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The Effect of Heat Treatment on Microbiological Qualities of Bovine Colostrum, Passive Immune Transfer of Neonatal Calves, and Future Animal Performance

Inaugural-Dissertation

zur Erlangung der tiermedizinischen Doktorwürde

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“Erfolgreich ist, wer weiß, was er nicht kann.”

Willy Haas (1891-1973)

Für René,

der glaubte, dass ich neben dem Doktor der Chemie an meiner Seite

auch noch einen Dr. vet. med. auf dem Papier brauchen würde.

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1 ABBREVIATIONS

BVD	Bovine Viral Diarrhea
CFU	Colony Forming Units
DA	Displaced Abomasum
Ed	Edwards Agar
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Mac	MacConkey Agar
mean	Arithmetic Mean
n/no.	Number
NY	New York
p/p-value	Probability
PI	Persistent Infection
QMPS	Quality Milk Production Services
R^2	Linear Correlation Coefficient
SD	Standard Deviation
sp.	Species
spp.	Species (Plural Form)
subsp.	Subspecies
TP	Total Protein
TPC	Total Plate Count
TRT	Treatment
TSAE	Trypticase Soy Agar with Esculin
VJ	Vogel Johnson Agar

2 INTRODUCTION

At birth calves have negligible levels of immunoglobulins (ARGÜELLO et al., 2005). This is because the ruminant placenta impedes the transfer of maternal antibodies into the fetal circulation during gestation (TIZARD, 2004). Subsequently, the uptake of immunoglobulin-rich colostrum plays a fundamental role in the acquisition of passive immunity. Intestinally absorbed immunoglobulins circulating in blood protect the newborn from systemic diseases, while immunoglobulins left in the gastrointestinal tract provide local immunity (ZAREMBA and HEUWIESER, 1984). Additionally, colostrum plays an important biological role supplying the calf with nutrients and various other biofunctional constituents (NAKAMURA et al., 2003).

At the same time, pathogens such as *Mycobacterium avium* subsp. *paratuberculosis* (STREETER et al., 1995; SWEENEY, 1996), *Mycoplasma* spp. (BUTLER et al., 2000; STABEL et al., 2004), *Escherichia coli* (CLARKE et al., 1989), *Campylobacter* spp. (LOVETT et al., 1983), *Listeria monocytogenes* (JAYARAO and HENNING, 2001), and *Salmonella* spp. (STABEL et al., 2004) have been cultured from lacteal secretions. These organisms originate from either an infected mammary gland or from a post-harvest bacterial contamination of colostrum. In general, the presence of live bacteria in the digestive tract of the calf reduces the absorption of immunoglobulins by the intestinal epithelium (JAMES et al., 1981; STALEY and BUSH, 1985) and increases the risk of failure of passive transfer. Inadequate immune protection can lead to fatal diseases (TYLER et al., 1998) and substantial economic losses for the dairy farmer (JENNY et al., 1981): A recent study from the United States determined the mortality rate of preweaned dairy heifer calves to be 7.8 % (NAHMS, 2007).

Heat treatment of colostrum is a new technology and focus of current research. The aim is to eliminate pathogens from colostrum. A disadvantage of this method is the potential heat denaturation of colostral immunoglobulins and other essential constituents which could increase calf morbidity and mortality rates.

This thesis was designed to answer the question of whether the benefits of heat-treating colostrum outweigh the detriments. Due to the complexity of this matter this project was divided into three parts that were accomplished in a field study.

The objectives in the first part of this trial were to:

- Identify the most common bacteria in bovine colostrum.
- Quantify the bacterial contamination of colostrum on the study farm.
- Illustrate the effect of on-farm heat treatment of colostrum on colostral bacterial counts.

To this end, the total plate counts of paired pre- and post-heat-treated colostrum samples were evaluated, and the detected bacteria classified by a microbiological laboratory.

In the second part serum total protein levels of calves that had been fed heat-treated versus raw colostrum at first feeding were determined and compared.

In the third part health data of animals fed raw versus heat-treated colostrum were analyzed to evaluate possible associations of colostrum processing, serum total protein levels, and animal performance, respectively.

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3 MICROBIOLOGICAL CHARACTERISTICS OF BOVINE COLOSTRUM AND THE EFFECT OF COLOSTRAL HEAT TREATMENT

3.1 Current Recommendations on Colostral Heat Treatment

Even though sanitary colostrum harvesting and feeding methods and proper colostrum storage can minimize bacterial contamination (STEWART et al., 2005), it was not until the introduction of pasteurization systems for the purpose of heat-treating colostrum and waste milk that the potential for a major improvement of calf health and significant economic returns for animal producers was created (JAMALUDDIN et al., 1996). While early attempts to heat-treat colostrum using conventional pasteurization methods and temperatures led to an increase in viscosity and immunoglobulin denaturation (MEYLAN et al., 1996; GODDEN et al., 2003; STABEL et al., 2004), the usage of a lower-temperature, longer-time approach to heat treatment solved these problems (JOHNSON et al., 2007). Now, suggestions are made to heat colostrum at 60 °C for 60 minutes to sustain immunoglobulin concentrations, immunoglobulin functions, and colostral fluid characteristics (GODDEN et al., 2006; MCMARTIN et al., 2006), while eradicating (or at least considerably reducing the numbers of) infectious agents.

3.2 MATERIALS AND METHODS

3.2.1 Commercial Dairy Site and Routine Cow Management

This field study was conducted between January 25, 2007, and June 8, 2007 at Sunnyside, a large commercial dairy in Upstate New York, where 2,600 Holstein cows were milked three times a day with a herd average of 11,340 kg of milk. The cows were fed a total mixed ration consisting of 55 % forage (corn silage, haylage, alfalfa hay, and wheat straw) and 45 % concentrates (cornmeal, soybean meal, canola, cottonseed, citrus pulp, and brewer's grain). When parturition was imminent, cows were moved from free stalls into shared maternity pens, which were bedded with shredded waste paper and supervised 24 hours per day.

3.2.2 Colostrum Management, Sample Collection, and Records

First milking colostrum was assembled from fresh cows on a daily basis. Milkers wore rubber gloves during milking. Milking hygiene further included predipping with a 1 % iodine solution and whipping off the teats with a moist clean towel after a contact time of 20 seconds with the predipping solution. The individual milkings were milked into a bucket milker and pooled after collection to create a batch of fresh colostrum. The number of contributing cows, their individual colostral volumes (in l), and the collection date were recorded. Then, the colostrum batch was thoroughly mixed before a 20 ml sample was collected into a sterile vial, labeled, and dated. Afterwards the entire batch was randomly allocated to be either fed as heat-treated or as raw in the course of this study (see 4.2.1).

Colostrum that had been designated to be used without further processing was stored in equal portions of 3.8 l in clean, covered containers. Batches that had been assigned to be heat-treated underwent heating to 60 °C for 60 minutes in a commercial on-farm batch pasteurization system (DT Platinum, Dairy Tech, Inc., Windsor, Colorado) immediately after collection. The colostrum was constantly agitated throughout the entire process and finally automatically cooled to feeding temperature. A second (post-heat-treated) 20 ml sample was collected from all heat-treated colostrum batches before it was stored analogously in portions of 3.8 l.

All colostrum containers were labeled with the original batch number, the date of preparation, and the method of treatment (heat-treated or unprocessed) before they were refrigerated for feeding within the next 24 hours in a commercially available fridge. The colostrum batch samples were labeled in the same manner and transported refrigerated to the Ambulatory and Production Medicine Clinic (Cornell University, Ithaca, NY), where they were frozen until further analysis.

3.2.3 Laboratory Analyses on Colostrum Batch Samples

After completion of the sampling process, all frozen colostrum batch samples were submitted to the Quality Milk Production Services (QMPS) Laboratory (Ithaca, NY), where they underwent microbiological procedures to culture and quantify bacterial populations. Edwards agar (Ed), MacConkey agar (Mac), Vogel Johnson agar (VJ), and trypticase soy agar with 5 % sheep blood and 0.1 % esculin (TSAE) were used due to their particular selective features: Ed medium selects for streptococci, Mac agar enhances the growth of Gram-negative lactose fermenting *Enterobacteriaceae*, VJ plates select for *Staphylococcus aureus* and coagulase-negative staphylococci, and TSAE plates support the growth of Gram-positive bacilli.

In the laboratory the colostrum samples were allowed to thaw at room temperature. After they had been thoroughly mixed, a micropipette was used to aseptically dispense 100 µl of each colostrum batch onto Ed, Mac, VJ, and TSAE agar plates. Sterile disposable loops were used to spread these aliquots evenly across the entire plate surfaces. Additionally, a 10 µl aliquot of each colostrum batch sample was pipetted onto a second TSAE plate, and streaked out using a sterile disposable loop. If a colostrum batch had been heat-treated during this study, pre- and post-heat-treated samples were plated in this manner. After all samples had been streaked, the agar plates were allowed to set (non-inverted) at room temperature. When the individual aliquots had fully dried onto the media surfaces, the plates were inverted and put into an aerobic incubator at 37 ± 1 °C.

The visible colonies could be identified 48 hours later due to the selectivity of the agar plates. Based on the previously plated volume, the overall number of bacteria was calculated for each sample and expressed in a total plate count as colony forming units per milliliter (CFU/ml). The results were recorded on the laboratory's 'Quality Milk Production Services Modified Bacteria Count' sheet that had pathogens pre-listed as follows: *Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Streptococcus canis,* other environmental streptococci, *Staphylococcus aureus, Staphylococcus* spp., *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Pseudomonas, Serratia, Proteus,* Gram-negative bacillus, Gram-positive bacillus, *Corynebacterium bovis, Corynebacterium* spp., *Arcanobacterium pyogenes, Prototheca*, yeast, and molds. Bacteria identification and quantification were conducted according to the routine procedures at the QMPS laboratory.

