 pens/treatment in the pullet house. During hen phase, birds were transferred to 4 AV rooms (1 room per treatment; 4 sections per room) at 144 birds per section with a total of 576 hens per treatment. Body weight, percent mortality, hen day percentage and welfare parameter assessment were evaluated on every 28-d period. Serum concentration of osteocalcin (OC) and pyridinoline (PYD) were measured as markers of bone absorption and resorption respectively. Data were analyzed for 24, 28, 32 and 40 wk using mixed ANOVA model including fixed effect of diets and random effect of AV rooms and sections. Welfare parameters were analyzed as categorical data with binary or multinomial distribution respectively to the nature of the observations. Basal (C) birds had marginally lower body weight compared with the treatment groups (1.8 vs 1.85 kg; $P < 0.05$). Overall hen day egg production was greater in C (89.5%) compared with FL (88.3%), FS (88%), and Vit (87.6%). Mortality was low in all groups (<2%) however, FL and FS had lower mortality compared with C and Vit birds ($P < 0.05$). At wk 24, > 90% of the birds had mild damage of keel bone (score ‘1’) with presence of tip fractures regardless of the dietary treatments. Prevalence of foot disorders were lower in FL compared with

other groups (52.3% vs. 59.43%; $P < 0.05$). Concentrations of serum bone markers were not influenced by dietary treatments.

Key Words: laying hen, aviary, cage-free, omega-3, vitamin D

611P Effect of valerian extract (*Valerian officinalis*) and density on performance, egg quality and stress indicators of Japanese quail hens. Teresita Huapaya, Daniel Huaranga, Ruben Cespedes, Liliana Jimenez, Otto Zea, and Carlos Vilchez*, *Universidad Nacional Agraria La Molina, Lima, Peru.*

Valerian (*Valerian officinalis*) is a well-known medicinal plant used since ancient times and the root of this plant has sedative and hypnotic effects so it is indicated in cases of nervousness or anxiety and sleep disorders. It has been shown, moreover, that it has antispasmodic and myorelaxant effects. On the other hand, Japanese quail are characterized for its aggressiveness and stressfulness behavior and these affect the productive performance during the laying period. Therefore, this study aimed to evaluate the effect of valerian extract (*Valerian officinalis*) and density on performance, egg quality and stress indicators Japanese quail hens. One-hundred 80 Japanese quail hens were randomly assigned into 18 experimental units (EU). Three EU received one of the 6 treatments that resulted from the combination of 3 density (1, 2 or 3 quails per cage) and 2 valerian levels in water (0 and 4 mg/ml). All the experimental animals received a common corn-SBM based quail layer diet during a 7-wk evaluation period. Feed (mash form) and water were offered *ad libitum*. Feed Intake (FI), egg production (EP), egg weight (EW), egg mass (EM), egg quality (EQ) and broken eggs (BE) were recorded on a weekly bases. Stress indicators as tonic immobility (TI), heterophil/lymphocyte (H/L) ratio and blood cortisol level (CL) were determined at the end of the experiment. The registered data was submitted to ANOVA under a Completely Randomized Design with a 3×2 factorial arrangement and means were separated by Tukey Test. The results showed that only FI and yolk diameter were significantly influenced ($P < 0.05$) by density and valerian extract, respectively. There was significant interaction ($P > 0.05$) between density and valerian levels for shell thickness. Quails without valerian extract and 3 animals per pen had higher ($P < 0.05$) CL than those from quails with valerian extract and maximum density. Of the 3 stress indicators, only the H/L was influenced ($P < 0.05$) by the level of valerian extract. It is concluded that both density and valerian extract influence performance and egg quality and that the addition of valerian extract in the drinking water can be used to reduce the stress of quail hens raised in high density conditions.

Key Words: Japanese quail, performance, valerian extract, stress, H/L ratio

612P Effect of different dietary zinc sources on keel bone deformation of commercial white laying hens. Lauren Nolan*¹, Anthony Pescatore¹, Tuoying Ao², Lizza Macalintal¹, Marquisha Paul¹, and Mike Ford¹, ¹University of Kentucky, Lexington, Kentucky, United States, ²Alltech Inc, Nicholasville, Kentucky, United States.

Keel bone damage is of major concern to the commercial egg laying industry because damage cannot only cause pain, but also reduce egg productivity, causing financial strain on the layer industry. This study was conducted to determine the effect of dietary ZnO (inorganic) versus Bioplex® Zn (Zn proteinate, Alltech Inc.) on keel bone damage. One hundred and 60 commercial white egg layers were divided among 5 diets. Diet 1 was a commercial corn-soy diet with no additional supplementation of Zn; diets 2 and 3 were a corn-soy diet supplemented with 80 or 30 mg Zn/kg diet (respectively) as ZnO; diets 4 and 5 were

corn-soy diet supplemented with 80 or 30 mg Zn/kg diet (respectively) as Bioplex® Zn. Hens were housed 2 birds per cage (25 × 41 cm) and photo-stimulated with 16L: 8D. Feed and water were provided *ad libitum*. Keel bones were scored by 3 judges every 4 weeks for 36 weeks, starting at 29 weeks of age. Scoring was conducted using palpation, outlined by Casey-Trott et al., 2015. At the end of the 36-week period, 12 birds per diet were randomly selected for keel bone samples. Breast muscles were removed and final cleaning was accomplished with exposure to Dermestidae beetles for 36 h. Digital pictures were taken of all bones, as well as, imaged using the Volscan Profiler. ImageJ was used to measure length and deviation of keel, as well as type of deviation. Keel bones were divided into 4 quadrants, and deviations from a straight keel was recorded. Keel bone scores were analyzed using the PROC FREQ function in SAS 9.4; deviation and type of deviation were analyzed using PROC GLM. Birds without any deviation from normal were given a score of 1, any deviation from “normal” a 2; while 3 was a deviation resembling the shape of an ‘S’. Keel bone scores increased from a score of 1 to a score of 3 over the 36-week trial, regardless of treatment. At the start of the experiment 63% birds were scored with a 1, 37% with a score of 2, and 0% a score of 3. By the end of the 36 weeks, 8% birds were given a score or 1, 55% a score of 2, and 37% a score of 3. An ‘S’ shape had a significantly higher deviation of 36.36 mm ($P < 0.05$), compared with 22.55 mm of a ‘U’ shape keel bone. Numerical differences were detected with lower deviation of keels from birds receiving 80 mg Zn/kg as Bioplex® Zn of 24.04 mm, compared with deviations from birds fed other diets. From this data set, it can be concluded that a higher percentage of birds displayed an ‘S’ shape curve as they advance through production cycles.

Key Words: keel bone, commercial white layers, Bioplex® Zn

613P Storage time and temperature effects on exterior and interior quality of eggs from Cobb 500 broiler breeders. Walter Blalock*¹, Kotoyo Suganuma¹, Anel Atencio², Tia O’Brien¹, Kayla Davis¹, Takashi Hamasaki¹, and Tomohiro Hamaoka¹, ¹Calpis America, Peachtree City, Georgia, ²Perdue Farms, Salisbury, United States.

Egg storage and environmental factors such as temperature, humidity, CO₂ level, and time can impact interior egg quality. These factors have mainly been studied using table eggs from laying hens; however, the relationship between storage factors and quality of eggs from broiler breeders is not fully known. The objective of the present study was to examine the effects of storage time and temperature on weight, breaking strength, and Haugh Units (HU) of eggs from Cobb 500 broiler breeders. Three trials were conducted storing eggs at 4 Celsius degrees (C), 23C and 35C. A total of 380 freshly laid Cobb 500 eggs were obtained from a US commercial farm at 39 and 40 weeks of age for each trial by collecting them fresh off the belt on the second run of the morning. All eggs were candled initially to remove any damaged eggs. In each trial, a total of 360 intact eggs were each weighed and randomly assigned into 4 treatment groups of 90 eggs with the same initial average weight. The days of storage (DAY) were 0 h, 24 h, 48 h, and 72 h. At the end of storage time for each treatment, eggs were analyzed for weight, breaking strength, and HU by use of a DET6000 digital egg tester (NABEL Co., Ltd., Japan). The data were analyzed with SAS for Windows Version 9.4 by using Proc CORR and Proc REG for Pearson Correlation Coefficients. A strong negative correlation was observed between HU and DAY at 35C ($r = -0.6950$, $P < 0.001$), which was moderate at 23C ($r = -0.5198$, $P < 0.001$), and weaker at 4C ($r = -0.2153$, $P < 0.001$). Linear regression was calculated to predict HU based on DAY at different temperatures. The DAY had a significant impact on HU at 35C ($P < 0.001$)

and 23C ($P < 0.001$). At 35C, the HU is equal to 86.7 – 6.0 (DAY) and 87.0 – 3.4 (DAY) at 23C. Based on these results, HU decreased by 6.0 per DAY at 35C and decreased by 3.4 per DAY at 23C for Cobb 500 breeder eggs. On the other hand, no significant correlation was confirmed between DAY and either egg weight or breaking strength at 4C, 23C, or 35C. The results of the current study indicated that increased DAY and temperature does affect current Cobb 500 breeder egg interior quality, specifically HU. The HU is considered one of the indicators correlated with hatchability, with higher HU associated with higher hatchability. Further investigation is needed to evaluate the impact of breeder egg storage time and Haugh Units on hatchability and chick quality.

Key Words: broiler breeder, egg quality, Haugh units, temperature, storage time

614P Evaluation of heat stress as a model to induce gastrointestinal leakage in broiler chickens. Jared Ruff*, Justin Blankenship, Howard Lester, Callie Selby, B. Graham, Christine Vuong, Sami Dridi, Elizabeth Greene, Billy Hargis, and Guillermo Tellez, *University of Arkansas, Fayetteville, Arkansas, United States.*

Enteric inflammation models can help researchers' study methods to improve health and performance and evaluate various growth promoters and dietary formulations targeted to improve performance in poultry. Oral administration of fluorescein isothiocyanate-dextran (FITC-d; 3–5 kDa) to evaluate pericellular mucosal epithelial leakage is an established biomarker to evaluate enteric inflammation in multiple species. The purpose of this study was to evaluate heat stress (HS) as a model to induce gastrointestinal leakage in broiler chickens. On day of hatch, 320 chicks were allocated into 8 environmental chambers, 4 thermoneutral (TN) and 4-heat stress (HS). Each chamber was divided into 2 pens containing separate feeders and water jugs (8 replicates per treatment, 20 birds/pen). The environment was established to simulate production setting as best possible for the first 21 d. Birds were housed in environmental chambers. A gradual reduction on temperature from 32°C to 24°C with relative humidity at $55 \pm 5\%$ for the first 21 d. At the time of heat stress, the heat stress groups were exposed to 35°C from d 21–42; while thermoneutral was maintained at 24°C from d 21–42. Chickens were equipped with a ThermoChron temperature logger for continuous monitoring of core body temperature. The environmental temperature and relative humidity were continuously recorded. Blood was collected on 21d before HS and then on 28d, 35d and at the end of the experiment on 42d to measure from 2 chickens/replicate ($n = 16$) were randomly selected, and given an oral gavage dose of FITC-d. After one h of oral gavage, blood samples were collected to determine the passage of FITC-d. Tibias were removed from all chickens to evaluate break strength (kg) only on 21d and 42d (before HS and at the end of the trial). Performance parameters were evaluated weekly from 21d to the end of the trial. Chronic HS caused an increase in core body temperature which decreased feed intake, body weight, and feed efficiency (28d, 35d and 42d) when compared with control TN chickens ($P < 0.05$). Similarly, serum FITC-d was significantly increased in HS chickens at all points of evaluation. Chronic HS also caused a significant reduction on bone strength at 42d when compared with the control chickens. Body temperature was significantly increased after 2 h of starting HS and remained that way until the end of the study. The results from the present study suggest that HS can be a robust model to induce gut leakage in broiler chickens.

Key Words: heat stress, serum FITC-d, chickens, performance, enteric inflammation

615P Evaluating water consumption, performance parameters, and yield of broilers reared with different water treatment programs under high stocking density and heat stress.

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Water is both essential and one of the most important nutrients for broiler production. A beneficial water source will improve digestion and metabolism, reduce stress, and improve overall bird health and welfare. Chronic heat stress has been shown to have a variety of effects in poultry production, such as lowering overall performance increasing mortality, producing inferior quality meat, lowering antibody production, and lowering thyroid activity. In addition, high stocking density has also been linked to decreased health, growth performance, and carcass yields in broilers. Thus, the objective of this study was to evaluate the effects of 4 different treatment groups, where 3 water treatment programs, comprised of unique combinations of herbal essential oils, *Yucca schidigera* extract, and/or vitamins and electrolytes, were delivered to commercial broilers reared at a high stocking density (0.85 ft²/bird) and compared with a Control group of birds reared at a lower stocking density (1.00 ft²/bird) with no water treatment. A total of 2,724 Ross 708 × Ross 708 as hatched broiler chickens were reared at the SFASU Poultry Research Center in 50 ft² pens for 49 d. Control group birds were stocked under standard conditions at 50 birds/pen, and water treatment group birds were stocked at a higher density of 59 birds/pen. For growth phase d 28 to 49, birds were reared under heat stress at 3.89°C above optimal growing temperature, from 12:00 to 6:00 p.m. daily. Average body weight (BW), feed intake (FI) and feed conversion ratio (FCR) were measured at 13, 33, and 49 d. Mortality and water consumption were measured daily. Carcass yield was performed at 49 d using 4 birds/pen. All data were analyzed using one-way ANOVA in SAS, with means separated using Tukey's range test, at a P-value of 0.05. Results show that all 3 treatment groups receiving a unique combination of essential oils, *Yucca schidigera* extract, and vitamins/electrolytes had a significantly heavier BW, lower FCR, and higher overall water consumption at 49 d when compared with the Control group. No significant differences were detected among the treatment groups for meat yield. In conclusion, the supplementation of these products via drinking water mitigated the negative effects of chronic heat stress, even at a higher stocking density, and increased both performance parameters and water consumption.

Key Words: broiler, essential oils, heat stress, performance, water

616P Evaluating water consumption, performance parameters, and yield of broilers reared on different water treatment programs and under high stocking density and optimal temperature conditions.

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Water is one of the most important nutrients for *Gallus gallus domesticus*, especially those quickly-growing commercial broiler chickens. Adequate water source can be linked to improved digestion and metabolism, reduced stress, and improved overall bird health and welfare. High stocking density has been related to increased bacterial challenges, decreased flock health and growth performance, and reduced carcass yield in broilers. Conversely, rearing broilers at optimal temperature

can maximize their genetic potential through performance, health, and welfare. Therefore, the objective of this study was to evaluate the effects of water treatments comprised of a control group plus 3 different water programs incorporating unique combinations of herbal essential oils, *Yucca schidigera* extract, and/or vitamins and electrolytes. These treatments were delivered to commercial broilers reared at a high stocking density (0.85 ft²/bird) and compared with a Control group of birds reared at a lower stocking density (1.00 ft²/bird) with no water treatment. For the 4 treatment groups, a total of 2,724 Ross 708 × Ross 708 as hatched broiler chickens were reared at the SFASU Poultry Research Center in 50 ft² pens for 49 d. In the Control group, birds were stocked under standard conditions at 50 birds/pen, and for the water treatment groups, birds were stocked at a higher density of 59 birds/pen. Average body weight (BW), feed intake (FI) and feed conversion ratio (FCR) were measured at 13, 33, and 49 d. Mortality and water consumption were measured daily. Carcass yield was performed at 49 d using 4 birds/pen. All data were analyzed using one-way ANOVA in SAS. Means were separated using Tukey's range test at a P-value of 0.05. Results show that a combination of essential oils, *Yucca schidigera* extract, and vitamins/electrolytes statistically increased BW, FCR, and water consumption at 49 d compared with the Control group. No significant differences were observed for meat yield analyses. In conclusion, results suggest that the addition of these products to the water line, in their unique combinations, will significantly improve broiler performance when reared under higher stocking densities.

Key Words: broiler, essential oil, performance, water consumption, yield

617P Effect of growth rate and age on the severity of white striping and woody breast and meat quality characteristics of broilers. Mireille Arguelles-Ramos*¹, Aixa Rivera-Serrano², Paola Cruz-Ramos², Zamara Hernandez², and Francisco Garcia², ¹Clemson University, Clemson, South Carolina, United States, ²University of Puerto Rico, Mayaguez, Puerto Rico, United States.

This study aimed to investigate the effect of growth rate and age of broilers on the severity of certain myopathies, and meat quality characteristics. A total of 400, 1 d-old CobbHubbard chicks were randomly distributed among 20 floor pens and fed one of 2 treatments. The starter, grower, finisher 1 and finisher 2 diets were fed from 1 to 14, 15–28, 29–35 and 36–42 d-of-age, respectively. Treatments consisted of 2 feed presentations: mash (slow growth) or pellet (fast growth). Feed intake (FI), body weight (BW), mortality and adjusted feed conversion ratio (FCR) were determined at the end of each feeding phase. At 33 and 40 d, 2 males per pen were randomly selected, weighted and processed for a meat quality assessment (24 h postmortem), resulting in a randomized complete block design (RCBD) design with a 2x2 factorial (age x feed presentation). Data were analyzed using a 2-way ANOVA. For the performance data, a one-way ANOVA using an RCBD with 10 replicate pens per treatment was employed. The GLM procedure of SAS was used to analyze live performance and meat quality, and means were partitioned by Tukey's test. Statements of statistical significance were based on $P < 0.05$. As expected, broilers fed pelleted feed had higher FI and BW gains ($P < 0.05$). From 29 to 35 d, birds fed pelleted feed had better AdjFCR than mash fed (1.87 vs. 2.35, respectively), and the opposite was observed for the 36–42 d period (2.58 vs. 2.11, respectively). White striping (WS) score was higher for broilers fed pelleted feed (1.43 vs. 1.13; $P < 0.05$). Woody breast (WB) was less severe for younger birds (0.90 vs. 1.75) and those fed mash feed (1.15 vs. 1.50; $P < 0.05$). L* and b* values were higher for 40-d-old birds (63.38 vs. 53.51, and 13.77 vs. 11.16, respectively). pH values were lower for 33

d-old birds (5.82 vs. 5.96), while a* (4.76) and WB shear force (1.13 kg) were higher ($P < 0.05$). Birds fed mash had higher breast moisture content at 33 d-of age (73.42%) and lowest at 40 d-of-age (74.23%), while the other 2 treatments were intermediate. Breast crude protein content was higher for 40 d-old birds fed with mash (21.82%), and lowest for 40 d-old broilers fed pellet (20.96%; $P < 0.05$). No differences were observed on breast ash or crude fat content. It was concluded that under the conditions of this study, growth rate and age could have an impact on the severity of WS and WB and quality characteristics.

Key Words: growth rate, white striping, woody breast, broilers, meat quality

618P Predicting the growth performance of broiler chickens based on the body weight of one day old chicks. Noel Mendoza-Cesareo*², Sergio Gomez-Rosales^{1,2}, Maria de Lourdes Angeles³, and Beatriz Ibarra-Macari⁴, ¹INIFAP, Queretaro, Mexico, ²Autonomous University of Queretaro, Juriquilla, Mexico, ³INIFAP, Mexico City, Mexico, ⁴Excelling SA de CV, Juriquilla, Mexico.

The objective of this research was to evaluate the growth performance of broilers at different phases of growth and carcass traits based on the body weight (BW) of one-d old chicks using a real database (RDB) and a predicted database (PDB). In the RDB, the growth responses of broiler chickens from 8 floor-pen experiments with 330 experimental units were used. Males, females and mixed sexes broilers in groups of 25–30 were allocated in 1x2 m pens provided with a drinker, a feeder and wood shavings. At d 1, 14, 28 and 42 of age the BW of each group of chicks was registered. The feed intake (FI), body weight change (BWC) and the feed conversion ratio (FCR) were calculated for each phase of growth: 1–14, 15–28, 29–42 and 1–42 d of age. Predicting equations to calculate the weight of the breast, thighs and legs from the final BW were added to the RDB. Additionally, the RDB was used to develop a predicting model based on equations, mainly linear, quadratic and potential, for each variable response within each phase of growth to generate the predicted database (PDB). Both the RDB and PDB were assorted in 8 groups according to the BW of chicks at d one of age: from 40.0 to 40.99, 41.0–41.99, 42.0–42.99, 43.0–43.99, 44.0–44.99, 45.0–45.99, 46.0–46.99 and 47.0–47.99 g. The data was subjected to ANOVA and linear regression analysis. All variable responses were significantly ($P < 0.01$) affected by the initial BW and in all of them a highly significant quadratic response was observed in the RDB and PDB. The maximum BW at 42 d of age, the BWC from 1 to 42 d of age, the carcass and the sum of the carcass components weight were observed at an initial BW between 47.57 and 47.66 g in the RDB and between 45.51 and 46.34 in the PDB. The maximum FI from 1 to 42 d of age was observed at a similar BW in one-d old chicks in the RDB (45.4 g) and the PDB (45.7 g). But the BW of one-d old chicks to reach minimum FCR was lower for the RDB (42.3 g) compared with the PDB (45.1 g). Using the results of the linear portion of the slope of each variable response, it was estimated that for each extra gram of BW in one-d chicks the BW at 42 d of age was improved by 63.2 and 76.2 g, the breast weight was improved by 11.4 and 13.9 g and the carcass components weight was improved by 22.1 and 26.9 g using the RDB and the PDB, respectively. In summary, the initial BW of chicks significantly affected the growth response and carcass measurements at 42 d of age. The initial BW necessary to reach maximum growth and carcass responses was underestimated by about 4% and the improvements in the growth and carcass traits were overestimated by about 21% when using a PDB compared with the RDB.

Key Words: growth performance, carcass traits, one-day old, real database, predicted database

619P Evaluating the effects of XPC, TASC0, and Betaine fed to Pekin ducks reared in heat-stressed and non-stressed environments. Eric Sobotik*, Gabrielle House, Jill Nelson, and Greg Archer, *Texas A&M University, College Station, Texas, United States.*

High ambient temperatures can have a major impact on the performance of commercial poultry. Heat stress can result in lower body weight, decreased feed consumption, higher feed conversion, and increased stress. A study was conducted to evaluate the effects of XPC, TASC0, and Betaine fed to Pekin ducks reared in heat-stressed and non-stressed environments. A study with 384 MLF Pekin ducks, housed in 32 floor pens (1.67m²) from 1 to 35 d of age was conducted to evaluate the effects of XPC, TASC0, and Betaine fed to Pekin ducks reared in heat-stressed and non-stressed environments. Ducks were randomly allocated to one of 4 dietary treatments: Control (CON); XPC (XPC, 1.25kg/MT); TASC0 (TS, 0.50kg/MT); Betaine (BT, 2.0kg/MT). All birds were weighed on d35 to obtain performance parameters: body weight (BW), feed consumption (FC), feed conversion ratio (FCR), and mortality percentage (MORT). Beginning on d21, each dietary treatment was heat-stressed (+HS) or non-stressed (+NS) for a period of 14 d. Heat-stressed birds experienced elevated temperatures (32°C-35°C) for 16hrs/day. On d35, blood was collected from 20 birds per treatment; blood was analyzed for plasma corticosterone (CORT), and heterophil to lymphocyte ratios (H/L). On d35, ileum sections were collected from 10 birds per treatment to assess villus height (VH), crypt depth (CD), and villus/crypt ratio (V/C). All data was analyzed via One-Way ANOVA using GLM with treatment means deemed significantly different at $P < 0.05$. No differences were observed in d35 BW between dietary treatments. Heat-stressed birds weighed less (2.79 ± 0.03 kg, $P < 0.05$) than non-stressed birds (3.10 ± 0.04 kg, $P < 0.05$). From d1 to d35, XPC consumed more feed (131.65 ± 2.59 g/b/d, $P < 0.05$) than CON (126.96 ± 2.10 g/b/d, $P < 0.05$). From d1 to d35, heat-stressed birds consumed less feed (123.93 ± 1.18 g/b/d, $P < 0.05$) than non-stressed birds (136.89 ± 1.72 g/b/d, $P < 0.05$). No differences were observed in FCR from d1 to d35 between dietary treatments, heat-stressed, or non-stressed birds. No differences were observed in CORT levels between dietary treatments. CORT levels were higher in heat-stressed (9087.46 ± 605.05 , $P < 0.05$) than non-stressed birds (6531.67 ± 478.67 , $P < 0.05$). H/L was higher in CON (0.46 ± 0.04 , $P < 0.05$) than all dietary treatments (0.35 ± 0.01 , $P < 0.05$). Heat-stressed birds had higher H/L (0.42 ± 0.02 , $P < 0.05$) than non-stressed birds (0.33 ± 0.02 , $P < 0.05$). V/C was lower in heat-stressed (4.39 ± 0.20 , $P < 0.05$) than non-stressed birds (4.74 ± 0.20 , $P < 0.05$). Results from this study indicate supplementation with XPC, TASC0, and Betaine may reduce the negative attributes associated with heat stress, ultimately improving performance and welfare in Pekin ducks.

Key Words: duck, heat stress, performance, intestinal health, corticosterone

620P Evaluating the effects of safmannan, green tea extract, and Poultry Star fed to Pekin ducks reared in heat-stressed and non-stressed environments. Eric Sobotik*, Gabrielle House, Jill Nelson, and Greg Archer, *Texas A&M University, College Station, Texas, United States.*

High ambient temperatures can have a major impact on the performance of commercial poultry. Heat stress can result in lower body weight, decreased feed consumption, higher feed conversion, and increased stress. A study was conducted to evaluate the effects of Safmannan, Green Tea Extract, and Poultry Star fed to Pekin ducks reared in heat-stressed and non-stressed environments. Day-old MLF Pekin ducklings were randomly allocated to one of 4 dietary treatments: Control

(CON); Safmannan (SAF, 0.25kg/MT); Green Tea Extract (GT, 0.50kg/MT); Poultry Star (PS, 0.55kg/MT). All birds were weighed on d35 to obtain performance parameters: body weight (BW), feed consumption (FC), feed conversion ratio (FCR), and mortality percentage (MORT). Beginning on d21, each dietary treatment was heat-stressed (+HS) or non-stressed (+NS) for a period of 14 d. Heat-stressed birds experienced elevated temperatures (32°C-35°C) for 16hrs/day. Blood was analyzed for plasma corticosterone (CORT), and heterophil to lymphocyte ratios (H/L). On d35, ileum sections were collected from 10 birds per treatment to assess villus height (VH), crypt depth (CD), and villus/crypt ratio (V/C). All data was analyzed via One-Way ANOVA using GLM with treatment means deemed significantly different at $P < 0.05$. No differences were observed in d35 BW between dietary treatments. Heat-stressed birds weighed less (2.82 ± 0.02 kg, $P < 0.05$) than non-stressed birds (3.08 ± 0.02 kg, $P < 0.05$). From d1 to d35, no differences were observed in FC between dietary treatments. From d1 to d35, heat stressed birds consumed less feed (131.16 ± 1.34 g/b/d, $P < 0.05$) than non-stressed birds (137.93 ± 1.53 g/b/d, $P < 0.05$). No differences were observed in FCR between dietary treatments, heat-stressed, or non-stressed birds. No differences were observed in CORT levels between dietary treatments. CORT levels were higher in heat-stressed (6594.61 ± 576.62 , $P < 0.05$) than non-stressed birds (4536.50 ± 376.00 , $P < 0.05$). H/L ratios were higher in CON (0.62 ± 0.03 , $P < 0.05$) than SAF (0.48 ± 0.03 , $P < 0.05$). H/L ratios were higher in heat-stressed (0.59 ± 0.03 , $P < 0.05$) than non-stressed birds (0.50 ± 0.02 , $P < 0.05$). No differences were seen in VH, CD, or V/C between dietary treatments, heat-stressed, or non-stressed birds. Results from this study indicate supplementation with Safmannan, Green Tea Extract, and Poultry Star may reduce the negative attributes associated with heat stress, ultimately improving performance and welfare in Pekin ducks.

Key Words: duck, heat stress, performance, corticosterone

621P Evaluating the effects of MicroSaf, and essential oils fed to Pekin ducks reared in heat-stressed and non-stressed environments. Eric Sobotik*, Gabrielle House, Jill Nelson, and Greg Archer, *Texas A&M University, College Station, Texas, United States.*

High ambient temperatures can have a major impact on the performance of commercial poultry. Heat stress can result in lower body weight, decreased feed consumption, higher feed conversion, and increased stress. A study was conducted to evaluate the effects of MicroSaf, and essential oils fed to Pekin ducks reared in heat-stressed and non-stressed environments. Day-old MLF Pekin ducklings were randomly allocated to one of 4 dietary treatments: Control (CON); Essential Oil #1 (EO1, 0.09kg/MT); MicroSaf (MS, 0.50kg/MT); Essential Oil #2 (EO2, 0.50kg/MT). All birds were weighed on d35 to obtain performance parameters: body weight (BW), feed consumption (FC), feed conversion ratio (FCR), and mortality percentage (MORT). Beginning on d21, each dietary treatment was heat-stressed (+HS) or non-stressed (+NS) for a period of 14 d. Heat-stressed birds experienced elevated temperatures (32°C-35°C) for 16hrs/day. Blood was analyzed for plasma corticosterone (CORT), and heterophil to lymphocyte ratios (H/L). On d35, ileum sections were collected from 10 birds per treatment to assess villus height (VH), crypt depth (CD), and villus/crypt ratio (V/C). All data was analyzed via One-Way ANOVA using GLM with treatment means deemed significantly different at $P < 0.05$. No differences were observed in d35 BW between dietary treatments. Heat-stressed birds weighed less (2.74 ± 0.03 kg, $P < 0.05$) than non-stressed birds (3.06 ± 0.03 kg, $P < 0.05$). From d1 to d35, no differences were observed in FC between dietary treatments. From d1 to d35, heat stressed birds consumed less feed (119.09 ± 1.31 g/b/d, $P < 0.05$) than non-stressed

birds (131.60 ± 1.83 g/b/d, $P < 0.05$). From d1 to d35, EO1 had a lower FCR (1.51 ± 0.02 , $P < 0.05$) than CON (1.59 ± 0.02 , $P < 0.05$). No differences were observed in FCR from d1 to d35 between heat-stressed and non-stressed birds. No differences were observed in CORT levels or H/L ratios between dietary treatments, heat-stressed, or non-stressed birds. VH was greater in EO1 (775.82 ± 19.07 , $P < 0.05$) compared with CON (686.00 ± 37.73 , $P < 0.05$). VH was reduced in heat-stressed birds (689.64 ± 22.24 , $P < 0.05$) compared with non-stressed (753.60 ± 15.49 , $P < 0.05$). V/C was greater in EO2 and MS (5.29 ± 0.15 , $P < 0.05$) compared with CON (4.38 ± 0.36 , $P < 0.05$). Results from this study indicate supplementation with MicroSaf and essential oils may reduce the negative attributes associated with heat stress, ultimately improving performance and welfare in Pekin ducks.

Key Words: duck, heat stress, performance, intestinal health, corticosterone

622P Evaluating the effects of XPC, TASCO, and Betaine fed to broilers reared in heat-stressed and non-stressed conditions.

Eric Sobotik*, Gabrielle House, Jill Nelson, and Greg Archer, *Texas A&M University, College Station, Texas, United States.*

High ambient temperatures can have a major impact on the performance of commercial poultry. Heat stress can result in lower body weight, decreased feed consumption, higher feed conversion, and increased stress. A study was conducted to evaluate the effects of XPC, TASC0, and Betaine fed to broilers reared in heat-stressed and non-stressed environments. Day-old Cobb 500 male broiler chicks were randomly allocated to one of 4 dietary treatments: Control (CON); XPC (XPC, 1.25kg/MT); TASC0 (TS, 0.50kg/MT); Betaine (BT, 2.0kg/MT). All birds were weighed on d14, d28, and d42 to obtain performance parameters: body weight (BW), feed consumption (FC), feed conversion ratio (FCR), and mortality percentage (MORT). Beginning on d28, each dietary treatment was heat-stressed (+HS) or non-stressed (+NS) for a period of 14 d. Heat-stressed birds experienced elevated temperatures (32°C - 35°C) for 16hrs/day. Blood was analyzed for plasma corticosterone (CORT), heterophil to lymphocyte ratios (H/L), and anti-KLH IGG titers (KLH). On 42 d, bilateral metatarsal traits were measured from 60 birds per treatment to assess physical asymmetry (ASYM). On d42, ileum sections were collected from 10 birds per treatment to assess villus height (VH), crypt depth (CD), and villus/crypt ratio (V/C). All data was analyzed via One-Way ANOVA using GLM with treatment means deemed significantly different at $P < 0.05$. Heat-stressed birds weighed less (2.85 ± 0.05 kg, $P < 0.05$) than non-stressed birds (3.01 ± 0.07 kg, $P < 0.05$). From d28 to d42, heat-stressed birds consumed less feed (173.34 ± 5.52 g/b/d, $P < 0.05$) than non-stressed birds (194.58 ± 5.80 g/b/d, $P < 0.05$). No differences were observed in FCR from d28 to d42 between heat-stressed and non-stressed birds. CORT levels were higher in CON (4107.93 ± 811.05 , $P < 0.05$) compared with all dietary treatments (2110.82 ± 273.88 , $P < 0.05$). Heat-stressed birds had higher CORT levels (3224.59 ± 471.31 , $P < 0.05$) than non-stressed birds (1995.61 ± 345.10 , $P < 0.05$). H/L was greater in heat-stressed birds (0.92 ± 0.05 , $P < 0.05$) compared with non-stressed birds (0.69 ± 0.05 , $P < 0.05$). ASYM was greater in CON (1.58 ± 0.08 , $P < 0.05$) compared with all dietary treatments (1.35 ± 0.90 , $P < 0.05$). No differences were observed in V/C or CD between dietary treatments, heat-stressed, or non-stressed birds. VH was reduced in heat-stressed birds (910.86 ± 23.59 , $P < 0.05$) compared with non-stressed birds (1010.64 ± 27.36 , $P < 0.05$). Results from this study indicate supplementation with XPC, TASC0, and Betaine may reduce the negative attributes associated with heat stress, ultimately improving performance and welfare in broilers.

Key Words: broiler, heat stress, performance, intestinal health, corticosterone

623P Evaluating the effects of safmannan, green tea extract, and Poultry Star fed to broilers reared in heat-stressed and non-stressed conditions. Eric Sobotik*, Gabrielle House, Jill Nelson, and Greg Archer, *Texas A&M University, College Station, Texas, United States.*

High ambient temperatures can have a major impact on the performance of commercial poultry. Heat stress can result in lower body weight, decreased feed consumption, higher feed conversion, and increased stress. A study was conducted to evaluate the effects of Safmannan, Green Tea Extract, and Poultry Star fed to broilers reared in heat-stressed and non-stressed environments. Day-old Cobb 500 male broiler chicks were randomly allocated to one of 4 dietary treatments: Control (CON); Safmannan (SAF, 0.25kg/MT); Green Tea Extract (GT, 0.50kg/MT); Poultry Star (PS, 0.55kg/MT). All birds were weighed on d14, d28, and d42 to obtain performance parameters: body weight (BW), feed consumption (FC), feed conversion ratio (FCR), and mortality percentage (MORT). Beginning on d28, each dietary treatment was heat-stressed (+HS) or non-stressed (+NS) for a period of 14 d. Heat-stressed birds experienced elevated temperatures (32°C - 35°C) for 16hrs/day. Blood was analyzed for plasma corticosterone (CORT), and anti-KLH IGG titers (KLH). On 42 d, bilateral metatarsal traits were measured from 60 birds per treatment to assess physical asymmetry (ASYM). On d42, ileum sections were collected from 10 birds per treatment to assess villus height (VH), crypt depth (CD), and villus/crypt ratio (V/C). All data was analyzed via One-Way ANOVA using GLM with treatment means deemed significantly different at $P < 0.05$. No differences were observed in BW at d42 between dietary treatments, heat-stressed, or non-stressed birds. From d28 to d42, no differences were observed in FC or FCR between dietary treatments, heat-stressed, or non-stressed birds. CORT was reduced in GT (3451.85 ± 454.80 , $P < 0.05$) when compared with CON (10861.68 ± 3303.62 , $P < 0.05$). VH was greater in SAF (1002.85 ± 36.48 , $P < 0.05$) compared with GT and PS (865.63 ± 29.72 , $P < 0.05$). V/C was greater in SAF, GT, and CON (5.76 ± 0.19 , $P < 0.05$) compared with PS (4.66 ± 0.31 , $P < 0.05$). KLH titers were higher in SAF, GT, and PS (1797.48 ± 24.97 , $P < 0.05$) compared with CON (1524.18 ± 170.22 , $P < 0.05$). Heat-stressed birds had lower KLH (1680.28 ± 60.32 , $P < 0.05$) than non-stressed birds (1796.87 ± 49.66 , $P < 0.05$). Results from this study indicate supplementation with Safmannan, Green Tea Extract, and Poultry Star may reduce the negative attributes associated with heat stress, ultimately improving performance and welfare in broilers.