3.3 RESULTS

A total of 51 colostrum batch samples (pooled from 109 individual milkings) was collected over the entire study period. As opposed to conventional methods, sample size had not been previously determined, but depended on the number of opportunities that opened up to the researcher to visit the study farm.

Therefore, the samples consisted of 19 colostrum batches (first milkings from 39 individual cows) that were used without further processing, and 32 colostrum batches (first milkings from 70 individual cows) that were heat-treated. Final statistics, however, were only completed for 29 of these 32 heat-treated colostrum batches. Three batches (nos. 12, 15, and 24) had to be omitted from the study after colonies of coagulase-negative staphylococci (in batch no. 12) and Gram-positive bacilli (in batches no. 15 and no. 24) appeared in the heat-treated samples that had not been present in their raw equivalents which led to concerns about cross contamination.

Streptococcus agalactiae or *Staphylococcus aureus* bacteria were not found in any of the examined colostrum batch samples. Normal inhabitants of bovine skin and mucosa, namely environmental streptococci, coagulase-negative staphylococci, and *Corynebacterium* spp. predominated. Gram-positive and Gram-negative bacilli were also frequently present in this study's colostrum batch samples. Representatives of the family *Enterobacteriaceae*, which included *Escherichia coli*, *Enterobacter* spp., and *Serratia* spp., rarely appeared. To facilitate figures and statistics, the latter three genera were summarized and listed under the family of *Enterobacteriaceae*.

3.3.1 Colostrum Batches Fed Unprocessed: Total Plate Counts and Detailed Microbiological Classification of Bacterial Contents

Figures and tables were created for the 19 pooled colostrum batch samples that were used without previous heat treatment in the course of this study.

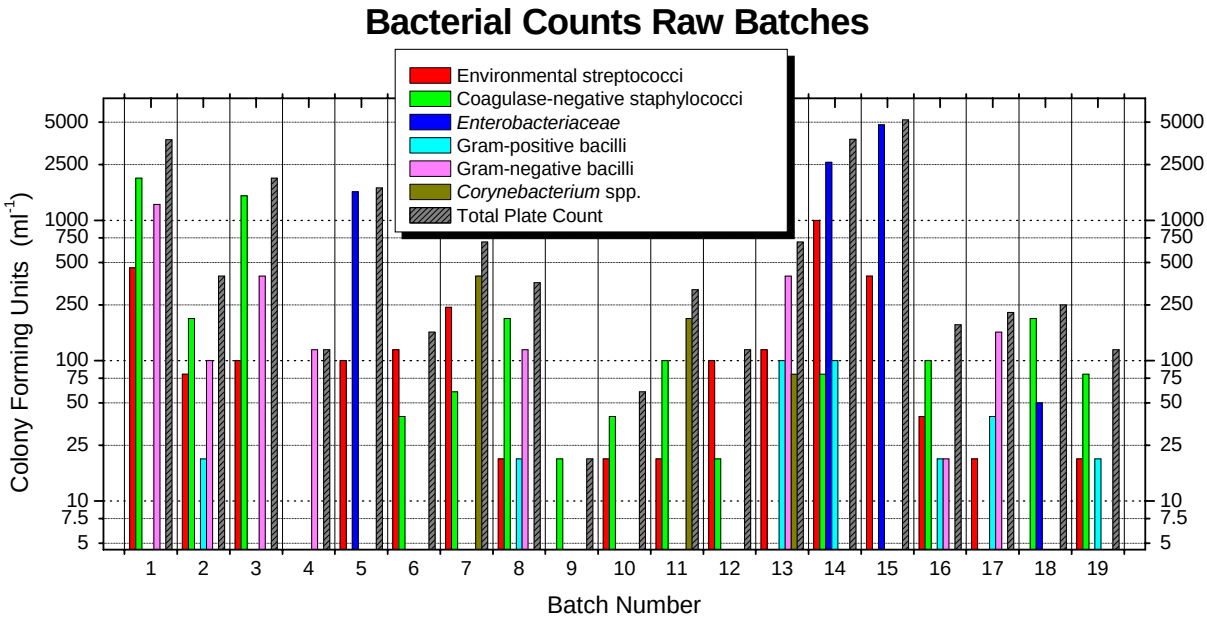

Figure 1: Microbiological characteristics per batch sample for the 19 colostrum batches fed as raw to the study calves (see 4.2.1). The shares of individual pathogens, as identified through selective media, are illustrated by differently colored columns. Note the logarithmic ordinates.

Figure 1 illustrates that the total plate counts of these colostrum batches varied from 20 to 5200 CFU/ml. The mean total plate count amongst the raw colostrum batches was 1062 CFU/ml with a standard deviation of 1537 CFU/ml (see Figure 2 on page 12).

As displayed in Table 1 below, environmental streptococci and coagulase-negative staphylococci were the most common contaminants in these 19 colostrum batches. Gram-negative and Gram-positive bacilli followed quantitatively. Only a small proportion of samples contained *Corynebacterium* spp. or representatives of the family *Enterobacteriaceae*.

Table 1: Proportions and percentages of microbiologically contaminated batches amongst the 19 colostrum batch samples used as raw in this study.

	Raw Samples
Pathogen Classification	**Proportions (Percentages)**
Environmental streptococci	16/19 (84.2)
Coagulase-negative staphylococci	14/19 (73.7)
Enterobacteriaceae	4/19 (21.1)
Gram-positive bacilli	7/19 (36.8)
Gram-negative bacilli	8/19 (42.1)
Corynebacterium spp.	3/19 (15.8)
Total	**19/19 (100.0)**

All 19 colostral samples contained at least one of the bacterial genera listed under 'Pathogen Classification' in Table 1. Four out of the five genera were present in a single colostrum batch sample. Nine colostrum batches contained bacteria of three different genera. Pathogens of two genera could be cultured from seven colostrum samples. Finally, a single bacterial genus was found in two colostrum batches.

3.3.2 Colostrum Batches Fed Unprocessed: Average Bacterial Contents

Figure 2 shows the average bacterial composition of the 19 colostrum batch samples that were fed unprocessed in the course of this study (see 4.2.1).

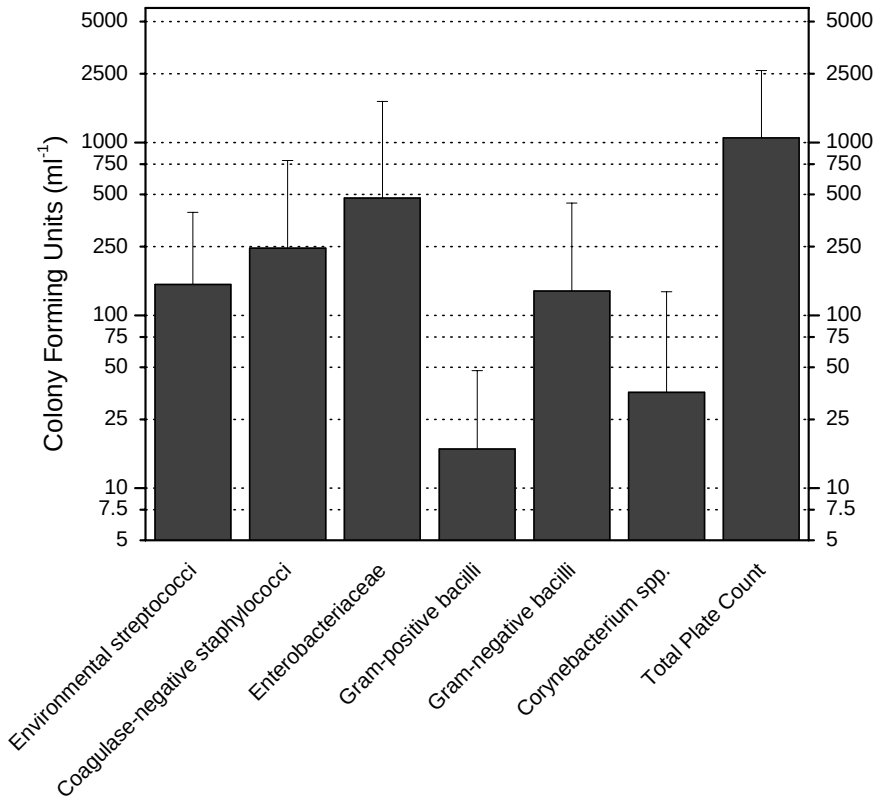

Figure 2: Average bacterial contamination (CFU/ml) of colostrum batches fed without previous heat treatment. Note the logarithmic ordinates.

Enterobacteriaceae predominated with 476 CFU/ml, followed by coagulase-negative staphylococci with 244 CFU/ml, environmental streptococci with 151 CFU/ml, and Gram-negative bacilli with 138 CFU/ml. *Corynebacterium* spp. and Gram-positive bacilli were the least numerous with 36 CFU/ml and 17 CFU/ml, respectively.

3.3.3 Colostrum Batches Fed Heat-Treated: A Comparison of Total Plate Counts and Detailed Bacterial Contents Pre versus Post Heat Treatment

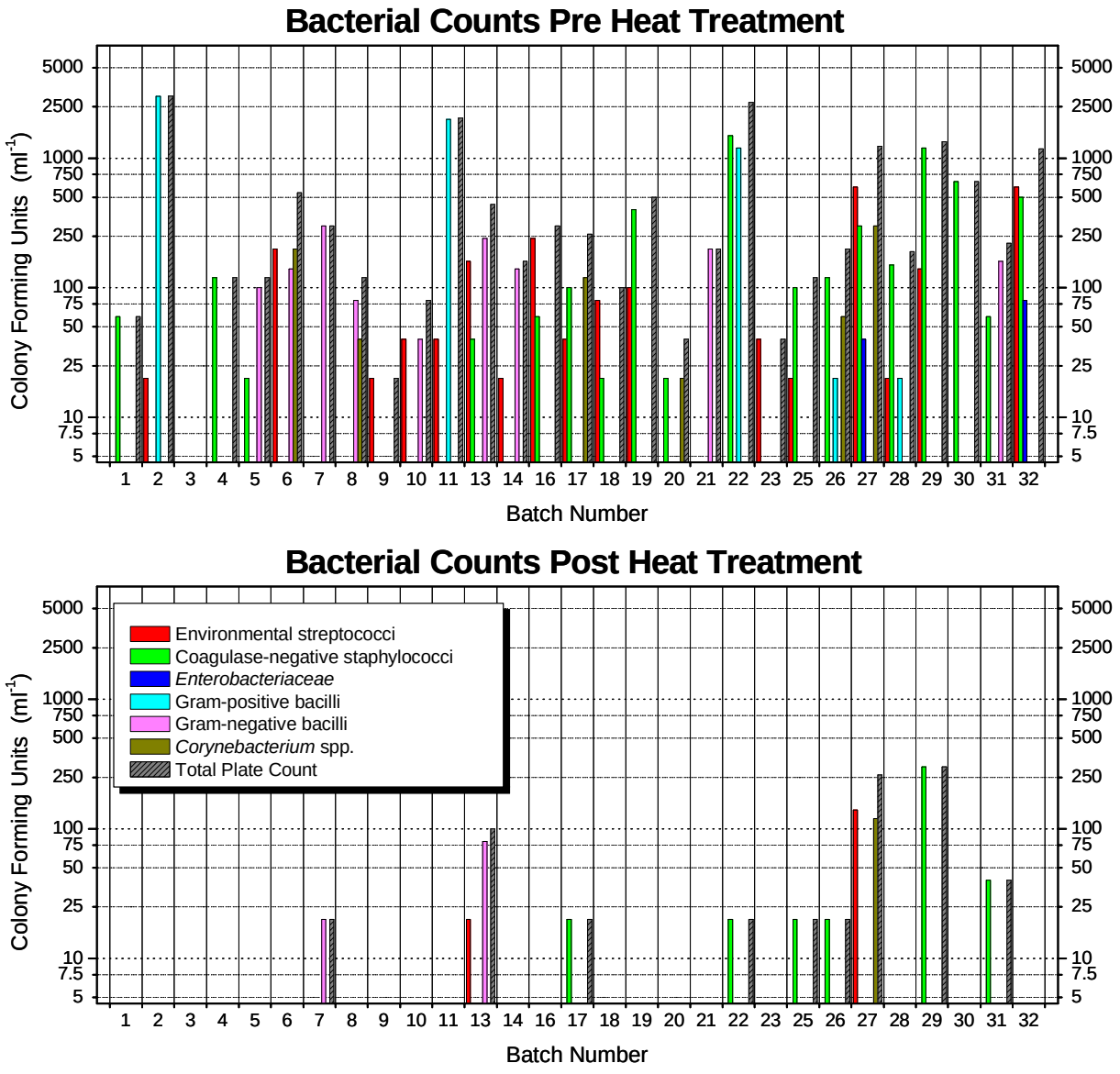

Figure 3: Microbiological characteristics per batch for the 29 utilized colostrum batch samples prior to and after their heat treatment. The contribution of individual pathogens, as identified through selective media, is illustrated by differently colored columns. Note the logarithmic ordinates.

Figure 3 compares the bacterial contents of the 29 colostrum batches that were fed heat-treated to the study calves (see 4.2.1). The total bacterial counts before heat treatment varied from 0 to 3020 CFU/ml, with a mean average of 562 CFU/ml and a standard deviation of 798 CFU/ml. Heat processing led to total plate counts ranging from 0 to 300 CFU/ml, a reduced mean average of 28 CFU/ml, and a standard deviation of 73 CFU/ml (see Figure 4 on page 16).

As shown in Table 2, coagulase-negative staphylococci and environmental streptococci were the most common contaminants in these 29 colostrum batch samples, quantitatively followed by Gram-negative bacilli, *Corynebacterium* spp., and Gram-positive bacilli. Only a small proportion of samples contained representatives of the family *Enterobacteriaceae.*

Table 2: Proportions and percentages of microbiologically contaminated batch samples prior to and after their heat treatment amongst the 29 pooled colostrum batches fed heat-treated. The significance of each bacterial reduction is listed in the last column.

	Pre Heat Treatment	**Post Heat Treatment**	**Statistical Significance**
Pathogen Classification	**Proportions (Percentages)**		**One-sided P-Values**
Environmental streptococci	17/29 (58.6)	2/29 (6.9)	< 0.0001
Coagulase-negative staphylococci	18/29 (62.1)	6/29 (20.7)	0.0009
Enterobacteriaceae	2/29 (6.9)	0/29 (0)	0.2
Gram-positive bacilli	5/29 (17.2)	0/29 (0)	0.03
Gram-negative bacilli	9/29 (31.0)	2/29 (6.9)	0.008
Corynebacterium spp.	6/29 (20.7)	1/29 (3.4)	0.03
Total	**28/29 (96.6)**	**9/29 (31.0)**	**0.0002**

Prior to heat treatment a single batch sample showed no bacterial growth, while at least one genus of pathogens could be cultured from the remaining 28 colostrum batches. Seven batches contained one genus of bacteria, 14 colostrum batches contained bacteria of two different genera, six colostrum samples contained bacteria of three genera, and a total of four bacterial genera was present in one colostrum batch.

After heat processing, 20 of the 29 colostrum batch samples were sterile. The 9 samples that still showed bacterial growth mainly contained coagulase-negative staphylococci, while few contained Gram-negative bacilli, environmental streptococci, and *Corynebacterium* spp.. Two of these bacterial genera were present in 2 of the contaminated batches, whereas the remaining 7 colostrum samples contained pathogens of one genus only.

Statistical Significance of the Results:

The McNemar's test for two related proportions was conducted using SAS (Statistical Analysis System, Version 9.2, SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513). The results are presented in Table 2 (above). A p-value cut-off of 0.05 was used to establish significance. While the one-sided p-value shows that the reduction of *Enterobacteriaceae* was not significant, heat treatment significantly reduced environmental streptococci, coagulase-negative staphylococci, Gram-negative bacilli, Gram-positive bacilli, and *Corynebacterium* spp.. Overall, heat processing significantly eliminated bacteria from the colostrum samples ($p = 0.0002$).

3.3.4 Colostrum Batch Samples Fed Heat-Treated: Average Bacterial Contents Pre versus Post Heat Treatment

Figure 4 (below) depicts the average bacterial composition of the 29 colostrum batches that were fed to calves as heat-treated in the course of this study (see 4.2.1). Initially, Gram-positive bacilli dominated with 215 CFU/ml, followed by coagulase-negative staphylococci with 187 CFU/ml, environmental streptococci with 82 CFU/ml, Gram-negative bacilli with 48 CFU/ml, *Corynebacterium* spp. with 26 CFU/ml, and *Enterobacteriaceae* with 4 CFU/ml. Gram-positive bacilli were most powerfully diminished and were undetectable after heat processing. *Enterobacteriaceae* were also fully eliminated through the impact of heat. Environmental streptococci and Gram-negative bacilli were reduced by 93 % each, coagulase-negative staphylococci by 92 %. The effectiveness of the heat-treating device was lowest for *Corynebacterium* spp. with a reduction of 84 %. Overall, the total bacterial count of these 29 batches was decreased by 95 %.

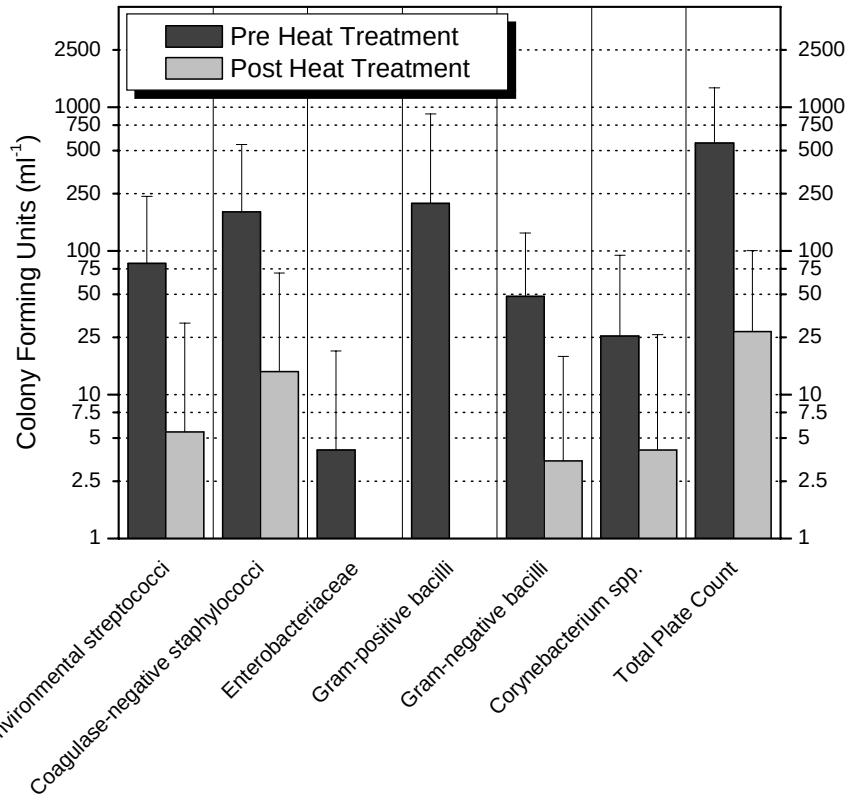

Figure 4: Average bacterial contamination of colostrum batches before and after heat treatment. Note the logarithmic ordinates.