Key Words: broiler, heat stress, performance, intestinal health, corticosterone

624P Evaluating the effects of MicroSaf, and essential oils fed to broilers reared in heat-stressed and non-stressed conditions.

Eric Sobotik*, Gabrielle House, Jill Nelson, and Greg Archer, *Texas A&M University, College Station, Texas, United States.*

High ambient temperatures can have a major impact on the performance of commercial poultry. Heat stress can result in lower body weight, decreased feed consumption, higher feed conversion, and increased stress. A study was conducted to evaluate the effects of MicroSaf, and essential oils fed to broilers reared in heat-stressed and non-stressed environments. Day-old Cobb 500 male broiler chicks were randomly allocated to one of 4 dietary treatments: Control (CON); MicroSaf (MS, 0.50kg/MT); Essential Oil #1 (EO1, 0.09kg/MT); Essential Oil

#2 (EO2, 0.50kg/MT). All birds were weighed on d14, d28, and d42 to obtain performance parameters: body weight (BW), feed consumption (FC), feed conversion ratio (FCR), and mortality percentage (MORT). Beginning on d28, each dietary treatment was heat-stressed (+HS) or non-stressed (+NS) for a period of 14 d. Heat-stressed birds experienced elevated temperatures (32°C-35°C) for 16hrs/day. Blood was analyzed for plasma corticosterone (CORT), heterophil to lymphocyte ratios (H/L), and anti-KLH IGG titers (KLH). On 42 d, bilateral metatarsal traits were measured from 60 birds per treatment to assess physical asymmetry (ASYM). On d42, ileum sections were collected from 10 birds per treatment to assess villus height (VH), crypt depth (CD), and villus/crypt ratio (V/C). All data was analyzed via One-Way ANOVA using GLM with treatment means deemed significantly different at $P < 0.05$. From d28 to d42, no differences were observed in FC or FCR between dietary treatments. From d28 to d42, heat-stressed birds consumed less feed (162.59 ± 1.50 g/b/d, $P < 0.05$) than non-stressed birds (199.55 ± 1.90 g/b/d, $P < 0.05$). From d28 to d42, FCR was higher in heat stressed birds (2.14 ± 0.02 , $P < 0.05$) than non-stressed birds (1.80 ± 0.02 , $P < 0.05$). From d1 to d42, FC was greater in MS, EO1 and EO2 (116.88 ± 1.52 g/b/d, $P < 0.05$) compared with CON (111.82 ± 1.90 g/b/d, $P < 0.05$). From d1 to d42, heat-stressed birds consumed less feed (109.95 ± 0.53 g/b/d, $P < 0.05$) than non-stressed birds (121.28 ± 1.50 g/b/d, $P < 0.05$). From d1 to d42, FCR was lower in EO1 (1.59 ± 0.02 , $P < 0.05$) compared with EO2 (1.62 ± 0.02 , $P < 0.05$). CORT levels were reduced in EO2 (2912.71 ± 376.30 , $P < 0.05$) compared with CON (5374 ± 1205.49 , $P < 0.05$). Heat-stressed birds had higher CORT levels (4865.35 ± 731.04 , $P < 0.05$) than non-stressed birds (3195.28 ± 346.06 , $P < 0.05$). Results from this study indicate supplementation with MicroSaf and essential oils may reduce the negative attributes associated with heat stress, ultimately improving performance and welfare in broilers.

Key Words: broiler, heat stress, performance, intestinal health, corticosterone

625P Evaluating the timing of phosphatidic acid supplementation on growth performance and carcass yield in broilers. Eric Sobotik^{*1}, Gabrielle House¹, Austin Stiewert¹, Austin Brown¹, Scott Hagerman², and Greg Archer¹, ¹Texas A&M University, College Station, Texas, United States, ²Cheminutra, Austin, Texas, United States.

With a growing increase in further processing of poultry, there has been an increased interest factors including feed additives that may improve broiler performance increase growth, and influence dressing percentage. Mammalian target of rapamycin (mTOR) is known to play vital roles in protein synthesis; mTOR controls the anabolic and catabolic signaling of skeletal muscle mass, resulting in the modulation of muscle hypertrophy. Exogenous phosphatidic acid (PA) can stimulate the mTOR pathway via its activation of the substrate S6 kinase. A study with 648 Cobb 500 male broilers, housed in 36 floor pens (1.11m²) from 1 to 42 d of age was conducted to evaluate the timing of PA (Mediator[®] 50P, Chemi Nutra) supplementation on the growth performance and carcass yield of broilers. Dietary treatments included: T1, Control (CON), T2, 5mg/bird/day of PA for 42 d (d1-42), T3, 5mg/bird/day of PA for 28 d (d14-42), T4, 5 mg/bird/day of PA for 14 d (d8-42). All birds were weighed on d14, 28, and 42 to obtain BW (body weight), FC (feed consumption), FCR (feed conversion ratio), and MORT (mortality percentage). On d42, 8 birds per pen were processed to determine carcass and breast meat yield. All data was analyzed via One-Way ANOVA using GLM with treatment means deemed significantly different at $P < 0.05$. No differences were observed in BW at d14 or d28. At d42, birds fed T2 were heavier (3.73 ± 0.02 , $P < 0.05$) than all dietary treatments (3.68 ± 0.02). From d1 to d28, birds fed T2 had the lowest FCR (1.423 ± 0.005 , $P < 0.05$) compared

with all dietary treatments (1.441 ± 0.005). From d1 to d42, birds fed T2 and T3 had a lower FCR (1.545 ± 0.014 , $P < 0.05$) when compared with the CON (1.609 ± 0.013). No differences were observed in FC or MORT between treatments during growout. Increased BW observed in birds fed T2 translated to increased breast fillet weight (0.772 ± 0.009 kg, $P < 0.05$) when compared with the CON (0.743 ± 0.008 kg). Carcass yields were increased in birds fed T2 (77.48 ± 0.32 kg, $P < 0.05$) when compared with all dietary treatments (76.24 ± 0.16 kg). Utilizing PA for 42 d increased live weights, improved FCR increased carcass yield, and increased breast fillet weight at processing. Results from this study indicate supplementation of PA during all phases of growth may increase the production efficiency of broilers.

Key Words: phosphatidic acid, performer, growth, broiler, yield

626P Not Presented

627P Use of Alquernat Immuplus (botanical product) to improve the efficacy of vaccination against hemorrhagic enteritis in turkeys. David Díez, Carlos Domenech, Júlia Pié Orpí*, Maria Soriano, and Anna Tesouro, Biovet, S.A., Constantí, Tarragona, Spain.

Background: Alquernat Immuplus is a natural product based on active botanical molecules called pronutrients that improve the physiology of the immune system (innate and adaptative) and optimizes response to vaccination to achieve a better protection against infections. Objective: An experiment was conducted to evaluate whether Alquernat Immuplus improved the response to the vaccination against hemorrhagic enteritis (HE) in turkeys. Methods: The trial was carried out in an experimental farm with 536 one-day-old male turkeys (B.U.T. 6). Turkeys were raised for 21 weeks and allotted to 2 treatments: 1) "Control" (vaccinated animals that did not receive any immunobooster); and 2) "Immuplus" (vaccinated animals that received Alquernat Immuplus at 1 mL/l the 5 d prior and post vaccination). Antibody titers were analyzed the day before, 21 and 63 d after HE vaccination. Higher antibody titers are correlated to a greater protection against HE. Results: Pre-vaccination, average antibody titers for HE in the Control and Immuplus were 0.450 and 0.325, respectively. On the 21st day post-vaccination, titers were 2.550 (Control) and 2.200 (Immuplus), and on the 63rd day post vaccination, titers were 6.200 and 6.775, respectively. Analysis of the results: Despite Immuplus group started with lower antibody titers against HE (27.78% lower than the control), it had higher titers than the control group on the 63rd post-vaccination (9.27% higher), thanks to a significantly greater increase of the antibody titers. Conclusions: Water supplementation with Alquernat Immuplus the days before and after vaccination markedly improved vaccine response in turkeys, which is correlated with a better protection against HE.

Key Words: immunity, vaccination, turkeys, adenovirus, hemorrhagic enteritis

628P Choline supplementation enhances production performance, tocopherol content, and oxidative stability in layer hens fed flaxseed. M. H. Behesti Moghadam, A. Aziza, and G. Cherian*, Oregon State University, Corvallis, Oregon, United States.

Choline is an essential nutrient in laying hen diets and is needed for the formation of phosphatidylcholine (PC), that serves as a rich source of long chain ($\geq 20C$) n-3 fatty acids (FA) in eggs. Methionine (Met) is the first limiting amino acid in layer hen diets and serves as a lipotropic

agent with antioxidant properties. The objectives of the current study is based on the hypothesis that choline and Met supplementation will enhance egg PC and tocopherol content, n-3 FA status, lipid stability, and production indices in layer hens fed flaxseed. A total of 96, 40 week-old laying hens (White Leghorns) were randomly allocated to 4 treatment groups, with 6 replicates containing 4 hens per each replicate. The hens were kept in individual cages and were fed corn-soybean meal-based diet containing 0% flaxseed (Control), 15g/100g flaxseed (Flax), Flax+50% more methionine (Flax+ Met), or Flax+0.15g /100g choline chloride (Flax+Cho). All the diets were isocaloric and isonitrogenous. The experimental diets were fed for a period of 120 d. Egg mass and hen-day egg production was highest for Flax+Cho ($P < 0.05$). Addition of choline chloride led to an increase in egg weight in flaxseed-fed hens ($P < 0.05$). Choline supplementation increased α -tocopherol content ($P < 0.05$) while reducing lipid oxidation products measured as thiobarbituric acid reactive substances in egg yolk ($P < 0.05$). Feeding flaxseed led to over 6-fold increase in total n-3 FA. Neither Met or choline had any impact on α -linolenic (18:3 n-3), or docosahexaenoic (22:6 n-3) acid incorporation in hens fed flaxseed. However, addition of Met and choline led to an increase in docosapentaenoic acid (22:5 n-3) in hens fed flaxseed ($P < 0.05$). The PC content was lowest in Flax+Met ($P < 0.05$) and was not different in Control, Flax and Flax+Cho ($P > 0.05$). No difference was found in total lipid or phosphatidylethanolamine content of eggs ($P > 0.05$). The results from the current study suggest that n-3 FA content of eggs can be greatly increased by feeding flaxseed and supplementing choline chloride can improve production performance, egg quality, tocopherol content and oxidative stability of eggs from hens fed flaxseed. As consumer demand for n-3 FA-rich eggs are increasing, choline could be used as a feed additive for enhancing egg antioxidant status and lipid stability while increasing hen productivity.

Key Words: choline, flaxseed, hen, eggs, tocopherol

629P Not Presented

630P Effect of protected organic acids + essential oils on intestinal health and performance of broiler chickens. Margot Poujol*, Roxann Scott-Delaunay, Jean-Christophe Bodin, Mariana Lemos de Moraes, Ludovic Lahaye, and Elizabeth Santin, *Jefo Nutrition Inc., Saint-Hyacinthe, Quebec, Canada.*

The poultry industry has been reducing antibiotic use over the last years. However, it is not without consequences on gut health when broilers face intestinal challenges. Most of the intestinal disorders cause inflammation, which may negatively impact the digestive processes or nutrient absorption. As a result, body weight gain (BWG) or/and feed conversion ratio (FCR) may be impaired. To improve gut health and minimize performance losses, an experiment was conducted to evaluate the effects of Protected Organic Acids + Essentials Oils P (OA+EO – Jefo) on broiler chickens under an intestinal challenge. A total of 1,080 male chickens (Ross 308) were raised until 35 d of age and distributed into 3 treatments (T) with 12 replicates. They were - T1: Negative Control (NC) – not challenged, not supplemented; T2: Positive Control (PC) – challenged, not supplemented; T3: P(OA+EO) – challenged, supplemented with P(OA+EO). The intestinal challenge was performed at 17 d using a coccidiosis challenge model (200 000 oocysts/bird of *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella* administrated in the feed). The birds were reared on floor pens (30/pen) and fed *ad libitum*. Each pen represented an experimental unit. On d 22 (5 d after challenge), a jejunum sample from one bird per pen was collected and analyzed by the *I see Inside* (ISI) scoring system methodology. In this

methodology, an impact factor (IF) ranging from 1 to 3 is defined for each alteration on tissue according to the reduction of the organ's functional capacity, where 3 is the most impactful alteration for the organ function. In addition, the intensity and extension of each alteration is evaluated and a score from 0 to 3 is assigned, 0 being the absence of lesion. To obtain the final value of the ISI index, the IF of each alteration is multiplied by the respective score number, and the results of all alterations are summed according to the formula $ISI = \sum(IF * S)$, where IF = impact factor and S = Score. Data was analyzed using one-way ANOVA, followed by post hoc Fisher LSD test. The challenge significantly increased ($P < 0.05$) ISI score of inflammatory parameters (lamina propria thickness, inflammatory cell infiltration in the lamina propria and epithelium). As a result, BWG and FCR were impaired at 21, 28 and 35 d. The P(OA+EO) group had a healthier gut, represented by a lower ISI score, and better FCR than PC group at 28 and 35 d ($P < 0.05$). These results demonstrate the progressive gut health and performance benefits of the evaluated Protected Organic Acids + Essential Oils on broiler chickens undergoing an intestinal challenge.

Key Words: broiler, ISI, intestinal mucosa, challenge

631P *Bacillus amyloliquefaciens* CECT 5940 improved intestinal morphology and performance in broiler submitted to coccidial vaccine challenge . Juliano Cesar De Paula Dorigam*, *Evonik Nutrition & Care GmbH, Hanau, Hessen, Germany.*

Coccidiosis is responsible for substantial economic losses to the poultry industry. The reduction in performance is also attributed to the loss of intestinal integrity resulting in shorter and thicker villi and a reduction in the intestinal absorptive area. Possible solutions can be found in organic acids and probiotics, which can enhance nutrient utilization by improving gut integrity. Thus, this study was designed to investigate the effect of the probiotic strain *Bacillus amyloliquefaciens* CECT 5940 fed alone or in combination with butyrate in broiler. Six hundred 72 Ross 308 male chicks were randomly assigned to 5 treatments with 8 pen replicates and 14 birds each. Basal diets were formulated to meet nutritional recommendations for starter (1–21 d) phase. The treatments were: 1) Positive control (PC, Basal diet without feed additives and no challenge), 2) negative control (NC, Basal diet without feed additives and challenge), 3) NC + *B. amyloliquefaciens* CECT 5940 (10^6 cfu/g feed), 4) NC + calcium butyrate (300g/ton feed) and 5) NC + calcium butyrate (300g/ton feed) + *B. amyloliquefaciens* CECT 5940 (10^6 cfu/g feed). The challenge model consisted in the application of a multi-strain anticoccidial vaccine at 10 times the dosage at d 14. Parameters evaluated from 1 to 21 d were weight gain (WG), feed intake (FI), and feed conversion ratio (FCR). On d 21, ileal tissues were collected to measure villus height (VH), crypt depth (CD) and VH:CD ratio. Data were analyzed by one-way ANOVA followed by mean comparison with SNK test at 5% of probability. There were no significant differences ($P > 0.05$) between treatments for FI or VH. Birds fed butyrate, *B. amyloliquefaciens* CECT 5940 or its combination had no significant difference ($P > 0.05$) in WG (835, 865 and 866 g, respectively) and FCR (1.316, 1.240 and 1.242 g/g, respectively) when compared with PC (875 g and 1.276 g/g, respectively). However, only birds fed *B. amyloliquefaciens* CECT 5940 alone or in combination with butyrate had significantly higher ($P < 0.05$) WG and FCR compared with the NC (808 g and 1.336 g/g, respectively). In addition, *B. amyloliquefaciens* CECT 5940 alone resulted in significantly shorter ($P < 0.05$) crypts depth (130 μ m) and higher VH:CD ratio (4.99) when compared with NC (150 μ m and 4.16, respectively), but no significantly different ($P > 0.05$) from PC (144 μ m and 4.46, respectively). In conclusion, feeding *B. amyloliquefaciens* CECT 5940 alone or in combination with butyrate is able to

improve feed conversion and weight gain as the result of better VH:CD ratio provided by *B. amyloliquefaciens* CECT 5940 supplementation.

Key Words: butyrate, broiler, probiotic, coccidial, vaccine

632P Dietary impact of an additive based on canthaxanthin/polyphenols and egg storage time on the quality and oxidative stability of table eggs. Jorge Tay*¹, Dany Rueda¹, Jennifer Toskano¹, Josselyn Serrano², and Carlos Vilchez², ¹Montana S.A., Lima, Peru, ²Universidad Nacional Agraria La Molina, Lima, Peru.

The effect of an additive based on canthaxanthin and polyphenols (CP) on the quality and oxidative stability (OS) of eggs of laying hens were evaluated. 240 38 wk-old laying hens, with similar body weight and rate of egg production were randomly distributed into 24 experimental units (EU) with 10 birds each. Eight (8) EU received, for 4 weeks, one of the following treatments: T₁, Diet control; T₂, Diet with 30 g of CP/TM, and T₃, Diet with 60 g of CP/TM. Feed (mash form) and water were provided ad libitum. A total of 144 eggs were used for the determination of the quality and oxidative stability. Egg weight (EW), albumen height (AH), Haugh Units (HU), and the yolk index (YI) were determined by using the Egg Digital Meter (DET6000, Nabel Co., Ltd.); additionally, both the pH of the albumen (ApH) and pH of the yolk (YpH) were measured. The OS of egg yolks were determined by measuring the malonaldehyde content (ug MDA/g Y). The measurements were carried out at 0, 7 and 14 d of storage. The data was submitted to ANOVA under a Completely Randomized Design with a 3 × 3 factorial arrangement; for the separation of the means the test LSD was used. With exception of EW, all the variables were influenced significantly ($P < 0.01$) by the time of storage. The dietary treatments did not influenced significantly ($P > 0.05$) the evaluated variables, with exceptions of the YI and of OS. The oxidative stability of the egg yolk (ug MDA/g Y) was significantly lower ($P < 0.01$), both the yolks of 0 d of storage and those from laying hens that received the T₂. In conclusion, the time of storage affects the quality and oxidative stability of the egg yolks and that the inclusion of 30 g of CP/TM improves the oxidative stability of the egg yolks, which will allow a longer shelf life.

Key Words: canthaxanthin, polyphenol, egg quality, oxidative stability, laying hens

633P Comparison of mode of action of dietary enramycin, salinomycin and bacitracin in broiler chickens. Seiichi Saiki*, Yosuke Tsuneyoshi, Kazuki Nakagawa, Akinori Uehara, and Izuru Shinzato, *Ajinomoto Co. Inc., Kawasaki-Shi, Kanagawa-Ken, Japan.*

As strict regulation has been imposed on the use of antibiotics growth promoters (AGPs) in animal feeds, alternatives to AGPs are demanded. Because it is important to understand the mode of action of AGPs for efficient development of AGPs alternatives, this study was conducted to evaluate the difference in the mode of action of 3 AGPs. A total 600 male broiler chicks of 1-d old were employed for a 3-week experiment with 3 replicates of 50 birds per pen. Chicks were allotted to the following 4 dietary treatments; a basal diet without AGPs (CON), enramycin-supplemented diet (EN, 10ppm), salinomycin-supplemented diet (SAL, 50ppm), and zinc bacitracin-supplemented diet (ZB, 168ppm). During the experiment, feed and water were provided ad libitum. Feed intake and body weight per pen were measured weekly. Four chicks with the median weight from each pen were sacrificed to collect plasma, liver, jejunum, ileum, and the intestinal contents on the 7th and 21st day. Another 4 chicks per pen were used to examine the FITC-dextran leakage as a marker of paracellular transport and mucosal barrier dysfunction on

the 22nd day. Plasma and cecal contents were provided for measurement of the metabolites. Cecal and ileal contents were provided for identifying the microbiota. Jejunum and ileum were provided for analyzing gene expressions of parameters related to the gut integrity. Data were analyzed for a one-way ANOVA, and significances were examined using Dunnett's Tests ($P \leq 0.05$). The body weight gain and feed intake were significantly higher and the feed conversion ratio was better for all AGPs-fed groups compared with CON. The FITC-dextran concentration in plasma was lower ($P < 0.05$) for ZB than CON with EN and SAL in-between. Those results suggest that the dietary supplementation of 3 AGPs (Enramycin, Salinomycin and Bacitracin) improved the growth performance, at least in part, through the improvement of the intestinal barrier function in broilers.

Key Words: antibiotics growth promoters, gut health, microbiota, paracellular transport, broiler

634P Encapsulated butyrate supplementation of broiler diets leads to improved feed efficiency and growth performance associated with histomorphological changes of the gastrointestinal tract. Jason Payne*, Oscar Tejada, Justin Fowler, and Laura Ellestad, *University of Georgia, Athens, Georgia, United States.*

With pressure to remove antibiotic growth promoters from animal production, there is a need to investigate alternative feed additives to aide production. Butyrate, a fermentation by-product, is one supplement that has been shown to stimulate cellular growth along the gastrointestinal (GI) tract, reduce inflammation, and have anti-microbial activity. Currently, various forms of butyrate are commercially available in a plethora of protections to target specific segments of the GI tract. To date, few direct comparisons of butyrate presentations have been conducted; therefore, our objective was to evaluate effects of different sources of butyrate on broiler performance and gene expression associated with intestinal integrity, nutrient transport, and the innate immune response. A floor pen trial was conducted with Cobb 500 broilers fed standard rations (Ctrl) or standard rations supplemented with sodium butyrate protected with sodium salts of palm fatty acid (But-FA) or encapsulated with hydrogenated stearin (But-Hyd), with 8 replicate pens per treatment ($n = 8$). Body weight gain (BWG) and feed intake (FI) were determined on days (D) 14, 21, 28, and 35. On D21 and D35, 2 birds from each pen were euthanized and jejunum and cecal tonsils were collected for analysis of mRNA transcripts. Data were analyzed by ANOVA, and differences were determined using Tukey's multiple comparison test. From D21 onwards, both butyrate treatments had a lower cumulative feed conversion ratio (FCR; g FI/g BWG) compared with Ctrl birds (D21 and D28, $P < 0.05$; D35, $P = 0.068$). Moreover, on D35, But-FA ($2441 \text{g} \pm 32$) and But-Hyd ($2414 \text{g} \pm 43$) showed numerical increases in bird weight above the Ctrl ($2367 \text{g} \pm 57$) diet. In jejunum, no significant differences in mRNA expression profiles for genes regulating barrier integrity (e.g., claudin 1 and 2, occludin, zona occludins 1) or carbohydrate transport (e.g., glucose transporters 2, 5, 12, sodium-glucose transporter 1) were observed; however, there was a 5-fold reduction of the antimicrobial factor Cathelicidin-2 in the But-Hyd diet compared with Ctrl on D21 ($P < 0.05$). These data suggest that in the absence of enteric challenge, sodium butyrate supplementation improves feed efficiency regardless of encapsulation. Moreover, this improved feed efficiency may be associated with a reduction in energy needed for mounting an innate immune response.

Key Words: butyrate, encapsulation, histomorphology, epithelium, gastrointestinal tract

635P The effect of a standardized blend of isoquinoline alkaloids on broiler performance under practical conditions.

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Numerous university studies have shown that a standardized blend of isoquinoline alkaloids (IQs) improves broiler performance. As conditions between research facilities and field conditions can vary greatly, it is recommended to reassess former findings from university trials under practical conditions. **Objective** The objective of the study was to evaluate the effects of a standardized formulation of isoquinoline alkaloids (IQs) on performance of broiler chickens under field conditions in Hungary. **Material and Methods** The field report was conducted on a broiler integration in Hungary. Ross 308 and Cobb 500 as-hatched broiler chickens were equally distributed between 2 treatments: 1| Negative Control (NC; 83,610 birds; n = 1); 2| IQs (120 ppm, provided as Sangrovit® Extra; 72,090 birds; n = 1). For both treatments, birds were obtained from the same parent flock and same hatchery. Trial duration was 42 d (d) and IQs were supplied over the whole trial period. Feed was based on corn and soybean meal and birds had *ad libitum* access to feed and water. Feed was supplemented with coccidiostats in both treatment groups (d 0–20: Maxiban, 90 ppm; d 21–32: Elancoban, 100 ppm). **Results** Due to a bad chick quality, mortality was increased in the first week of life, leading to an overall mortality of 5.7% and 6.0% in NC and IQs, respectively. Supplementation of IQs led to a 230 g higher final body weight (2.7 kg and 2.93 kg, respectively) and an improved FCR (1.62 and 1.74, respectively). The improvement was reflected in a higher European Broiler Index for birds fed IQs (343 and 399, respectively). **Conclusion** The inclusion of a standardized formulation of IQs improved performance in broiler chickens under practical conditions. Therefore, IQs offer a beneficial and natural solution to support an efficient and sustainable broiler production.

Key Words: alkaloids, plant extract, field trial, broiler, performance

636P Effect on the vaccination success of a dietary supplementation with a dry grape extract in broilers in conditions of thermic discomfort. Paul Engler¹, Agathe Demortreux*¹, and Christophe Alleno², ¹Nor-Feed, Beaucaouze, France, ²Zootests, Ploufragan, France.

Vaccination is a key aspect of biosecurity in farms. Several studies have shown the negative impact that heat stress can have on immunity in *Gallus gallus domesticus*. The use of natural antioxidants, such as grape polyphenols, is more and more common to help livestock cope with oxidative stress caused by heat stress. The aim of this study was to assess the impact of supplementing broilers with a Dry Grape Extract (Nor-Grape® 80) on the success of vaccination. 360 d-old male chicks were randomly divided in 2 groups (12 cages of 15 birds each), control (CTL, standard diet) and supplemented (NG, diet CTL + 30ppm of Nor-Grape®). Broilers were vaccinated against Gumboro (D17, drinking water) and Infectious Bronchitis (BI, D0 and D12, aerosol spraying). All birds were exposed to a period of thermic discomfort by raising the building temperature to 30°C from D14 to D19 included. On slaughter day (D35), all birds were individually weighed and blood samples were drawn to measure specific antibody titers. The titers were then compared with a threshold reference value to determine whether each bird was considered as immunized against the antigen. Growth performances did not differ significantly between groups (*t*-test) nor did the overall European Poultry Index (405 vs. 407 for CTL and NG respectively, ns, *t*-test). Antibody titers did not differ significantly between the NG and the CTL groups for Gumboro (3602 vs. 2624 respectively, ns, *t*-test)

or BI (444 vs. 405 respectively, ns., *t*-test). However, these results also showed that the supplementation tended to increase the success of vaccination against Gumboro ($P < 0,10$, Khi2) and the proportion of birds immunized against at least one of the 2 vaccines was significantly higher in supplemented chickens ($P < 0,05$, Khi2). These results evidence the potential interest of such a supplementation to support animals in periods of thermic discomfort, to improve the success of vaccination and therefore participate to improving biosecurity in farms.

Key Words: vaccination, chicken, immunity, grape, polyphenols

637P Performance responses and gut bacterial pathogens in broiler chickens raised with either antibiotics or an antibiotic alternative product based on a microencapsulated blend of organic acids and essential oils. Niwat Chansiripornchai*², Supornchai Sri-Nhonghang¹, Jean-Christophe Bodin¹, and Glenmer Tactacan¹, ¹Jefo Nutrition Inc., Saint-Hyacinthe, Quebec, Canada, ²Chulalongkorn University, Bangkok, Thailand.

The issue of antibiotic resistance along with the increasing public demand for antibiotic-free poultry products have led to the emergence of natural-based alternatives used as substitutes to antibiotics in countering bacterial pathogens adversely affecting broiler chickens raised commercially. To this end, a study was conducted to compare the effects of antibiotics with an antibiotic alternative product based on a microencapsulated blend of organic acids and essential oils in broiler performance and gut bacterial pathogens. In a completely randomized design, a total of 80-d-old female broiler chicks were divided into 2 treatment groups of 4 replicates consisting of 10 birds each. Treatment 1 was supplemented with antibiotics during the starter (3–7 d Tilmicosin at 20 mg/kg body weight (BW) via drinking water), grower (14–21 d Bacitracin at 35 g/kg BW in feed), and finisher period (36–42 d Doxycycline at 20 mg/kg BW via drinking water). Treatment 2, was supplemented with a microencapsulated blend of organic acids and essential oils (1–42 d at 300 g/ton of feed). Production performance (final BW, feed conversion ratio (FCR) and mortality) and gut bacterial pathogens were determined at d 42. Cecal samples were collected from each treatment group for *E. coli*, *Salmonella spp.* and *Clostridium perfringens* isolations. In terms of performance, final BW and FCR were not significantly different ($P > 0.05$) between Treatment 1 and 2. Compared with Treatment 1, the final BW was numerically lower (2392.6 vs. 2362.4 g) but FCR was slightly better (1.75 vs. 1.74) in Treatment 2. No mortality was recorded throughout the duration of the study. There was no difference in cecal *E. coli* and *Salmonella spp.* levels, however, cecal *Clostridium perfringens* was reduced significantly in Treatment 2 compared with Treatment 1. Overall, the supplementation of a microencapsulated blend of organic acids and essential oils delivered similar production performance as broilers raised with antibiotics but appeared to offer additional benefits in terms of gut health by reducing the intestinal colonization of a potential bacterial pathogen like *Clostridium perfringens* which can contribute to the risk of necrotic enteritis development in field conditions.

Key Words: microencapsulated, organic acid, essential oil, *Clostridium perfringens*, broiler

638P Not Presented

639P Effect of an organic approved feed additive on broiler performances reared under challenged conditions. Bertrand Medina¹, Ashley Wagner*¹, James McNaughton², and Ivan Girard¹,

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Conversion to no-antibiotics-ever (NAE) or organic broiler systems is often associated with lower growth performances and a slow but consistent decline of feed efficiency during production cycles. Thus, the transition to antibiotic-free poultry operations has to be done carefully with adjusted practices (expended empty periods, litter quality management) and proper nutrition (highly digestible feedstuffs, alternative and helpful feed additives). The objective of this study was to test the efficacy of a novel, proprietary feed additive (Alterna®OP, a blend of phytonutrients, organic acids and functional oils) formulated by Probiotech International Inc. to support broiler performances reared under the National Organic Standard (NOS) guidelines. One-day old Cobb chicks (n = 1872) were randomly assigned to 3 treatments: a basal group (CTL) and 2 groups with distinct inclusion patterns: AOP1 with Alterna®OP at 1.5kg/MT in starter (0–10d) and 1.0kg/MT in grower-finisher (11–39d); AOP2 with Alterna®OP at 1.0kg/MT in starter (0–10d), finisher (30–39d) and 1.5kg/MT in grower (11–29d). Each treatment was fed to 12 replicates of 52 mixed-sex broilers. Diets were in mash form. Birds received a coccidiosis vaccine at the hatchery and were reared on built-up litter (moisture > 25%): 70% new kiln-dried mixed with 30% used litter which contained controlled bacteria and coccidia counts. Individual live weights (LW) were recorded after 10, 29 and 39 d. Feed intake (FI) was measured for each feeding phase, dead birds were counted daily and reported as percentage per time period. Treatment means were compared with a multi-factorial procedure ANOVA. Means were further separated using a Duncan's LSD test and $P < 0.05$ as significant threshold. Cumulative mortality (CM) of the CTL group averaged 5.56%, confirming challenged conditions, and was elevated ($P < 0.05$) compared with treatment groups. CM was also affected by level of AOP ($P < 0.05$; AOP1: 0.87; AOP2: 2.43%). Over the 39d trial, FI was not different ($P > 0.05$) between groups. However, final LW affected ($P < 0.05$; CTL: 2444; AOP1: 2557; AOP2: 2508g) by both AOP supplementation and inclusion pattern. Flock uniformity as coefficient of variation of LW was also significantly improved ($P < 0.05$) with supplementation, but not impacted ($P > 0.05$) by level (CTL: 15.89; AOP1: 12.15; AOP2: 12.09%). Consequently, a better ($P < 0.05$; CTL: 1.72; AOP1: 1.63; AOP2: 1.66) feed conversion was reported for the supplemented groups compared with CTL one with no effect ($P > 0.05$) of level. Collectively, the results indicate that the supplementation of AOP is beneficial for broiler performance under NOS and challenged conditions, but feeding level must be considered.

Key Words: organic poultry operations, phytochemical, functional oils, growth performances, challenged conditions

640P Enzymatically hydrolyzed yeast carbohydrates improve broiler chicken growth performance within a commercial organic production system. Anhao Wang^{*1}, Paul Garvey¹, Mike Edwards², and Rob Patterson¹, ¹Canadian Bio-systems, Calgary, Alberta, Canada, ²Jones Feed Mill, Linwood, Ontario, Canada.

The aim of this study was to evaluate the beneficial effects of dietary supplementation of an enzymatically hydrolyzed yeast carbohydrates product (Maxi-Nutrio; MN) on the growth performance of broiler chickens raised under a commercial organic production system. A total of 16,320 newly hatched Ross x Ross 708 broiler chicks were placed at a certified organic commercial farm (Ontario, Canada). At placement, a subsample of 360 birds were randomly selected from the main flock and placed in 18 identical floor pens with 20 birds/pen for a 28 d feeding study. The experimental pens, which provided 966 cm²/bird floor space, were located within the barn and were fitted with individual feeders and

waterers. Floor pens were randomly assigned to 3 dietary treatments: T1, corn-soy based control diet, without MN supplementation; T2, formulated as T1 with MN added at 0.1%, 0.075% and 0.05% during the starter (1 - 14 d), grower (15 - 24 d) and finisher stages (25 - 28 d) respectively; T3, formulated as T1 with MN added at 0.1% throughout the entire feeding period. Birds had *ad lib* access to feed and water for the entire study period. Body weight (g), average daily gain (ADG, g/d), average daily feed intake (ADFI, g/d) and feed conversion ratio (FCR) were measured at 7, 14, 21 and 28 d. The effects were analyzed as a completely randomized design with 6 replications. During 1 - 28 d, birds fed 0.1% MN throughout the entire study had greater ADG than T1 birds (41.9 vs. 38.2 g/d, $P = 0.02$). During 22 - 28 d, birds in T2 had higher ADFI than the T1 birds (89.8 vs. 78.0 g/d, $P = 0.03$). At 28 d, birds in T3 were heavier than T1 birds (1250.6 vs. 1152.2 g, $P = 0.04$). Over the entire study, FCR for T1, T2 and T3 were 1.74, 1.67 and 1.61 respectively and did not differ among treatments ($P = 0.26$). The current study demonstrated that supplementing organic broiler chicken feeds with 0.1% MN can improve growth performance and may result in greater economic returns.