The mean total plate counts (in Figure 2 and Figure 4) were added to the results to show that the colostrum batches fed as raw in this study initially contained higher bacterial counts than the raw batches that were to be fed heat-treated. While the average total plate count was 1062 CFU/ml for the 19 colostrum batches fed as raw, it was 562 CFU/ml previous to heat treatment for the other 29 utilized batches.

3.4 DISCUSSION

3.4.1 Total Bacterial Counts in Raw Lacteal Secretions – Legal Regulations, Suggested Limits, and the Prevailing Situation in New York State and on the Study Farm

Bacteria in raw milk may endanger human health. Consequently, raw milk destined for pasteurization is subject to numerous quality regulations including limits on the tolerable number of total bacteria. To encourage the production of low bacteria count milk, dairymen are rewarded with bonuses for maintaining total bacteria counts below certain levels. The legal maximum for raw milk in the United States, based on the Grade A Pasteurized Milk Ordinance, is 100,000 CFU/ml, while the suggested standard is a total plate count of less than 10,000 CFU/ml (PMO, 2007). Many producers, however, constantly attain numbers below 5,000 CFU/ml (MILKPRODUCTION, 2002). A study conducted in 1998 on New York State bulk tank samples showed that out of the 854 analyzed milk samples 26.8 % reached total bacterial counts below 5,000 CFU/ml, 49.8 % contained less than 10,000 CFU/ml, and only 5.4 % of all samples exceeded the limit of 100,000 CFU/ml (BOOR et al., 1998).

In contrast to this, 82 % of all colostrum samples on U.S. dairies exceed the industry goal of a total plate count of 100,000 CFU/mL (POULSEN, 2002, cited by STEWART et al., 2005). The average raw colostrum on the study farm had extremely low bacterial counts during this trial with 100 % of all analyzed samples meeting the goal for the maximum total plate count to be fed to calves at < 100,000 CFU/ml (STEWART et al., 2005). A single raw colostrum batch sample had a bacterial count of 5200 CFU/ml, while all other raw samples in this study ranged between 0 and 3780 CFU/ml.

3.4.2 Classification of Colostral Contaminants and its Informative Value

The subsequent determination of the actual genera or families of bacteria present in the colostrum batch samples was an important step towards the correction of the prevailing minor quality problems on the study farm.

Streptococcus agalactiae and *Staphylocccus aureus* could not be detected in any of the analyzed colostrum samples. Apparently, these major causative organisms of contagious mastitis had already been successfully excluded from the herd.

The actual types of pathogenic organisms found indicated that mastitis was most likely not the reason for colostral contamination. In fact, microbiological results implied that for troubleshooting of high bacterial counts the farm's colostrum harvesting and processing practices in regard to udder and equipment hygiene needed to be scrutinized.

3.4.2.1 Detection of Environmental Streptococci in Colostrum

In contrast to mastitis caused by contagious pathogens, mastitis of environmental genesis cannot be eradicated from a herd and generally is the major mastitis problem on modern, well-managed farms (SMITH and HOGAN, 1993). In this study, environmental streptococci were the most frequently isolated bacteria with 33 out of the 48 (68.8 %) analyzed raw colostrum batch samples positive for these organisms. The term 'environmental streptococci' summarizes streptococci other than *Streptococcus agalactiae*. This group includes well known pathogens like *S. dysgalactiae*, *S. uberis* (the most commonly identified streptococcal species (ZADOKS et al., 2004)), *S. canis*, as well as all *Enterococcus* species. These pathogens prosper in the environment of cows appearing in organic bedding materials, manure, soil, and on various body sites on the animal (JONES and SWISHER, 1998). Buildups of these bacteria can be found in teats and on teat ends, if cows lie in wet, contaminated areas. Additionally, environmental streptococci grow on milk filters and survive in milk films on hard-to-clean areas of milking machines and feeding equipment. Elevations of bacterial counts are caused by either infected or wet and dirty cows at the time of milking, by contaminated milking equipment or by improperly working cooling devices for milk or colostrum (MILKPRODUCTION, 2002). Generally, counts of 750 CFU/ml should not be exceeded in bulk tank milk (MILKPRODUCTION, 2002). A single raw colostrum batch in this study topped this goal reaching a count of 1000 CFU/ml, while all other positive batches had counts between 20 and 600 CFU/ml.

3.4.2.2 Coagulase-negative Staphylococci as Colostral Contaminants

In this trial, coagulase-negative staphylococci were detected in 32 out of the 48 (66.7 %) analyzed raw colostrum batch samples. It is common in well-managed herds to find 10 to 20 % of quarters infected with coagulase-negative staphylococci (NMC, Staphylococcus). Compared to *Staphylococcus aureus,* which is coagulase-positive, these staphylococci are only mildly pathogenic. They include a variety of species like *S. chromogenes, S. hyicus, S. simulans, S. epidermidis, S. hominis, S. xylosus,* and *S. sciuri*. Coagulase-negative staphylococci are either found free-living in the environment or represent normal resident skin flora and can cause mastitis when they enter the mammary gland from the teat skin. An achievable goal for coagulase-negative staphylococci in bulk tank milk is < 1,000 CFU/ml (NMC, Bulk tank) - higher counts usually indicate poor udder preparation and teat sanitation rather than mastitis. Four colostrum batches in this study exceeded this limit, all other positive samples ranged between 20 and 660 CFU/ml further suggesting udder preparation before colostrum harvest as an area of concern.

3.4.2.3 Presence of Gram-positive and Gram-negative Bacilli in Colostrum

Germs classified as 'Gram-negative bacilli' were isolated from 17 of the 48 (35.4 %) raw colostrum batch samples. The bacterial counts in these positive samples ranged from 20 to 1300 CFU/ml. This group includes a variety of aerobic Gram-negative rods (other than *Enterobacteriaceae*) like the family of *Pasteurellaceae* (including the genera *Actinobacillus*, *Haemophilus (Histophilus)*, *Mannheimia,* and *Pasteurella*) or the genera of *Acinetobacter*, *Burkholderia*, and *Pseudomonas*. Sporadic mastitis cases in ruminants caused by species of these genera have been reported (BARNUM, 1954; OSBORNE et al., 1981; ALSENOSY and DENNIS, 1985; RAHMAN and BAXI, 1985; KIPER and PAULSEN, 1988; WATKINS et al., 1992; GRINBERG et al., 1993; BERRIATUA et al., 2001). Bacteria belonging to the family of *Pasteurellaceae* live on mucosal surfaces especially in the upper respiratory tract (CHRISTENSEN and BISGAARD, 2008). *Acinetobacter* spp. are widely distributed in nature (BERGOGNE-BEREZIN et al., 1996), *Burkholderia* spp. (VANDAMME et al., 2007) and *Pseudomonas* spp. (BROOKS et al., 2007) can commonly be found in soil and water.

The laboratory had used the term 'Gram-positive bacilli' to categorize a number of aerobic Gram-positive spore-forming bacilli and aerobic Gram-positive rods that were found in 12 out of the 48 (25 %) analyzed raw colostrum batch samples. Aside from *Bacillus anthracis*, aerobic Gram-positive spore-forming members of the *Bacillus* species are considered to be non-pathogenic under most circumstances. They are commonly found in nature, existing in soil, water, dust, air, feces, and on vegetation (NMC, Bacillus). *B. subtilis* has seldom been associated with pathological conditions (FOSSUM et al., 1986) as opposed to *B. cereus*, which can cause severe mastitis, but usually does not represent a natural pathogen for the bovine udder (SCHIEFER et al., 1976). In fact, strains of commensal *Bacillus* spp. isolated from the mammary glands of healthy cows have displayed strong in vitro activity against Gram-positive mastitis pathogens through the production of broadly active inhibitors (AL-QUMBER and TAGG, 2006). Aerobic Gram-positive rods include, for example, *Lactobacillus* spp. and *Listeria monocytogenes*. *Lactobacillus* spp. are a major part of the lactic acid bacteria group and contribute to the healthy microflora of mucosal surfaces. They have shown to be able to repress the growth of *Staphylococcus aureus* and *Escherichia coli* on agar medium and may play a role in mastitis control through their antagonistic activity on these pathogenic bacteria (FANG et al., 1996). *Listeria monocytogenes* was isolated from 12.6 % of the milk filters of New York State dairy herds enrolled in a 1-year-long study of Cornell's Quality Milk Production Services laboratory (HASSAN et al., 2000). As a saprophytic bacterium, the source of this organism could be environmental, or it could be shed by infected animals in their feces or milk. The numbers of Gram-positive bacilli in this study's positive colostrum batches varied from 20 to 3000 CFU/ml.

3.4.2.4 Discovery of Corynebacterial Species in Colostrum

The genera *Corynebacterium* and *Arcanobacterium* comprise Gram-positive, aerobic or facultatively anaerobic, non-motile, non-sporulated, rod-shaped bacteria. They can be found on the skin, on mucous membranes, and in many other environments, including soil and trees. *Arcanobacterium pyogenes*, the causative organism of summer mastitis, was not found in any of this study's colostrum samples. *Corynebacterium* spp. were present in 9 out of the 48 (18.8 %) analyzed raw colostrum batch samples with counts ranging from 20 to 400 CFU/ml. In sheep, *C. mastitidis* (FERNANDEZ-GARAYZABAL et al., 1997) and *C. camporealensis* (FERNANDEZ-GARAYZABAL et al., 1998) have been described as causative organisms of mastitis. A

subspecies of *C. diphtheriae* has been isolated from ulcerated teats and from milk of cows with mastitis (CORBOZ et al., 1996). *C. bovis*, which is very common in milk samples and generally considered to be a harmless commensal (BROOKS and BARNUM., 1984), can - under certain conditions - be a highly contagious pathogen (PANKEY et al., 1985). Its primary reservoir is the udder of infected cows, where it is found in teat canals and cisterns of the mammary gland. The transmission from cow to cow is accomplished at milking via milking machines when insufficient milking hygiene is utilized. Infection rates can be lowered tremendously when teat disinfection and dry cow therapy are used (BROOKS et al., 1983). Moreover, it has been postulated that an infection with *C. bovis* protects the mammary gland against infections with *Staphylococcus aureus* (PANKEY et al., 1985).

3.4.2.5 Enterobacteriaceae as Colostral Contaminants

The family of *Enterobacteriaceae* represents primary environmental pathogens. In this study, the group comprised *Escherichia coli*, *Serratia* spp., as well as *Enterobacter* spp.. With 6 out of the 48 (12.5 %) analyzed raw colostrum batch samples positive for *Enterobacteriaceae,* they formed the smallest group of bacteria detected. Nevertheless, 30 to 40 % of all clinical mastitis cases are caused by coliform bacteria (NMC, Environmental). Sources of these environmental pathogens include manure, bedding, mud, dirt, and water. Counts of less than 100 CFU/ml in bulk tank milk are considered acceptable, but counts of 10 CFU/ml or less would be desirable (JONES and SUMNER, 1999). In a 1998 study on New York State bulk tank samples (BOOR et al., 1998) 23.3 % of the 855 analyzed samples contained less than 10 CFU/ml, and 76.5 % were below 100 CFU/ml. Three of the 6 positive colostrum samples in this trial had counts of 40, 50, and 80 CFU/ml, while the other 3 exceeded the target values with significantly higher counts of 1600, 2600, and 4800 CFU/ml, respectively. These elevations of coliform counts can originate from mastitis, but in general indicate an insufficiency of milking hygiene.

3.4.3 Possible Ways of Colostral Contamination and Prevention Methods

Minimizing bacteria counts in colostrum requires the knowledge of conditions and practices that cause these elevations of counts. Therefore, it is important to identify proactive procedures that avoid the occurrence of these problems. In this particular case, special training on

premilking udder sanitation, milking and colostrum harvesting, on proper usage and cleaning of the heat-treating equipment, as well as on adequate colostrum storage would be included. The degree of colostral contamination is known to vary due to personnel changes or the available time to perform the tasks. It has been shown, for example, that microbiological qualities of colostrum are worse in warm months (FECTEAU et al., 2002). This might be either due to faster bacterial growth or due to an increase in seasonal workload that leaves less time for calf care and equipment cleaning. Since non-hygienic working methods and a deficiency of knowledge of approaches to improve microbiological colostrum qualities greatly endanger the success of a colostrum feeding program, it is important to mind the information given in the following sections.

3.4.3.1 Influence of the Housing System

The exposure to environmental pathogens can easily be minimized by keeping housing and calving environments clean, dry, and cool (SMITH and HOGAN, 1993). Wet and soiled bedding should be removed on a daily basis (JONES and SWISHER, 1998). The use of tie stalls is especially problematic since it raises the prevalence of infections with environmental pathogens (BARTLETT et al., 1992) due to the animal's inability to avoid contact with dirty bedding areas. The incidence of clinical mastitis can be lowered, and the chances of colostral contamination decreased by an augmentation of cow cleanliness and by the clipping of udders (JARRETT, 1988).

3.4.3.2 Colostrum Harvest as a Critical Control Point in Quality Protection

Good udder preparation takes center stage to reduce the contamination risk at the time of colostrum harvest. Milkers should wear rubber gloves during milking and should forestrip each quarter, thereby checking for abnormal milk (JONES and SWISHER, 1998). Teats should be wiped with a damp cloth or predipped, and finally dried off with a disposable paper towel (GALTON et al., 1984). Water hose wash of the whole udder leads to an elevation of total plate counts (BARTLETT et al., 1992) due to dripping water and is not advisable. It has been shown that the prevalence of environmental streptococci can be reduced through the removal of teat end soil at the time of teat preparation (MILKPRODUCTION, 2002). Premilking teat care that

includes predipping is controversially discussed (PANKEY and DRECHSLER, 1993; RUEGG and DOHOO, 1997) meaning that individual conclusions must be drawn by each dairy farmer on whether the investment in predipping products and the extra amount of time spent in the milking parlor outweigh the benefits. Predipping does reduce the microbiological population of major mastitis pathogens (PANKEY et al., 1987; OLIVER et al., 1993) on the teat end and thereby minimizes the probability of mastitis (PANKEY, 1989). However, it did not prove to be more effective against Gram-negative bacteria, *Corynebacterium bovis*, and coagulase-negative staphylococci (OLIVER et al., 1993) than postdipping alone. Teats should not be touched after they have been sanitized and dried (JONES and SWISHER, 1998). During the act of milking, fall-offs and liner slips of the milking machine must be avoided (JONES and SWISHER, 1998) to prevent the transfer of bacteria into the milking system. Dirty teat cups and pipelines also introduce bacteria into the system, while milk films on the gaskets and milkstone buildups in milk liners allow them to multiply. Milking equipment malfunctions, which lead to incomplete milkouts and elongated herd milking times, are known to increase the incidence of coliform infections (BARTLETT et al., 1992). The milking system should be regularly cleaned, maintained, and evaluated (JONES and SWISHER, 1998) to minimize these risks. The routine use of a germicidal postmilking teat dip has been shown to prevent spread of coagulase-negative staphylococci (JONES and SUMNER, 1999), but has only shown limited effects on the incidence of mastitis infections caused by environmental streptococci and coliform exposures (JONES and SWISHER, 1998).

3.4.3.3 Risks during Colostrum Treatment and Storage

Proper handling of freshly collected colostrum, like transportation in sanitized, clean, and covered containers from the milking parlor to the heat-treating device, is essential to prevent bacterial pollution after colostrum harvest. After heat processing, adequate storage in plastic containers (MANOHAR et al., 1997) at recommended temperatures, and later, appropriate feeding techniques (STEWART et al., 2005) help to preserve good microbiological colostrum characteristics. Finally, thorough cleaning and disinfection of the pasteurizer and other equipment after its usage must not be neglected.

3.4.3.4 Additional Methods to Lower the Prevalence of Mastitis Causing Agents

New mastitis infections can be reduced through maximizing the immune resistance of cows by feeding balanced diets with adequate levels of vitamin E, selenium (SMITH and HOGAN, 1993), vitamin A and beta-carotene, as well as by supplementation with balanced dietary copper and zinc (JONES and SWISHER, 1998). Arranging an optimal, stress-free environment should decrease the number of teat-end injuries to the absolute minimum (SMITH and HOGAN, 1993) and prevent the invasion of mastitis causing agents. The transmission of such organisms can also be lowered through fly control and the elimination of fly breeding sites (JONES and SWISHER, 1998). Vaccines against environmental mastitis pathogens are being developed, but, through the number and heterogeneity of causative agents, this seems to be particularly problematic (YANCEY, 1999). One commercially available vaccine, however, which includes bacterins of rough mutants of *Escherichia coli* strain J 5, seems to be highly effective (YANCEY, 1999) in reducing the incidence and severity of clinical coliform mastitis. The general use of dry cow treatment is able to cure up to 100 % of quarters infected with coagulase-negative staphylococci and coliform bacteria, and up to 94 % of all intramammary infections caused by environmental streptococci (OWENS et al., 1994). Reinfections, however, can occur during the dry period (NMC, Staphylococcus) since these organisms are normal inhabitants of the teat skin and ubiquitously present.

3.4.4 Total Bacterial Counts in Heat-Treated Milk – Legal Regulations, Suggested Limits, and the Prevailing Situation in New York State and on the Study Farm

While the Pasteurized Milk Ordinance of the U.S. Food and Drug Administration grants pasteurized milk a bacterial limit of 20,000 CFU/ml (PMO, 2007), others desire laboratory-pasteurized counts with less than 300 CFU/ml (JONES and SUMNER, 1999). In the New York State bulk tank trial (BOOR et al., 1998) with 853 examined samples 65.7 % showed laboratory-pasteurized counts below 250 CFU/ml, 43.7 % of them were even below 100 CFU/ml. The heat-treating device used in this trial managed to achieve levels between 0 and 300 CFU/ml which is absolutely acceptable in an on-farm environment.

3.4.4.1 Possible Explanations for the Presence of Viable Bacteria After Heat Processing

Previous studies have shown that lower-temperature, longer-time approach pasteurizers are efficient in eliminating various kinds of pathogens (GODDEN et al., 2006). While 60 °C for 60 minutes were needed to reduce *Mycobacterium avium* subsp. *paratuberculosis* to levels below the detection limit, 30 minutes were sufficient to kill *Mycoplasma bovis*, *Listeria monocytogenes,* and *Salmonella enteritidis*. Fifteen minutes at 60° C were long enough to eradicate *Escherichia coli*.

The comparison of bacterial counts pre and post heat treatment (see Figure 3) raises the question why some batches (especially nos. 27 or 29) in this study did not experience a greater bacterial reduction through the influence of heat.

One possible explanation for this phenomenon could be that the remaining bacteria belonged to a thermal resistant species, thus withstanding heat processing. Thermoresistance has been described as a characteristic of certain representatives of the genera *Streptococcus*, *Enterococcus*, *Bacillus*, and *Clostridium* (MÜLLER and WEBER, 1996). However, among the bacteria that survived heat treatment at 60 °C for 60 minutes in this study were, according to Figure 4, mainly coagulase-negative staphylococci, some environmental streptococci, some *Corynebacterium* spp., and a few Gram-negative bacilli. Interestingly, none of the spore-forming Gram-positive bacilli, which are known to be heat-stable, could be detected after heat processing.