Key Words: enzymatically hydrolyzed yeast carbohydrates, broiler chickens, organic production

641P Standardized natural citrus extract effect on blood biochemical parameters and zootechnical performances of broilers chickens. Sekhou Cisse^{1,2}, Assia Boumezrag³, Anne Burel^{*1,2}, Agathe Demortreux², Pierre Chicoteau^{1,2}, and David Guilet^{1,4}, ¹Labcom Feed In Tech, Maine et Loire, France, ²Nor-Feed, Beaucauze, France, ³Institute of Veterinary Science, Ibn-Khaldoun University of Tiaret, Tiaret, Algeria, ⁴EA 921 SONAS, Beaucauze, France.

Objective: The objective of this study was to evaluate the effect of a standardized natural citrus extract (SNCE, Nor-Spice® AB, Nor-Feed SAS, France) on broiler chickens zootechnical performances and blood biochemical parameters. **Experimental design:** Two trials were performed in an experimental farm located at 1000 m above sea level in Algeria. In the first one, 360 one day old Arbor Acres were divided into 2 groups. Each group contained 6 replicates of 30 birds: Group 1: CTL group: Standard diet without supplementation; Group 2: SNCE group: Standard diet supplemented with 250 ppm of SNCE. In the second one, 650 one day-old Arbor Acres were randomly assigned to 5 groups of 130 chickens: Group 1: Standard diet without supplementation (CTL group), Group 2: Standard diet supplemented with 100 ppm of avilamycin (AVI group), Group 3: Standard diet supplemented with 250 ppm of the SNCE (SNCE group), Group 4: standard diet supplemented with 250 ppm of dried sweet orange peel (ORA group), Group 5: Standard diet supplemented with 250 ppm of dried lemon peel (LEM group). On the first trial, birds were reared until d 46 and were put under thermic stress at 30°C for 4 h per day, from d 26 to d 30. On the second trial, chickens were reared to 42 d of age without stress induction. Zootechnical performances were recorded weekly and per replicate of 30 birds (group monitoring) for trial n°1 and twice a week (individual monitoring) in the second trial. In addition, blood biochemical parameters and carcass quality were assessed on the second trial. **Statement of statistical analysis:** Statistical analysis were performed using GraphPad Prism V7, per replicate (Trial n°1) and per individual (Trial n°2). Statistical significance was considered at $P < 0.05$. **Results:** First trial results didn't showed statistical difference between final weight, feed intake and mortality of bird from the 2 groups. However, the FCR of broilers from SNCE group was significantly lower than FCR from CTL group ($P < 0.05$, *t*-test). Regarding the second trial, only SNCE group demonstrated statistical

difference on live weight and carcass yield, compared with CTL group ($P < 0.05$, ANOVA). The intestinal length of chickens from AVY group was significantly lower ($P < 0.05$, ANOVA) compared with CTL group. Concerning blood biochemical parameters, Triglycerides concentration increased significantly ($P < 0.05$, ANOVA) in SNCE and LEM group broilers compared with control group. **Conclusion:** According to these data, SNCE allow to increase zootechnical performances of broilers. Previous studies realized on poultry has already shown similar effects on poultry. Supplemental analysis have to be done to better understand the effect of SNCE on blood triglycerides concentration.

Key Words: citrus extract, Arbor Acres, zootechnical performances, blood chemical parameters, trial results

642P Influence of marine red seaweed supplementation on growth performance, blood metabolites, breast muscle meat quality, fecal consistency score, excreta microbial shedding and noxious gas emission in broilers. Balamuralikrishnan Balasubramanian*¹, Jin Su Koo¹, Deun Sol Ko¹, Ju Hee Park¹, Neeraja Recharla¹, Sungkwon Park¹, and In Ho Kim², ¹Sejong University, Seoul, Korea (the Republic of), ²Dankook University, Cheonan, Korea (the Republic of).

Marine macroalgae have a long history of use as food and traditional remedy in Asia but are much less communal in Europe and North America, as a part of the diet. Only a few species are harvested for direct human eating in limited coastal regions. The present experiment was conducted to evaluate the *in vitro* analysis and the influence of supplementation of marine red seaweed (MRS) on performance in broilers fed corn-soybean meal based diet. Seven hundred and 20 1-d old ROSS 308 broiler chicks (45 ± 0.50 g) randomly sorted into pens (18 birds/cage, 8 replications/treatment and 144 broiler/treatment) and housed ($1.55 \times 0.75 \times 0.55$ m/cage) in an environmentally controlled room (32–24°C and 65% relative humidity). All broilers were allowed *ad libitum* access to water and feed. Dietary treatments followed as supplemented 0, 0.05%, 0.1%, 0.15% and 0.25% MRS in diet for 42 d feeding trail period. The commercial MRS, *Halymenia palmata* (Organic Whole Leaf-Dulse, Vitaminsea®) was used. All results were analyzed using the general linear model procedure of SAS/STAT® (Version 9.2) for a randomized complete block design with pen as experimental unit. Linear and quadratic polynomial contrasts were performed to determine the effect of MRS at inclusion of potent level in diet. Statistical significance was considered when $P < 0.05$. The MRS was analyzed for moisture (10.44%), crude proteins (18.48%), crude lipids (0.22%), carbohydrates (50.32%), crude ash (19.04%), crude fiber (1.83%), minerals, and amino acids. Phytochemical analysis of MRS methanol extract has confirmed the presence of saponin, phenolic compounds, sugar, alkaloids, steroids, flavonoid, glycoside and oils. The antibacterial activity of methanolic extract of MRS has shown potent inhibitory activity against *E. coli*, *Salmonella Spp.*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Our *in vivo* results demonstrated that inclusion of 0.15% MRS improved growth performance ($P < 0.05$) of ADG without effects on ADFI and FCR ($P > 0.05$) at wk 2 and improved ADG and FCR ($P < 0.05$) without effects on ADFI at wk 6. Dietary inclusion of MRS had beneficial effects on excreta microflora counts as reduced *E. coli* and increased *Lactobacillus* but not significant and reduced level of fecal consistency score at wk 6. However, no significant effects were found for meat quality, blood metabolites and noxious gas emissions. In conclusion, dietary inclusion of 0.15% had beneficial effects on growth performance, cooking loss, drip loss, diarrhea score and fecal microflora counts in broilers. It is anticipated that further studies will lead to

improved feed industries strategies directed toward the production of higher and healthy livestock in the future.

Key Words: marine red seaweed, broiler, growth performance, meat quality

643P A blend of protected organic acids + essential oils improves growth performance, nutrient digestibility, and intestinal health of broiler chickens undergoing an intestinal challenge. Mariana Lemos de Moraes*¹, Marcia de Souza Vieira¹, Jean-Christophe Bodin¹, Elizabeth Santin¹, and Catarina Stefanello², ¹Jefo, Saint-Hyacinthe, Quebec, Canada, ²Federal University of Santa Maria, Santa Maria, Brazil.

We investigated the dietary supplementation of Protected Organic Acids + Essential Oils P(OA+EO) in challenged broiler chickens. A total of 1,080 Cobb 500 male chickens were reared in pen-floors in a climate-controlled experimental poultry house. Chicks were allocated using a completely randomized design of 4 treatments and 10 replicates (27 birds/pen) for a 42 d trial. Treatments (T) were as follow: T1: negative control (NC), T2: positive control (PC), T3: antibiotic growth promoter (AGP, Enramycin at 10 ppm), T4: P(OA+EO), at 300 g/t (Jefo). Except those on the NC group, all birds were challenged with *Eimeria* spp. at 1 d and *Clostridium perfringens* challenged at 11, 12 and 13 d. Feed intake, body weight gain (BWG) and feed conversion ratio (FCR) were evaluated weekly. At 17 d, one bird per pen was orally gavaged with of fluorescein-isothiocyanate labeled dextran (FITCd). FITC-d is a molecule with high molecular weight that is only detected in the blood when the intestinal mucosa is damaged. One h after, blood samples were collected for FITC-d detection. Nutrient and energy ileal digestibility were evaluated at 21 d. Macroscopic and microscopic alterations in the intestine were evaluated at 21 d by the I See Inside Scoring System methodology (Kraieski et al., 2017). Data was subjected to one-way ANOVA and Fisher LSD using the XLSTAT software. From 1 to 42 d, birds on P(OA+EO) group had respectively 6.5 and 4.7% better ($P < 0.001$) BWG and FCR compared with PC, while there was no significant difference to the NC group. For the same period, birds on P(OA+EO) had 2.9% even better BWG ($P < 0.001$) than birds on AGP. FCR was also better ($P < 0.05$) for birds on P(OA+EO) compared with AGP from 14 to 21, 21–28 and 35–42 d, while for the other feed phases they had similar results. The birds on P(OA+EO) group, compared with PC ($P < 0.05$), had higher ileal digestibility of dry matter (3.3%) and energy (110 kcal) and no difference compared with the AGP group. P(OA+EO) improved intestinal integrity, evidenced by the lower level of FITC-d in the blood compared with PC ($P < 0.001$) group. Compared with PC, P(OA+EO) also improved intestinal health based on: reduced macroscopic duodenum *Eimeria* lesion scores; reduced inflammation in the serosa and mucosa layer in the jejunum, ileum and cecum, associated with reduced thickness of intestinal wall in the jejunum at macroscopy and the reduced inflammatory cell infiltration in the epithelium ($P < 0.05$). In conclusion, the blend of Protected Organic Acids + Essential Oils evaluated showed better or similar effects to an antibiotic growth promoter in improving growth performance, nutrient digestibility, and intestinal health of broiler chickens undergoing an intestinal challenge.

Key Words: broiler, essential oil, intestinal health, nutrient digestibility, organic acid

644P Effects of three regulators on reproduction performance and ovarian function of broiler breeders with different egg production rate. Shuju Zhao*, Ying Zhang, Shi Bai, Mei Ding, Wei Su, and Ping Wang, Sichuan Agriculture University, Chengdu, China.

The aim of this study was to investigate the effects of 3 regulators on the reproduction performance and ovarian function of broiler breeders with different egg production rate. The animal study was conducted on broiler breeders (AA) for the period of 48–55wk using a completely randomized design at suining, China. A total of 512 broiler breeders (48-wk-old) were assigned to 8 treatments in a 2 × 4 completely randomized design, with 8 replicate per treatment. The 8 treatments included 2 egg production level (average egg production (AVE), 80%), low egg production (LOW, 70%), and 4 diets (basal diet (CON), basal diet + 30mg/kg *Enterococcus faecium* (EF, Probiotic), basal diet + 200mg/kg apple pectin oligosaccharide (APO, Prebiotic), basal diet + 1000mg/Kg tributyrin (TRI, short-chain fatty acid). Data were analyzed as a 2 × 4 (egg laying rate × regulators) factorial arrangement of treatments by 2-way ANOVA with a model that included the main effects of egg laying rate and feed additive, as well as their interaction. When an effect was significant ($P < 0.05$), means were compared by using Duncan's multiple comparison test to determine significant differences among means. The reproduction performance shows that the egg production rate and qualified egg rate in AVE group were higher than those in LOW group ($P < 0.05$), and the FCR and egg weight were lower than those in LOW group ($P < 0.01$). Compared with CON, the diet that was supplemented with 3 regulators significantly increased egg weight ($P \leq 0.01$). The expression of ovarian reproductive regulatory gene and hormone receptor gene shows that the expression of BMP15, ESR2 and AMH in AVE group was significantly higher than these in LOW group ($P < 0.05$), and compared with EF and TRI, the addition of APO to diet significantly increased the expression of BMPR2 and BMP15 in the ovary ($P < 0.05$). And there was an interaction between egg laying rate*regulators for BMP15 ($P < 0.05$). The effect of APO on increasing the expression of BMP15 were more pronounced in AVE group. Compared with CON and APO, diet supplemented with TRI significantly increased the expression of BMPR1B in the ovary ($P < 0.01$). And there was an interaction between egg laying rate*regulators for BMPR1B ($P < 0.05$). The effect of TRI on increased the expression of BMPR1B were pronounced in AVE group. The results showed that the reproduction performance and ovarian function of AVE group were better than these of LOW group. Dietary addition of 3 regulators increased egg weight. Diet supplemented with APO and TRI might improve ovarian function of broiler breeders.

Key Words: broiler breeder, regulator, egg production rate, egg quality, ovarian function

645P Effects of three intestinal regulators on the intestinal health of broilers breeders with different egg laying rate. Ru Jia*, Ying Zhang, Shi Bai, Feng Zeng, Mei Ding, and Ping Wang, *Sichuan Agricultural University, Chengdu, SiChuan, China.*

Abstract: The aim of this study was to investigate the effects of 3 intestinal regulators on the intestinal health of broiler breeders with different egg laying rate. The animal study was conducted on broiler breeders (AA) for the period of 48–55wk at suining, China. 512 AA broiler breeders were assigned to 8 treatments in a 2 × 4 completely randomized design, with 8 replicate per treatment. The 8 treatments included 2 egg production level (average egg production (AVE), 80%, low egg production (LOW), 70%) and 4 diets (basal diet (CON), basal diet + 30mg/kg *Enterococcus faecium* (EF), basal diet + 200mg/kg apple pectin oligosaccharide (APO), basal diet + 1000mg/Kg tributyrin (TRI, short-chain fatty acid). Data were analyzed as a 2 × 4 (egg laying rate × regulators) factorial arrangement of treatments by 2-way ANOVA with a model that included the main effects of egg laying rate and feed additive, as well as their interaction. When an effect was significant ($P < 0.05$),

means were compared by using Duncan's multiple comparison test to determine significant differences among means. The results showed that the crypt depth in the AVE group was significantly lower than that in the LOW group ($P < 0.05$). The effect of APO on decreasing the crypt depth were more pronounced in AVE group. The villus height/crypt depth ratio of the AVE group was significantly higher than that of the LOW group ($P < 0.01$). Compared with EF and APO, the addition of TRI significantly increased the content of acetic acid, propionic acid, and total VFAs in the cecal chyme ($P < 0.05$). The addition of TRI increased the content of Butyrate than other groups ($P = 0.01$). Microbial diversity (Shannon index), community richness (Chao1 index and ACE index) and observed species in the AVE group were significantly higher than those in the LOW group ($P \leq 0.01$). The relative abundance of Bacteroidetes, Firmicutes, and Euryarchaeota was lower in the AVE group than in the LOW group ($P < 0.05$), and Proteobacteria, Spirochaetes and Kiritimatiellaeota was significantly higher than in the LOW group ($P < 0.05$). The relative abundance of Fecalibacterium in the AVE group was significantly higher than that in the LOW group ($P = 0.03$), Methanobrevibacter was significantly lower than that in the LOW group ($P < 0.01$). Bacteroidetes, Firmicutes, Bacteroidia and Methanobrevibacter-woesei species in the LP group (the control group with low egg laying rate) were significantly higher than these in AP group (the control group with average egg laying rate), and Proteobacteria and Gammaproteobacteria in LP group were significantly lower than these in AP group. The results showed that the intestinal morphology and microbial diversity and richness of AVE were better than these in LOW.

Key Words: broiler breeder, intestinal regulator, intestinal health, different egg laying rate

646P The efficacy of AB20® in broiler chicks exposed to diets contaminated with aflatoxin and ochratoxin A. Miriam Garcia*, Dennis Nuzback, James Harness, Melissa Collins, and Derek Mclean, *Phibro Animal Health Corporation, Corvallis, Oregon, United States.*

The aim was to evaluate the efficacy of a bentonite, containing hydrated sodium calcium aluminosilicates (AB20, Phibro Animal Health Corporation) included in broiler diets. Forty-two 0-d old chicks obtained from a local hatchery were randomly allocated in pairs of 2 to battery cages. Cages (7 per treatment) were randomly assigned to 1 of 3 dietary treatments (soy-corn base diets): 1) a diet without mycotoxin (CTL), 2) a diet with mycotoxin but without AB20 (MYC), and 3) a diet with mycotoxin and 0.5% AB20 (AB20). The mycotoxin concentrations in the last 2 diets were 1.88 ppm aflatoxin B1 and 1.13 ppm ochratoxin A. Birds were on experimental diets from 0 to 17 d of age, and were provided mash feed and water ad libitum. For measures of growth, body weight (BW) was recorded on d 0, 3, 7, 10, and 14; intake was recorded for the same day-periods; and mortality was recorded daily. Birds were harvested for blood collection and for liver and spleen weights on d 17. Data were analyzed as repeated or non-repeated measures using the MIXED procedure of SAS with treatment as a fixed effect and cage as a random effect, and the PDIF statement was used for multiple comparisons. For repeated measures, age and its interaction with treatment were included as fixed effects. At 14 d of age, chicks fed MYC were lighter, and AB20 was able to ameliorate numerically the detrimental effect of mycotoxins (374, 294, 321 g; CTL, MYC, and AB20, respectively; treatment × age, $P < 0.01$). During the period of 11 to 14 d, average daily gain was the lowest for chicks fed MYC and intermediate for chicks fed AB20 (36.5, 24.8, and 30.4 g/d; CTL, MYC, and AB20, respectively; treatment × age, $P < 0.01$). Feed intake was also lower with MYC diets and AB20 recovered feed intake to CTL levels (41.1, 31.6, and 37.0 g/d; CTL, MYC, and AB20, respectively; treatment × age, $P = 0.01$). Relative liver weight was

greater with mycotoxin diets (2.7, 3.9, and 3.6% BW; CTL, MYC, and *AB20*, respectively; treatment \times age, $P < 0.01$). Interestingly, biomarkers of liver damage, aspartate- and alanine-aminotransferases did not differ with treatments ($P > 0.10$). Nevertheless, a reduction ($P < 0.01$) in plasma concentrations of cholesterol, total protein, and albumin in broilers fed diets with mycotoxins (MYC and *AB20*) suggest a degree of impaired hepatic function. Plasma concentrations of aflatoxin and ochratoxin A were increased ($P < 0.01$) with MYC, and the feeding of *AB20* reduced aflatoxin concentrations, but not ochratoxin A, to that of CTL. Our findings demonstrate that 0.5% *AB20* is efficacious in preventing the detrimental effects of mycotoxin-containing diets on broiler performance.

Key Words: broilers, mycotoxins, growth, blood biomarkers, *AB20*

647P The efficacy of *AB20*[®] in laying quail hens exposed to diets contaminated with aflatoxin and ochratoxin A. Miriam Garcia*, James Harness, Melissa Collins, and Derek Mclean, *Phibro Animal Health Corporation, Corvallis, Oregon, United States.*

This study was conducted to evaluate the efficacy of a bentonite, containing hydrated sodium calcium aluminosilicates (*AB20*, Phibro Animal Health Corporation) included in mycotoxin-containing diets fed to Japanese quail. Thirty 28-d old female quail obtained from a local hatchery were randomly allocated in pairs of 2 to battery cages. Quail were fed a single soy-corn base diet during the first week of arrival to acclimate to a mash diet and facilities. Cages (5 per treatment) were randomly assigned to 1 of 3 dietary treatments (soy-corn base diets): 1) a diet without mycotoxin (CTL) a diet with mycotoxin but without *AB20* (MYC), and 3) a diet with mycotoxin and with 0.5% *AB20* (*AB20*). The mycotoxin concentrations in the last 2 diets were 3 ppm aflatoxin and 2 ppm ochratoxin A. Quail were fed experimental diets from 35 to 83 d of age and mash feed and water provided ad libitum. Body weight (BW) and intake were recorded weekly, and mortality and egg production were recorded daily. Birds were slaughtered for blood collection and liver weight. Data were analyzed as repeated or non-repeated measures using the MIXED procedure of SAS with treatment as a fixed effect and cage as a random effect, and the PDIF statement was used for multiple comparisons. For repeated measures, week and its interaction with treatment were included as fixed effects; and BW at enrollment was included as covariate factor for analysis of weekly BW measures. Throughout the study, hens fed MYC were lighter ($P < 0.01$) compared with hens fed CTL or *AB20* (148 vs. 157 and 159 g BW, for MYC, CTL, and *AB20*, respectively). Dietary treatments did not affect feed intake ($P > 0.10$). Hens fed MYC were numerically older at the onset of egg production (38, 46, and 40 ± 3 d of age for CTL, MYC, and *AB20*, respectively, $P = 0.17$). Across the study, egg production was drastically impaired ($P < 0.01$) by MYC but the addition of 0.5% *AB20* returned egg production to similar levels as CTL. Egg size tended ($P = 0.06$) to be smaller with MYC, while *AB20* and CTL produced similar size eggs. Hens fed CTL had smaller ($P = 0.02$) relative liver weight compared with other diets, but plasma biomarkers of liver inflammation did not differ with treatments ($P > 0.10$). Plasma concentrations of aflatoxin and ochratoxin A were increased ($P < 0.01$) with MYC, and the feeding of *AB20* reduced aflatoxin concentrations, but not ochratoxin A, to that of CTL. The findings of this study demonstrate that *AB20* at 0.5% inclusion rate is efficacious on preventing the detrimental effects of feeding mycotoxin-contaminated diets on quail hen live performance.

Key Words: quail hen, mycotoxins, growth, egg production, *AB20*

648P The efficacy of *VECTOR*[™] in mitigating the adverse effects of aflatoxins, fumonisins, and T-2 toxins in broilers. Dennis Nuzback, Derek Mclean*, Paulo Rezende-Napier, and Miriam Garcia, *Phibro Animal Health Corporation, Corvallis, Oregon, United States.*

The objective was to evaluate the impact of *VECTOR* (Phibro Animal Health Corporation) on performance of broilers fed diets containing mycotoxins. Three-hundred, day-old male Cobb 500 broiler chicks were assigned to 30 pens of 10 chicks each over 5 treatments with 6 replicates per treatment. Birds were fed commercial corn-soy based starter diets for 21 d. Dietary treatments were: 1) a negative control diet (no mycotoxins); 2) a control with 0.5% *VECTOR* (no mycotoxins); 3) a mycotoxin diet with no adsorbent; 4) a mycotoxin diet with 0.25% *VECTOR*; 5) a mycotoxin diet 0.5% *VECTOR*. Total dietary mycotoxin concentrations in the contaminated diets were 1.4 ppm aflatoxins, 50 ppm fumonisins, and 0.75 ppm T-2 toxins. Performance variables (BW, ADG, and ADFI) were measured and feed conversions calculated. Carcass and liver weights were used to determine relative liver weights. Serum chemistries were measured and the sphinganine to sphingosine ratios (Sa:So) calculated. Data were analyzed as a complete randomized design, with repeated or non-repeated measures using the MIXED procedure of SAS with treatment as a fixed effect and cage as a random effect. The PDIF statement was used for multiple comparisons. For repeated measures, age and its interaction with treatment were included as fixed effects. As expected, BW, ADG and ADFI decreased ($P < 0.05$) in broilers fed the mycotoxin diet compared with those fed the negative control and control with 0.5% *VECTOR* diets. The BW at d 21 for broilers fed the mycotoxin diet with 0.5% *VECTOR* was numerically higher than the mycotoxin diet group (756 vs. 729 g, PDIF = 0.15). During the last week of the study, broilers fed the mycotoxin diets with 0.25 and 0.5% *VECTOR* had greater ($P < 0.05$) ADFI when compared with the mycotoxin diet but were lower ($P < 0.05$) than the 2 diets containing no mycotoxins. Relative liver weights were lower ($P < 0.01$) in broilers fed *VECTOR*, with or without mycotoxins, when compared with the mycotoxin diet (2.98, 3.11, 4.03, 3.72, and 3.64%, treatments 1 through 5, respectively). The mycotoxin diet with 0.5% *VECTOR* prevented the reduction in serum total proteins ($P < 0.01$), a response consistent with mycotoxicosis, when compared with the mycotoxin diet (2.66, 3.04, 2.21, 2.52, and 2.86 mg/dL, treatments 1 through 5, respectively). Broilers fed mycotoxins and 0.25% or 0.5% *VECTOR* had lower ($P < 0.01$) Sa:So, biomarkers for fumonisin mycotoxicosis, than those fed the mycotoxin diet (0.16, 0.17, 0.74, 0.26 and 0.18, treatments 1 through 5, respectively). In conclusion, *VECTOR* was effective in mitigating the mycotoxin challenge in this study and had a significant impact on the mitigation of dietary fumonisins.

Key Words: broilers, fumonisin, aflatoxin, T-2 toxin, *Vector*

649P Not Presented

650P Cashew nut shell liquid-based supplements improve growth performance and show anticoccidial effect in broilers. Yan Martel Kennes¹, Stephanie Dion¹, Sonia Yacini¹, Caroline Decaux², and Carl Julien*¹, ¹CRSAD, Deschambault, Quebec, Canada, ²Ceresco Nutrition, Saint-Urbain-Premier, Quebec, Canada.

Drugs alternatives are needed for the poultry industry not only for organic production, but also for limiting drug resistance and for complying with upcoming regulations and consumer demands. Two pen trials were conducted to evaluate the effect of Calica+ and other formulations from cashew nut shell liquid (CNSL) on broiler performance in coccidio-

sis conditions. The first trial was conducted in *Eimeria* vaccination conditions with commercial Coccivac-B52 (2 doses per bird at d3 of age). The second one was realized in *Eimeria* challenge conditions, induced by an oral administration of 200,000 sporulated oocysts of mixed *Eimeria* species harvested from field isolates. Trials were performed with 8 or 9 pens of 45 birds per treatment. Performance data and oocyst counts were analyzed by a mixed model including treatment as fixed effect and pen as random effect. Intestinal lesion scores were analyzed by category analyses. In *Eimeria* vaccination conditions, Calica+ improved average daily gain (ADG) (+4.5%, $P < 0.05$) during the overall growing period and decreased levels of oocysts per gram of feces (OPG) (-44.1%, $P < 0.05$), at *Eimeria* excretion peak (d 17–20), compared with untreated broilers. However, no significant effect of Calica+ on *Eimeria* intestinal lesions was observed. Overall ADG in Calica+-treated birds was not significantly different compared with nicarbazin-monensin anticoccidial medication ($P = 0.8362$) but showed higher feed conversion ratio (FCR; +6.0%, $P < 0.05$). In *Eimeria* challenge conditions, Calica+ feeding treatment improved ADG after infection, during the period from d20 to d36 of age (+4.4%, $P < 0.05$) compared with untreated birds. Compared with nicarbazin-monensin medication, Calica+-treated birds showed higher FCR (+3.5%, $P < 0.05$), but comparable ADG ($P = 0.3145$). Dual energy x-ray absorptiometry (DEXA) measurements with prediction equations developed by Hambi et al., 2018 and Salas et al., 2012 revealed that Calica+ increased total carcass protein (+6.2%, $P < 0.05$) at d36, compared with untreated birds. However, no effect of Calica+ or other CNSL-based formulations was observed on body ash content or total carcass fat at d10, d20 and d36. Interestingly, birds fed the treatment with Calica+ showed a trend of increased plasmatic levels of IL-1 β compared with untreated broilers (+25.6%, $P = 0.0676$), suggesting an immune activation as a potential mechanism of action for Calica+. In summary, Calica+ and other CNSL-based formulations showed beneficial growth performance and anticoccidial effects in these 2 different trial conditions. These feed additives could be involved in a strategy to reduce the use of anticoccidial medication in broiler production.

Key Words: coccidiosis, organic, broilers, osteodensitometry, *Eimeria*

651P Not Presented

652P Enzymatically digested food waste influence lipid parameters in broiler meat. Linda Truong*, Shiva Emami, Zhichao Zhang, Ameer Taha, and Annie King, UC Davis, Davis, California, United States.

Dietary fats in poultry diets could affect both chicken and human health. The fatty acid (FA) composition of nonpathogenic food waste (FW, containing fruits, vegetable, meats, and bakery waste; California Safe Soil, LLC, McClellan Park, CA, USA), diets containing FW, and variously processed thigh meat of 2-week-old broiler chicks fed diets containing FW were evaluated. Diets were a basal corn/soy control (Con), 75% Con + 25% FW (75:25), and 50% Con + 50% FW (50:50). Cages containing 6 birds were considered replicates as well as the experimental units ($n = 24$ /replication). Due to lipid content in food waste we hypothesized that thigh meat from birds fed a diet containing 50% FW would be less oxidative than that from birds fed a Con diet. Generally, results showed that the FW and diets containing FW had a lower n-6: n-3 polyunsaturated fatty acids (PUFA) ratio and a higher saturated fatty acids (SFA): PUFA ratio. For thigh meat FA and lipid oxidation, a 2-way ANOVA was used to test significance at $P < 0.05$. Results showed that thigh meat of broilers fed 50:50 had numerically lower n-6: n-3 PUFA ratio and significantly higher SFA: PUFA ratio

when compared with Con and 75:25 ($P < 0.001$). The Con diet and thigh meat had the highest concentration of PUFA in comparison to 75:25 and 50:50. The n-6: n-3 PUFA ratios for Con, 75:25, and 50:50 diets were 23.09, 16.70, and 15.24, respectively. Those for and SFA: PUFA were 0.27, 0.61, and 1.12, respectively. There was no significant difference in lipid oxidation among treatments; however, Con raw and cooked thigh meat, with higher concentrations of PUFA, had numerically higher lipid oxidation values than that of 75:25 and 50:50 thigh meat. Results indicated that FW substitution in broiler feed at 50% had the potential to increase SFA intake. Therefore, it is recommended that the 75:25 diet be fed to broiler chicks for the first 2 weeks of life.

Key Words: broiler, by-products, lipids, oxidation

653P Not Presented

654P Effect of beetroot juice (*Beta vulgaris*) on growth performance, blood profile and carcass characteristics of broiler chickens. Oluwatoyin Tayo*, Babcock University, Ilishan-Remo, Ogun, Nigeria.

This study was conducted to determine the effect of beetroot juice (*Beta vulgaris*) as an additive on the performance, carcass characteristics and blood profile of broiler chickens. One hundred and 20 d old Marshal strain of broiler chickens were assigned to 4 treatments at 30 birds per treatment with 3 replicates of 10 birds in a completely randomized design. Birds were kept in movable pens with feed and water offered *ad libitum*. Beetroot was administered in their drinking water every other day at 0.00mL/l (T1), 10.00mL/l (T2), 20mL/l (T3), and 30mL/l (T4) for 8 weeks. Daily feed intake, weekly weight gain and mortality were recorded. Blood samples were collected through the wing web from one bird per replicate to determine hematological and serum biochemical parameters following standard procedures. Carcass parameters of one bird per replicate treatment measured included live weight, bled weight, eviscerated weight, dress weight, primal cuts and internal organs. Results were subjected to one-way ANOVA. Significant differences ($P < 0.05$) were observed in the average daily feed intake and the average daily weight gain. Birds on T4 had the best feed conversion ratio of 1.65. Treatments effects on packed cell volume (PCV), hemoglobin (Hb), and red blood cells (RBC) were significant ($P < 0.05$) with T4 having the highest values of 40.33%, 11.96g/dL and 3.82×10^6 /ul respectively. Birds on T3 had the highest value (4.0%) of monocytes while those on T1 had the lowest value (2.33%). The highest value of Eosinophil (4.33%) was observed in birds on T1 while the lowest (0.33%) was observed in birds on T4. Treatments had no effect on White blood cells, Platelets, Lymphocytes, Neutrophils, Basophil. Serum biochemical parameters were different ($P < 0.05$), birds on T4 had the lowest glucose (150.58mg/dL), cholesterol (82.54mg/dL) and triglyceride (122.29mg/dL) values. With the exception of eviscerated liver and heart percentages no differences were observed in the carcass characteristics. Findings from the study indicated that inclusion of beetroot (*Beta vulgaris*) in the drinking water of broiler chicken at 30mL/l can improve the growth performance, PCV, RBC and Hb of birds and also reduce the Glucose, Cholesterol and Triglyceride levels of birds.

Key Words: broiler, *Beta vulgaris*, carcass characteristics, blood profile, biochemical parameters

655P Salmonella colonization in hens fed a yeast cell wall supplement with high levels of mannans and beta-glucans. John Padgett*¹, Paul Price^{2,3}, Thomas Gaydos², Kimberly Gardner¹, and

Christopher Bailey¹, ¹Texas A&M University, Bryan, Texas, United States, ²Phileo Lesaffre Animal Care, Milwaukee, Wisconsin, United States, ³Clemson University, Clemson, South Carolina, United States.

Although *Salmonella* is often regarded as a commensal organism within the intestinal tract of poultry, it is responsible for approximately 1.2 million illness cases annually, with 1 million of those being foodborne illnesses (CDC, 2018). Previous studies with birds fed feeds supplemented with yeast cell wall (YCW) containing high levels of mannans show a reduction in *Salmonella* counts in broiler ceca (Kiros, 2019). To understand the effects of this YCW on *Salmonella* in egg producing hens, a study was conducted at the USDA Southern Plains Agricultural Research Center in College Station, Texas. At 18 weeks of age, a total of 48 Hy-Line W36 laying hens were transferred to the USDA facilities and randomly assigned to raised wire cages (1 bird/cage). Each individual bird (cage) serves as the experimental unit for replication. Birds were divided into 2 groups of 24, and challenged with *Salmonella* Typhimurium or *Salmonella* Braenderup respectively. Within each of the 2 groups, 12 birds were fed basal diet only and 12 birds were fed basal diet plus treatment of 500ppm cell wall. Birds were challenged at approximately 22 weeks of age. One week post challenge all birds were humanely euthanized, cecal and ovary samples were taken, and prevalence and enumeration were recorded. Data were analyzed via a one-way ANOVA and differences were deemed significant at $P < 0.05$. Cecal counts on birds challenged with *S. Typhimurium* showed a final count of $4.71 \log_{10}$ cfu/mL, while YCW samples were $3.71 \log_{10}$ cfu/mL ($P = 0.015$). A 1 log reduction of cecal *Salmonella* indicates there may be potential for yeast cell wall to impact levels of *S. Typhimurium* in the ceca. Reductions in prevalence of *Salmonella* Braenderup and Typhimurium in the ovaries in the YCW treated groups, as well as a significant reduction in cecal counts in the *S. Typhimurium* treated groups indicates that there may be potential for yeast cell wall to impact levels of *Salmonella* colonization in both the ovaries and the ceca.

Key Words: *Salmonella* Typhimurium, *Salmonella* Braenderup, laying hens, yeast cell wall, mannans

656P Administration of prebiotics and direct-fed microorganisms to broiler chickens improves growth performance, reduces *Campylobacter*, and increases lactic acid bacteria. Lindy Froebel^{*1}, Sangita Jalukar², Theresia Lavergne², Craig Coufal¹, and Tri Duong¹, ¹Texas A&M University, College Station, Texas, United States, ²Arm & Hammer Animal and Food Production, Princeton, New Jersey, United States.