Another explanation for viable bacteria in processed colostrum samples could be an inadequate pasteurizer function due to excessive bacterial counts in the raw product. The complete elimination of large amounts of bacteria in batches no. 2 or no. 11 through heat treatment, however, proves that the heat-treating device can successfully reduce high initial bacterial counts.

Besides the possibility of a post-harvest contamination, the most likely explanation for this peculiarity could be an inconsistent performance of the pasteurizer. It is known that peak operating temperature and the logarithmic reduction of the microbial load are significantly correlated (CAPEL et al., 2006). The pasteurizer might, due to logistical or technical problems, not heat its contents up to the correct target temperature.

Another problem that may occur with an on-farm heat-treating device is that colostrum is not maintained long enough at the target temperature either unintentionally through operator errors or on purpose due to time pressure. Therefore, it is necessary to monitor pasteurizer efficacy through a routine quality control protocol. An easy way to assure that target temperatures are being reached for the appropriate duration is to equip pasteurizers with a time-temperature control chart or at least a thermometer that is checked on a regular basis. Unfortunately, Dairy Tech does not currently market on-farm heat-treating devices that seal during the heat-treating process which would inhibit attempts to open the machine and remove contents before the target temperature has been reached.

After heat processing, colostrum should be cooled rapidly to prevent bacterial proliferation. Pasteurizer adequacy can be monitored by measuring the activity of alkaline phosphatase in the heat-treated batches or by determining bacterial counts post heat treatment (GODDEN and CHESTER-JONES, 2005). Colostrum that has not been effectively treated prior to its use as calf feed should be regarded with caution as it may still contain live pathogens, which could endanger calf health.

3.5 STUDY FLAWS

First, freezing of colostrum was conducted without the addition of an adequate preserving agent. Also, depending on the collection date, samples had been frozen for varying time periods until microbiological analysis was performed. It is not known how this influenced the microbiological composition of the colostrum batch samples.

Second, it would have been a more accurate approach to sample the colostrum batches shortly before feeding than right after batch preparation since the microbial load that calves actually received might have changed during storage.

These facts should be considered in future studies to receive more accurate data on the microbiological composition of bovine colostrum and on colostral bacterial growth.

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4 A COMPARISON OF THE TOTAL PROTEIN STATUS OF CALVES FED RAW VERSUS HEAT-TREATED COLOSTRUM

4.1 LITERATURE REVIEW

4.1.1 Genesis and Transport of Colostral Immunoglobulins

The transfer of passive immunity in ruminants is based upon the uptake of immunoglobulins through the ingestion of colostrum. Normally, bovine colostrum contains 50 to 150 g/l of immunoglobulins that are composed of about 85 to 90 % IgG (IgG1 accounting for about 80 to 90 % of total IgG), about 7 % IgM, and about 5 % IgA (LARSON et al., 1980). These immunoglobulins originate not only from an increase in the immunoglobulin synthesis in the dam, but are transferred from storage centers like spleen and lymph nodes into the maternal blood circulation around parturition (LARSON, 1958). Supported by an elevation of the blood flow into the mammary gland, immense amounts of immunoglobulins of the IgG class leave the maternal blood stream and accumulate in the udder shortly prepartum (SASAKI et al., 1976).

Due to an increase in the gene expression of a surface membrane receptor in the cytoplasm of acinar and ductal epithelial cells of the ruminant udder (MAYER et al., 2002), strong specific binding sites for IgG1 emerge on bovine mammary cells three to one days prepartum and support the already existing slightly weaker binding sites for IgG1 and IgG2 (SASAKI et al., 1976). These Fc receptors are able to selectively bind the Fc region of IgG1 and facilitate its transport from the basal side of the mammary acinar epithelial cell to the luminal side (basolateral-to-apical transport), thus secreting IgG1 into colostrum (MAYER et al., 2002). Even though maternal blood serum contains diverse immunoglobulin classes, only IgG is transferred in large amounts from blood to lacteal secretions (LARSON et al., 1980). This highly selective transport mechanism (SASAKI et al., 1977a; MAYER et al., 2005) in the bovine mammary epithelial cell provokes a decrease in the IgG1 concentration of maternal blood and accounts for the large accumulation of IgG1 (along with some IgG2) in colostrum (SASAKI et al., 1976).

Hence, colostrum mainly consists of immunoglobulins derived from serum (NEWBY and BOURNE, 1977) - local synthesis in the udder only accounts for immunoglobulins of the quantitatively less important IgA and IgM classes (LARSON et al., 1980). Plasmacytes located adjacent to the secretory epithelium and in mammary secretions produce these small amounts of IgA and IgM (LARSON et al., 1980). An additional translocation of IgA from gut-associated lymphoid tissue into the mammary gland (SALMON, 1999) is granted by the entero-mammary cell circulation, which enables the mammary gland to provide the neonate with specific lacteal IgA antibodies against enteric agents (BUTLER, 1979).

4.1.2 Factors Affecting Colostral Immunoglobulin Content

The concentration of colostral immunoglobulins is subject to high fluctuations. Ranges from 0 to 120 g of immunoglobulins per liter of colostrum have been reported in the past (LONA and ROMERO, 2001).

According to previous studies, colostral immunoglobulin concentrations are apparently neither influenced by the length of the dry period nor by the level of feeding during this time (LOMBA et al., 1978; MORIN et al., 2001). The season of calving (PRITCHETT et al., 1991; MORIN et al., 2001; GULLIKSEN et al., 2008) and the breed (LOMBA et al., 1978; MULLER and ELLINGER, 1981; GUY et al., 1994; TYLER et al., 1999a; MORIN et al., 2001) are controversially discussed in this regard. The volume of colostrum produced (PRITCHETT et al., 1991), disease history (JASTER, 2005), lactation number (MULLER and ELLINGER, 1981; DEVERY-POCIUS and LARSON, 1983; MORIN et al., 2001), and vaccination program of the dam prove to be of prime importance in regulating colostral immunoglobulin concentrations.

Theoretically, the effect of the latter three influences can be reduced by feeding colostrum pooled from several dams. Since data showed that large volume colostrums will lower the pool immunoglobulin concentration (PRITCHETT et al., 1991), only colostrum tested by specific gravity measurement is recommended for usage to ensure the successful transfer of passive immunity in neonates.

4.1.3 Critical Control Points in a Colostrum Feeding Program

KRUSE estimated by computer simulation that a certain frequency of hypogammaglobulinaemia in neonatal calves could not be avoided under practical farm conditions due to variations in the birth weight, the immunoglobulin concentration of colostrum, the ingested dose of colostrum, the age at first feeding, and the genetically determined ability to absorb immunoglobulins (KRUSE, 1970, cited by BUSH and STALEY, 1980). Good farm management, however, should be able to control colostrum quality, its quantity fed, and the time of its first feeding.

The mean interval from birth to feeding was 0.86 hours for both treatment groups in this study which allows direct comparison of raw versus heat-treated colostrum in resulting total protein. In general, the overall goal is to feed calves with colostrum as soon as possible after birth (NOCEK et al., 1984) to prevent the loss of absorptive sites in their intestine and to avoid bacterial colonization of the naturally sterile neonatal gastrointestinal tract (QUIGLEY, 1997). Especially when the newborn is exposed to a substandard calving environment containing large numbers of pathogens, the chances of septicemia through intestinal bacterial colonization will be increased (QUIGLEY, 1997). Colostrum that is fed prior to an infection has beneficial effects preventing the adhesion of bacteria (BROOKS et al., 2006), the exfoliation of microvilli, and the transepithelial migration of pathogens (CORLEY et al., 1977) in the gut.

The next essential factor, colostrum quality, can easily be controlled. Commercially available colostrometers use the specific gravity of colostrum as a method of estimating relative colostrum quality (MORIN et al., 2001). This technique is based on the existence of a significant relationship between colostral specific gravity and its immunoglobulin concentration (FLEENOR and STOTT, 1980) and allows for the determination of immunoglobulin levels prior to colostrum feeding or its processing.

The total immunoglobulin mass available for absorption by the calf's intestine is a combination of the immunoglobulin concentration present in colostrum and of the volume of colostrum fed. The colostrum given at first feeding should provide a minimal immunoglobulin mass of 100 g to the newborn (GARRY) which means that a high colostral immunoglobulin concentration would allow feeding of a smaller volume (FLEENOR and STOTT, 1980). To

ensure adequate immunoglobulin masses, the study farm routinely used a feeding program where a fixed volume of 3.8 l of colostrum was administered to each calf with an esophageal tube feeder. Therefore, the colostral immunoglobulin concentration must exceed 26.3 g/l to provide an adequate immunoglobulin mass to the newborn.

Through the selection of colostrum with adequate immunoglobulin concentrations (LOMBA et al., 1978), through the controlled administration of sufficient colostral volumes, and through the determination of the point in time when colostrum is actually given to the progeny, the dairy farmer can lower the incidence of failure of passive transfer (PRITCHETT et al., 1991) and therefore, reduce the morbidity and mortality rates amongst his future replacement heifers (MAUNSELL et al., 1998).

4.1.4 Tube Feeding of Colostrum - A Matter of Dispute?

When comparing different feeding methods, the administration of colostrum by tube feeder proved to be the safest way to achieve satisfactory serum immunoglobulin concentrations in newborn calves. In a previous study, esophageal tube feeding created failure of passive transfer in only 10.8 % of animals, while 19.3 % of calves that had been bottle fed, and 61.4 % of calves that had been allowed to nurse their dams had insufficient serum immunoglobulin concentrations (BESSER et al., 1991). Force feeding ensures that all calves receive sufficient colostral volumes, whereas voluntary consumption can be inadequate due to a deficiency of the cow's mothering abilities, bad udder confirmation, dam sickness or poor calf vigor.