Regulatory and consumer pressures have led to decreased administration of sub-therapeutic antibiotics and increased interest in the development of alternatives to antibiotic growth promoters (AGP). Prebiotics and Direct-Fed Microorganisms (DFM) have been demonstrated to improve growth performance of broiler chickens and the microbial food safety of poultry and poultry products and are thought to be potentially important alternatives to the use of AGP. We investigated the effects of prebiotic and DFM administration to broiler chickens. Birds were fed rations supplemented with *Saccharomyces*-derived refined functional carbohydrates (RFC) as a dietary prebiotic, spores from one of 2 *Bacillus* cultures as DFM, or a combination of RFC and a *Bacillus* culture as a synbiotic. Broilers fed untreated or bacitracin methylene disalicylate (BMD)-treated rations served as control groups. Experimental animals ($n = 2280$) were allocated to the 6 experimental treatment groups with 10 replicate pens of 38 birds arranged as a randomized complete block design. Birds were raised in floor pens on built-up litter; provided age-appropriate heat, ventilation, and lighting; and given access to potable water and experimental feed ad libitum for the duration of the

study. Growth performance was evaluated through 42 d of production, and gastrointestinal microbiota were evaluated on d 42. Performance measures and bacterial counts were analyzed using ANOVA. Prebiotic RFC, direct-fed *Bacillus*, and synbiotic administration improved d 14 BW ($P = 0.026$), starter ($P = 0.022$) and cumulative ($P = 0.030$) ADG, and starter FCR ($P = 0.002$), when compared with the untreated control and to levels similar to BMD-treated broilers. Administration of RFC, either DFM, or the synbiotic reduced counts of *Campylobacter* spp. in the cecum ($P = 0.05$) by up to $1 \log_{10}$ cfu g^{-1} when compared with the untreated control. Additionally, $1 \log_{10}$ cfu g^{-1} more total Lactic Acid Bacteria were recovered from the ileum ($P = 0.001$) when broilers were administered prebiotic RFC compared with the untreated control. These data suggest that administration of RFC as dietary prebiotics, *Bacillus* spores as DFM, and a combination of RFC and *Bacillus* as a synbiotic can be used to improve growth performance, reduce the colonization of human foodborne pathogens, and promote the colonization of beneficial bacteria in poultry.

Key Words: direct-fed microorganisms, prebiotics, *Bacillus* spp, refined functional carbohydrates, *Campylobacter*

657P Effects of Red Osier Dogwood extracts as alternatives to in-feed antibiotics on growth performance, gut health and meat quality in broiler chickens. Marion Mogire^{*1}, Janghan Choi², Deborah Adewole³, Shangxi Liu¹, Chongwu Yang¹, Peng Lu¹, Argenis Rodas-Gonzalez¹, and Chengbo Yang¹, ¹University of Manitoba, Winnipeg, Manitoba, Canada, ²University of Manitoba Animal Science, Winnipeg, Manitoba, Canada, ³Dalhousie University, Truro, Nova Scotia, Canada.

Red Osier dogwood is an indigenous North American shrub that is rich in the phenolic compounds rutin, quercetin, gallic and ellagic acids and has demonstrated antioxidative, anti-inflammatory and antimicrobial properties. The plant has been shown to mitigate ruminal acidosis and increase protein efficiency when fed to heifers and increase the absorptive capacity of piglets challenged with *E. coli* K88+. This study investigated the effects of Red Osier Dogwood extracts (ROD) in the feed on growth performance, intestinal morphology and meat quality in broilers. The trial was conducted with a total of 320 0-d old male broilers (Cobb 500) allocated 32 pens with 8 replicate pens per treatment. The treatments included: 1) Corn-wheat-soybean meal basal diet (negative control); 2) basal diet with 30 ppm avilamycin premix (positive control); 3) basal diet with 0.1% ROD extracts (ROD1); 4) basal diet with 0.3% ROD extracts (ROD2). Statistical analysis was done using one-way ANOVA (SAS 9.4 statistical software). There were no significant differences in growth performance at each feeding stage between treatments ($P > 0.05$) but feed conversion ratio tended ($P = 0.07$) to increase at the grower phase in ROD1 and ROD2. Similar organ weights of heart, bursa, liver and spleen were observed among treatments ($P > 0.05$). A lower length of jejunum crypt depth ($58.06 \mu\text{m}$) was observed in ROD1 compared with negative control ($77.69 \mu\text{m}$). Birds fed ROD1 or ROD2 showed the highest villus height to crypt depth ratio (VCR; $P 0.05$) in the jejunum with an average $14.5 \mu\text{m}$ and $15.0 \mu\text{m}$, respectively. White stripping and woody breast scores were normal (scores 1) in all treatments ($P > 0.05$). The values of pH, purge loss (%) and meat color including L* and b* values were similar among treatments ($P 0.05$); but, a reduced a* value was observed in ROD2 ($P = 0.04$). In conclusion, dietary supplementation with ROD extracts improved gut health by increasing the VCR without affecting growth performance and meat quality.

Key Words: broilers, intestinal morphology, gut health, meat quality, Red Osier Dogwood

658P Effect of synthetic emulsifier based on polyethylene glycol glyceryl ricinoleate on nutrient digestibility and broiler performance. Thais Stefanello*¹, Everton Luis Krabbe², Valdir Avila², Edenilse Gopinger³, Andréa Ribeiro¹, and Eduardo Xavier⁴, ¹Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, ²Embrapa Swine and Poultry, Concórdia, Brazil, ³Federal Institute of Education Science and Technology of Rio Grande do Sul, Sertão, Brazil, ⁴Federal University of Pelotas, Pelotas, Brazil.

The addition of synthetic emulsifiers to diets have been considered for years, however recently this practice has been reassessed, as a consequence of inconsistency of efficacy, indicating the need for further applied studies. This study evaluated the utilization of a fat emulsifier based on glyceryl polyethylene glycol ricinoleate in chicken diets on productive performance and nutrient digestibility. The diets were formulated based on corn and soybean meal, then mixed and pelleted under normal feed mill conditions (80 ± 2°C and 9 s conditioning) using a die with 4,75 mm hole diameter and 50 mm length. Two different feed form, mash and crumble, with and without the use of emulsifier (400g/ton) were produced, resulting 4 different diets/treatments. All other nutrients were set to meet animal requirements. The pelleted diets were ground to eliminate the physical form factor but maintaining the effect of conditioning and processing. In total, 480-d-old broiler were used, Cobb 500, male, housed in metabolic cages, distributed in completely randomized block design with 12 replicates and 10 birds per cage. The experimental performance (feed intake-FI, body weight gain-BWG, feed conversion ratio-FCR) period was from 1 to 14 and 15 to 28 d age, and the nutrient digestibility (dry matter-DM, nitrogen-N, ether extract-EE, apparent energy metabolizable-AME) was evaluated from 24 to 28 d age, consisting of the total excreta collection method. The data were submitted to ANOVA (ANOVA) and the presence of interaction between the factors were studied applying Tukey test ($P < 0.05$). No interaction ($P > 0.05$) between emulsifier and processing was observed for performance in the periods analyzed, however, birds fed with crumbled diets ($P < 0.05$) had a higher FI and BWG, which reflected better FCR compared with those that received the mashed form. Although not significant ($P > 0.05$), the addition of the emulsifier affects positively the FI and BWG in both stages. For nutrient digestibility, an interaction ($P < 0.05$) between the factors was observed for N. The presence of the emulsifier increased N digestibility when supplied in the mashed diet. The main effect of emulsifier was significant ($P < 0.05$) for DM, N and AME, whereby diets containing the additive presented improved DM and N digestibility and increased AME efficiency. As well as, physical form showed effect ($P < 0.05$) for the same nutrients, whereby crumbled diets improved this. In conclusion, the improvements as consequence of emulsifier use were not clearly observed on the broilers' performance data, however, the nutrient digestibility was increased.

Key Words: feed additive, nutrition, pelleting

659P Effects of a specific blend of oleoresins of spices and essential oils on growth performance of broilers: Insight from a meta-analysis approach. Marie-Pierre Létourneau-Montminy*¹, Farouk Messad¹, Bertrand Medina², and Jean-François Gabarrou³, ¹Laval University, Quebec, Quebec, Canada, ²Probiotech International, Saint Hyacinthe, Quebec, Canada, ³Phodé, Tersac, France.

Considering that the simple removal of antibiotic growth promoters might have a negative economic impact in broilers production by reducing growth performance and jeopardizing broiler health, search for alternative growth promoter has been intensified. The main goal of this study was to evaluate the effects of a blend of essential oils and oleoresins of spices, Oleo, on growth performances of broilers from

a meta-analysis approach. An exhaustive database including 25 trials performed from 2001 to 2018 with Oleo was built to quantify its impact on average daily gain (ADG) and feed conversion ratio (FCR) of broilers. The database includes both commercial ($n = 11$) and experimental ($n = 14$) trials. Dietary treatments were either a positive control (C+) with antibiotic growth promoter, a negative control (C-) without antibiotic or a treatment consisting in an addition of Oleo at 100 ppm. Regarding the range of trial durations and slaughter ages of broilers, the Y variables were converted as relative difference in FCR and ADG expressed in percent between Oleo and C+ (ADG_Oleo/C+, FCR_Oleo/C+) and Oleo and C- (ADG_Oleo/C-, FCR_Oleo/C-). When C+ and C- were both present in the same trial ($n = 9$), the relative difference between them (C+/C-, %) was also calculated as Challenge Acuity Index and used as an X variable. Metabolizable energy and crude protein has been recalculated based on diet composition using table of feedstuff composition and expressed based on requirement of the genetic line studied. The mixed procedure of Minitab (Version 18.0) was used with the effect of the trial as random effect and the dose of Oleo and C+/C- as X variables. The trial was never significant, as well as dietary metabolizable energy and crude protein ($P > 0.05$). When comparing Oleo and C- treatments, results showed $2.8 \pm 0.70\%$ ($P < 0.001$; $R^2 = 65\%$) reduction of FCR and $3.6 \pm 1.2\%$ ($P = 0.006$; $R^2 = 56\%$) improvement of ADG with 100 ppm of Oleo. However, when comparing Oleo with C+ treatment there was no significant effect on FCR or ADG, indicating that Oleo and C+ are equal. The FCR_Oleo/C- was negatively correlated (Linear, $P = 0.03$; Quadratic, $P = 0.02$; $R^2 = 85\%$) with the Challenge Acuity Index (C+/C-) indicating that when C+ performed better than C-, so in challenge condition, the reduction of FCR with Oleo addition was higher. The effect was not significant in terms of ADG. This work helps quantifying the average effect of Oleo. However, more data are needed to highlight factor that modulate the response of the birds.

Key Words: oleoresins of spice, essential oil, growth, broiler

660P The effect of a Bacillus-based direct-fed microbial on broiler chickens subjected to a necrotic enteritis challenge. Jessica Edward*¹, Charles Hofacre², Nathan Augspurger³, and Firmin Delago¹, ¹Microbial Discovery Group, Franklin, Wisconsin, United States, ²Southern Poultry Research Group Inc., Watkinsville, Georgia, United States, ³United Animal Health Inc., Sheridan, Indiana, United States.

The objective of this experiment was to quantify the effects of a mild necrotic enteritis challenge on performance of broilers, and the corresponding effect of a direct fed microbial feed additive comprised of multiple strains of *Bacillus subtilis* and *Bacillus pumilis* in the presence of that challenge. A total of 1,800 straight-run broilers (Ross × Ross) were used in a 4-treatment experiment to compare a non-challenged control to a necrotic enteritis challenge alone or combined with dietary supplementation of BMD (55 mg/kg) or the direct-fed microbial (3.67×10^8 cfu/kg). Birds were allotted on day-of-hatch into 36 pens of 50 birds (0.092 m²/bird) to achieve 9 replicate-pens per treatment. All birds were administered a commercially-available coccidia vaccine via a spray cabinet upon arrival to the experimental facility and housed in dirt-floor pens on reused litter. Feed and water were available ad libitum throughout the experiment through one tube feeder and one bell waterer per pen. Experimental diets were based on corn, soybean meal, and DDGS, formulated to be adequate in nutrients (NRC, 1994), pelleted at 80°C, and fed in 3 phases (d 0–13, d 13–35, and d 35–42). The necrotic enteritis challenge was administered through *E. maxima* addition to pens on d 14 followed by *C. perfringens* delivered through the drinking water (approx. 4×10^8 cfu/bird) following a 4-h withdrawal of feed

and 2-h withdrawal of water on d 18, 19, and 20. Data were analyzed by ANOVA procedures with 4 treatments and 9 replicates, and treatment means were separated using the LSD test where the main effect of treatment was significant ($P < 0.05$). The necrotic enteritis challenge increased ($P < 0.05$) NE-related mortality and intestinal lesion scores compared with the non-challenged control. Dietary supplementation of BMD and *Bacillus*-based DFM did not affect ($P > 0.05$) NE-related mortality but did reduce ($P < 0.05$) intestinal lesion scores compared with the challenge treatment. The NE challenge also negatively affected ($P < 0.05$) both 42-d body weight gain (–110 g) and feed conversion (0.11 kg/kg) compared with the non-challenged control group. Neither feed additive affected ($P > 0.05$) body weight gain, but both BMD and the *Bacillus*-based DFM reduced ($P < 0.05$) feed conversion (0.09–0.12 kg/kg) compared with the challenge control. In conclusion, both the *Bacillus*-based DFM and BMD ameliorated intestinal and growth performance effects of a necrotic enteritis challenge.

Key Words: *Bacillus*, DFM, necrotic enteritis, chickens

661P Not Presented

662P Effects of a liquid extract of humic substances in coccidia challenged growing broiler chickens. Maria de Lourdes Angeles^{*1}, Sergio Gomez-Rosales^{2,3}, Yair Lopez-Garcia¹, Eugenio Ruiz Marin¹, and Alejandra Dominguez-Negrete³, ¹INIFAP, Mexico City, Mexico, ²INIFAP, Queretaro, Mexico, ³Autonomous University of Queretaro, Juriquilla, Mexico.

The objective of the study was to evaluate the growth performance, carcass main components weight and yield and the oocyst excretion in broilers chickens from 21 to 42 d of age added with a liquid extract of humic substances (LEHS) in the drinking water and challenged with sporulated *Eimeria* oocysts (SEO). One hundred and 80, Ross 308 males broilers were individually allocated in holding cages and were randomly assigned to 3 levels of a LEHS (0, 15 and 30%) mixed with tap water. The LEHS was obtained from a wormcompost made of sheep manure. Within each treatment, half of the birds were inoculated with 11.5×10^4 of SEO. Broilers were weighed at 21, 28 and 42 d of age and the weight gain (WG) was calculated. The feed intake (FI) and feed conversion ratio (FCR) were also registered. On d 28 and 42, excreta were collected for oocyst counting and 10 chicks from each treatment were killed to get the carcass main components. The data was subjected to ANOVA and orthogonal contrasts. At 28 d of age, the body weight ($P < 0.05$), FI and WG ($P < 0.01$) and the breast and carcass main components weight were higher in broilers receiving 30% LEHS. The FI and WG ($P < 0.05$) were depressed in SEO challenged broilers. The oocyst excretion was higher in 30%LEHS:SEO challenged broilers compared with the rest of the treatments (LEHS and SEO interaction, $P < 0.01$). At 42 d of age, the FI and FCR ($P < 0.01$) were lower in broilers receiving 30% LEHS. The oocyst excretion was lower in broilers receiving 15 and 30% LEHS. The body weight ($P < 0.05$), WG ($P < 0.10$) and the breast and carcass main components weight ($P < 0.05$) were lower in SEO challenged broilers. In summary, the addition of 30% of a LEHS in growing broilers challenged with a 11.5×10^4 dose of SEO, showed increases in production variables and carcass weight at 28 and 42 d of age, as well as reduction of the oocyst excretion at 42 d of age. The SEO challenged broilers at 21 d of age had reductions on the performance responses at 28 and 42 d of age.

Key Words: humic substances, *Coccidia* challenge, productive responses, carcass measurements, oocyst excretion

663P Dietary supplementation of dried plum: A novel strategy to mitigate heat stress in poultry. Sanjeev Wasti^{*}, Donna Kuehu, Nirvay Sah, Amit Singh, Rajesh Jha, and Birendra Mishra, *University of Hawaii at Manoa, Honolulu, Hawaii, United States.*

Heat stress is a significant problem in the poultry industry causing a severe economic loss due to its detrimental effect on health and performances of chickens. A well adoption of effective strategies to mitigate this effect remains limited. Dried plum (DP) is a good source of minerals, vitamins, antioxidants, and phenolic compounds, and plays a role in calcium homeostasis and cardiovascular dysfunctions. Based on the health benefits of DP, we hypothesized that the dietary supplementation of DP would minimize the detrimental effects of heat stress on broilers. To test the hypothesis, day-old broiler chicks ($n = 72$) were randomly allocated to 3 treatment groups ($n = 24$ /group): no heat stress (NHS), heat stress (HS), and heat stress with dried plum (HS+DP), and reared under standard condition. After 21 d, birds in the HS and HS+DP groups were exposed to heat stress condition (33°C for 8 h during day-time) for 3 weeks while those in the NHS group were reared under normal condition. The inclusion of 2.5% DP in the feed of HS+DP group was made during the treatment period, while birds in other groups were provided with a standard diet. Weekly body weight and feed intake were recorded to calculate the average daily growth rate (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). On d 42, chickens were euthanized, a portion of ileum tissue was collected for gene expression and histomorphometric analysis. Similarly, cecal digesta was collected to analyze the microbial population by using 16s rRNA sequencing. The data were analyzed using the SAS and comparisons between different groups were carried out by Tukey-Kramer test after performing one-way ANOVA. Statistical significance was set at $P < 0.05$. Supplementation of DP in the heat stressed birds significantly increased the final body weight, ADG, ADFI, and FCR. Furthermore, supplementation of DP significantly increased the expression of antioxidant-related genes (*SOD1*, *SOD2*, *GPX1*, *GPX3*, *PRDX*, *TXN*), tight junction-related genes (*CLDN1*, *OCLN*), and immune-related genes (*IL-4*, *MUC2*) in the ileum. Additionally, villus height and villus height to crypt depth ratio in ileum was found to be significantly lower in the HS group while DP supplementation was found to improve the villus height and villus height to crypt depth ratio in heat-stressed broilers. At phylum level, the composition of Firmicutes was high and Bacteroidetes was low in HS group compared with NHS group. HS+DP group had reduced composition of Firmicutes compared with HS group. These results support that DP is effective in improving body weight, FCR, gene expression and gut microbial diversity. Thus, dried plum can be a prospective remedy for heat stress in the poultry.

Key Words: cecal microbiota, dried plum, gene expression, mitigation, heat stress

664P An in vitro analysis of the interaction affinity between a dacitic (rhyolitic) tuff breccia and three different mycotoxins. Oscar Tejada^{*1}, Haci Bayir², Matthew Jones³, Nickki Tillman⁴, and Justin Fowler¹, ¹University of Georgia, Athens, Georgia, United States, ²Republic of Turkey, Ankara, Turkey, ³AZOMITE Mineral Products Inc., Nephi, Utah, United States, ⁴Nutritional Statistics, LLC, Buford, Georgia, United States.

Mycotoxin contamination of feed grain is a perennial issue for poultry producers. Exposure can cause impairments in immune function, gut integrity, growth and production. AZOMITE® is a hydrated sodium calcium aluminosilicate (HSCAS), described as a dacitic tuff breccia that is volcanic in origin. HSCAS are known to improve feed manufacture parameters such as flowability, pellet quality, and pellet throughput and

have also been shown to adsorb certain mycotoxins (especially aflatoxin) with a relatively high affinity. For this study, titrated amounts of aflatoxin B₁ (AFB₁), fumonisin B₁ (FUM) and deoxynivalenol (DON) were mixed with a suspension of Azomite (at a concentration of 0.2%) for 24 h to reach an equilibrium concentration. Mycotoxin concentrations used were 0.4, 1.6, 3.2, 6.4, 9.6, and 16.0 ppm. After mixing, mycotoxin concentrations of the supernatant were analyzed via UV spectrophotometry (in duplicate) to determine the amount of unadsorbed toxin. The data were fit to a regression model to evaluate adsorption affinity and capacity. Affinity was determined by the slope of the ascending portion of the curve, and capacity was determined by the break-point of the model. The adsorption affinity for AFB₁ was linear throughout the range of doses evaluated. A saturation point characteristic of the Langmuir Isotherm was not achieved. There was a consistent degree of adsorption for AFB₁ (66% across the range of doses). The binding affinity for DON appeared to be general non-specific binding, averaging 11% adsorption across the range of doses. For FUM, the model was best fit by an exponential response. Between the 0 - 3.2ppm range, the average adsorption was 5%, whereas as the 6.4, 9.6, 16.0 ppm doses there was adsorption of 16%, 38%, and 57% (respectively). The FUM, like AFB₁, also did not reach a saturation point at a 0.2% inclusion. These data suggest that the adsorption capacity of Azomite is not reached when levels of both AFB₁ and FUM were as high as 16.0 ppm in solution.

Key Words: aflatoxin, fumonisin, deoxynivalenol, AZOMITE

665P Effect of an organic approved feed additive to regulate the intestinal pathogenic microflora of broilers reared under challenged conditions. Ashley Wagner^{*1}, Bertrand Medina¹, James McNaughton², and Ivan Girard¹, ¹Probiotech International Inc., *St-Hyacinthe, Quebec, Canada*, ²AHPharma Inc., *Salisbury, Maryland, United States*.

No-antibiotics-ever or organic poultry systems are often associated with more pronounced enteric dysbiosis; and thus, investigation of organic approved commercial additives is necessary. The objective of this trial was to test the efficacy of a proprietary feed additive (Alterna®OP (AOP), blend of phytogenics, organic acids and functional oils, Probiotech International Inc.) to regulate broiler intestinal microflora reared under National Organic Standard (NOS) guidelines. One-d old Cobb chicks (n = 1872) were randomly assigned to 3 treatments: basal group (CTL) and 2 AOP treatments: AOP1- 1.5kg AOP/MT in starter (0–10d) and 1.0kg/MT in grower-finisher (11–39d); AOP2- 1.0kg AOP/MT in starter (0–10d), finisher (30–39d) and 1.5kg/MT in grower (11–29d). Each treatment had 12 replicates of 52 mixed-sex broilers. Birds received coccidiosis vaccine at the hatchery and were grown on built-up litter (moisture >25%): 70% new kiln-dried litter mixed with 30% previously used contaminated with bacteria and coccidia. Intestinal lesion scores and bacteria counts (*C. perfringens*, *E. coli*) were completed at both 10d (2M + 2F) and 39d (20M + 20F) from each pen. *Salmonella* incidence was conducted at both 10d (2M + 2F) and 39d (5M + 5F) from each pen. A multi-factorial procedure using Duncan's LSD post-tests were used on log₁₀-transformed microbial counts, *Salmonella* incidence, and lesion scores. At 10d (CTL:91.7; AOP1:37.5; AOP2:58.3%) and 39d (CTL:83.3; AOP1:44.2; AOP2:58.3%), *Salmonella* incidence gradually reduced ($P < 0.05$) with elevated AOP in the starter where incidence was least in AOP1 vs. AOP2 vs. CTL. The same pattern ($P < 0.05$) was seen for 10d *E. coli* (CTL:6.84; AOP1:5.76; AOP2:6.32 log₁₀ of cfu/g) and *C. perfringens* (CTL:4.54; AOP1:3.41; AOP2:3.87 log₁₀ of cfu/g). However, 39d *E. coli* was reduced for AOP treatments compared ($P < 0.05$) to the CTL (6.75 log₁₀ of cfu/g), but not ($P > 0.05$; AOP1:5.97; AOP2:6.03 log₁₀ of cfu/g) within AOP treatments. Although there was

no ($P > 0.05$) difference in *C. perfringens* between AOP2 (4.31 log₁₀ of cfu/g) and CTL (4.47 log₁₀ of cfu/g), the counts in AOP1 (3.58 log₁₀ of cfu/g) treated birds were lower ($P < 0.05$). Lesion scores at d 10 and 39 demonstrated a graded ($P < 0.05$) reduction by AOP level in the starter with higher, moderate, and lower scores in CTL (10d:1.250; 39d:1.308), AOP2 (10d:0.958; 39d:0.567), and AOP1 (10d:0.354; 39d:0.367), respectively. The results demonstrate the pathogenic microbial inhibition within the gastrointestinal of AOP. Although level within the starter feed may have a greater impact on gastrointestinal health, future research is warranted to determine if these results correspond to growth performance in a NOS and challenged environment.

Key Words: *E. coli*, *Clostridium perfringens*, *Salmonella*, organic poultry systems, feed additives

666P Not Presented

667P Not Presented

668P Evaluation of nutrient supplementation for chickens after hatching. Lucio Araújo*, Luis Barbosa, Cristiane Araujo, and Nara Consolo, *University of Sao Paulo, Pirassununga, Brazil*.

The aim of this study is to evaluate the impact of different nutrient supply for broilers immediately after hatching. Five hundred and 4 birds, 1d old, male, Cobb 500, were distributed in a completely randomized design composed of 6 treatments (T1 -Control - fasting; T2 - hydrolyzed yeast: 7.5 g; T3 - fish oil: 0.8 mL; T4 - vitamins: 0.9 g; T5 - zinc: 1.2 mg; T6 - manganese: 0.48 mg) of 7 replicates with 12 birds per experimental unit. Hydrolyzed yeast, vitamins, zinc and manganese were previously diluted in 1 L of water, properly homogenized and supplied in 0.5mL dose of this solution orally to chicks. The fish oil was provided, also orally, with a syringe. The nutrients were given to the birds after hatching. Then, the chicks were kept in transport boxes for a period of 5 h to simulate the period between the departure from the hatchery and the arrival at the farm. Right after this period, the birds were housed in pens and received a common diet. The performance was evaluated by weight gain, feed intake and feed conversion. At the end of the experiment (41 d), 2 birds per replicate were slaughtered to evaluate the carcass characteristics (carcass, breast, legs yield and abdominal fat). Data were analyzed using the GLM procedure of SAS (2010) and means were compared by Tukey test at 5% probability. Birds fed nutrient supplementation had better weight gain and feed conversion than control treatment ($P = 0.035$ and $P = 0.034$, respectively). Birds fed manganese supplementation showed the best breast yield ($P = 0.003$). The results showed that the supply of nutrients in the post-hatching phase influenced positively the development of broilers.

Key Words: fish oil, manganese, vitamins, zinc, yeast cells

669P Not Presented

670P Not Presented

671P Not Presented

672P Star anise oil effects on antioxidant status in muscle of birds. Xiao Ding* and Zaibin Yang, *Animal Nutrition, Tai'an, China*.

Objective Poultry are exposed to many stressors such as disease and enteric pathogens. Birds suffering from *Escherichia coli* (*E.coli*) use their immune system to eliminate *E.coli* but, as a result, accumulate free radicals. Star anise (*Illicium verum* Hook.f.) has long been used in traditional Chinese medicine and has been shown to possess many beneficial properties. The experiment was conducted to investigate the antioxidant effects of star anise oil in birds during subclinical *E.coli* challenge. Experimental design This experiment uses 512 male birds (White Leghorn) 30 wk of age randomly allocated to 8 treatments with 8 replicates, following a 4 × 2 factorial arrangement to evaluate dietary Star anise oil supplementation (0, 200, 400, 600 mg/kg of basal diet), *E.coli* challenge (with or without oral gavage of *E.coli*) and their interactions. The experiment comprised 1 wk of adaptation and 4 wk of measurement. Materials and methods On the last day of the experiment, 1 bird per replicate was weighed after fasting for 8 h and killed by cervical dislocation. Breast muscle samples were collected and frozen in liquid nitrogen immediately after sampling for chemical and antioxidant assay. Statement of statistical analysis Data were analyzed statistically by 2-factors ANOVA with the use of the general linear model procedure from SAS (version 8.2). Polynomial contrasts were conducted to determine linear and quadratic responses of the birds to dietary Star anise oil dosages. Results There were interactions ($P < 0.05$) between star anise oil supplementation and *E.coli* challenge on the activities of SOD, GSH-Px and CAT and MDA content. Meanwhile, the interactive effects were also significant ($P < 0.05$) on mRNA levels of SOD1, SOD2, Nrf2 and GPX4. With *E.coli* challenge, most birds initially became dull and depressed. *E.coli* challenge also resulted in a significant decrease in final body weight ($P < 0.05$). Birds challenged with *E.coli* also had lower ($P < 0.001$) mRNA expression of CAT than unchallenged birds. Among the groups challenged with *E.coli*, dietary addition of Star anise oil increased ($P < 0.001$) mRNA level of CAT in muscle of birds over those not fed the oil. As the levels of star anise oil increased from 0 to 400 mg/kg of diet, the activities of SOD, GSH-Px and CAT were quadratically increased ($P < 0.05$) in muscle of birds. Meanwhile, the mRNA level of SOD1 in muscle was also linearly and quadratically increased ($P < 0.05$). Conclusions The mechanisms by which star anise oil exerts its antioxidant effect during subclinical *E.coli* challenge correlates with the activation of CAT and Nrf2 in muscle. Our experimental findings provide scientific support for the use of star anise oil in the diets of 30 wk old male leghorn chickens.

Key Words: star anise oil, muscle, antioxidant, enzymes, mRNA levels

673P Metagenomic analysis of cecal microbiota of broiler chickens fed with bacitracin supplemented diet. Muhammad Rehman^{*1}, Quail Das², Xianhua Yin¹, Teri-Lyn Hasted¹, Kelly Ross³, Yan Martel Kennes⁴, Hassina Yacini⁴, Rashedul Islam¹, and Moussa Diarra¹, ¹Agriculture and Agri-Food Canada, Guelph, Ontario, Canada, ²University of Guelph, Guelph, Ontario, Canada, ³Summerland Research and Development Centre, Summerland, British Columbia, Canada, ⁴Centre de Recherche en Sciences Animales de Deschambault, Deschambault, Quebec, Canada.

The gut microbiota affects poultry well-being and productivity. Bacitracin is a polypeptide antibiotic used in chicken feed to prevent necrotic enteritis (NE) caused by *Clostridium perfringens* (CP) that can be translated to improvement of birds' performance, presumably by modulation of gut microbiota. The objective of this study was to investigate microbiome changes in the ceca of broiler chickens fed with bacitracin. A total of 540 male day-old broiler Cobb 500 chicks vaccinated against coccidiosis were fed a diet with or without bacitracin at 55 ppm. Each group consisted of 6 pens of 45 birds/pen. At d 21, 2 birds per pen (12/

treatment group) were sampled for necropsy and internal health as well as for blood and cecal samples collection. Intestine of sacrificed birds were scored for coccidiosis and NE lesions. Blood sera were analyzed for immunoglobulin (IgM) concentrations and DNA was extracted from ceca for metagenomic analysis using an Illumina HiSeq 4000 platform. Galaxy based workflows were used to determine the resistance and microbiome in the metagenomic reads. Performance data were analyzed using the GLM procedure of SAS. Microbiota and microbiome data were analyzed by MANOVA. Body weight (BW), average daily gain (ADG), feed conversion ratio and the mortality rate were significantly improved ($P < 0.05$) in bacitracin-fed group. IgM concentration was significantly higher in the bacitracin treated group ($P < 0.05$). *Firmicutes*, *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* comprised the major bacterial phyla in both groups representing ~97% of all reads. The bacitracin was associated with substantial decrease in relative abundances of the genus *Bacteroides* ($P < 0.05$). Of the 127 bacterial families detected there were significant differences between treatments for *Hafniaceae*, *Oceanospirillaceae*, *Orbaceae*, *Nitrosomonadaceae*, *Nitrospiraceae* ($P < 0.05$). A total of 14 antimicrobial resistant genes (ARGs) were detected regardless of treatment ($P > 0.05$), however a significant correlation was found between bacitracin resistant gene and multidrug efflux pump and tetracycline resistance genes ($P < 0.05$) suggesting bacitracin could promote resistance to tetracycline. Efmamycin resistance gene was also correlated with rifampin, sulfonamides and tetracycline resistance ($P < 0.05$). This study provide new insights to the current knowledge on how bacitracin influence cecal microbiota of chicken with significant changes in the abundance of bacterial species carrying specific ARGs. Further, these findings demonstrates that beside its beneficial effects on performance, bacitracin-feeding has a profound impact on the dynamic of the chicken ceca microbiota and microbiome.

Key Words: broiler chickens, microbiomes, bacitracin, metagenomics, antimicrobial resistance

674P The effects of sodium bisulfate on poultry litter microbiome diversity and pathogen viability. Justin Blair^{*1}, Josh Payne², Hong Li¹, and Amy Biddle¹, ¹University of Delaware, Philadelphia, Pennsylvania, United States, ²Jones-Hamilton Co., Walbridge, Ohio, United States.

Objective: To measure abundance of live/dead populations of *Enterococcus cecorum*, *Clostridium perfringens*, and *Staphylococcus aureus* in poultry litter following treatment with sodium bisulfate (SB). Experimental design: Sodium bisulfate is a litter treatment that lowers the litter pH and ammonia. This experiment measured secondary effects on the poultry litter microbiome including commensal and pathogenic organisms. Materials and methods: Broiler litter from a treatment and a control house was sampled at -1, 2, 24, and 27 d. The treatment house received SB at a rate of 100 lb/1000 ft² on days -1 and 24. Samples were mixed with sterile phosphate-buffered saline (PBS) to suspend bacterial cells, and then washed by centrifugation and resuspended in PBS. Supernatants (500 mL aliquots) were collected and treated with either PMAxx or sterile PBS buffer (control), or autoclaved and PMA treated (heat-killed control) to monitor PMA binding efficiency. The samples were dark/light incubated for PMA activation. These treated solutions were centrifuged, DNA was extracted from the pelleted cells using QIAmp Powerfecal kit. Differential qPCR (40 cycles) comparing PMA-treated and untreated DNA with species specific primers to target bacteria of interest was used to differentiate between live/dead cells. DNA from a composite sample from each house at each time point was sequenced using Illumina MiSeq for comparison. Statement of statistical analysis: Data from the averages of each time point's PMA treated and

untreated cycle threshold (Ct) values were compared with create a ΔCt to represent relative dead levels of bacteria, the PMA treated and PMA treated heat killed data was used to create a ΔCt to help determine PMA binding efficiency for each target organism. MiSeq data was normalized to absolute abundance before comparison. Results and conclusions: The treatment house had consistently slightly higher levels of dead bacteria overall. *S. aureus* was not found in either house. *C. perfringens* may require further investigation into PMA binding efficiency. *E. cecorum* shows a consistent increase in levels of dead bacteria in the treatment house demonstrating it may be reduced by SB. Some bacterial families present in the survey data for the control house were found to be eliminated in the treatment house.

Key Words: sodium bisulfate, poultry litter, microbiome, cell viability, PMA

675P Withdrawn

676P Development of an antimicrobial cold plasma treatment for raw chicken breast meat. Hong Zhuang* and Brian Bowker, *US National Poultry Research Center, Athens, Georgia, United States.*

Microbiological safety, spoilage, and meat quality of fresh chicken meat are main challenges to the poultry industry. The objective of this study was to develop an in-package antimicrobial cold plasma treatment for retaining safety and quality of fresh chicken breast meat (*pectoralis major*). Boneless skinless broiler breast meat was collected from a local commercial plant. Non-inoculated meat samples and meat samples inoculated with *Campylobacter jejuni* and *Salmonella* Typhimurium were packed in polymeric trays under ambient air conditions. The samples were treated with a dielectric barrier discharge cold plasma at different voltages (55, 70, or 85 kV) for 180 s or at 70 kV for different times (60, 180, or 300 s). Treated meat was stored at 4°C for 5 d. Microbial counts (psychrophiles, *C. jejuni*, and *S. Typhimurium*) and meat color ($L^*a^*b^*$) were measured before cold plasma treatments and after 5 d of post-treatment storage. One-way ANOVA was used for data analyses. Psychrophilic growth was inhibited ($P < 0.05$) and both foodborne pathogen populations were reduced ($P < 0.05$) by the cold treatments regardless of treatment voltage or time. No differences in psychrophilic and *S. Typhimurium* counts were observed between the 3 treatment voltages. However increasing treatment voltage beyond 55 kV resulted in additional inactivation of *C. jejuni*. Treatment time did not influence *C. jejuni* and *S. Typhimurium* counts; however, elongating treatment time beyond 60 s further reduced psychrophilic counts. In terms of meat color, there were no differences ($P > 0.05$) in a^* (redness) or b^* (yellowness) values between pre-treatment and post-treatment plus storage measurements. However, cold plasma treatments resulted in increased L^* (lightness) values ($P < 0.05$). Results indicate that cold plasma treatments can be used to reduce both microbial spoilage and foodborne pathogen risks; however, it may increase pale color in raw chicken breast meat.

Key Words: broiler, psychrophile, *Salmonella*, *Campylobacter*, color

677P Establishing an in ovo challenge model for Salmonella Enteritidis. Tomohiro Hamaoka*, Jason Marshall, and Andrew Van Kessel, *University of Saskatchewan, Saskatoon, Saskatchewan, Canada.*

To establish a novel *in ovo* *Salmonella* Enteritidis (SE) challenge model, 2 trials were conducted to investigate the effect of dose and timing of SE

inoculation into amniotic fluid on the chick physiology. All experiments used fertilized eggs (White Leghorn, University of Saskatchewan Poultry Centre) externally sanitized with 0.5% vol/vol of sodium hypochlorite, weighed, and incubated in HEPA-filtered incubators under standard conditions. After the inoculation, all eggs were incubated in a HEPA-filtered sterilized hatcher until 22 d of incubation (E22). In experiment 1, at d 17 of incubation (E17), a total of 95 eggs were randomly assigned to 5 treatments balanced for weight at E0. Control eggs received 100 μ L of sterilized saline and the other 4 groups received 6×10^2 , 6×10^4 , 6×10^6 or 6×10^8 cfu SE per bird in saline (100 μ L). In experiment 2, 8 treatment groups balanced for weight at E0 were inoculated with either saline (100 μ L) or saline containing 10^2 cfu SE at E17, E18, E19 or E20. At E22, the number of pipped, hatched and live birds were recorded. Birds alive at E22 (hatched or pipping) were euthanized before recording body weight and yolk sac weight. Cecal contents and blood were collected from 5 birds per treatment to enumerate SE by culture on DHL agar (NISSUI) at 37°C for 24h. In experiment 1, 63–84% of SE birds were pipped and 74–95% were dead by E22, with no clear dose response relationship. Healthy birds hatched from all control eggs whereas no SE-inoculated eggs hatched. Counts of SE ranged from 9.9 to 10.3 log cfu/g in cecum and from 4.0 to 5.6 log cfu/g in blood also without dose response relationship. No SE were observed in control birds. For experiment 2, responses became less severe as inoculation age increased. Hatching rate of SE treatment was 0, 23, 85 and 100% on E17, E18, E19 and E20 respectively. Mortality rate was 92% and 85% for SE group on E17 and E18. Remaining yolk sac increased ($P < 0.05$) and egg yolk-free body mass was decreased ($P < 0.05$) in SE group at E17, E18, and E19, but E20 was not different from control. In SE treatment, SE in cecum ranged from 9.4 to 10.0 log cfu/g whereas in blood SE ranged from 6.9 to 7.3 on E17 and E18. SE was cultured in only 1 of 5 birds on E19 and was not from blood on E20. SE was recovered in blood of 1 of 5 control birds at E19 and E20. *Salmonella* Enteritidis inoculation in amniotic fluid was lethal before E19 associated with translocation and high recovery from blood. The response to SE declined as embryonic age at inoculation increased such that no gross effects were observed by E20. Results are consistent with previous findings following inoculation with non-pathogenic *E. coli* and suggest maturation of intestinal barrier function at E20.

Key Words: *Salmonella*, in ovo, barrier function, chick quality

678P Withdrawn

679P Hot water at high pressure reduces the number of bacteria on broiler carcasses pre-scald, post scald and post-pick. Douglas Cosby^{*1}, Joshua DeVoll², Susan Mize¹, Michael McIntyre², Osman Koyun³, Todd Callaway³, Mark Berrang⁴, and Arthur Hinton¹, ¹USDA, ARS, PMSPRU, Athens, Georgia, ²Spray Systems Company, Wheaton, United States, ³University of Georgia, Athens, United States, ⁴USDA, ARS, Athens, United States.

Reducing the number of bacteria on carcasses on the kill line would be expected to reduce number of bacteria entering the evisceration line and chill tank. This study was designed to evaluate the efficacy of a high pressure (250 psi), low volume, fluidic nozzle equipped wash cabinet using heated tap water (160°F) to remove bacteria from pre-scald, post-scald and post picked carcasses. Carcasses (n = 5) from each location were obtained from a commercial processing plant and transported to the laboratory pilot plant. Carcasses were hung in standard shackles within an experimental spray cabinet and sampled by sponge swab of the breast skin. All carcasses were washed with water at 250 psi and 160°F; post-

wash breast sponge samples were collected. Ten mL of buffered peptone water was added to plastic bags containing sponges, and sponges were stomached for 60 s. Total aerobic bacteria (TA), *Enterobacteriaceae* (Ent) and *E. coli* (EC) were enumerated by plating serial dilutions of sponge rinsates on the appropriate PetriFilm® cards, and *Campylobacter* presence or absence was determined by streaking on Campy-Cefex agar (CCA) plates. All PetriFilm® were incubated at 37°C for 24 h, and CCA were incubated in a microaerobic environment at 42°C for 48 h. Three replications (n = 15) were conducted on separate dates. Paired *t*-tests were used to compare numbers recovered from breast samples before and after high pressure spray wash. Statistical significance is reported at $P < 0.05$. The wash treatment caused reductions of 2.00 and 1.29 log₁₀ colony forming units/mL (cfu/mL) were observed for TA on post-scald and pre-scald carcasses, respectively. Additionally, a 1.23 log₁₀ cfu/mL reduction in the counts for EC was observed on post-scald carcasses. These data indicate that heated water and high pressure combined can reduce bacterial numbers on carcasses before entering the evisceration area of a processing plant. This reduction might decrease the need for chemical treatments in the chiller and on the finished carcasses.

Key Words: bacterial load, processing, carcasses, broilers, wash

680P Identification of Gram-positive cocci isolated from RWA poultry with undefined enteritis or necrotic enteritis. Audrey Charlebois*, Eric Parent, and Martine Boulianne, *University of Montreal, St-Hyacinthe, Quebec, Canada.*

Presence of *Clostridium perfringens*, but also of numerous Gram-positive cocci was observed histologically in intestines from raised without antibiotics (RWA) broilers affected with undefined enteritis or necrotic enteritis. These flocks were part of a year-long field trial where RWA and conventionally raised chickens were compared. The objective of this study was to identify Gram-positive cocci isolated from these flocks. Sixty-six fecal samples recovered from healthy birds or birds with undefined enteritis or necrotic enteritis were submitted to our laboratory for isolation of Gram-positive cocci. Purified isolates were then identified by the API rapid ID 32 Strep and ID 32 Staph. In all, 207 colonies corresponding to Gram-positive cocci were isolated. After biochemical identification, the most predominant species were: *Aerococcus viridans* (n = 45), *Globicatella sanguinis* (n = 25) and *Staphylococcus saprophyticus* (n = 21). *Globicatella sanguinis* is closely related to *A. viridans*. As so, catalase, oxydase and production of H₂S were tested on all *G. sanguinis* isolates. All tests were negative which is consistent with *G. sanguinis*. Since this bacterial species has never been described in poultry, additional genetic 16S rRNA sequencing analyses were carried out. Results showed that all *G. sanguinis* isolates were identified as *Aerococcus* sp. (98–99%). *A. viridans* is believed to be a normal inhabitant of the chicken gut flora as it has been isolated in poultry meat and egg shell membrane both likely contaminated by fecal material. In addition to bacterial identification, intestinal lesion scoring will be performed on samples from all flocks and scores will be compared and linked to the presence of Gram-positive cocci. In conclusion, this study showed that a large number of *A. viridans* isolates were recovered from poultry with undefined enteritis or necrotic enteritis. Moreover, this study highlights the importance of using genetic tools for bacterial identification.

Key Words: Gram-positive cocci, enteritis, 16S rRNA sequencing, API strips, *Aerococcus viridans*

681P Therapeutic effect of boric acid against *Salmonella* Enteritidis infection, intestinal permeability, total IgA

concentration, and cecal microbiome composition in broilers chickens. Daniel Hernandez-Patlan¹, Margarita Arreguin-Nava^{*2}, Bruno Solis-Cruz¹, Bishnu Adhikari³, Juan Latorre³, Xochitl Hernández-Velasco⁴, Rubén Merino-Guzmán⁴, Billy Hargis³, Raquel López-Arellano¹, and Guillermo Tellez⁵, ¹Facultad de Estudios Superiores (FES) Cuautitlan, Universidad Nacional Autónoma de México (UNAM), Cuautitlan Izcalli, Estado de México, Mexico, ²Eco-Bio LLC, Fayetteville, Arkansas, United States, ³University of Arkansas, Fayetteville, Arkansas, United States, ⁴Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Coyoacán, Ciudad de México, Mexico, ⁵University of Arkansas, Fayetteville, Arkansas, United States.

Recently, our laboratories have published in vitro studies showing the bactericidal properties of BA against *Salmonella* Enteritidis (SE). Therefore, the objectives of the present study were to evaluate the antimicrobial effect of BA on SE colonization, intestinal permeability, total intestinal IgA levels, and cecal microbiota composition in broiler chickens. For this purpose, 60 d-old-chicks were randomly allocated to one of 2 groups: 1) nontreated control group and 2) 0.1% (wt/wt) BA in the feed. All chicks were challenged with 10⁴ cfu of SE per bird at 1-d old. On d 3 and 10 post-SE challenge, samples of crop and ceca-cecal tonsils (CCT) were aseptically collected to evaluate overall aerobic, lactic acid bacteria and SE recovery. However, only on d 10 post-SE challenge, blood and intestinal samples to evaluate intestinal permeability of fluorescein isothiocyanate-dextran (FITC-d) and total intestinal IgA production, respectively, as well as the content of the left ceca for microbiota analysis were collected. Data from bacterial counts (Log₁₀ cfu/g), total IgA levels and serum FITC-d concentration were subjected to ANOVA (ANOVA) and significant differences among the means were determined by Duncan's multiple-range test at $P < 0.05$. Microbiota was analyzed using Quantitative Insights into Microbial Ecology, QIIME version 1.9. at Jetstream cloud computing platform. No significant differences were found on d 3 post-SE challenge between the groups, whereas on d 10 post-SE challenge, the concentration of SE in crop and CCT significantly decreased ($P < 0.05$) by 2.60 log and 5.26 log, respectively, in the BA group compared with the control group. Furthermore, chickens treated with BA had a significant ($P < 0.05$) reduction in serum FITC-d concentration and lower total intestinal IgA levels by 0.17 µg/mL and 10.1 µg/mL, respectively, when compared with the control group. Interestingly, in the microbiota analysis, only the abundance of Actinobacteria phylum was significantly lower ($P < 0.01$) in the BA group (0.038%) in comparison with the control group (0.14%) and significant differences ($P < 0.05$) in β diversity were also observed between the groups. The results of the present study suggest that BA could maintain intestinal homeostasis, as well as the balance in the microbiota due to its effectiveness in controlling SE infection.

Key Words: boric acid, chicken, inflammation, microbiota, *Salmonella* Enteritidis

682P Characterizing gastrointestinal microbial communities from laying hens housed in conventional and aviary housing types. Maddison Wiersema*, Stephan Schmitz-Esser, Lucas Koester, and Dawn Koltes, *Iowa State University, Ames, Iowa, United States.*

Recent changes in consumer preference have shifted egg production toward cage-free housing. However, the physiological impact of the cage-free setting on bird health is not well understood. The gastrointestinal microbiome of laying hens plays a vital role in maintaining hen health and productivity. Therefore, this study was conducted to determine if microbial communities were different between hens housed in an aviary compared with conventional cages, and to determine diversity of micro-

bial communities between housing types. Ileal digesta was collected from hens in at a single commercial site. Forty-one hens were housed in conventional cages, and 49 hens were housed in an aviary system. Bacterial DNA was extracted using the Qiagen Powerlyzer Powersoil kit and sequenced for the 16S rRNA gene via Illumina MiSeq. All sequences were prepared, aligned, and classified using MOTHUR software. After undergoing quality control, 6,474,777 were generated. Sequences were grouped into 2,658 operational taxonomic units (OTU's) with 99% similarity with a minimum of 10 reads. Diversity values calculated from MOTHUR yielded highest species diversity from hens housed in the conventional cage systems (Shannon = 2.11) compared with hens from the aviary system (Shannon = 1.92). Differences were observed ($P < 0.05$) in microbial communities between housing type as determined by MOTHUR. The most abundant microbial populations from hens in the conventional cage housing system were *Romboutsia ilealis* (30.80%), *Lactobacillus avarius* (11.15%), and *Candidatus Arthromitus* (5.18%). These microbes are commonly found in adult broilers and are often associated with normal intestinal health. The most abundant microbial populations from hens housed in aviaries were *Lactobacillus kitasatonis* (34.29%), *Clostridium colinum* (4.55%), and *Gallibacterium anatis* (1.91%). These microbial communities have been associated with intestinal diseases with *Gallibacterium anatis* recently identified as an opportunistic pathogen in chicken. While we observed microbial populations that are associated with disease states, all birds appeared healthy at time of sampling and inferences regarding function should be made sparingly. While these data indicate that hens from the conventional cage systems may have a more suitable intestinal microbiome overall, additional research is needed to elucidate the true function and contribution of these microbes to the overall intestinal health.

Key Words: microbiome, laying hens, housing type, conventional cage, aviary

683P Efficacy of a novel, all-natural bioproduct to reduce microbial populations within poultry litter. Michael Rothrock*¹, Kimberly Cook¹, Ashley Wagner², and Ivan Girard², ¹USDA-ARS, Athens, Georgia, United States, ²Probiotech International Inc., Quebec, Quebec, Canada.

Due to the recent implementation of the Veterinary Feed Directive, antibiotic use in poultry production can no longer be used for prophylactic purposes. Therefore, there is a direct need to develop alternative to antibiotics to control microbial populations inherent throughout the poultry production chain. Poultry litter acts as a microbial reservoir during the pre-harvest period and represents a critical control point for the contamination/re-contamination of a commercial flock. The objective of this study was to test the efficacy of a novel, proprietary biological product to reduce general microbial populations and pathogenic populations. In a laboratory scale microcosm study, 5 different rates of the bioproduct (1X application on d0, 10X application on d0, 100X application on d0, 1X application weekly, 0X control) were added to triplicate jars each containing 350g of poultry litter collected from commercial poultry houses after 2–3 flocks, and the litter moisture content was raised to ~55% to emulate conditions found under the waterer lines. On a weekly basis (to d42) homogenous samples were taken from each jar (~15g) and analyzed culturally (total aerobic, total Enterobacteriaceae, coliforms), molecularly via qPCR (*Salmonella*, *Campylobacter*, *Clostridium*), and physiochemically (pH, EC, moisture content), and 2-way ANOVAs using Fisher's LSD post-tests were used to look at the effect of sampling time and treatment on the microbial (log₁₀-transformed) and physiochemical data. While sampling time significantly affected all microbial populations tested, the bioproduct significantly reduced total

Enterobacteriaceae ($P < 0.0001$), coliforms ($P < 0.0001$) and *Clostridium* ($P = 0.0128$), while not significantly affecting litter physiochemistry. In terms of rate of bioproduct application, the 10X rate was the most effective at reducing bacterial populations, resulting in significantly lower total Enterobacteriaceae ($P < 0.0002$), coliforms ($P < 0.0197$) and *Clostridium* ($P < 0.0474$) than the other application rates and the control. The natural *Salmonella* and *Campylobacter* levels in the litter were quite low throughout (<1 log copies/qPCR), therefore future spiking studies will be needed to assess the true efficacy of this bioproduct against these major foodborne pathogens. These data suggest that a 10X application rate of this novel, proprietary bioproduct has the potential to significantly reduce general and potentially pathogenic microbial populations with poultry litter, and these studies need to be expanded to house-scale tests with live birds to further assess their efficacy to be considered an antibiotic alternative used within the poultry industry to improve product safety.

Key Words: antibiotic alternative, poultry litter, pre-harvest, food safety

684P Bacteriocins produced by commensal *Clostridium perfringens* strains as a new alternative to control necrotic enteritis.

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With the current increase in antimicrobial resistance and consumers' concern, the Canadian poultry industry has opted for the preventive withdrawal of antibiotics considered of human importance by 2020. New alternatives are needed to control necrotic enteritis (NE), a disease that causes billions of dollars in economic losses to the poultry industry worldwide. Bacteriocins are antimicrobial peptides produced by bacteria to kill or inhibit the growth of other bacterial competitors. These peptides may become a potential candidate as alternatives to fight pathogenic bacteria in the future. Perfrin is the only reported bacteriocin associated with pathogenic strains of *Clostridium perfringens*, the causal agent of NE. The aims of this study were to screen for commensal *Clostridium perfringens* strains with an antimicrobial activity against *C. perfringens* pathogenic strains and to identify and characterize the produced bacteriocins. Commensal *C. perfringens* strains were selected from our isolate bank. Antimicrobial activity of those selected strains was tested against *C. perfringens* pathogenic strains using the agar spot test method. Commensal strains showing antimicrobial activity were cultured in BHI broth medium and centrifuged to retain the supernatant containing bacteriocins. Proteins were precipitated using the ammonium sulfate precipitation method and purified by an ion exchange chromatography followed by a hydrophobic interaction chromatography. After each chromatography, antimicrobial activity of each fraction was tested using the method described above to choose fractions containing bacteriocins. Heat stability of the bacteriocin was characterized by boiling the sample for one hour. Proteinase K susceptibility was also characterized by incubating the sample with 2 different concentrations of proteinase K, 0.1 mg/mL and 1 mg/mL, at 37°C for one hour. Antimicrobial activity was tested after both tests to assess if the bacteriocin was inactivated. Among tested strains, 2 had an antimicrobial activity against various pathogenic strains. Bacteriocins from strain A were isolated by HPLC and will be analyzed by mass spectrophotometry. The bacteriocin activity was lost after an hour incubation at 100°C suggesting that it is thermolabile. No antimicrobial activity was observed after incubation with proteinase K at both tested concentrations suggesting that the bacteriocin is susceptible to proteases activity. In this study, commensal *C. perfringens* strains producing bacteriocins active against pathogenic strains have been identified. These bacteriocins could be an

interesting alternative to antibiotics for the control of necrotic enteritis but further data is needed.

Key Words: bacteriocins, *Clostridium perfringens*, antibiotic resistance, purification, bacteriocin characterization

685P Innate and adaptive immune responses in broilers to oral delivered chitosan-based *Salmonella* subunit nanoparticle vaccination. Yi Han*¹, Renu Sankar¹, Ramesh Selvaraj², and Renukardhya Gourapura¹, ¹The Ohio State University, Columbus, Ohio, United States, ²University of Georgia, Athens, Georgia.

Salmonella enterica serovar enteritidis (*S. enteritidis*) infection of poultry causes a significant risk to public health through contamination of meat from infected animals, especially broilers. Current *Salmonella* vaccines utilized in poultry are not effective. Considering the short life-span of broilers, an easy-to-apply and effective *Salmonella* vaccine is urgently needed for broilers. We designed a chitosan-based *Salmonella* subunit nanoparticle vaccine containing outer membrane proteins and flagella of *S. enteritidis*, and vaccinated broiler birds orally at 3 d of age and booster dose administered 2 weeks later. We describe here the temporal expression of important immune molecules associated with innate and adaptive responses at various days post vaccination (dpv). In the cecal tonsils of chitosan nanoparticle *Salmonella* vaccinates, we observed enhanced expression of mRNA coding toll-like receptors (TLRs)-2 (3 dpv), TLR-3 (7 dpv) and TLR-21 (7 dpv) after the first dose of vaccination; and cytokines interleukin-1beta (IL-1 β) and IL-4 at 7 dpv after booster inoculation. Outer membrane proteins specific lymphocytes proliferation response in spleen were enhanced at 7 dpv after booster vaccination with chitosan nanoparticle *Salmonella* vaccine. However, the overall antibody response was low, maybe due to the young age of birds. In conclusion, we showed that chitosan nanoparticle *Salmonella* vaccine augments innate and cellular immune responses following oral vaccination in broilers. Studies are in progress to determine the candidate vaccine efficacy in a bacterial challenge infection trial in vaccinates.

Key Words: *Salmonella*, broiler, vaccine, nanoparticle

686P Susceptibility of *Salmonella* strains in feed to a formaldehyde based product. Jose Ramirez*, Anitox Corp., Lawrenceville, Georgia.

Feed and feed ingredients are considered fomites for microbial pathogens such as mold, bacteria and viruses, causing concern for food safety, animal welfare and production efficiency. Various intervention strategies have been utilized to reduce this risk including exclusion of high-risk ingredients, heat treatment and chemical preservatives. Exclusion of high-risk ingredients was a common strategy for reducing risk in feed, however research has shown that no ingredient is devoid of risk. Pelleting and extrusion have been reported to reduce *Salmonella* levels in feed. The emergence of thermotolerant strains of *Salmonella* have required more intensive pelleting conditions to control. Chemical preservatives, such as organic acids and formaldehyde, are also used to control bacteria/viruses. Organic acids reducing the level of microorganisms in feed by 50 - 90% at usage rates of 5 to 10 kg/ton. Differences in the susceptibility of *Salmonella* strains to organic acids has been reported and long-term usage can result in acid-tolerant strains. Formaldehyde has been shown to be effective against *Salmonella*. Information on the sensitivity of *Salmonella* strains in feed to formaldehyde is limited. Experiments were conducted to determine the minimum effective dose (MED) of formaldehyde against *Salmonella* and the susceptibility of different strains of *Salmonella* to the MED. In the dose range finding

experiment, *S. typhimurium* was grown in aqueous meat and bone meal and a dry inoculum prepared. Poultry feed was treated with 0 (control), 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 kg/ton of the formaldehyde base product (FBP). At 24h and 7d post treatment, 10g samples of feed (3 replicates per treatment level) were transferred to 90 mL of buffered peptone water and serial dilutions plated on xylose lysine tergitol 4 agar and plates incubated at 35°C for 24h (3 replicate plates per dilution). After 24h of incubation, *Salmonella* colonies were enumerated, and average percent reduction compared with the control determined. At the 24h time interval, no *Salmonella* (<10 cfu/g) was isolated from the 1 kg/ton treatment level (100% reduction). At the 7d test interval, no *Salmonella* was detected at the 0.75 kg/ton treatment level. In the second set of experiments, the susceptibility of 10 strains of *Salmonella* to 1 kg of FBP was determined. Isolates were tested individually in separate experiments. Feed was treated with 1 kg/ton FBP in the lab mixer and percent reduction of *Salmonella* in feed determined at 24h. In these experiments, it was observed that that all strains tested were susceptible to the FBP at the 1 kg/ton treatment level.

Key Words: *Salmonella*, feed, formaldehyde, susceptibility

687P Development of an *in vitro* model for investigating inhibition of pathogen adhesion to chicken epithelial cells by dietary prebiotics. Laney Froebel* and Tri Duong, Texas A&M University, College Station, Texas, United States.

Prebiotics are selectively fermented feed ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota which confer benefits upon host health. *Salmonella* is a human foodborne pathogen commonly associated with the gastrointestinal (GI) tract of poultry that poses a significant burden to public health. The administration of mannan-oligosaccharides (MOS) as a dietary prebiotic has been demonstrated to improve microbial food safety by reducing GI colonization of *Salmonella* and other human foodborne pathogens in poultry. Although the ability of MOS and other dietary prebiotics to inhibit adhesion of pathogenic bacteria to epithelial cells is thought to be important to their functionally, the mechanisms responsible are not well understood. The goal of our study was to develop an *in vitro* model to investigate the inhibition of pathogen adhesion to chicken epithelial cells by dietary prebiotics. We evaluated the effect of a commercial *Saccharomyces*-derived MOS product on the adhesion of *Salmonella* to the LMH chicken epithelial cell line to develop a standard set of conditions for use in future assays. *Salmonella* was co-incubated with chicken epithelial cells in 0.0% to 2.5% MOS (% wt/vol), and the number of adherent bacteria was enumerated. Counts of adherent *Salmonella* were log₁₀ transformed for analysis using ANOVA with significance being evaluated at $P \leq 0.05$. Inhibition of *Salmonella* adhesion by MOS was determined to be dose-dependent ($P < 0.01$). Additionally, 2.5% MOS was observed to reduce *Salmonella* adhesion by up to 3.9 log₁₀ cfu. Our results suggest this assay using the LMH chicken epithelial cell line to be a suitable method to evaluate the inhibition of pathogen adhesion by dietary prebiotics. Future studies will extend this model to investigate additional dietary prebiotics, such as products that contain fructo-oligosaccharides or β -glucans, and other human foodborne pathogens including *Campylobacter* spp. and poultry pathogens, such as *Clostridium perfringens* and avian pathogenic *E. coli*.

Key Words: dietary prebiotics, mannan-oligosaccharides, *Salmonella*, adhesion

688P Next generation sequencing of subsamples used for traditional microbiological analysis of *Campylobacter* in broiler

carcass rinsates collected with neutralizing buffered peptone water or buffered peptone water. Jennifer Wages^{*1}, Kristina Feye², B. J. Bench^{3,1}, and Steven Ricke¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²USDA, Fayetteville, Arkansas, United States, ³Tyson Foods Inc., Springdale, Arkansas, United States.

Campylobacter is one of the most prevalent foodborne microorganisms in the world. The poultry industry monitors for this pathogen throughout harvesting using rinsates. Traditional microbiological monitoring and molecular methods are perpetually improved to ensure suitable sensitivity and specificity. This study set out to determine if buffered peptone water (BPW) or neutralizing buffered peptone water (nBPW) increased the recovery of *Campylobacter* from poultry carcasses monitored in a commercial facility. Eighteen broiler carcasses collected in pairs at the exit of chiller were individually rinsed with 400 mL of nBPW or BPW. Rinsates were direct plated onto *Campylobacter* Chromogenic Plating Medium (CCPM) agar plates to determine *Campylobacter* cfu/mL counts. Aliquots (30 mL) of rinsates were enriched in Bolton broth for prevalence counts. After either the initial sampling point or post-enrichment, rinsates were pelleted, washed, re-suspended in PBS, and placed in -80°C . *Campylobacter* typical growth obtained from CCPM plates were also removed from the agar, suspended in 2.0 mL PBS and placed in -80°C freezer. Genomic DNA extraction of the frozen Bolton broth suspensions were performed using a QIAamp DNA Stool Mini Kit (Qiagen). Genomic DNA extraction of the frozen *Campylobacter* isolates obtained from CCPM agar plates were performed using DNeasy Blood and Tissue Kit (Qiagen). Extracted genomic DNA was amplified based on V4 region of 16S rRNA gene sequences, and resulting sequences were multiplexed, pooled and sequenced using MiSeq[®] v2 (500 cycle) Reagent cartridge (Illumina) on the Illumina MiSeq[®]. Sequences were analyzed using QIIME 2 pipeline version 2018.11. No significant differences were observed in sequences observed from culturing methods when rinsates were direct plated on CCPM agar plates. A significant difference in OTUs belonging to the genus *Vibrio* were observed in the Bolton broth enrichments from nBPW rinsates when compared with the BPW rinsates. Prevalence levels of *Campylobacter* in nBPW and BPW rinsates were 78% and 89%, respectively. Taxonomic profiles of the 2 rinsates identified genus *Lactobacillus* to be more dominant in BPW samples compared with nBPW. Genus *Escherichia* *Shigella* OTU was more dominant in nBPW compared with BPW. The differences in OTUs observed indicate that the choice of rinsate influences results of culturing methodologies.

Key Words: broilers, microbiome, nBPW, processing, *Campylobacter*

689P Evaluation of the antimicrobial and anti-inflammatory properties of *Bacillus*-DFM (NorumTM) in broiler chickens infected with *Salmonella* Enteritidis. Bishnu Adhikari^{*1}, Daniel Hernandez-Patlan³, Bruno Solis-Cruz³, Young Min Kwon¹, Margarita Arreguin-Nava², Juan Latorre¹, Billy Hargis¹, and Guillermo Tellez¹, ¹University of Arkansas, Fayetteville, Arkansas, United States, ²Eco-Bio LLC, Fayetteville, Arkansas, United States, ³Universidad Nacional Autónoma de México (UNAM), Cuautitlán Izcalli, Estado de México, Mexico.

Restrictions of in-feed antibiotics use in poultry has pushed researches toward finding their appropriate alternatives such as Direct-Fed Microbials (DFM). In this study, previously tested *Bacillus* isolates (*B. subtilis* and *B. amyloliquefaciens*) were used to evaluate their therapeutic and prophylactic effects against *Salmonella* Enteritidis in broiler chickens. For this purpose, initial antibacterial activity of *Bacillus*-DFM (10^4 spores/g or 10^6 spores/g) against *S. Enteritidis* colonization in crop, proventriculus and intestine was investigated using in vitro digestive

model. Furthermore, to evaluate therapeutic and prophylactic effects of *Bacillus*-DFM against *S. Enteritidis* ceca-cecal tonsils (CCT) and crop colonization, 25 broiler chickens were randomly allocated to either DFM or Control group depending upon whether they received *Bacillus*-DFM (10^4 spores/g) or not, respectively. Chickens were orally gavaged with 10^4 cfu of *S. Enteritidis* per chicken at 1-d old and samples were collected at 3 and 10 d later during therapeutic study, whereas they were orally gavaged with 10^7 cfu of *S. Enteritidis* per chicken at 6-d old and samples were collected 24 h later from 2 independent trials during prophylactic study. Serum SOD, FITC-d and intestinal IgA levels were reported for both chicken trials, in addition of cecal microbiota analysis from therapeutic study. DFM significantly reduced *S. Enteritidis* colonization in intestine, and in both proventriculus and intestine as compared with the Control when used at 10^4 spores/g and 10^6 spores/g, respectively ($P < 0.05$). In addition, there was a clear tendency of numerical reductions of *S. Enteritidis* CCT and crop colonization by DFM in both therapeutic and prophylactic studies. DFM significantly reduced FITC-d and IgA, and SOD and IgA levels as compared with the Control in therapeutic and prophylactic studies, respectively ($P < 0.05$). Interestingly, there was significant difference in bacterial community structure between DFM and Control. Likewise, phylum Actinobacteria and the genera *Bifidobacterium*, *Roseburia*, *Proteus*, and cc_115 were decreased, while the genus *Streptococcus* was enriched significantly in DFM group as compared with the Control (MetagenomeSeq, $P < 0.05$). Moreover, bacterial genes that are predicted to be involved in various metabolic pathways such as bile acid synthesis, carbohydrate and purine metabolism were enriched in the Control, while aminoacid metabolism and alkaloid biosynthesis were enriched in the DFM. Thus, the overall results suggest that the *Bacillus*-DFM can reduce *S. Enteritidis* colonization and improvement of intestinal health in chickens through mechanism(s) that might involve the modulation of gut microbiota and their metabolic pathways.

Key Words: *Bacillus*-DFM, *Salmonella* Enteritidis, inflammation, microbiota, broiler chickens

690P Sanitizing broiler hatching eggs with lysozyme in incubators provided with light. Vicki Mackintosh^{*}, Bruce Rathgeber, Janice MacIsaac, and Murray Clements, Dalhousie University Faculty of Agriculture, Truro, Nova Scotia, Canada.

Early chick quality is crucial for positive poultry performance. Commercial egg sanitizers are currently utilized in hatcheries to reduce potential bacterial contamination, and improve overall chick quality. The current egg sanitizer widely used in the hatchery industry is formaldehyde-based. This threatens the safety of hatchery employees as well as the health of newly hatched chicks. The toxicity of this substance has led to the experimentation of novel sanitizers. Evidence suggests that providing light during incubation influences embryo development, and may impact the effectiveness of in-hatchery sanitation procedures. The objective of this experiment was to determine whether the addition of LED lighting would interact with the use of 3% lysozyme as a sanitizer. Three thousand 2 hundred, Cobb 500, eggs were placed into 8, chick master incubators. The experimental design was a completely randomized factorial (2x2x2) with lighting, breeder age, and sanitizer as factors. Of the 8 incubators, 4 were equipped with red LED lights, and exposed to 12h of light per day. The remaining 4 were left in darkness. For each light treatment, 2 of the incubators, were treated with 3% lysozyme sanitizer, while the other 2 were exposed to water as a control. Half of the eggs in each incubator were acquired from 57-week-old hens; the remaining 200 were obtained from 27-week-old hens. Eggs were incubated for a total of 512 h, and mortalities were monitored. On d 1 and 18, the incubators were treated with the lysozyme sanitizer or the control. Post hatch,

chicks were measured for quality indicators such as; navel score, chick length, and chick weight. Navel score was determined using a scale of 1 to 3 (Molenaar, 2011). PROC MIXED in SAS was used to conduct ANOVA to investigate interactions between the treatments ($\alpha = 0.05$). Tukey-Kramer multiple range comparison was performed to determine differences between treatments and treatment conditions. There was no significant difference ($P > 0.05$) in the level of mortalities during incubation between provision of light or incubation in the dark. Hatchability, chick weight, and chick length did not differ between the light and dark treatments. Eggs treated with lysozyme and incubated in the dark had improved navel scores. While LED lighting did not increase chick quality, the lysozyme treatment provided an effective preventative measure for navel infection and contamination.

Key Words: broiler, chick quality, lighting, lysozyme incubation

691P Effects of multiple alternative-to-antibiotic interventions on multidrug-resistant *Salmonella* Heidelberg in commercial turkeys. Divek V. T. Nair, Jijo Vazhakkattu Thomas, Grace Dewi, Sally Noll, Timothy Johnson, and Anup Kollanoor Johny*, *University of Minnesota, St. Paul, Minnesota, United States.*

Salmonella Heidelberg (SH) colonizes the cecum of turkeys and can potentially contaminate the carcasses through faulty evisceration during processing. The objective of this study was to determine the efficacy of turkey-derived *Lactobacillus* (*L. ingluviei* and *L. salivarius* in combination; PRO), a mannanoligosaccharide prebiotic (PRE) and a *Salmonella*-specific vaccine (VAC) against cecal colonization of multidrug-resistant (MDR) SH in 12-week old commercial turkeys. The 9 treatments included in the study were: negative control (-SH, -PRO, -PRE, -VAC), SH control (+SH, -PRO, -PRE, -VAC), PRO group (+SH, +PRO, -PRE, -VAC), PRE group (+SH, -PRO, +PRE, -VAC), VAC group (+SH, -PRO, -PRE, +VAC), PRO+PRE group (+SH, +PRO, +PRE, -VAC), PRO+VAC group (+SH, +PRO, -PRE, +VAC), PRE+VAC group (+SH, -PRO, +PRE, +VAC) and PRO+PRE+VAC group (+SH, +PRO, +PRE, +VAC). Each treatment had 10 turkeys, and the study included a total of 180 turkeys in 2 experiments. Application of VAC was made as a coarse spray on d 1 and at 3 weeks of age through drinking water to the turkeys. Treatments PRO (10^8 cfu/ml) and PRE (0.2%) were administered to the turkeys for 12 weeks through water and feed, respectively. Turkeys in all groups except negative control were inoculated with MDR SH at wk 11 (10^8 cfu/turkey). After 2 and 7 d of pathogen challenge, MDR SH colonization in the cecum and its dissemination to liver and spleen were determined. The experiment followed a completely randomized design, and the data were analyzed using ANOVA. The treatments resulted in a significant reduction of MDR SH colonization in the cecum of turkeys 2 d after challenge ($P < 0.05$). The combination of VAC with PRO or PRE and PRO+PRE+VAC group resulted in a consistent $2.0 \log_{10}$ cfu/g reduction of MDR SH 2 d after challenge compared with SH control ($P < 0.05$). The PRO+PRE group also resulted in $2.0 \log_{10}$ cfu/g reduction of MDR-SH in the cecum ($P < 0.05$). The magnitude of MDR SH reduction in turkey cecum was higher 7 d after challenge ($P < 0.05$). All the treatments resulted in at least $2.0 \log_{10}$ cfu/g reduction of MDR SH in the cecum whereas VAC, PRO+VAC or PRO+PRE+VAC groups resulted in $\geq 2.5 \log_{10}$ cfu/g reduction ($P < 0.05$) at d 7 post challenge. The treatments significantly reduced MDR SH dissemination to the liver and spleen. The results revealed that combination treatments could be an effective strategy to control MDR SH in turkeys (Minnesota State Legislature Rapid Agricultural Response Fund project #RR-212).