Through the use of this artificial feeding method, however, the esophageal groove reflex is not induced and, therefore, the fluid is deposited into the forestomachs (CHAPMAN et al., 1986). The subsequent outflow of colostrum from the preruminant rumen into the abomasum and the small intestine occurs within 3 hours after feeding (LATEUR-ROWET and BREUKINK, 1983) which does allow an adequate intestinal absorption of colostral proteins before the loss of the gastrointestinal permeability sets in (MOLLA, 1978). As a consequence, a slightly delayed increase in serum immunoglobulin concentrations can be observed in drenched versus bottle-fed calves (KASKE et al., 2005). Eventually, though, drenched calves reach significantly higher serum immunoglobulin concentrations than bottle-fed calves (KASKE et al., 2005).

4.1.5 Feeding Pooled Colostrum – A Growing Trend?

A widespread practice in large dairy operations is the commingling of colostrum from various fresh cows. The proportion of operations that pool colostrum grows as herd size increases. The numbers range from 16.0 % of small operations to 56.9 % of large operations that use pooled colostrum (NAHMS, 2007). This practice saves time and labor when colostrum is to be heat-treated. Moreover, pooled colostrum can be extended over a greater number of neonatal calves by adjusting the amount fed to a minimal volume that delivers a fixed mass of immunoglobulins.

Pooling colostrum, however, is not recommended without previous control through a colostrometer since the overall batch quality might be reduced due to the dilution of excellent colostrum with colostrum of low value. Also, feeding pooled colostrum is a quick way to spread pathogens like *Mycobacterium avium* subsp. *paratuberculosis* to those animals that will represent the future adult herd (NAHMS, 2002) and are most susceptible to persistent infection with this pathogen. This risk of disease transmission might account for the decrease in operations that pool colostrum - from 2002 to 2007 the percentage dropped from 27.0 % to 21.0 % (NAHMS, 2007).

4.1.6 Kinetics of Immunoglobulin Absorption

Following the ingestion of colostrum, the kinetics of the absorptive process of colostral immunoglobulins from the gut lumen into the circulation of the calf can be characterized by the following stages (STALEY and BUSH, 1985). First, colostral immunoglobulins are bound by the enterocytes' microvillous border. Subsequently, these highly vacuolated, immature mucosal epithelial cells (KRUSE, 1983) of the small intestine (mainly the jejunum) incorporate binding sites and immunoglobulins through endocytosis. In this process, the endocytosed membrane at the binding site extends into the cytoplasm, initially creating a tubule, which then transforms into a vacuole. A protein named clathrin seems to assist the formation of these coated vesicles (JOCHIMS et al., 1994).

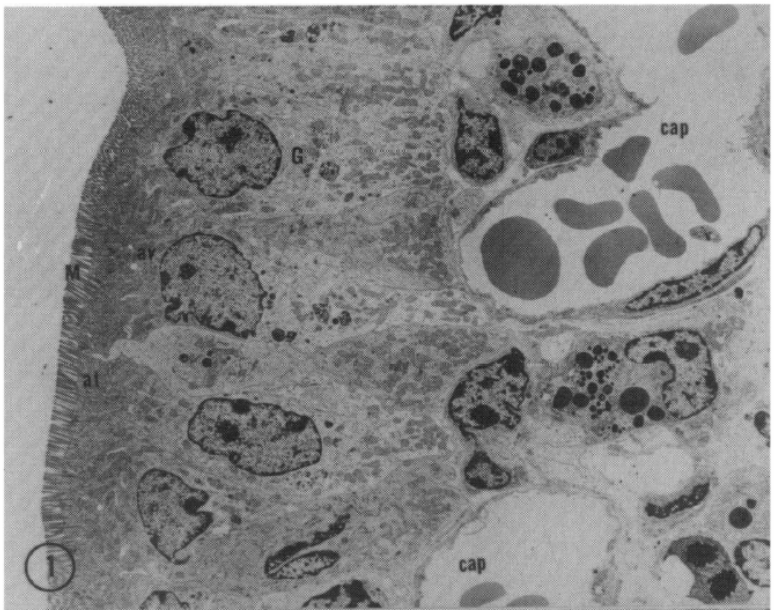

Figure 5: "Columnar intestinal absorptive cells from the jejunum of a newborn calf. Well-developed microvilli (M), tubules (at), and vacuoles (av) are in the apical end of the cell. Nuclei also are located in the apical end of the cell. The area of the Golgi apparatus (G) and multilaminated bodies are subnuclear. Capillaries (cap) are in close apposition to the intestinal epithelial cells, and lamina proprial cells with bizarre granules are interspersed between capillaries." (Figure and legend taken from STALEY and BUSH, 1985, originally from STALEY et al., 1968)

Besides this nonspecific method of absorption, Fc receptors on the intestinal epithelial cell, which fit the Fc portions of IgG molecules, are known to play a crucial role in the selective transport of IgG in different species (JAKOI et al., 1985; ISRAEL et al., 1997; KACSKOVICS, 2004). Binding to these receptors might shield immunoglobulins from proteolytic digestion, while unbound protein is degraded in phagolysosomes by the absorbing cell (STALEY and BUSH, 1985). In a study on neonatal sheep, however, Fc receptors could not be found in enterocytes (MAYER et al., 2002) which supports the hypothesis that the uptake and transport of macromolecules in ruminants may be qualitatively nonspecific (LARSON et al. 1980; KRUSE, 1983) and include a variety of homologous and heterologous proteins (KRUSE, 1983; STALEY and BUSH, 1985).

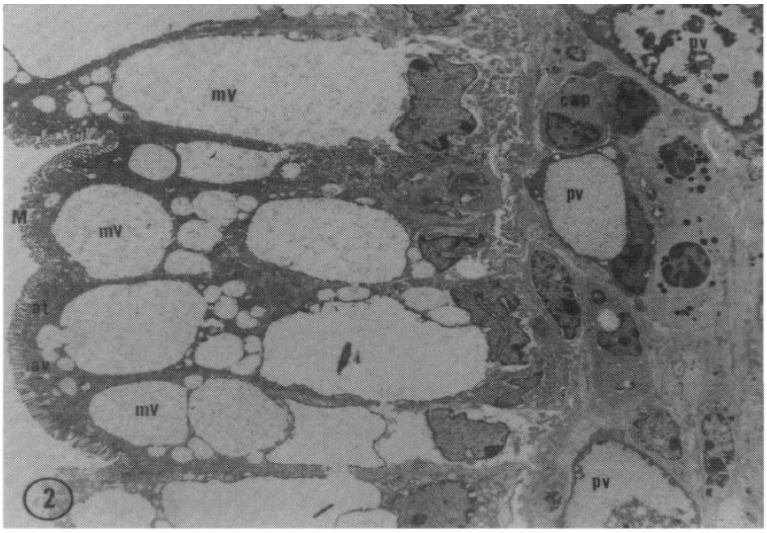

Figure 6: "Columnar intestinal absorptive cells from the ileum of a newborn calf. The most apparent feature is the highly vacuolated cytoplasm. A flocculent polysaccharide material is dispersed throughout most of the supranuclear vacuoles (mV)."…"Large cells with periodic-acid-Schiff-positive vacuoles (pv) fill the lamina propria." (Figure and legend taken from STALEY and BUSH, 1985, originally from STALEY et al., 1968) (Periodic acid-Schiff is a staining method used to identify the carbohydrate portion of intracytoplasmic immunoglobulins. (IOACHIM and MEDEIROS, 2008))

The intracellular transport of the created vacuole with the enclosed immunoglobulin ends at either the lateral or basal cell membrane, where, upon contact with the cell membrane, vacuolar contents are exocytosed into the lamina propria. Subsequently, the cellular freight is released into the lymphatics and blood capillaries of the intestinal villus (JASTER, 2005).

4.1.7 Gut Closure and Factors Contributing to this Phenomenon

The overall transfer of material from epithelial cells to blood decreases progressively after 12 hours of age (BUSH and STALEY, 1980). The mean closure time of the intestine to absorption is assumed near 24 hours postpartum (STOTT et al., 1979) with a standard deviation of approximately 4 hours (BUSH and STALEY, 1980). The exact mechanisms leading to gut closure are still unknown – it seems to be a multifactorial event comprising various endocrine

influences (STOTT et al., 1976; KRUSE, 1983), the maturation of fetal intestinal epithelial cells, the onset of the cessation of transport at the basal and lateral cell membranes of the enterocytes, and the enhancement of the intracellular proteolytic activity of the lysosomal system (JOCHIMS et al., 1994).

The secretion of digestive enzymes into the gastrointestinal tract of the calf remains suppressed for a short period after birth (GUILLOTEAU et al., 1983) to prevent the digestion of immunoglobulins and to allow their absorption. This suppression is slowly revoked by about 12 hours postpartum which reduces the chances of immunoglobulins to be absorbed prior to their degradation (QUIGLEY, 1997). Further, the proteolytic activity in the digestive tract is minimized by the presence of a trypsin inhibitor in colostrum (KRUSE, 1983).

4.2 MATERIALS AND METHODS

4.2.1 Routine Calf Management, Calf Enrollment, and Record Keeping

Personnel at the study farm routinely removed newborn calves from their dams before suckling could occur. The neonates were initially placed in a group pen within the maternity barn, their navels were treated with iodine solution, and ear tags for identification were placed. Within 90 minutes after birth all calves received 3.8 l of previously collected colostrum (see 3.2.2) via esophageal tube feeder.

Female Holstein calves (n = 166), born between January 25, 2007, and June 8, 2007, were assigned on alternate days to receive 3.8 l of either unprocessed (n = 74) or previously heat-treated (n = 92) colostrum at first feeding. More calves received heat-treated colostrum because of erratic worker compliance. Afterwards, they were moved to the calf barn, where they were housed in individual pens. They received 3 l of pasteurized waste milk twice daily for the first two weeks of life which was then increased to 4 to 6 l (depending on appetite and milk supply) of pasteurized waste milk twice daily until weaning at approximately six weeks of age. One week after weaning they were moved to group pens of 25 calves each with a bedded pack lying area and a scraped feed area. At about six months of age they were stabled in a freestall barn. While all heifer calves were raised to maturity on site, bull calves left the property at a few days of age.