Key Words: turkeys, *Salmonella* Heidelberg, *Lactobacillus*, mannanoligosaccharide, vaccination

692P The antimicrobial effects of buffered vinegar and lauric acid against *Salmonella* spp. on commercial broiler poultry. Clarissa Harris*, *University of Florida, Gainesville, Florida, United States.*

Food safety is an ongoing struggle and studies have proven that poultry is the leading commodity that is responsible for many foodborne outbreaks. To reassure human health and reduce economic losses, numerous studies have tested a wide variety of approaches to reduce pathogens on processed meat and poultry products. These methods include but are not limited to organic acids, medium chain fatty acids with or without surfactants, and essential oils. The experimental approach in this study included the evaluation of a buffered dried vinegar ingredient and a lauric acid rinse for controlling *Salmonella* spp. in retail ground poultry. The antimicrobial effects of buffered dried vinegar and lauric acid were investigated in vitro on the initial day to determine if the solutions were effective. *Salmonella* spp. (10^6 cfu/ml) was inoculated into the dried vinegar solutions (0% positive control, 0% negative control, 5, 6, 7, 8, 9, and 10%) and lauric acid solutions (0% positive control, 0% negative control, 0.25, 0.5, and 1.0%). The treatments were analyzed for initial bacterial total plate count, *Salmonella*, and pH. The controls for the total plate counts did not contain any inoculum. The results revealed that buffered vinegar (10%) and lauric acid (0.5%) resulted in significant reductions ($P \leq 0.05$) in *Salmonella* at 1.45 and 1.89 log reductions, respectively, when compared with the control. Lauric acid (1.0%) had the largest reduction (1.12 logs) ($P \leq 0.05$) in total plate count bacteria, and there were no significant differences among the buffered vinegar solutions.

Key Words: buffered vinegar, lauric acid, *Salmonella*

693P A survey of *Clostridium* species in US broilers and turkeys. Samantha Anderson*, Jodi Delago, Tom Rehberger, and Alexandra Smith, *Arm & Hammer, Waukesha, Wisconsin, United States.*

A growing concern among poultry companies is the potential for an increase in necrotic enteritis (NE) caused by *Clostridium perfringens*. Generally, NE rates increase following the removal of antibiotic growth promoters (AGP) in commercial poultry systems, as observed after banning AGPs in Scandinavian countries and then in the European Union in 1995 and 1999, respectively. In order for US poultry producers to understand the growing NE challenge, it is important to characterize and track *C. perfringens* populations within commercial production systems. In this 2018 survey, we collected samples from 1802 commercial birds (916 broilers and 886 turkeys) from 32 different complexes across the US market in 2018 to enumerate and isolate *Clostridium* species and *C. perfringens* from the GIT. Results showed that the average level of *Clostridium* species across turkeys was 5.29×10^4 cfu/g, whereas the level across broilers was 1.30×10^6 cfu/g; the average level of *C. perfringens* across turkeys was 1.65×10^4 cfu/g, whereas the level across broilers was 1.29×10^6 cfu/g. *C. perfringens* (2414 isolates) were genotyped by RAPDs. The *C. perfringens* strains retrieved from turkey and broiler GIT samples were highly diverse separating into over 180 genotypes. There was greater diversity in the isolates from turkeys than broilers, with a Shannon-Weiner index of 4.35 compared with 3.89 respectively. Sequencing of the 16S RNA determined that the major species found among turkey day-of-hatch birds was *C. paraputrificum* and *C. tertium*. The results of this survey identified some of the underlying *Clostridium* species that inhabit the GIT and that may impact the overall health and performance of broilers and turkeys. According to the results of this study, the majority (57%) of *Clostridium* species isolated from the GIT were *C. perfringens* Type A and the remaining 43% include other clostridia such as *C. paraputrificum* and *C. tertium*.

Key Words: *Clostridium*, *C. perfringens*, necrotic enteritis, commercial, 16S sequencing

694P Efficacy of sodium selenite and sodium picolinate in reducing *Salmonella* Heidelberg on chicken eggs. Abraham Joseph Pellissery¹, Poonam Vinayamohan*¹, Oliver Perrine¹, Atacan Zeran¹, Abhinav Upadhyay¹, Annie Donoghue², and Kumar Venkitanarayanan¹, ¹University of Connecticut, Storrs Mansfield, Connecticut, United States, ²ARS, USDA, Fayetteville, Arkansas, United States.

Salmonella enterica serovar Heidelberg (SH) is a significant foodborne pathogen causing human outbreaks primarily linked to poultry-derived foods. Besides its high invasiveness in the host, SH is resistant to multiple antibiotics including aminoglycosides, fluoroquinolones and β -lactams. Chicken eggs can get contaminated with SH from a variety of sources on the farm, highlighting the need for effective egg surface disinfection methods. This study investigated the efficacies of a GRAS (Generally Recognized as Safe) -status compound, sodium selenite (NaSe) and, sodium picolinate (NaPC) applied as an antimicrobial spray or pectin-based coating for reducing SH on shell eggs. Sodium selenite and picolinate have proven anticancer, antioxidant and immunomodulatory properties, however, its efficacy in reducing foodborne pathogens on eggs is unknown. Surface sterilized white-shelled eggs were spot inoculated with a 3-strain mixture of SH (8.0 log cfu/mL) and coated evenly with pectin-based NaPC (0, 2 and 3%). Similarly, SH-inoculated eggs were sprayed with ~0.5 mL of NaPC (0, 2 and 3%) or NaSe (0, 1 and 2%). The treated eggs were stored at 4°C and SH populations enumerated on d 0, 1, 3, 5, 7, 14, 21 and 28 of storage. The study was replicated twice (5 eggs/treatment/day), and data were analyzed using Student's *t*-test with significance tested at $P < 0.05$. On d 0, ~8 log cfu and 5.0 log cfu/egg SH counts were recovered from control eggs sprayed with deionized water or coated with pectin, respectively. At the end of refrigerated storage (d 28), NaPC spray treatment reduced SH population on eggs to undetectable levels compared with controls, which yielded ~3 log cfu of SH/egg ($P < 0.05$). The pectin-based NaPC coating was also effective in reducing SH on eggs, where the pathogen load was decreased by ~2.5 log cfu/egg compared with controls on d 28 ($P < 0.05$). Furthermore, NaSe spray treatment significantly reduced SH counts by ~3.0 log cfu/egg compared with the untreated controls on the last day of storage. Results indicate the potential efficacy of NaPC and NaSe in reducing SH on shell eggs; however, further studies investigating the industrial feasibility of their treatment and effects on sensory attributes of eggs are warranted.

Key Words: *Salmonella* Heidelberg, sodium selenite, sodium picolinate, spray treatment, pectin-based coating

695P Reduction of *Salmonella* Heidelberg in ground turkey using plant-derived antimicrobials. Grace Dewi*, Shijinaraj Manjankattil, Claire Peichel, Shiliang Jia, Divek V. T. Nair, and Anup Kollanoor Johnny, University of Minnesota, Saint Paul, Minnesota, United States.

Salmonella Heidelberg (SH) is a highly invasive human pathogen for which turkeys can serve as reservoir hosts. Colonization of turkeys with SH could result in potential contamination of valuable products such as ground turkey. Therefore, interventions to reduce contamination of ground turkey are necessary to ensure a microbiologically safe product. With the rise in popularity of the clean label among consumers and significant antimicrobial property, plant-derived alternatives have garnered interest for food industry applications including their use in turkey production and processing. The objective of this study was to

evaluate the antimicrobial efficacy of 3 GRAS-status plant-derived antimicrobials (PDAs), lemongrass essential oil (LG), citral (CIT), and trans-cinnamaldehyde (TC), against SH in ground turkey. Fifteen-gram samples of ground turkey (93% lean, 7% fat) were treated with either LG, CIT, or TC at 2% (vol/wt). The samples were inoculated with a 3-strain cocktail of nalidixic acid-resistant *Salmonella* Heidelberg to achieve ~3.50 log₁₀ cfu/g of ground turkey. Positive (PC; inoculated but not treated with PDAs) and negative (non-inoculated and not treated with PDAs) controls were included. The samples were stored at 4°C in sterile Whirl-Pak bags and SH populations were enumerated on d 0 (30 min after inoculation), 1, 3 and 5, and enriched. The experiments were conducted in duplicates and repeated thrice. Populations of SH were logarithmically transformed for analysis and samples from which no bacteria were recovered after spread plating but were positive after enrichment were assumed a value of 0.95 log₁₀cfu/g. The data were analyzed using the lmerTest package of R and significant difference considered at $P < 0.05$. All PDAs significantly reduced SH populations in ground turkey by d 1 ($P < 0.05$). Among the PDAs, TC resulted in reductions of 2.50- and 3.09- log₁₀ cfu/g ($P < 0.05$), respectively on d 1 and 3. By d 5 of storage, a reduction of 2.38- and 2.64- log₁₀ cfu/g was observed with CIT and LG ($P < 0.05$) and no SH was detected in samples treated with TC even after enrichment (3.34- log₁₀ cfu/g reduction). Results of this study indicate that the 3 tested plant-derived antimicrobials, LG, CIT, and TC are effective against SH in ground turkey, indicating their potential as post-harvest interventions to reduce the risk of *Salmonella* contamination in ground turkey.

Key Words: *Salmonella* Heidelberg, plant-derived antimicrobials, ground turkey, food safety, processing

696P Characterization of bile tolerance and putative bile salt hydrolases in *Lactobacillus*. Timothy Broderick* and Tri Duong, Texas A&M, College Station, Texas, United States.

Administration of probiotic *Lactobacillus* has been demonstrated to improve animal health, growth performance, and preharvest food safety in poultry production. Although survivability and persistence is thought to be important to probiotic functionality, the mechanisms important to these traits have not been well characterized. Deconjugation of bile salts has been used as a criterion for the selection of probiotic cultures and has been linked to bile tolerance and survival of probiotic cultures in the gastrointestinal tract. In this study, we investigate bile tolerance and bile salt hydrolase activity of *Lactobacillus crispatus* and *Lactobacillus gallinarum* strains isolated from the gastrointestinal tract of chickens. The bile tolerance of 2 *L. crispatus* strains (JCM 5810 and ST1) and 2 *L. gallinarum* strains (ATCC 33199 and JCM 8782) was evaluated using growth in medium supplemented with glycocholic acid (GCA), glycodeoxycholic acid (GDCA), taurocholic acid (TCA), and taurodeoxycholic acid (TDCA). Cultures were analyzed in triplicate for both biological and technical replicates with maximum growth rate calculated using a modified gompertz equation. Data were analyzed using ANOVA with means being deemed significant at $P \leq 0.05$. Maximum growth rate of ST1 and JCM 8782 was significantly reduced by the 4 bile salts ($P < 0.001$). JCM 5810 was able to achieve similar growth rates in the presence of 0.2% TCA and GCA as it did in MRS, while ATCC 33199 was only able to maintain similar maximum growth after exposure to TCA. Hydrolysis of individual bile salts was characterized using the precipitation in bile acid-containing overlay medium. ST1 was able to deconjugate all 4 bile acids, while JCM 5810 had activity against only the cholic acid derivatives. JCM 8782 demonstrated chologlycine hydrolase activity while ATCC 33199 did not demonstrate the ability to deconjugate bile salts. Two open reading frames (ORF) encoding

putative orthologs of the *Lactobacillus acidophilus* NCFM chologlycine hydrolase, bshA, with sequence homology of 77.9% (E = 0.0) and 78.8% (E = 0.0) identity were identified in the genome sequences of *L. crispatus* ST1 and *L. gallinarum* ATCC 33199, respectively. The putative chologlycine bile hydrolase of *L. gallinarum* ATCC 33199 was targeted for the construction of an isogenic mutant using the pORI28 chromosomal insertion system. Hydrolase activity will be characterized using liquid chromatography-mass spectrometry to evaluate the deconjugation of bile salts. The results of our study will inform future research investigating the role of bile tolerance and bile salt hydrolase activity in the persistence of probiotic *Lactobacillus* cultures in the gastrointestinal tract of poultry.

Key Words: *Lactobacillus*, bile salt hydrolase, probiotic, survivability, deconjugation

697P Lesson from whole genome analyses: *Salmonella enterica* possess a highly diverse genome. Gerardo Nava and Carolina Rendiz-Nava*, *Universidad Autonoma de Queretaro, Queretaro, Oro, Mexico.*

Salmonella remains one of the most important foodborne pathogens worldwide and poultry meat is considered one of the main sources of contamination. To design effective strategies for *Salmonella* prevention and control, it is fundamental to understand its genomic flexibility and potential. Today, comparative genomics provide a unique opportunity to explore genomic features of foodborne pathogens. The objective of the present study was to analyze genomic diversity of *S. enterica* to identify molecular traits of genome evolution. To accomplish this goal, we analyzed the whole genome of 3,074 *S. enterica* isolates. Comparative genomic analyses were performed using genomes archived in the Integrated Microbial Genomes and Microbiomes (IMG/M) system. Gene annotations were downloaded and analyzed to identify variations in genome size, gene content and horizontal gene transfer. Interestingly, the analyses uncovered that *S. enterica* possess a highly diverse genome, ranging from 2.64 to 5.93 Mbp. Interestingly, gene repertoire showed a high variability, ranging from approximately 3,700 to 9,900 genes. Also, it was uncovered that some serotypes have acquired up to 17% of their genome via horizontal gene transfer (HGT). The highest rate of HGT was observed in serotype Typhi and Typhimurium ($P < 0.05$). These results indicate that *S. enterica* possess a very promiscuous genome and an extensive genomic repertoire. These genomic features provide *S. enterica* with a versatile genome to express numerous mechanisms of virulence and resistance. Also, these data suggest that more comprehensive analysis are required to classified *S. enterica* isolates. Base on these results, conventional classifications of *S. enterica* must be revised and updated.

Key Words: *Salmonella*, genome, evolution, gene

698P Antimicrobial susceptibility of pathogenic and indicator bacteria from edible offal and muscle meat in Nashville, Tennessee. Agnes Kilonzo* and Samuel Nahashon, *Tennessee State University, Nashville, Tennessee, United States.*

Prevalence and antimicrobial resistance of *Salmonella enterica* serovar, *Campylobacter*, *E. coli* O157:H7, *E. coli*, and *Enterococci* from edible offal and muscle meats obtained from Davidson County, Tennessee were determined. A total of 348 (160 edible offal and 188 muscle) meats were purchased from retail stores and analyzed for *Salmonella enterica* serovar, *Campylobacter*, *E. coli* O157:H7, *E. coli*, and *Enterococci*. Presumptive pathogenic and indicator bacteria were confirmed using

PCR. *Salmonella enterica* serovar (4.4 and 4.3%), *Campylobacter* (1.9 and 1.1%), *E. coli* (79.4 and 89.4%), and *Enterococci* (88.1 and 95.7%) were identified in edible offal and muscle meats, respectively. Chicken liver (9.7%) was most frequently contaminated with *Salmonella enterica* serovar, followed by ground chicken (6.9%) and chicken wings (4.2%), while no *Salmonella enterica* serovar was isolated from beef liver, beef tripe, and ground beef. The prevalence of *Campylobacter* in beef livers was 6.9%, ground beef (2.3%) and ground chicken (1.4%). None of the meat samples tested positive for *E. coli* O157:H7. A total of 41 multi-drug resistance profiles were identified among pathogenic and indicator bacteria, *Enterococci* presented the most multidrug resistance patterns ($n = 22$). Results show that resistance was significantly ($P < 0.05$) higher in erythromycin (98.3–99.1%), tetracycline's (94.0–98.3%), and vancomycin (88.8–92.2%) as compared with chloramphenicol (43.1–53.9%), amoxicillin/clavulanic (43.5–45.7%), and ciprofloxacin (51.45.7–55.7%). Imipenem showed the lowest resistance (0–0.9%). This baseline data on the occurrence and antimicrobial resistance of bacteria on meats, especially edible offal is desirable as food preferences are continuously changing in cities due to immigration and cultural influence on food choices.

Key Words: antimicrobial susceptibility, pathogenic and indicator bacteria, edible offal and muscle meat

699P Features of laying behavior before onset of egg incubation in the Silkie hens. Norio Kansaku*^{1,2}, Emi Nakamura¹, and David Zadworny², ¹*Azabu University, Sagami-hara, Kanagawa, Japan*, ²*McGill University, Ste Anne de Bellevue, Quebec, Canada.*

In chickens, the mechanism initiating egg laying after photo-stimulation is well documented. Photo-stimulation induces Dio2 mRNA expression and increases content of T3 in the hypothalamus. Increases of T3, in turn, induce the morphological changes of GnRH neurons. In broody strains, PRL shows anti-gonadal effects and termination of egg laying is induced by increased concentration of PRL. From various experiments, VIP is the physiological PRL releasing factor. However, regulatory factor(s) for control of VIP mRNA expression have not been identified. Interestingly, previous work clearly demonstrated that the increase of plasma PRL preceded the onset of incubation behavior by 4 to 7 d. Therefore, this suggested that hypothalamic content of VIP mRNA or VIP also preceded incubation behavior by the same interval. To identify the regulator(s) for VIP, precise estimation of stages which show no decrease of food and water intake, plasma LH or estrogen concentrations but increased PRL concentration is necessary. To characterize features of each hen before displaying incubation behavior, the laying behavior of Silkie hens was recorded and analyzed over a 1 to 2 year interval. A total of 11 silkies hen (6 to 7 mo old) were assigned to individual floor pens (0.9 m × 0.6 m) containing a nest box. Hens were kept under 14L10D lighting schedule with freely available food and water. The physiological status and incubation behavior were monitored by a video recording system over 1–2 years. Eggs were replaced with mock eggs made of wood after oviposition. Ten out of 11 hens showed incubation behavior multiple times during the experiment. Hens which showed incubation behavior revealed 2 different features. In a typical clutch, hens spent 10 to 15 min in the nest to lay an egg. Interestingly, once hens spent over 1 h in the nest, nest occupancy incrementally increased and significant difference was observed. This shift in nest occupancy occurred 7 to 10 d before the onset of incubation and was highly repeatable. Furthermore, 1 or 2 d before the onset of incubation, hens starting egg incubation before the light was off. Results of this study indicate that spending over 1 h in the nest may initiate an increase of VIP and hence PRL content in the Silkie. This change occurs in precedence of the decrease of LH, food

and water intake observed in incubating hens. Hypothalamic or higher brain centers may be providing inputs that regulate the expression of VIP associated with the transition from egg laying to egg incubating behavior. In conclusion, egg laying behavior before the onset of incubation behavior was highly repeatable and may provide new insights into the reproductive cycle of Silkies.

Key Words: incubation, laying, behaviour

700P The isolation and characterization of chicken oviduct epithelial cells. Hyeon Yang*, Bo Ram Lee, Hwi-Cheul Lee, Sun Keun Jung, Ji-Youn Kim, Jingu No, Suresh Kumar Shanmugam, Yong Jin Jo, and Sung June Byun, *National Institute of Animal Science, Jeonju-si, Korea (the Republic of).*

Chicken is an attractive bioreactor model owing to their remarkable benefits. To generate transgenic chickens, several technologies have been widely used, typically viral vector or chicken primordial germ cells (pGCs) injection. In viral injection system, chicken ovalbumin promoter was mostly used to produce recombinant proteins, which was led to developing various lines of chicken ovalbumin promoters. To evaluate exact promoter activity, these ovalbumin promoters should be analyzed in cells isolated from chicken oviduct tissues. However, reliable chicken oviduct epithelial cells (cOECs) were merely obtained and no one has been commercially available. Here we isolated and characterized the cOECs, and finally investigated the ovalbumin promoter activity in the cells. The oviduct tissue from infundibulum to magnum was separated from 35 wks egg-laying hens. The tissue was washed, trimmed, and gently torn off to expose inner surface of the oviduct. The inner surface was delicately scraped and the scraped tissue fragments were minced into around 2 mm, and digested with collagenase P. The digested tissue was seeded into collagen treated cell culture dish with keratinocyte complete medium supplemented with 5% chicken serum and cOECs were observed in a 48 h, approximately. The chicken oviduct marker, ovalbumin protein, was clearly detected in the cOECs with Western blot and immunofluorescence assay. Next, the 4.4 kb mutated chicken ovalbumin promoter was constructed and cloned into pGL4.11 reporter vector. The promoter analysis showed that the ovalbumin promoter had 7.1-fold ($P < 0.001$) higher activity than a negative control in cOECs, but 1.8-fold ($P < 0.001$) differences between ovalbumin promoter and the negative control were also observed in chicken fibroblast cells. In conclusion, these isolated cells can be utilized to demonstrate the chicken ovalbumin promoter activity in vitro.

Key Words: chicken oviduct epithelial cells, chicken ovalbumin promoter

701P Wnt/ β -catenin signaling pathway regulates the feather follicle morphogenesis and development in broiler chickens.

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The feather growth is an important commercial characteristic of poultry in commercial environments, and feather follicles serve as the control center for feather growth and regeneration. However, the molecular signaling involved in regulating the development feather follicles is unknown. The purpose of this study was to investigate the morphogenesis of feather follicles, and to investigate the role of Wnt/ β -catenin pathway in regulating the feather growth and development in broiler chickens. In experiment I, skin tissues of 12 embryos were collected

on embryonic (E) d 7, 8, 9, 10, 11, 12, 13, 15, 17, 19 and the day of hatching (DOH), respectively, to observe the proteins expression of Wnt/ β -catenin pathway during the morphogenesis of feather follicles. In experiment II, a total of 120 fertilized embryo eggs were randomly divided into 4 treatments including a non-injection group (Control group), injected 100 μ L PBS/egg (PBS control group), injected 100 μ L PBS/egg, contains of 600 ng DKK1/egg (600 ng DKK1 group), and injected 100 μ L PBS/egg, contains of 1,200 ng DKK1/egg (1,200 ng DKK1 group). Feathers and skin tissues were sampled on DOH to examine feather mass, diameter and density of feather follicles, and the proteins expression of Wnt/ β -catenin pathway. Results showed that: 1) Skin appeared protrusion during E7-E9, feather buds elongated during E10-E11, epidermal cells rapidly aggregated and invaginated to form feather follicles on E13. Feather follicle morphological development was basically completed on E15. 2) The proteins expression of β -catenin, TCF4, Cyclin D1, and c-Myc significantly increased during E8-E12, then decreased during E13-DOH ($P < 0.05$). 3) Compared with CON and PBS group, DKK1 injection groups significantly decreased the relative mass of feathers ($P < 0.05$), and the diameter and density of feather follicles. 4) DKK1 injection significantly downregulated the proteins expression of β -catenin, TCF4, Cyclin D1, and c-Myc ($P < 0.05$). In conclusion, feather follicles gradually formed during E7-E15, and the proteins expression of Wnt/ β -catenin pathway were significantly upregulated during feather follicle morphogenesis. The feather follicle morphogenesis and feather growth are regulated by Wnt/ β -catenin pathway in broiler chickens.

Key Words: feather growth, in ovo injection, DKK1, Wnt/ β -catenin, broiler chicken

702P Target of rapamycin complex 1 signaling pathway mediates crop milk protein synthesis in domestic pigeons (*Columba livia*). Nengxia Pan, Wenyan Xie, Shiguang Jiang, Mengjie Chen, Xiuqi Wang*, Huichao Yan, and Chunqi Gao, *College of Animal Science, South China Agricultural University/Guangdong Provincial Key Laboratory of Animal Nutrition Control, Guangzhou, 510642, China.*

Pigeon crop milk is the major source of nutrition that supports squabs during the period of rapid growth from hatching to the end of the first week to 10 d of life. However, the molecular signaling involved in regulating the crop milk protein synthesis is unclear. The objective of this study was to determine the role of target of rapamycin complex 1 (TORC1) signaling pathway in regulating the crop milk protein synthesis in domestic pigeon (*Columba livia*). In experiment I, 10 pairs of breeder pigeons that in the non-breeding period Incubation period, and lactation period were respectively selected, and their crop tissues were collected to determine the expression of TOR signaling pathway related proteins. In experiment II, 30 pairs of breeding pigeons were randomly subjected to one of the following treatments for 7 d: Control (injected with PBS), low-dose rapamycin (RAPA, a specific inhibitor of TOR; an intravenous injection of 0.6 mg/kg body weight), high-dose RAPA (1.2 mg RAPA/kg body weight). Results showed that, compared with the pigeons in the non-breeding and incubation period, the crop weight and thickness of the crop were increased, and a large amount of crop milk was synthesized in the lactation period ($P < 0.05$). Meanwhile, the proteins expressions of TORC1, 4EBP1, S6K1, S6, eIF4E in the crop of breeder pigeon were upregulated during the lactation period ($P < 0.05$). As aspects, intravenous injected with RAPA injection decreased the thickness and weight of the crop, protein content in crop milk of pigeon breeder, and inhibited the growth of squabs ($P < 0.05$). The RAPA treatment also significantly decreased the proteins expression of

TORC1, 4EBP1, S6K1, S6, eIF4E ($P < 0.05$) in crop tissue of breeder pigeons. Together, the TORC1 signaling pathway is upregulated during lactation period, and RAPA decreases the content of crop milk protein and the growth of squabs, suggesting the TORC1 signaling is involved in pigeon crop milk protein synthesis.

Key Words: pigeon, crop milk, protein synthesis, growth performance, TOR signaling pathway

703P Delayed access to feed affects small intestinal development and mRNA abundance of the stem cell marker Olfactomedin 4 and proliferating cell marker MKi67 in early posthatch broilers. Kuan-Ling Liu* and Eric Wong, *Virginia Polytechnic Institute and State University, Blacksburg, Virginia, United States.*

Newly hatched chicks are often deprived of access to feed and water up to 48 h posthatch. Delayed access to feed (DAF) can inhibit small intestinal development, compromising growth of the chick. The objective of this study was to determine the effect of DAF on small intestinal villus height (VH), crypt depth (CD), and the mRNA abundance of Olfactomedin 4 (Olfm4) and Marker of Ki67 (MKi67) in early posthatch broilers. The Olfm4 mRNA is exclusively expressed in stem cells, which allows easy identification of the intestinal crypt and measurement of VH and CD. The MKi67 is a proliferating cell marker. Cobb 500 chicks, hatching within a 12-h window, were randomly allocated into 3 experimental groups: control with no feed delay (ND), 24-h feed delay (D24), and 36-h feed delay (D36). Quantification and cell localization of Olfm4 and MKi67 mRNA were investigated by quantitative PCR and in situ hybridization, respectively, in duodenum (DUO), jejunum (JEJ), and ileum (ILE) at hour (h) 0, h24, h36, h72, h120, and h168 posthatch. Statistical analysis was performed using JMP Pro 14, and significant differences were determined by *t*-test and one-way ANOVA. In the DUO at h0, the CD in ND was greater than all other time points ($P < 0.001$). A similar pattern was seen in the JEJ, except CD at h36 was not different from h0. With DAF, VH was lowered in all intestinal segments but there were no differences between groups by h168. The CD was greater in the DUO of D24 chicks at h24 ($P < 0.0001$) and h36 ($P = 0.039$) compared with ND. In contrast, D36 chicks at h36 had reduced CD in the JEJ ($P = 0.006$) and ILE ($P = 0.017$), and D24 chicks had reduced CD in the ILE at h24 ($P = 0.003$). The mRNA abundance of Olfm4 ($P = 0.006$) and MKi67 ($P = 0.0003$) were both increased at h120 in D36 chicks, which could be a delayed compensatory effect due to DAF. In addition, the same increase in Olfm4 ($P = 0.005$) and MKi67 ($P = 0.008$) mRNA abundance were observed in D24 birds, but not until h168. The VH/CD ratio was reduced in the DUO in D24 chicks at h24 ($P = 0.049$) and h36 ($P = 0.006$), and in D36 birds at h72 ($P = 0.003$). The D36 chicks also had lowered VH/CD ratios in JEJ ($P = 0.002$) and ILE ($P = 0.007$), but not until h120. Interestingly, before refeeding, ILE displayed an increased VH/CD ratio in D24 at h24 ($P = 0.030$) and D36 at h36 ($P = 0.003$) compared with ND. In summary, as a form of adaptive response to DAF, duodenal CD and jejunal VH were both transiently increased to enhance villus growth and to increase absorptive surface area, respectively. However, DAF ultimately reduced small intestinal development in early posthatch broilers with the impact more pronounced in D36 than D24 chicks.

Key Words: delayed access to feed, Olfm4, MKi67, villus height, crypt depth

704P Thyroid hormone action in ovarian small white follicle cells from turkey hens with differential egg production. Kristen Brady*¹, Julie Long², Hsiao-Ching Liu³, and Tom Porter¹,

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Ovarian small white follicles (SWF) are prehierarchal follicles that respond to follicle stimulating hormone (FSH) to produce estradiol (E_2) in avian species. The hypothalamo-pituitary-thyroid (HPT) axis plays a role in the initiation and cessation of egg production. Recently, the HPT axis was shown to influence ovarian steroid hormone production, where nuclear and cell membrane thyroid hormone receptors (THR_s) were expressed in SWF, indicating possible genomic and non-genomic effects of thyroid hormone on the reproductive axis. In the present study, characterization of HPT axis gene expression and in vitro response to thyroid hormone treatment was performed in SWF cells isolated from low egg producing hens (LEPH) and high egg producing hens (HEPH) ($n = 4$). Nuclear THR_s, *THRA* and *THRB*, plasma membrane THR_s, *ITGAV* and *ITGB3*, and the main enzyme involved in thyroid hormone metabolism, *DIO2*, were measured in untreated SWF from LEPH and HEPH through RT-qPCR. Isolated SWF cells from LEPH and HEPH were subjected to no pretreatment (NPT) or 1.5 ng/mL of thyroid hormone (T_3) for 12 h, followed by FSH treatment at 0, 10, and 100 ng/mL for 5 h. After incubation, media from the SWF cell cultures were assayed for E_2 production by radioimmunoassay. Gene expression and cell culture data were analyzed by a one-way and 3-way ANOVA, respectively, using the mixed models procedure of SAS, with significance indicated at $P < 0.05$. SWF cells from HEPH showed increased mRNA levels for *THRA* and *ITGB3*. Cells from HEPH also displayed higher mRNA levels for *DIO2*. Basal E_2 production in vitro did not differ between SWF cells from LEPH and HEPH, regardless of pretreatment. However, at 10 and 100 ng/mL treatment of FSH, HEPH SWF cells showed significantly higher E_2 production when compared with LEPH. SWF cells from LEPH did not respond to either FSH treatment in terms of E_2 production. Pretreatment with T_3 , both at 10 and 100 ng/mL of FSH, decreased E_2 production in SWF cells from HEPH, reducing E_2 production to levels seen in LEPH. At the transcript level, SWF cells from HEPH showed increased expression of THR_s and thyroid hormone metabolism genes. Additionally, in vitro, SWF cells from HEPH were more responsive to T_3 treatment, with T_3 treatment significantly decreasing FSH induced E_2 production. Furthermore, the addition of T_3 pretreatment caused SWF cells from HEPH to respond to FSH in a similar manner to that seen in cells from LEPH. These results suggest that the HPT axis may play a role in differentially regulating E_2 production in the SWF of LEPH and HEPH. Further studies will be necessary to pinpoint the role of the HPT axis in the regulation of ovarian steroid hormone production and egg production rates in turkey hens.

Key Words: turkey, egg production, HPT axis, estradiol, small white follicles

705P Characterization of the somatotrophic axis in low and high egg producing turkey hens. Kristen Brady*¹, Julie Long², Hsiao-Ching Liu³, and Tom Porter¹, ¹University of Maryland, College Park, Maryland, United States, ²ARS, USDA, Beltsville, Maryland, United States, ³North Carolina State University, Raleigh, North Carolina, United States.

The hypothalamo-pituitary-somatotropic (HPS) axis regulates growth and body composition, while the hypothalamo-pituitary-gonadal (HPG) axis regulates reproduction. Selection for carcass traits has negatively impacted the reproductive efficiency of the turkey hen. At the transcript level, low egg producing hens (LEPH) show decreased mRNA levels for genes associated with HPG axis stimulation and increased mRNA levels for genes associated with HPG axis inhibition, when compared

with high egg producing hens (HEPH). This study examined if mRNA levels for genes related to growth were differentially regulated between LEPH and HEPH and if growth-related genes were negatively correlated to previously seen trends in reproduction-related genes. Six LEPH and 6 HEPH were sampled, taking the hypothalamus, pituitary, as well as the granulosa layer of the largest follicle (FIG) and small white follicles (SWF) ($n = 3$ per group). Hens were sampled outside and during the preovulatory surge that triggers ovulation to examine changes in the HPS axis during the ovulatory cycle. The FIG and SWF are responsible for progesterone and estradiol production, respectively, and were sampled to determine the impact of the HPS axis on ovarian steroid hormone production throughout follicle maturation. The mRNA levels of key genes in the HPS axis involved in growth regulation were examined using RT-qPCR. A 2-way ANOVA using the mixed models procedure of SAS compared normalized mRNA levels between LEPH and HEPH, both outside and during the PS. Hypothalamic mRNA levels of somatostatin (*SST*) and growth hormone receptor (*GHR*) were higher in HEPH than in LEPH ($P < 0.05$). HEPH also exhibited increased mRNA levels of growth hormone (*GH*) in the pituitary ($P < 0.05$). LEPH displayed upregulation of growth hormone releasing hormone receptor (*GHRHR*) and insulin-like growth factor receptor (*IGFR*) in the pituitary when compared with HEPH ($P < 0.05$). *IGFR* gene expression was higher in LEPH in the FIG but higher in HEPH in the SWF ($P < 0.05$). HPS axis gene expression not only differed in LEPH and HEPH but also changed during the ovulatory cycle and during follicle maturation. HEPH displayed increased mRNA levels in the hypothalamus for genes related to HPS axis inhibition and feedback (*SST* and *GHR*). LEPH showed increased mRNA levels in the pituitary related to HPS axis stimulation and feedback (*GHRHR* and *IGFR*). *IGFR* expression was increased in more mature follicles in LEPH and increased in less mature follicles in HEPH. These results indicate crosstalk between the HPS and HPG axes and may implicate a role for the HPS axis in ovulation frequency and follicle development, ultimately impacting egg production levels.

Key Words: turkey, egg production, somatotrophic axis, gene expression, ovulation

706P Differential effects of constant supplemental light wavelength on plasma estradiol and melatonin, body weight, and egg production in female broiler breeders. Kayo Takeshima¹, Martin Zuidhof², Charlene Hanlon¹, and Gregoy Bedecarrats^{*1}, ¹University of Guelph, Guelph, Ontario, Canada, ²Martin Zuidhof, Edmonton, Alberta, Canada.

Uniform broiler breeder body weight (BW) synchronizes the initiation of sexual maturation, maximizing flock reproductive performance. To achieve target BW precisely, a precision feeding (PF) system was developed to allocate feed to individual birds based on real-time BW measurements. However, to allow access to the feeding station beyond the standard 8 h (h) photoperiod during pullet rearing, 24 h of dim illumination is required, but such illumination must not interfere with reproductive potential. The objectives were to investigate the effect of long and short wavelength 24 h supplemental feeder light (SFL) on estradiol (E_2), and the photoperiodic hormone melatonin (Mel), responsible for circadian rhythm. Ross 708 chicks ($n = 480$) were housed in 4 identical rooms (12 pens per room, $n = 10$ birds per pen), and each pen was assigned to one of 3 continuous SFL treatments: pure red, pure green, or dark as control ($n = 16$ pens each; 10 lx), with SFL exposure from 2 weeks of age (woa). Feed was allocated to all birds daily using a conventional feeding approach, starting from 2 woa. Daytime rearing photoperiod was 8L:16D from 2 woa until photostimulation at 22 woa with an abrupt change to 14L:10D. Blood samples were collected at 13,

18, 21 woa, weekly from 23 to 28 woa, biweekly from 30 to 36 woa, and at 40, 44, 48, 52 and 58 woa for E_2 profiles ($n = 32$ birds per treatment) and, at 58 woa ($n = 8$ birds per treatment) for Mel profiles during the scotophase (22:00, 2:00) and photophase (8:00, 14:00, 20:00). Data were analyzed using a mixed model. Tukey's post-hoc test was used to separate means, which are reported as different where $P < 0.05$. Age at first egg was delayed in the red SFL treatment (192.8 ± 2.4 d) compared with green (178.1 ± 1.3 d) and dark (177.4 ± 1.0 d; $P < 0.001$). The shift in sexual maturation between SFL treatments was reflected in the E_2 profile, which concentration increased at 26 woa for green and dark treatments, while the increase was delayed by 4 weeks (30 woa) in the red SFL treatment. A positive correlation was observed between BW and E_2 ($r = 0.40$; $P < 0.001$), indicating that a BW threshold may be required for initiation of an E_2 increase. Plasma Mel increased during the scotophase compared with photophase ($P < 0.001$), however, no Mel differences were observed in response to SFL treatments. In conclusion, continuous pure red supplemental light delayed sexual maturity and its use is discouraged. Pure green light appears to be an acceptable approach to providing continuous illumination of the PF system. As well, no difference in Mel between SFL indicates that 24 h of dim SFL does not alter the circadian rhythm

Key Words: broiler breeders, light spectrum, reproduction, estradiol, melatonin

707P Delayed feeding alters transcriptional and post-transcriptional programming of hepatic metabolic pathways in peri-hatch broiler chicks. Julie Hicks², Tom Porter^{*1}, Nishanth Sunny¹, and Hsiao-Ching Liu², ¹University of Maryland, College Park, Maryland, United States, ²North Carolina State University, Raleigh, North Carolina, United States.

Hepatic fatty acid oxidation of yolk lipoproteins provides the main energy source for chick embryos. Post-hatching these yolk lipids are rapidly exhausted and metabolism switches to a carbohydrate-based energy source. We recently demonstrated that a large number of microRNAs (miRNAs) are key regulators of hepatic metabolic pathways during this metabolic switching. MiRNAs are small non-coding RNAs which post-transcriptionally regulate gene expression in most eukaryotes. To further elucidate the roles of miRNAs in the metabolic switch we used delayed feeding for 48 h post-hatching to impede the hepatic metabolic switch in Ross 708 broilers. Real-Time quantitative PCR (RT-qPCR) analysis demonstrated that hepatic expression of several miRNAs including miR-33, miR-20b, miR-34a, and miR-454, and their metabolic targets was significantly ($P < 0.05$, ANOVA, $n = 6$ males) affected by delaying feed consumption for 48 h. For example, we found that delayed feeding resulted in increased miR-20b expression and conversely reduced expression of its target FADS1, an enzyme involved in fatty acid synthesis. Interestingly, the expression of a previously identified miR-20b regulator FOXO3 was also higher in delayed fed chicks. FOXO3 also functions in protection of cells from oxidative stress. Delayed fed chicks also had significantly ($P < 0.05$, ANOVA, $n = 6$ males) higher levels of plasma ketone bodies (β -hydroxybutyrate colorimetric assay, Cayman Chemical) than their normal fed counterparts. This suggests that delayed fed chicks rely almost exclusively on lipid oxidation for energy production and are likely under higher oxidative stress. Thus it is possible that FOXO3 may function to both limit lipogenesis as well as to help protect against oxidative stress in peri-hatch chicks until the initiation of feed consumption. This is further supported by evidence that the FOXO3-negatively regulated histone deacetylase, HDAC2, was found to recognize the FASN (involved in fatty acid synthesis) chicken promoter in a yeast one-hybrid assay. Expression of both HDAC2 and

FASN mRNAs was lower in delayed fed chicks until feed consumption. Taken together the results of the present study indicate that a complex transcriptional and post-transcriptional hepatic regulatory system is triggered by nutritional/physiological cues to facilitate the metabolic transition in peri-hatch chickens.

Key Words: delayed feeding, metabolic switch, microRNA, gene expression, oxidative stress

708P Developmental changes of goblet cells in the small intestine of broiler chicks. Krista Reynolds and Eric Wong*, *Virginia Tech, Blacksburg, Virginia, United States.*

The major role of the small intestine is to digest feed and absorb nutrients, but it also plays a crucial role in protecting the bird from outside pathogens. The gastrointestinal tract contains goblet cells, which line the intestinal villi and secrete mucus primarily made up of mucin and water. This mucus coats the small intestine creating a barrier between pathogens and the intestinal epithelial cells. The primary mucin present in the small intestine is Mucin 2 (Muc2). The objectives of this study were to use in situ hybridization to identify goblet cells and determine the development- and intestinal segment-specific changes in the number and distribution of goblet cells. Small intestinal samples from the duodenum (Duo), jejunum (Jej), and ileum (Ile) were collected from Cobb 500 broilers at embryonic d 19 (e19), day of hatch (doh), d2, and d4. Tissue samples were processed by in situ hybridization using the RNAscope method to identify goblet cells expressing Muc2 mRNA. The number and distribution of goblet cells were counted in the crypt and villi using Image J. A one-way ANOVA was performed using JMP v14.0. The density of goblet cells (number of goblet cells/unit area of intestine) was compared using a full factorial between the 3 small intestinal segments at each time point. Further comparison was performed using a Tukey test ($P < 0.05$). In the villi at d4, the Ile showed a greater number of goblet cells than the Duo ($P = 0.004$). There was also an increase in the density of goblet cells in the Jej from e19 to doh ($P = 0.03$) and d2 to d4 ($P = 0.008$), and in the Ile from e19 to doh ($P = 0.0004$) and d2 to d4 ($P = 0.03$). The density of goblet cells in the Duo remained constant from e19 to d4. In the crypt at doh, the Ile had a greater density of goblet cells than the Duo ($P = 0.003$), and at d4 the Ile ($P < 0.0001$) and Jej ($P = 0.002$) both had a greater density of goblet cells compared with the Duo. There was also an increase in the density of goblet cells in the Jej from e19 to doh ($P < 0.0001$) and d2 to d4 ($P = 0.0002$), and in the Ile from e19 to doh ($P < 0.001$) and d2 to d4 ($P = 0.009$). While the density of goblet cells increased in the Jej and Ile posthatch, the density in the Duo stayed constant. This may be because the duodenum is the first part of the intestine to encounter a pathogen and therefore must be the first to complete cellular maturation.

Key Words: broiler, small intestine, goblet cell, mucin 2, in situ hybridization

709P Development of an *in vitro* system to evaluate intracellular signaling cascades regulated by somatotrophic hormones in chickens. Brett Marshall*¹, Trevor McClure¹, Tom Porter², and Laura Ellestad¹, ¹*University of Georgia, Winterville, Georgia, United States,* ²*University of Maryland, College Park, Maryland, United States.*

Genetic selection of modern broiler chickens has yielded birds with greater muscle mass, faster growth rates, and improved feed efficiency. Despite these advancements, there is a paucity of data regarding signaling mechanisms used by hormones including growth hormone (GH) and insulin-like growth factor 1 (IGF1), which serve as key mediators for

growth and development in vertebrates. To better define how signal transduction of GH and IGF1 drive growth and development of metabolically relevant tissues such as liver and muscle, we sought to establish an *in vitro* model to evaluate pathways involved in signal transduction of these hormones. Using LMH cells, a chicken hepatocyte cell line expressing both GH and IGF1 receptors, cultures were transfected with reporter constructs expressing luciferase driven by cascade-specific response elements known to be involved in GH or IGF1 signaling in other animals including signal transducer and activator of transcription (STAT) 1, STAT3, STAT5, p38 mitogen-activated protein kinase (MAPK), jun n-terminal kinase (JNK) MAPK, extracellular signal-regulated kinase (ERK) 1/2 MAPK, protein kinase A (PKA), and phosphoinositide 3-kinase (PI3K). Cells were co-transfected with a normalization plasmid constitutively expressing an alternate luciferase. Serum-starved, transfected cells were treated with pharmacological or biologically relevant activators of each pathway at different concentrations for one hour, at which time luciferase activity of normalization and pathway-specific reporter luciferases were measured sequentially. Normalized response element activation was determined from 3 independent reporter assays for each pathway ($n = 3$ experimental replicates), and one-way ANOVA was used to determine differences in normalized luciferase values by treatment. Reporters for STAT3, ERK1/2, and PKA were activated in a dose-dependent manner by interleukin-6 ($P < 0.0089$, 6.7-fold maximum increase over vehicle, effective range from 1 to 100ng/mL), epidermal growth factor ($P < 0.0001$, 4.8-fold maximum increase over vehicle, effective range from 100pg/mL to 1 μ g/mL), and forskolin ($P < 0.0001$, 2.7-fold maximum increase over vehicle, effective range from 5 μ M to 75 μ M), respectively. Together, these data demonstrate the efficacy of this *in vitro* system to resolve intracellular signaling pathway activation and establish it as a valuable tool which can be used to further our understanding of somatotrophic hormone signaling within target tissues of chickens.

Key Words: growth hormone, insulin-like growth factor 1, signaling, growth, metabolism

710P Analysis of gene expression in the hypothalamus and pituitary gland related to the HPG axis in a turkey line genetically selected for high egg production and a random bred control line. Catherine Galleher*¹, Kristen Brady¹, Julie Long², and Tom Porter¹, ¹*University of Maryland, College Park, Maryland, United States,* ²*Animal Biosciences and Biotechnology Laboratory, BARC, ARS, USDA, Beltsville, Maryland, United States.*

Previous studies involving commercial flocks of turkeys have demonstrated that mRNA levels in the pituitary and hypothalamus of high egg producing hens differ from those of low egg producing hens for genes associated with the hypothalamic-pituitary-gonadal (HPG) axis. High egg producing hens express higher levels of mRNA for genes associated with stimulation of the axis, while expressing lower levels of mRNA for genes associated with repression of the axis, when compared with low egg producing hens. The objective of this study was to determine if these results would be similar in a line of turkeys genetically selected for high egg production (HEP) when compared with a random bred control line (RBC). Three hypothalamic and 6 pituitary genes were selected based on expression differences seen in commercial flocks. The hypothalamus and pituitary were dissected from HEP and RBC hens at 48 weeks of age ($n = 6$ /group). Hens were selected based on eggs laid/day throughout the production period. The average of the HEP hens selected was 0.9 ± 0.0171 eggs/day. The average of the RBC hens selected was 0.53 ± 0.0619 eggs/day. Hens were also selected to be outside of the preovulatory surge of progesterone (P_4) and luteinizing hormone (LH). A radioimm-

muoassay measuring plasma levels of P_4 revealed that one hen from the HEP line was within the preovulatory surge based on elevated levels of P_4 ; samples from this hen were excluded from further analysis. RNA was extracted from the tissue samples using QIAGEN RNeasy kits and analyzed for mRNA levels using reverse transcription quantitative PCR. Expression levels of the genes in HEP samples compared with RBC samples were analyzed using the PROC *t*-test protocol in SAS. Within the hypothalamus, no significant differences were found between the 2 groups when comparing mRNA levels of P_4 receptor (PGR), GnRH, and neuropeptide VF precursor, which encodes for GnIH. In the pituitary, mRNA levels for LH β , common α subunit, PGR, and growth hormone were found to have no significant difference. However, a significant difference ($P < 0.05$) was observed in mRNA levels for prolactin (PRL) and FSH β (FSHB). Expression of PRL and FSHB was downregulated in HEP hens. Prolactin inhibits ovarian production, and FSHB stimulates follicle growth. Based on the selected genes tested, a significant difference in expression levels was not present in the hypothalamus and only present in 2 of the genes tested in the pituitary of HEP and RBC line hens. This suggests a possible difference in mRNA levels of genes associated with the HPG axis in the HEP line compared with high egg producing hens in a commercial line.

Key Words: turkey reproduction, mRNA, gene expression, pituitary, hypothalamus

711P Effects of genetic selection on endocrine mechanisms regulating growth and metabolism in the modern broiler. Lauren Vaccaro* and Laura Ellestad, *University of Georgia, Athens, Georgia, United States.*

The contribution of endocrine systems regulating growth and metabolism to the improved production performance resulting from over a half-decade of genetic selection in the broiler industry is not fully understood. This study's objective was to evaluate effects of commercial selection on gene expression and circulating hormones in 3 endocrine axes that are primary regulators of growth and metabolism: the somatotrophic, thyrotrophic, and adrenocorticotrophic axes. Gene expression levels and plasma hormone concentrations of a commercial line (Ross 708) were compared with those of the Athens-Canadian Random Bred (ACRB), a legacy population that has remained unselected since 1956. Muscle, liver, and plasma were collected from 8 male birds of each line on days (d) 10, 20, 30, and 40 post-hatch. Total RNA was extracted from 6 birds ($n = 6$) to evaluate mRNA expression levels of hormones and hormone receptors regulating growth and body composition by reverse transcription real-time quantitative PCR. Plasma was collected from 8 birds ($n = 8$). Levels of corticosterone (CORT) and insulin-like growth factor (IGF) 1 were determined using ELISAs, and triiodothyronine (T_3) and thyroxine (T_4) plasma levels were determined using RIAs. Data were analyzed for significant effects of line or line-by-age interaction with a 2-way ANOVA followed by the test of least significant difference. Ross birds were found to be significantly heavier than ACRB birds at all ages ($P < 0.01$). In muscle, growth hormone receptor (GHR), IGF binding protein (IGFBP) 3, and thyroid-hormone receptor α (THRA) mRNA exhibited line effects, with elevated expression levels in ACRB ($P < 0.01$). Expression of IGFBP7 mRNA also demonstrated a line effect and was reduced in ACRB ($P < 0.01$). A line-by-age interaction was observed for IGF2, with mRNA levels higher in Ross on d40 ($P < 0.05$). Levels of IGF receptor (IGFR) mRNA demonstrated a line-by-age effect, with increased expression in ACRB on d10 and in Ross on d40 ($P < 0.05$). Elevated IGFBP5 mRNA was seen on d40 in Ross as a result of another line-by-age effect ($P < 0.05$). In liver, a line effect appeared with higher GHR and IGFBP7 mRNA in Ross ($P < 0.01$), whereas a line-by-age

effect was observed with increased IGFBP1 mRNA in ACRB on d20 ($P < 0.01$). Main effects of line were also observed for CORT, T_3 , and T_4 . Plasma CORT and T_3 levels were higher in ACRB ($P < 0.01$), while T_4 levels were higher in Ross ($P < 0.01$). Differential expression of hormone receptors between lines suggests that target tissue sensitivity might play a role in improved growth performance, while expression patterns of IGFBPs indicate that hormonal ligand availability for receptor activation may also be important.

Key Words: growth, metabolism, hormones, gene expression, genetic selection

712P Hormonal regulation of visfatin gene in avian leghorn male hepatoma (LMH) Cells. Alison Ferver, Elizabeth Greene, and Sami Dridi*, *University of Arkansas, Fayetteville, Arkansas, United States.*

Visfatin, an adipocytokine and a nicotinamide adenine dinucleotide-related enzyme, has been extensively studied in mammals and has been shown to play an important role in obesity and insulin resistance. However, there is a paucity of information on visfatin regulation in non-mammalian species. Therefore, we undertook this study to determine its hormonal regulation in avian liver cells. LMH cells were cultured in McCoy 5A medium supplemented with FBS, chicken serum, penicillin-streptomycin, and amphotericin B at 37°C in a 5% CO_2 humidified incubator. At the exponential phase of growth, the complete medium was removed and replaced by a serum-free medium or a culture medium supplemented with thyroidectomized FBS and chicken serum (for the thyroid hormone study) overnight to synchronize the cells. Cells were treated with 5 and 10 ng/mL of recombinant human IL-6, TNF α , orexin A or orexin B, and 10 and 100 ng/mL of recombinant ovine leptin or triiodothyronine (T_3) for 24h. Untreated cells were used as controls, and $n = 8$ for all treatments. The doses and duration of treatments were chosen based on pilot and previous studies. Data were subjected to one-way ANOVA with hormonal treatments (doses) as the fixed effect. If ANOVA revealed significant effects, means were compared by Tukey's multiple range test, and differences were considered significant at $P < 0.05$. Addition of 5 ng/mL TNF α , 100 ng/mL leptin, 10 or 100 ng/mL T_3 for 24h significantly upregulated visfatin gene expression by 20%, 85%, 13%, and 11%, respectively, compared with untreated LMH cells. Administration of 10 ng/mL of orexin A significantly downregulated visfatin gene expression by 26% compared with control cells. In contrast, treatment with IL-6 or orexin B for 24h did not influence visfatin mRNA abundance. Hepatic CRP gene expression was significantly upregulated by IL-6, TNF α , and orexin B and downregulated by leptin, orexin A, and T_3 . LXR mRNA abundance was increased by orexin A and T_3 , decreased by orexin B, and did not affected by IL6, TNF α , or leptin. The expression of FXR gene was induced by IL-6, leptin, and T_3 , but it was not influenced by TNF α , orexin A or B. CXR gene expression was upregulated by TNF α , leptin, orexin B, and T_3 , downregulated by 5 ng/mL orexin A, and did not affected by IL-6. INSIG2 mRNA levels were increased by TNF α (5 ng/mL), leptin (100 ng/mL), and T_3 (100 ng/mL), decreased by orexin A, and remained unchanged with IL-6 or orexin B treatment. Together, this is the first report showing hormonal regulation of visfatin in avian hepatocyte cells and suggesting a potential role of CRP, INSIG2, and nuclear orphan receptor LXR, FXR, and CXR in mediating these hormonal effects.

Key Words: LMH Cells, visfatin, hormonal regulation

713P New transcriptomic insights into processes associated with formation of egg-white in the magnum of laying hens.

Nirvay Sah*, Donna Kuehu, Sanjeev Wasti, Rajesh Jha, and Birendra Mishra, *University of Hawaii at Manoa, Honolulu, Hawaii, United States.*

The egg-white makes up more than 60% of an egg and is formed in the magnum of the hen's oviduct. The magnum synthesizes, stores, and secretes the constituent proteins of the egg-white. Therefore, the functionality of the magnum determines the egg size and quality. However, selection and breeding based on quantitative traits in the hen are yet to be employed due to the lack of information on genes/proteins governing egg formation. The objectives of this study were to 1) identify the differentially expressed genes and biological pathways in the magnum (laying vs non-laying) involved in the egg-white synthesis and secretion, and 2) validate the identified novel genes in the laying, non-laying, and molting hens. Hy-line white hens (45–60 weeks) including 10 laying, 4 non-laying, and 5 molting hens were randomly housed in individual pens. Laying hens were euthanized at 2 different time points (3h, and 15–20h postovulation, p.o.) for the sampling of magnum tissues. Non-laying and molting hens were euthanized randomly, and magnum tissues were collected for RNA extraction. Total RNA from laying hens at 3h p.o. (n = 3) and non-laying (n = 3) hens were subjected to high-throughput sequencing. The mapped genes with FDR adjusted P-value < 0.05 and fold change > 3 were considered differentially expressed. In the laying hens at 3h p.o., a total of 540 genes were differentially expressed, of which, 152 and 388 were up- and downregulated, respectively. Glycine, serine and threonine metabolism was the most-enriched pathway. Temporal effects on gene expression was determined by qPCR assay in the magnum of laying hens at 3h p.o. (n = 5, egg in the magnum), 15–20h p.o. (n = 5, egg in the shell gland), non-laying (n = 4), and molting hens (n = 5, 60 weeks of age). Proteases such as CAPN2, TMPRSS9, MMP1, and MMP9 (protein maturation, ECM degradation, and angiogenesis); enzymes such as PHGDH, PSPH, and PSAT1 (amino-acid biosynthesis); RLN3, REN, and ACE (albumen synthesis, secretion, and egg transport); AVD, AvBD11, and GPX3 (antimicrobial and antioxidants); and solute carriers (SLC26A4, SLC22A3, SLC51B, SLC7A17, SLC1A4, SLC7A11) were recognized as essential molecules linked to egg-white formation in the magnum. In conclusion, the findings of this study revealed some novel genes that participate in the signaling pathways for synthesis and secretion of egg-white. These genes can be used as markers for formulating strategies to improve the size and quality of eggs produced.

Key Words: egg-white, gene expression, layers, magnum, RNA sequencing

714P Comparative transcriptomic analysis of the liver in laying hens at different ages. Gi Ppeum Han*¹, Jong Hyuk Kim¹, Jomari Delos Reyes¹, Hyeon Seok Choi¹, Geun Hyeon Park¹, Hwan Ku Kang², and Dong Yong Kil¹, ¹*Chung-Ang University, Anseong-Si, Korea (the Republic of)*, ²*Rural Development Administration National Institute of Animal Science, Pyeongchang, Korea (the Republic of)*.

The liver plays a central role in nutrient metabolism of animals. However, the molecular regulations for hepatic nutrient metabolism in laying hen at different ages have not been investigated. Therefore, the objective of the current experiment was to compare transcriptomic analysis of the liver in laying hens at different ages (30 vs. 46 weeks of age). A total of 128 30-week-old Hy-line Brown laying hens were selected at the start of this experiment. Laying hens were fed a commercial layer diet during 16 weeks of experiment. Five hens with similar body weight at

each age (i.e., 30 and 46 weeks of age) were chosen and euthanized to collect liver tissues. The collected samples were utilized to construct cDNA library for RNA-seq and sequenced using the Illumina Nextseq 500 sequencer. Genes with False Discovery Rate (FDR) < 0.05 were defined as differentially expressed genes (DEGs). Results indicated that a total of 4,650 DEGs were identified with 2,387 being upregulated and 2,263 being downregulated in the liver of hens at 46 weeks of age than hens at 30 weeks of age. The KEGG pathway analysis revealed that the DEGs belong to several biological pathways such as endocytosis, regulation of actin cytoskeleton, ubiquitin mediated proteolysis, insulin signaling pathway, calcium signaling pathway, lysosome pathway, etc. Specifically, some genes related to the metabolic pathway associated with glycogen degradation, taurine synthesis, folate metabolism, and bile acid synthesis were downregulated in hens at 46 weeks of age. Interestingly, genes associated with fatty acid biosynthesis (e.g., ACSL1, ACSL3, ACSL4, FASN, ACACA) were upregulated, whereas those related to fatty acid degradation (e.g., ADH6, ECI1, ALDH3A2) were downregulated in the liver of hens at 46 weeks of age, indicating increasing fat accumulation in the liver with age. In addition, genes related to amino acid metabolism were altered by age. In particular, genes related to the oxidation of branched chain amino acid (i.e., leucine, isoleucine, and valine) were upregulated in hens at 46 weeks of age. These results provide the molecular insights for hepatic nutrient metabolisms of the liver in laying hen between different ages, which may be used in the development of new dietary recommendations for laying hens.

Key Words: age, comparative transcriptomic analysis, laying hen, liver

715P Semen variables in Creole roosters of Mexico. Rosalía Ordaz-Contreras*¹, Diego Zárate-Contreras¹, Miguel Ángel Matus-Aragón¹, Fernando González-Cerón², Arturo Pro-Martínez¹, Juan Manuel Cuca-García¹, Jaime Gallegos-Sánchez¹, Ítai Martínez-Martínez², Jessica Almeraya-Carreón², and Yolanda Osorio-Marín¹, ¹*Colegio de Postgraduados, Texcoco, Estado de México, Mexico*, ²*Universidad Autónoma Chapingo, Texcoco, Estado de México, Mexico*.

The aim of this study was to evaluate the physical and seminal characteristics of Creole roosters (*Gallus gallus domesticus*) from an experimental flock under random mating of 4 ecoregions of the state of Campeche, Mexico. Twelve roosters of 97 weeks of age were evaluated weekly for 11 weeks. Six roosters were from the mating of birds between ecoregion I (ECR1) and ECR2. The remaining birds belonged to the crossing of ECR3 and ECR4. Two types of roosters were evaluated according to provenance, classifying them into 2 treatments T1 (ECR1ECR2) and T2 (ECR3ECR4). The physical characteristics were analyzed: body weight (BW), body condition (BC), ejaculate time (ET) and seminal characteristics: volume (VOL), consistency (CS), mass motility (MOT), percentage of alive (LIV) and percentage of deaths (D), abnormalities (AB). Semen (SE) was collected by dorsal-abdominal massage, then diluted with saline (SS) 2:1 (SE:SS), to evaluate VOL, CS, MOT, LIV, D and AB. The variables: BW, ET, VOL, LIV, D and AB were analyzed with a mixed model using PROC MIXED SAS 9.4 Tukey test with $\alpha = 0.05$, while the BC, CS and MOT variables were used in the Chi-squared test by PROC FREQ contingency tests with $\alpha = 0.05$. The results indicated interaction effect time x treatment ($P < 0.05$) in the variables BW, LIV, D and AB. The variables VOL and ET showed difference ($P < 0.05$) due to the age of the animal. The categorical variables CC, CS and MOT showed no difference ($P > 0.05$) due to the effect of the treatments. 60% of the birds presented adequate BC. Of the total semen samples, 25%

presented thick-creamy CS and 20% swirling fast wavy movement. In conclusion, the physical and seminal characteristics in roosters were influenced by the ecoregions of provenance, and the age of the animal.

Key Words: *Gallus gallus domesticus*, backyard poultry, avian semen, seminal quality, semen volume

WPSA Lecture: From the Jungle Fowl to Highly Performing Chickens: Are we Reaching the Limits?

716S From the jungle fowl to highly performing chickens: Are we reaching the limits? Michele Tixier-Boichard*, *INRA, Jouy-en-Josas, France*.

The lecture will present the evolution of the chicken as a species, since domestication, with a focus on the acceleration since 70 years due to modern breeding methods and intensification of management techniques, elaborated diets, sanitary protection... Can we continue at the same rhythm? Breeding did not reach yet the point where it could exhaust genetic variability and unfavorable correlated responses on physiology or health can be corrected by new techniques or new selection criteria, but there is always one defect coming after the other, until when? Ethics comes into play because of changing views of societies on how we handle animals. Environmental limits deal with the responsible use of resources, there is a growing concern about competition between animal and humans for access to plant resources for food. Poultry production has a great future, if done in a sustainable way. The option is to work for an optimum rather than a maximum.

Key Words: WPSA Lecture

Informal Nutrition Symposium: Toward Resolving Unsettled Issues in Poultry Nutrition

717S Digestible amino acids. Carl Parsons*, *University of Illinois, Champaign, Illinois, United States.*

The presentation will include a discussion of apparent versus standardized digestibility values for feed ingredients, and the most appropriate method or endogenous correction to use for standardized values. The most common methods for determining amino acid digestibility or bioavailability and their advantages and disadvantages will be discussed in addition to the best methods to use for determining the effect of feed enzymes on amino acid digestibility. The effects of bird age on amino acid digestibility and how to deal with this issue in practical feed formulation will also be covered. The topic of digestible amino acid requirements for commercial feed formulation will also be addressed.

Key Words: amino acids

718S Matching nutrient digestibility data with practical feeding strategies. Martin Zuidhof*, *University of Alberta, Edmonton, Alberta, Canada.*

Over the long history of animal nutrition, immense effort has been applied to characterize nutrient availability of feedstuffs (supply side), nutrient requirements (demand side), and optimal blending to most closely match nutrient supply to nutrient demand. These efforts have been applied at the flock level and have made chicken and egg production more efficient and profitable. However, we know that nutrient requirements of individual birds depend on their specific genome, environment, and stage of life. In the not too distant future, it will be possible to adjust nutrient intake to more closely match nutrient supply to individual birds based on their state-specific needs. This approach promises transformational benefits for sustainable production.

Key Words: digestibility

719S Matrix values for exogenous enzymes. Michael Bedford*, *AB Vista, Marlborough, United Kingdom.*

Exogenous enzymes are employed commercially as a result of their ability to improve the nutritive value of a diet via several mechanisms. In most cases the cost of the enzyme is more than offset by

assignment of a nutrient matrix relevant for the activity of the dose of enzyme employed. The actual nutrient release in any one instance is influenced by ingredient choice, nutrient density, husbandry conditions and bird related factors and as a result any matrix employed needs to be robust and deliver if shortfalls in performance are to be avoided. In many cases the nutrient matrix is estimated through a variety of techniques including digestibility, performance and carcass trials. The final application of the matrix by the end-user most often uses performance of the animal as measured by weight corrected FCR or even carcass yield of high value portions as the metric of success. Optimum commercial performance of an exogenous enzyme is achieved when as challenging matrix as possible is applied with no performance losses (or better still a slight uplift in animal performance). A matrix value of an enzyme therefore has to be evaluated over a significant number of trials to minimize the likelihood of a failure in delivery.

Key Words: enzymes

720S Precision nutrition—Applying enzyme matrix values in the real world. Aaron Cowieson*, *DSM Nutritional Products, Kirkwall, Orkney, United Kingdom.*

There is growing interest in improving the precision of delivery of digestible nutrients to poultry to improve economic and environmental sustainability. As with any feed ingredient, feed enzymes carry a degree of ambiguity into feed formulation with respect to overlapping matrices, variability and the breadth of effect across multiple nutrient domains. This presentation will consider some of the major factors that may influence feed enzyme response with the objective of improving precision in feed enzyme use in poultry production systems.

Key Words: enzymes

National Extension Workshop: High Risk and Vulnerable Poultry Producers

721S Educational outreach challenges and opportunities.

Anthony Pescatore*, *University of Kentucky, Lexington, Kentucky, United States.*

The number of small and medium-sized farms that are diversifying into poultry continues to grow. The desire to produce local products has created a new population of producers that are usually small scale and many times not located in traditional rural areas. These changes have expanded the potential audience for poultry outreach programs. This expanded audience may not be familiar with Extension or their state's land grant university. The challenge is to establish our outreach programs as a reliable source of information to an inexperienced audience that ultimately are involved in food production, marketing and distribution.

Key Words: extension

722S Successful niche market examples. Mark Harrison*, *White Oak Pastures, Bluffton, Georgia, United States.*

The discussion will focus on the impacts of pastured poultry for the process of regenerative farming and the need for better consumer education surrounding the difference between free range pastured poultry and commercial operations claiming the same thing. Talking points will highlight the challenges of mobile housing systems, predation control and the need for monitored field trials on food consumption and growth ratios for pastured poultry producers.

Key Words: extension

723S The importance of audits to gaining customer confidence.

Karen Christensen*, *Tyson Foods Inc., Springdale, Arkansas, United States.*

The vast majority of the US population is many generations removed from food production. Modern technology has added to the confusion and at the same time, access to information about how food is raised. Auditing of production practices is a critical part of establishing confidence between producers, customers and consumers.

Key Words: extension

724S Reaching the hard to reach: Model outreach programs;

Canada. Steve Laycock*, *Real Solutions Plus, Edmonton, Alberta, Canada.*

Education of all producers on proper bio-security, flock health, welfare and nutrition is of course the goal for all of us in extension. In our feather industry we have lots of people who are part-time producers with another full-time job or producers of non-mainstream birds likes ducks/geese and pheasants who simply aren't on the invitation list of the many meetings that we organize for the broiler, turkey and layer producers. And of course geography plays an important reason for even mainstream producers for not attending meetings in person. We believe that there is a role for simple technologies like YouTube and webinars to play in the education of our producers. This talk focuses on methods used and tried in Alberta.

Key Words: extension

725S Reaching the hard to reach: Model outreach programs; Mexico. Monica Vargas-Mendoza*, *Colegio de Postgraduados, Boca del Río, Veracruz, Mexico.*

Our program is called "International Symposium on Backyard Poultry Production." It started with the objective of provide a common platform to share and discuss the results of recent research conducted at different institutions of Mexico and USA. The government extension programs usually do not cover this sector of production, and producers rely on traditional knowledge to solve the problems that arise. From 2012 to 2019, we have held 8 meetings, on annual basis. We have reached more than a 1000 attendees, bringing together all the stake holders in back yard poultry production: producers, researchers, technicians and undergraduate and graduate students. Our symposia have become a means to share knowledge and experiences in back yard production with researchers beyond our borders, making this an international effort.

Key Words: extension

726S Reaching the hard to reach: Model outreach programs;

United States. Jacquie Jacob*, *University of Kentucky, Lexington, Kentucky, United States.*

In the United States there is an increasing number of people interested in keeping a few chickens in their backyard to supply the family with fresh eggs. In addition, several people are starting up small-scale poultry operations. All are in need of accurate poultry-related information. The online version of the cooperative extension service, known as eXtension, has an extension program for small and backyard poultry flock owners.

Key Words: Extension

Amino Acids: New Paradigms in Poultry Nutrition and Health

727S Maximizing amino acid nutrition under constantly changing dietary ingredient composition. Todd Applegate*, *University of Georgia, Athens, Georgia, United States.*

Often, bioassays for feed ingredient digestibility have revealed underlying variation due to ingredient composition and repeatability which may or may not directly accounted for in day-to-day formulation. Similarly, additivity of these bioassay results have recently been confirmed by various researchers. Thus, it is imperative that formulating nutritionists understand the nuances these bioassays have on digestibility results for their extrapolation. This is extremely difficult to do as primary feed ingredient nutrient composition changes occur on a day-to-day, weekly, monthly, and or seasonal basis.

Key Words: amino acids

728S Considerations for amino acid nutrition with all vegetable diets and antibiotic free production systems in broilers. Randy Mitchell*, *Perdue Farms, LLC, Salisbury, Maryland, United States.*

Most nutritional requirements for broilers have been developed in conventional production systems. The use of all vegetable diets brings new challenges to optimizing amino acid density. Wet litter can be a major challenge with all vegetable diets. Balancing the potential benefits of feeding high density diets with the negative aspects of wet litter in all vegetable diets will be discussed. This is further complicated in antibiotic free production systems where enteric challenges are a constant threat. Formulation strategies for utilizing synthetic amino acids and limiting problematic ingredients in antibiotic free systems will be discussed in context with other management changes to reduce the risk of enteric disease.

Key Words: amino acids

729S Beyond growth—The role of functional amino acids for immune functions. Rose Whelan*, *Evonik Nutrition & Care GmbH, Hanau, Germany.*

The advancements made to poultry genetics and nutrition in the last 50 years have resulted in exponential improvements to feed efficiency and rate of growth of broiler chickens. For example, while only a few decades ago feed was formulated on a crude protein level, nutrient requirements can now be defined to the specificity of SID amino acids. Despite the innovations in nutrition and genetics, the modern broiler is still susceptible to pathogens and other stressors that impair optimal growth performance. We aim to explore the underlying reasons why disease and environmental challenges impair growth, to understand how we can develop nutritional strategies to prevent this. We will discuss how we can support immune function and disease resistance through redefining nutrient requirements to not only meet the metabolic needs of a healthy animal, but to additionally optimize immune develop and function. One future aim may be to develop dynamic amino acid recommendations that support immune development, vaccination efficacy and response to potential threats. This will allow us to provide healthy sustainable nutrition solutions to improve resistance to stressors and optimize growth in sub-optimal conditions.

Key Words: amino acids

730S The effects of amino acids on microbiota and gut health of poultry. Elizabeth Santin*, *Jefo Nutrition Inc., Saint-Hyacinthe, Quebec, Canada.*

The intestinal mucosa is in constant development and they need for specific amino acids will be discussed during the talk. In the microbiome aspects, we will present some information from human and to understand the effect of the surplus of crude protein or specific amino acids on the growth of a specific microorganism and/or metabolome production that could be beneficial or not for the host.

Key Words: amino acids

731S Optimizing dietary protein levels in broiler breeder diets to improve production and chick's performance and health. Rick Van Emous*, *Wageningen University, Wageningen, The Netherlands.*

Nowadays, management issues in broiler breeders, associated with nutrition and reproductive characteristics, are becoming increasingly challenging. Due to genetic selection on broilers, body composition of breeders has changed dramatically during the last 50 years to less fat and more breast muscle. It is postulated that a certain amount of body fat in broiler breeders at the onset of lay is necessary for maximum performance. Feeding pullets a low protein diet during rearing decreased breast muscle and increased abdominal fat pad. The higher abdominal fat pad content resulted in an increased hatchability during the first phase of lay and a larger number of eggs during the second phase of lay. On the other hand, a low daily protein intake during the rearing and first phase of lay can lead to a poor feather cover. It is possible to decrease CP level of breeder diets with comparable reproduction from 22 to 46 wk; however, this is questionable for the rest of the production period. In the second phase of lay a low total CP intake is recommend to maximize hatchability of fertile eggs due to a decreased embryonic mortality. Little, no or opposite effects on performance and processing yields of the offspring has been reported by a change in maternal daily protein intake. More research is necessary to produce a more clear and consistent answer on this multifaceted question.

Key Words: amino acids

732S Challenges and opportunities for precise amino acid nutrition to improve health and performance of broilers. Peter Chrystal*, *Baiada Poultry, Pendle Hill, New South Wales, Australia.*

Challenges and opportunities for precise amino acids nutrition to improve health and performance of broilers will be discussed. In some instances, information is well known, so very little time would be spent on those aspects. More time would be spent on the lesser known aspects such as changes in blood plasma AA profiles and the possible implications thereof. Digestion dynamics is also worth covering as CP reduces and the practical implications explored.

Key Words: amino acids

Emerging Issues in Food Animal Welfare

733S What do birds think about gases? A. Webster*, *University of Georgia, Athens, Georgia, United States.*

Although controlled atmosphere stunning (CAS) of chickens was originated to get around carcass quality problems stemming from high current electrical stunning of birds, it quickly became apparent that CAS offered considerable animal welfare advantages over electrical stunning systems. Much of the rough handling, stress, fear, pain, and injury that is unavoidable in getting chickens properly presented to an electrical stunner can be eliminated with CAS. On the other hand, while a properly applied electrical stun appears virtually instantaneous, birds take some time to become unconscious in a controlled stunning atmosphere, raising the question of what they experience during that time. This presentation will review studies of the responses of poultry to different gases used for stunning, focusing on those in which birds were given an opportunity to avoid the stunning atmosphere, to offer a perspective on how aversive these atmospheres are to chickens and what their welfare experience may be when stunned in them.

Key Words: animal welfare

734S Using temperature sensors to better understand how management practices can affect broiler welfare. Brian Fairchild*, *University of Georgia, Athens, Georgia, United States.*

Many studies in the literature have used rectal temperature as the method of determining body temperature. Other studies have reported issues with rectal temperature methodology that could affect the measurement including, but not limited to, handling of the birds, probe insertion depth, time of day, and instrument performance. In addition, rectal temperature measurements are not continuous and are only recorded at specific times as outlined in the study's protocol. Some studies have utilized telemetry systems to avoid the issues associated with measuring rectal temperatures. These measurements had distance limitations between the transmitter and the receiver and the antennae usually protruded from the body which created a point for the measurement bird and pen mates to peck resulting in damage to the equipment and the animal. Another method of measuring body temperature is the use of data loggers. Several different data loggers have successfully been used in several studies over the past few years overcoming the issues associated with the rectal temperature and telemetry methods. This presentation will briefly review body temperature measurement methodology, focus on the advantages and disadvantages of data loggers and review the findings of recent studies that have been conducted.

Key Words: animal welfare

735S Is there a place for robots in our chicken houses? Colin Usher*, *Georgia Institute of Technology, Atlanta, Georgia, United States.*

Robotic solutions are starting to show up in operations across the world. Companies in Europe, Asia, and the United States have all developed different robots for operating inside of commercial houses. Some of these robots inspect the flock and the environment, some disinfect the flooring, and some can carry out utilitarian tasks such as automatic removal of floor eggs and mortality. This presentation

will highlight both the immediate impact these systems can have on animal welfare, as well as potential future impacts that such robots could have on the future of live operations.

Key Words: animal welfare

736S What does the consumer really want when it comes to animal welfare? Candace Croney*, *Purdue University, West Lafayette, Indiana, United States.*

It is increasingly acknowledged that although today's consumers are predominantly unaffiliated with animal agriculture, many have become interested in understanding how their food is produced and the extent to which current practices align with their conceptions of sustainable production. Relatedly, public interest in the welfare of food-producing animals has grown, creating market and social pressure for farmers to modify their production practices. In addition, corporations within the food producing sectors (e.g., processors and retailers) are now expected to provide assurance that animal welfare is duly considered and protected. Meeting consumer expectations about corporate social responsibility in regard to food production requires improved understanding of how animal welfare relates to people's notions of sustainability and the underlying ethical bases for their views and behaviors.

Key Words: animal welfare

737S When disaster strikes; Mass depopulation and welfare. Kenneth Anderson*, *NCSU, Raleigh, North Carolina, United States.*

Significant differences in management practices, bird type, and housing construction makes it difficult to prescribe the use of a single depopulation method across all farms in the event of a foreign animal disease outbreak. The primary methods 'conditionally approved' by USDA-APHIS and the AVMA for depopulation are CO₂ and water-based foam. If depopulation with these methods cannot be achieved within 24 h, alternative methods can be approved for use. One alternative method recently investigated for its effectiveness is ventilation shutdown (VSD). Ventilation shutdown (VSD) is defined as the cessation of natural or mechanical ventilation of atmospheric air in a building where birds are housed, with or without action to increase ambient temperature. The Department for Environment, Food, & Rural Affairs (DEFRA) set a precedence for VSD use within the United Kingdom with their "Guidelines for Killing Poultry Using Ventilation Shutdown (VSD)." However, until recently, no research has been published to support their instructions. Researchers at North Carolina State University have spent the past 3 years evaluating the effectiveness of VSD and ventilation shutdown with supplemental heat (VSDH) in commercial poultry, including egg-laying hens, turkeys, and broilers. Significant differences were observed in behavior, stress physiology, and environmental parameters between species, suggesting that the effect of heat is dependent on the age, body weight, and past management experiences. These differences have led researchers to question the effectiveness of VSD and VSDH in breeders, whose body weight and management experiences vary from commercially produced poultry.

Key Words: animal welfare

738S Update on cage free egg production and layer welfare. Darrin Karcher*, *Purdue University, West Lafayette, Indiana, United States.*

The continued move toward cage-free egg production has many positive and negative outcomes associated with layer welfare. There are numerous iterations of cage-free housing systems with each having associated challenges. This presentation will cover different types of cage-free housing, impacts on layer welfare and future directions for layer welfare in cage-free systems.

Key Words: animal welfare

739S Welfare implications of pullet rearing environments. Tina Widowski*, *University of Guelph, Guelph, Ontario, Canada.*

The early experience of pullets can have lifelong effects on their behavior, health and welfare. Rearing environment has been shown to affect spatial cognition, fearfulness and musculoskeletal development. This is particularly important for layer pullets destined for aviaries- where they must be calm, physically fit and able to negotiate highly complex environments. This presentation will review recent findings on how rearing environment and early opportunities for exercise affect physical development and behavior of different strains of layer pullets.

Key Words: animal welfare

Food Safety: Antimicrobial Interventions Used in Poultry Processing to Reduce Pathogens on Raw Meat

740S Addressing industry concerns. Christian Fuchs*, *Maple Leaf Foods, Mississauga, Ontario, Canada.*

The presentation will provide an overview of the company's multi-year fresh poultry pathogen reduction strategy. The supply chain approach to pathogen reduction will be reviewed, which will include a review of goals, progress, learnings/insights and the challenges the company has come across in the fresh poultry pathogen reduction journey.

Key Words: food safety

741S Microbial contamination in poultry processing (biomap) and development/validation of intervention strategies. Manpreet Singh*, *University of Georgia, Athens, Georgia, United States.*

This presentation will cover sources of microbial contamination at the processing plant and the importance of "biomapping" to determine high risk areas. There are several studies about incoming microbial loads of pathogens i.e., Salmonella and Campylobacter and how this relates to the contamination levels post-chill. Over the past decade we have observed several antimicrobials being used in poultry processing, their efficacies and their application in various processing steps. This presentation will also discuss the use of intervention technologies such as antimicrobials and in-plant validations to determine efficacy of antimicrobials.

Key Words: food safety

742S Canadian regulations and perspective. Jeff Farber*, *University of Guelph, Guelph, Ontario, Canada.*

This presentation will address the antimicrobial interventions used at the federal level for poultry processing in Canada to reduce pathogens on raw poultry, concentrating on Salmonella and Campylobacter. The federal process used to approve new antimicrobials in poultry slaughter facilities will be outlined. Also included will be discussions on the new federal requirements to reduce Salmonella to below detectable amounts in frozen raw breaded chicken products. Furthermore, an update will be given on initial discussions surrounding performance standards for raw poultry in Canada. Finally, we will discuss interventions for one of the provinces, as OMAFRA is currently rolling out mandatory interventions for poultry slaughter in provincial plants, and this will be discussed.

Key Words: food safety

743S USA regulations and government perspectives. Melanie Abley*, *USDA-FSIS, Washington, District of Columbia, United States.*

Processing aids are defined in the Food and Drug Administration's (FDA) regulation 21 CFR 101.100(a)(3)(ii) as substances that are added to a food during the processing of such food but are removed in some manner from the food before it is packaged in its finished form. FSIS Directive 7120.1 provides an up-to-date list of substances that may be used in the production of meat, poultry, and egg products. It also lists the approved On-Line Reprocessing (OLR) and

Off-Line Reprocessing (OFLR) Antimicrobial Intervention Systems. However, new uses of previously approved substances, including changes in concentration or application methods, are to be reviewed for safety and suitability by FDA and FSIS per MOU 225-00-2000 Amendment 1. The FSIS process for approving antimicrobials will be discussed.

Key Words: food safety

744S Harnessing potential bacteriophages to control and detect food borne bacterial pathogens. Hany Anany*, *Agriculture and Agrifood Canada, Guelph, Ontario, Canada.*

Over the past few years we have witnessed disturbing numbers of food recalls and food-borne outbreaks associated with an increased prevalence of antibiotic-resistant pathogens all over the world. This represents a formidable challenge to food industry in marketing safe food products. Hence, there is a growing interest in developing novel approaches to be implemented with or to replace existing protocols to control pathogens. The idea of capturing and destroying pathogens by natural interventions is attractive to the consumers. In this context, the use of bacteriophages (bacterial viruses) is considered as a promising green technology. The specificity interaction between phage and its host cell can be exploited to detect and control pathogenic bacteria, without affecting the viability of other microorganisms in the habitat. This presentation will briefly discuss bacteriophage biology and potential applications of bacteriophages in modern biotechnology. It will also shed light on some of the proposed approaches for using phages not only as harmless and natural biocontrol agents, but also as ultrasensitive and specific biosensing nanodevices capable of detecting and destroying bacterial pathogens in situ throughout the food supply chain.

Key Words: food safety

745S Novel approaches to chiller applications—Using high pH systems. Ingrid Cole*, *CMS Technology Inc., Bridgewater, New Jersey, United States.*

Chlorine and peracetic acid are common antimicrobial treatments used throughout poultry processing applications to reduce both Salmonella and Campylobacter. Chiller applications utilizing these interventions are typically adjusted to a pH range of 8.0–8.5. The efficacy of these treatments can be enhanced by adjusting the pH above 9.0. High pH reduces the surface tension of the antimicrobial and allows it to effectively wet out the fatty layer and skin. This leads to greater reductions in the level of Salmonella and Campylobacter within whole bird carcass rinses and on the skin's surface.

Key Words: food safety

746S Advancements in bio-based food safety interventions for the food industry. Saurabh Kumar*, *Corbion, Lenexa, Kansas, United States.*

Process and Antimicrobial Chemistry Synergy – Utilizing organization's proprietary scientific framework to assess mode of action of

antimicrobial solutions, chicken surface chemistry, process parameters interaction, sensory impacts and synergies to develop novel organic acid (with enhancers) based interventions for food safety and shelf life extension in poultry.

Key Words: food safety

Addressing Issues Facing the Poultry Industry

747S Excelling in the ABF world by emphasizing breeder and hatchery management. Jeanna Wilson*, *UGA, Athens, Georgia, United States.*

In recent years, the poultry industry has met the ever-increasing food safety guidelines by emphasizing processing plant contamination. Managing these critical control points in the processing plant has been very effective. However, the paradigm changed with the reduction of antibiotic use to all live birds. For most companies, antibiotic use is no longer a viable option to assist in meeting these goals. As an industry we must evaluate current practices and determine how to get cleaner, healthier chicks to the broiler house. Starting breeder chicks out in a newly cleaned and sanitized house is a must. The chicks need to develop their immune system not be overwhelmed by the bacteria, viruses and molds left from the last flock. The idea that we are reusing litter in rearing broiler breeders is a misnomer there is no litter remaining. Placing our newly hatched breeder chicks of the cleanest environment possible is the only acceptable method today. It is important to reduce all sources of stress for these birds. Evaluate bird density and feeder space, along with moving to every day feeding of a high fiber diet. Stressed breeders are less uniform, lay fewer eggs, hatch at a lower rate and the broilers have poorer bone strength and foot pad quality. The level of hatchery sanitation is only as good as the dirtiest breeder farm that is sending eggs to the hatchery. The best sanitation cannot keep up with continual shipments of dirty eggs and hatching those dirty egg once or more per week. A small number of contaminated eggs that crack or explode in the incubator contaminate the clean eggs, so there is a dramatic multiplication of contaminated egg numbers as the incubation process progresses. Do not cave to the pressure to bring dirty eggs to the hatchery or incubate them. Prevention of dirty egg production is essential. Be open to new hatching egg cleaning methods that involve chemical use (quaternary ammonium, liquid hydrogen peroxide, chlorine, acids and formaldehyde). Continual use of room and surface sanitizers like dry hydrogen peroxide, coupled with a good hatchery cleaning and sanitizing program can improve hatchability and chick quality. Reevaluating breeder grow-out and hatchery sanitation is a must as we move forward in producing clean and health broiler chicks.

Key Words: industry

748S Common misconceptions and errors in assuming a product is a viable alternative to an antibiotic. Greg Mathis*, *Southern Poultry Research, Athens, Georgia, United States.*

With the demand for poultry 'Raised Without Antibiotics' or 'No Antibiotics Ever' flocks, the number of alternative products has greatly increased. This has led to the development and use of several products such as probiotics, prebiotics, essential oils, organic acids, saponins, and tannins. One question we must consider in determining if the product is a viable alternative; does it provide the same or better disease control as an antibiotic? Research often demonstrates that most of the alternatives are not as effective as an antibiotic. Due to this, poultry producers should moderate expectations. However, alternatives do currently play a major role in maintaining bird health. Unfortunately, not all provide the same benefits. Just as each alternative category varies greatly, within each category there can be large differences. It would be an error to believe that one product is the same as another. For example, there are many types of Probiotics: feed or water based, non-heat stable or heat stable spore form, single species or mix, one-time or continuous administration, etc. Even strain variation for one species can be tremendous. Consistency and production of plant-based products have a similar issue. Just because plant extracts claim to be the same product, the extraction of active ingredients can vary from plant source to production facility. Like probiotics, dose levels vary with each plant-based product. Another misconception is that encapsulation processes provide the same degree of resistance to acid and enzyme breakdown. Thus the main misconception is that products are the same and will provide similar benefits.

Key Words: industry

Future of Data Analytics: Development and Application of Models and Decision Support Systems for Poultry Nutrition and Production

749S Concepts and development of growth and nutrient requirement models. Nilva Sakomura*, *Universidade Estadual Paulista—UNESP, Jaboticabal, São Paulo (SP), Brazil.*

Factorial models were developed to predict energy and essential amino acids requirement for an individual, considering the rates at which nutrients are being deposited in body. The models are a powerful tool to describe nutrient requirement dynamically enabling a better understanding of nutritional programs.

Key Words: data analytics

750S Application of nutritional models for estimating nutrient requirements. Rob Gous*, *University of KwaZulu-Natal, Pietermaritzburg, KZN, South Africa.*

Poultry nutritionists base their nutritional strategies on the concept of a 'nutrient requirement'. This is seen as a characteristic of the bird, and is the nutrient content required to support 'maximum' or 'optimum' production. Such requirements are published as tables by learned committees (e.g., NRC, 1994), by national extension services in many European countries, by breeding companies and by some universities and research institutes. Within a company the nutritionist will attempt to adapt these requirements to local circumstances taking some account of the objectives of the business and also of economic circumstances. The basis on which such adaptations are made is often tenuous and lacking in critical scientific rigor. The application of systems thinking and modeling to the problem of feed formulation leads to the replacement of the above approach with one in which nutritional decisions are made entirely in terms of the objectives of the business. Nutrient specifications are chosen that will maximize a desired objective function such as margin/m² per annum or number of hatching eggs per flock. Feeding animals to achieve some company objective is not the same as feeding them to meet a 'requirement'. Genetic potential as well as economic circumstances will change over time and different nutritional strategies will be needed to maximize margins. Also, nutritional decisions will depend on the stage in the production process at which margin is to be assessed. These are real differences, each requiring specific nutritional decisions. Simulation modeling enables these differences to be accommodated. Opportunities arising from changes in input costs or product value need to be grasped immediately if maximum benefit is to be gained, and this is only possible if performance can be predicted, rather than measured in the field. The role of nutritional modeling in this process is thus to predict nutritional responses so that the process of optimization may be speeded up. It should be made clear that the 'optimum' feeding strategy referred to throughout this discussion is that which will maximize or minimize the objective function defined by management. Nutritional models should thus be seen as making use of the nutrient requirements of an individual, which are relatively easy to calculate, to simulate the key outputs in performance of a population, such as food intake, weight gain and carcass yield, such that the economic optimum feeds and feeding program may be determined for each unique set of circumstances.

Key Words: simulation, optimisation, objective function, systems thinking, nutritional decisions

751S Industry perspective on the application of nutritional models. Jennifer Ellis*, *University of Guelph, Guelph, Ontario, Canada.*

Models for poultry production, which integrate nutrition and management knowledge into user-friendly applications, represent valuable 'decision support' tools for nutritionists as well as farmers and integrators. However, limitations may revolve around rapidly evolving genetics, the intensive nature of manually inputting required data, and the time required to become expert users. Here, there is an opportunity to embrace the next wave of digital agriculture in the form of sensors and artificial intelligence to marry these 2 approaches - to combine the speed and automation of big data with the cumulative biological knowledge present in mechanistic models. This presentation will review the successes and limitations of models applied in the field, and what the future path may look like.

Key Words: data analytics

752S Decision-making models for nutrition and poultry production. Edgar Oviedo*, *NC State University, Raleigh, North Carolina, United States.*

Mathematical modeling has been a common technique to understand and quantify complex systems affected by multiple factors in many areas of knowledge. Models can estimate optimum alternatives and guide better decision-making to achieve desired goals. Several models have been developed in the past decades for poultry nutrition and production becoming software. Some of these applications will be briefly described in this presentation. However, despite the well-known benefits of decision-making based on models, nowadays very few are broadly known or applied. The interest in modeling is greater in the industry than in academic institutions. The challenges that impose the growing, variable, and competitive markets rise the need to determine and constantly reevaluate economically optimal nutrition and management solutions. On the other side, very few poultry researchers direct their programs to develop information that could be used in models to quantify biological systems, determine impacts of nutrient levels and sources, variations in feed manufacturing, housing, health, management and processing in flocks and for the whole production system. A lack of training in this area, the preference for empirical data and basic statistics rather than mechanistic frameworks has limited the progress and use of models in poultry academic environments and some sectors of the industry. Most of the simulations made in the current models still do not have the accuracy and precision that poultry companies require. Presently, big data, analytics and artificial intelligence offer tools to develop more accurate models based on large amounts of real-time data produced in the industry. Many companies offer the infrastructure to collect, manage and summarize data. More data increases the need for poultry scientists trained on analytics and able to apply methodologies that link new information with the existing models or validate findings observed under controlled conditions. The understanding of relationships identified by analytics on big data could help to refine current mechanistic biological models. However, modeling cannot be limited to identify mechanisms of the main factors in the system based on theory. Many other unknown parameters could play a role and interactions among them may be complex and very dynamic. Methodologies of arti-

cial intelligence like neural and Bayesian networks, decision trees, fuzzy logic, algorithm generation, machine learning and automatic detection of anomalies could enhance current modeling capabilities for faster simulation, more reliable prediction and prescription with direct applications for decision-making in the poultry industry.

Key Words: decision-making, modelling, analytics, analytics, artificial intelligence

753S The future of data analytics and emerging technologies in protein production. Juan Pedro Fernandez Sanin*, *Asimetrix, Cary, North Carolina, United States.*

Animal protein offers one of the greatest opportunities of our time to increase efficiency by deploying new technologies on a large scale by combining data science, artificial intelligence and animal science

into tools that can be made available to protein producers around the world. The most impactful means for achieving these goals are based on capturing the data that farms already produce or can produce, and then deploying the resulting information to automate and improve the processes both within the farms and across their value chain. Capturing this data and deploying algorithms that combine animal science with machine learning via various complementary tools has helped our partners understand the effects of variables, such as temperature, humidity and water chemistry on animal development, while also tracking growth in precise, high frequency and non-invasive ways. This all allows for applying more precise management practices and nutrition techniques to increase health, uniformity and total output and improves human nutrition and animal welfare by producing more protein of better quality, at a lower cost.

Key Words: data analytics

The Bird and Its Ecosystem: A Global Approach for an Efficient Production

754S Intestinal functionality: The dichotomy between nutrient and barrier function. Todd Applegate*, *University of Georgia, Athens, Georgia, United States.*

The gut (GIT) is adaptive and responds to ingredients, to their nutritive and anti-nutritional values, and the microbial populations within the GIT's environment. However, the GIT's adaptive responses can alter endogenous maintenance as well as the digestibility of nutrients and energy. For instance, the GIT's response to pathogens depends on the quantity, virulence and other predisposing factors (e.g., cocci, mucin type, etc.). As a result, damage to the GIT and change in its immunological response can result in different nutrient "requirements" for tissue maintenance and alterations in how efficiently nutrients are used for growth and production.

Key Words: bird ecosystem

755S Nutrition rebalancing under antibiotic-free strategies and challenging conditions. Michael Persia*, *Virginia Tech, Blacksburg, Virginia, United States.*

Under antibiotic-free strategies, there is a need to revisit the nutrient supply. Under stressful conditions, the redox balance and the amino acid needs of the broilers have to be addressed. This presentation will provide a review of the energy vs. protein response by evaluating the lean and fatty tissue responses under coccidiosis infection.

Key Words: bird ecosystem

756S Feeding an ecosystem: How can we feed the beneficial gut bacteria. Filip Van Immerseel*, *Ghent University, Salisburylaan, Merelbeke, Belgium.*

Mucosa-associated bacterial communities are important for gut health. Butyrate-producing bacteria seem essential. Feeding strategies (e.g., prebiotics such as AXOS, probiotics) can steer bacterial population toward butyrogenic (mucosa-associated) taxa in the gut. Biomarkers of the host response are important tools for the development of future efficient gut health solutions.

Key Words: bird ecosystem

757S Butyrate: A tool for a better gut health? Joshua Gong*, *Agriculture & Agri-Food Canada, Guelph, Ontario, Canada.*

From a nutrient to a messenger, butyrate is essential for the enterocyte and serves as a marker for the metabolic activities of beneficial bacteria. Butyrate both produced by commensal bacteria, as well as supplemented through the feed appear essential as energy nutrient and as a messenger for a good gut function: from cell proliferation and differentiation, better gut frontier integrity, improved gut immune function and even effect on the local and central nervous system. Moreover, butyrate exhibits a potency to reduce the effect of pathogens in the intestine.

Key Words: bird ecosystem

758S Mechanistic understanding of intestinal barrier dysfunction induced by heat stress. Sami Dridi*, *University of Arkansas, Fayetteville, Arkansas, United States.*

Global warming and marked period of heat stress are affecting biological systems including birds, large animals, insects and crops. In broiler chickens, which play a key role in worldwide meat production and food security, the strong negative effect of heat stress on growth, feed efficiency, meat yield and mortality is well documented. Heat stress induces many cellular alterations such as protein misfolding and aggregation, cell cycle arrest, redox state, and transcription modulation, but underlying molecular mechanisms are not fully understood. Understanding and mediating the effect of heat stress on gut barrier function are described. Here, we will discuss the role of dsRNA dysmetabolism in mediating the effect of heat stress on gut barrier integrity and function.

Key Words: bird ecosystem

759S How various non-AGP strategies can reduce the systemic effects of inflammation. Doug Korver*, *University of Alberta, Edmonton, Alberta, Canada.*

When investigating non-AGP strategies, it is important to consider the potential to optimize the host inflammatory response. Indeed, reduction of inflammation is likely an important part of the growth-promoting effects of antibiotics. Non-antibiotic strategies to control gut microbes, may directly or indirectly affect systemic inflammation. Thus, only using different and complementary alternative solutions with different mechanisms would appear efficient to replace antibiotics and maintain or restore productivity.

Key Words: bird ecosystem

Mycotoxin Risk Management and Gut Health in Poultry, Importance in Modern Poultry Production and Innovative Solutions to Support It

760S Development of the immune system in poultry. Bernd Kaspers*, *University of Munich, Munich, Germany.*

The avian immune system presents some unique features that are genetically determined. Post hatch environmental conditions and bacterial colonization of the gut play an important role in the maturation of the immune system whereas the role of nutritional factors remains poorly understood.

Key Words: mycotoxins

761S Cost of impaired immunity and implication in the field. Filip Van Immerseel*, *Ghent University, Salisburylaan, Ingang, Belgium.*

Nowadays, the rating of the important organs is changing; the gut has become increasingly important, partly because of its implication in immune functions. To have a good performance, it is necessary to quantify both the cost of impaired immune response and the cost of activating the immune system.

Key Words: mycotoxin

762S Mycotoxin toxicity: Notion of risk level and interaction between fusariotoxins at the intestinal level. Isabelle Oswald*, *INRA, Toulouse, France.*

Among mycotoxins, fumonisin B1 and deoxynivalenol have a great impact on zootechnical parameters. These mycotoxins are not only locally toxic for the intestine, but also dysregulate many intestinal functions and impair the local immune response. This results in systemic toxicity leading to many symptoms. Feed contamination with mycotoxins also impair the barrier function of the intestine, leading to translocation of bacteria across the intestine and thus intestinal and systemic infections.

Key Words: mycotoxin

763S Managing mycotoxins at the speed of light: Application of the NIR technology for mycotoxin analysis. Carlos Mallmann*, *Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil.*

Mycotoxin analysis is a key tool in mycotoxin risk management in the feed. The control of raw materials must be accurate and quick to be an efficient decision-making tool. Recently, the fast, simple and low-cost technology for the detection of mycotoxins that employs near-infrared spectroscopy – NIR is developed and is a very promising alternative for the feed industry.

Key Words: mycotoxin

764S Mycotoxin detoxification strategies of feed in Europe, a review. Julia Laurain*, *Olmix SA, Brehan, France.*

One strategy for reducing animals' exposure to mycotoxins is to decrease mycotoxin bioavailability by incorporating various mycotoxin-detoxifying agents in the feed. Facing the abundance of available candidate detoxifying agents, screening methods are needed to evaluate their efficacy and select appropriate materials. A wide variability of results is observed, depending on the method and mycotoxin used.

Key Words: mycotoxins

Recent Advances in Calcium Nutrition of Broilers and Layers

765S Measurement of calcium digestibility in feed ingredients for poultry. Ravi Ravindran*, *Massey University, Palmerstown North, Manawatu, New Zealand.*

Determination of Ca digestibility in feed ingredients for poultry has not received any attention in the past due to the abundance and low cost of limestone, the major inorganic Ca source. The recent interest in the determination of digestible P necessitates the measurement of Ca digestibility because of the close relationship between the absorption and metabolism of these 2 minerals. In poultry, Ca digestibility needs to be measured at the ileal level because of the possible urinary Ca contamination at the excreta level. Ileal digestible data generated for the major Ca sources (limestone and meat and bone meal) indicate that the measured Ca digestibility values are different from bioavailability values reported in the past.

Key Words: calcium

766S Effect of calcium and phytate source and concentration on diet calcium and phosphorus digestibilities and phytase efficacy. Roselina Angel*, *University of Maryland, College Park, Maryland, United States.*

The work to be presented focuses on improving our understanding of some of the potential interactions between calcium and phytate source and phytate concentration on standardized ileal digestibility (SID) of Ca and P in diets with or without phytase. Results showed that phytate concentration from the same source influences % phytate hydrolysis achieved from a single dose of phytase. Phytate dose further altered the Ca and P contribution from phytase. There were also significant effects of phytate source, either from corn or soybean meal, on both SID Ca and P. Digestibilities of Ca and P in the diets were higher in diets containing 65% of its phytate from soybean phytate vs diets where phytate came solely from corn. At high inclusion of phytase these differences in digestibility between phytate sources disappeared. Calcium source had a profound effect of the interactions between calcium and phosphorus digestibilities, with bone calcium having a very small impact on phosphorus digestibility of the diet having a greater digestibility of Ca than that of the limestone tested.

Key Words: calcium

767S Predicting limestone Ca digestibility and impact on phytase efficacy: Where are we? Peter Plumstead*, *Chemuniqué, Randburg, South Africa.*

Recent research has shown that geological differences in the limestone rock, as well as differences in particle size of limestone resulted in large differences in ileal Ca digestibility in broilers in vivo. Further, as a result of interactions of Ca with phytate, the characteristics of the limestone used in feed formulation also affected ileal P digestibility and the contribution of P and Ca from exogenous phytase enzyme supplemented to feed. Our research focus has been the development of a dynamic model that could predict observed differences in Ca and P digestibility from an in vitro assessment of limestone quality, together with other influential factors. Results to-date have shown that including limestone solubility at multiple time points in a model was able to describe more variation in observed Ca and P digestibility than solubility at a single time point. Important interactions of lime-

stone characteristics with phytate level, phytate source, and added phytase are still required to be included in the final dynamic model that can be used to predict digestible Ca from limestone, as well as the expected contribution of Ca and P from exogenous phytase.

Key Words: calcium

768S Effect of age on ingredient calcium digestibility. Monika Proszkowiec-Weglarz*, *USDA-ARS, Beltsville, Maryland, United States.*

In developing a standardized methodology to determine calcium digestibility of feed ingredients for broilers, one of the questions asked was: Does broiler age impact calcium digestibility of ingredients. Standardized ileal digestibility (SID) of calcium in soybean meal, limestone, and monocalcium phosphate were determined at 11 and 25 d of age. There was an age effect ($P < 0.05$) on calcium digestibility from SBM and LM in 11 vs the 25 d old birds. Future work and predictions of digestibility of calcium from ingredients must consider this age effect.

Key Words: calcium

769S Modeling the fate of P and Ca in broilers—Prediction of digestible Ca and P requirements. Marie-Pierre Létourneau-Montminy*, *Laval University, Quebec, Quebec, Canada.*

Several phosphorus (P) response criteria can be used to assess dietary recommendation depending on the objective of production (e.g., profitability, welfare and environment) challenging the utilization of a single requirement. A robust multi-criteria model aiming to simulate actual calcium (Ca) and P retention within the different anatomical fractions and body ash of the broilers according to animal growth potential and dietary Ca and P intake has been developed. The proposed model simulating the metabolic fate of dietary P and Ca in growing broilers and the dynamics of body ash integrates the most relevant physiological processes involved in broiler P metabolism. It also simulates the impact feeding broilers according to a variety of feeding strategies and its inversion aim to estimate dietary digestible P and digestible Ca requirements over time.

Key Words: calcium

770S Calcium and P digestibility as affected by time after oviposition in laying hens. Micaela Sinclair-Black and Peter Plumstead*, *Chemuniqué, Johannesburg, Gauteng, South Africa.*

Laying hens undergo intense physiological changes throughout the day as medullary bone remodeling and eggshell formation occurs. These changes result in the hen requiring different quantities of Ca from her diet at different times in the daily bone-remodeling and egg formation process. Limestone provided in the laying hen diet contributes over 90% of this Ca requirement of the hen. Dietary Ca and P digestibility is greater 13h post oviposition during the time the egg is in the shell gland compared with 3h post oviposition when medullary bone replenishment is occurring. The change in ileal Ca and P absorption is likely modulated by shifts in parathyroid hormone (PTH) and 1,25 (OH) vitamin D₃. Higher particle size limestone can have greater benefits in increasing ionizable blood Ca during shell

formation. Based on this, the time of day, reproductive status, and limestone particle size can be influential in Ca and P digestibility.

Key Words: calcium

771S A global view of Ca absorption in laying hens: Complementarity between the transcellular and paracellular pathways. Michel Duclos*, Audrey Gloux, and Agnès Narcy, *INRA, Nouzilly, France*.

To meet the high calcium (Ca) demand for eggshell biomineralization, the absorption of Ca increases in laying hens after sexual maturity. Transcellular Ca absorption pathway and its major player CALB1 mediating a saturable transport across the epithelium of the small intestine has been extensively described. The paracellular pathway involving non-saturable Ca transport through intercellular tight junction has deserved little attention so far. The lecture will present a global view of the molecular actors involved in Ca absorption in laying hens before and after sexual maturity. It will draw the attention to a possible cooperation between transcellular and paracellular pathways of Ca absorption along the digestive tract.

Key Words: calcium

772S Development and evaluation of a dynamic model of calcium and phosphorus flows in layers. Rene Kwakkel*, *Wageningen University & Research, Wageningen, The Netherlands*.

In egg producing systems, a reduction of the P-content in the diet while maintaining production and health of the birds may reduce environmental pollution. The requirement for dietary P in the layer is

mainly due to the need to store Ca and P in the bones before egg shell formation. Therefore, a computer-based, dynamic model was developed, wherein a 100 d flow of Ca and P in a well-producing layer is being simulated on an hourly basis. Effects of management and nutritional strategies to reduce digestible P-requirements were tested and will be discussed. A second objective was to assess to which changes in input parameters the model was most sensitive.

Key Words: calcium

773S Calcium and phosphorous: More than just bones, more than just what is in the feed. Daniel Venne*, *Scott Hatchery, Scott, Quebec, Canada*.

Calcium (Ca) is a fundamental macro minerals in poultry feeding, especially during growth and egg production. Traditionally, bone requirements and egg production have been used to determine calcium and phosphorus (P) requirements. Blood Ca and P investigation showed that they can be modulated by more than just their content in the diet. Indeed, genetic selection, animal welfare, disease, phytase, vitamin D activated or not all impact the way we must look at these nutrients. Advances in portable biochemistry can help us in fine tuning the requirements for Ca and P to avoid costly deficiencies and excesses. The regulation of calcemia by ionized calcium and the effect of blood pH are critical indicators of Ca status. The P requirements may varied in relation to the use of phytase, during refeeding and fasting or when energy comes mainly from sugar or fat. Finally, the mechanisms of changing calcium phosphate from bone to calcium carbonate in the egg shell in layers will be discussed.

Key Words: calcium

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Numbers following names refer to abstract numbers. A number alone indicates an oral presentation; abstract numbers followed by P are posters and S are part of a symposium.

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