The following information was to be recorded for each calf involved in this study: calf identification number, date and time of birth, treatment allocation (unprocessed or heat-treated colostrum), time of feeding, and number of the colostrum batch fed.

4.2.2 Blood Sample Collection and Determination of TP Concentration

A 10 ml postcolostral blood sample was collected from each study calf at 20 to 72 hours of age via jugular venipuncture using a 10 ml serum (red top) Vacutainer tube (Becton-Dickinson, Franklin Lakes, New Jersey). The samples were refrigerated, centrifuged, and the serum separated from the clot within 24 hours of collection.

The serum total protein concentrations (g/l) were determined using a hand-held refractometer (VET 360, Reichert Inc., Depew, NY). Prior to usage, refractometer calibration was checked with distilled water and found to be accurate at room temperature. All TP concentrations were determined in the laboratory of the Cornell Ambulatory and Production Medicine Clinic where the same room temperature prevailed at all times.

4.2.3 Exclusion of Animals

Sixty-nine of the 166 calves that were initially enrolled in this study could not be considered for the final statistical analysis. Eight of these calves could not be included due to the post-harvest contamination of three colostrum batches (see 3.3). Three other calves were excluded because of a missing post heat treatment colostrum sample. A total of 58 calves had to be omitted from this study since individual values were lacking for the composition of a full data set. Main factors for exclusion were that the calves' exact times of birth or precise feeding times had not been recorded by the herdsmen, or that the blood samples could not be collected within the required time frame due to the working schedule of the researcher.

Therefore, the final statistical analysis was conducted on the remaining 97 animals only: 39 calves that had received unprocessed and 58 calves that had received heat-treated colostrum, respectively.

4.2.4 Data Analysis

A linear regression analysis was performed on serum TP concentration versus calf age at blood sampling for both treatment groups. (Exact as well as estimated birth times were considered for calves within the heat-treated group (see 4.3.2).) Additionally, least squares means of serum TP values were calculated with the help of the statistical program package of SAS (Statistical Analysis System, Version 9.2, SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513) to eliminate the influence of calf age at the time of blood sampling.

4.3 RESULTS

The following figures (Figure 7 and Figure 8) illustrate the detected serum total protein values at a certain age within the determined time frame of 20 to 72 hours postnatum for calves fed unprocessed and heat-treated colostrum, respectively. Trend lines are drawn as a guide.

4.3.1 Serum Total Protein Concentrations of Calves Fed Raw Colostrum

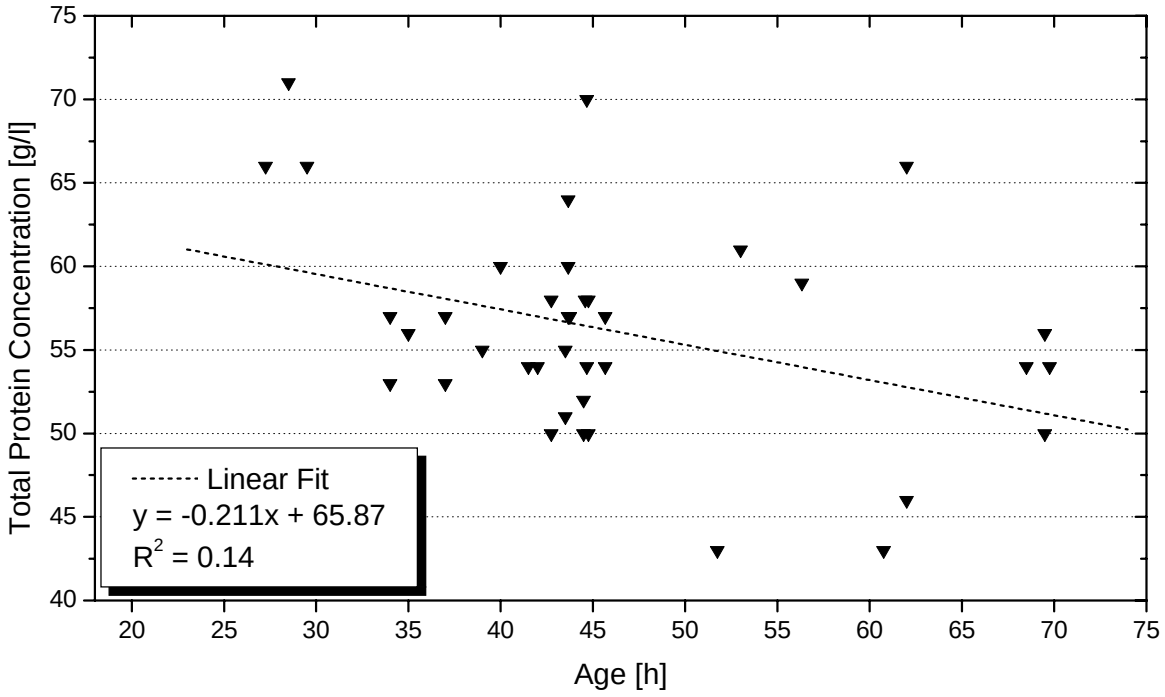

Figure 7: Serum total protein concentration (g/l) versus age at sampling (h) for calves fed unprocessed colostrum. Black triangles show values of calves with precise records of their birth times.

The sampling of calves' blood had been conducted between 27.3 and 69.8 hours postpartum. The mean collection time in this group was reached at 46.1 hours of age.

Total protein concentrations varied from 43 to 71 g/l. Values above 50 g/l were reached by 32 (82 %) of these calves. Four animals (10 %) had total protein values of 50 g/l implying a moderately successful passive transfer. The remaining 3 animals (8 %) showed insufficient total protein concentrations of 43 g/l to 46 g/l. The mean serum total protein concentration in this treatment group was 56 g/l.

With $R^2 = 0.14$ the linear correlation coefficient indicates a minimal correlation between serum total protein concentration and calf age for calves fed unprocessed colostrum.

4.3.2 Serum TP Concentrations of Calves Fed Heat-Treated Colostrum

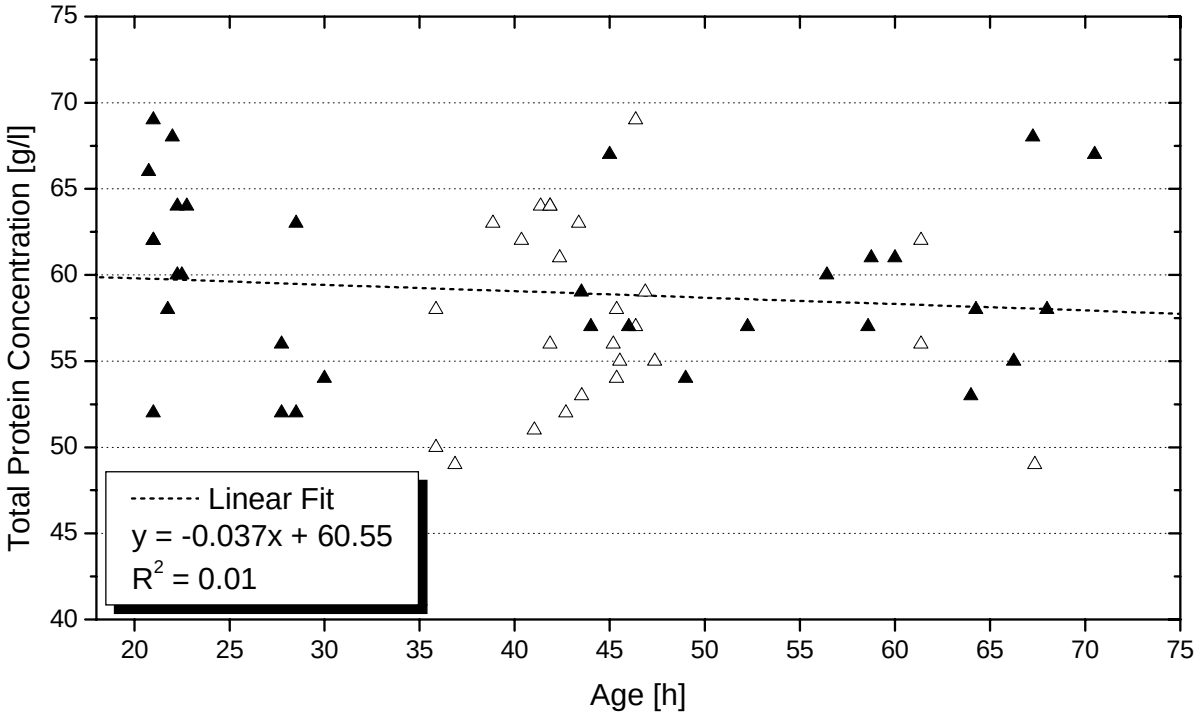

Figure 8: Serum total protein concentration (g/l) versus age at sampling (h) for calves fed heat-treated colostrum. Black triangles mark values of calves with exact records of their time of birth. White triangles represent values of calves with estimated birth times.

Blood collection times in these calves ranged from 20.8 to 70.5 hours postpartum. The mean duration from birth to sampling was estimated at 42.5 hours. (Exact birth times had been recorded for 32 of these 58 calves. For the remaining 26 calves only exact feeding times had been documented. Since the mean time from birth to feeding had been 0.86 hours for all other involved study calves, the birth times of these 26 calves were estimated using an interval of 0.86 hours.)

At the time of blood sampling, 55 animals (95 %) had reached total protein values of greater than 50 g/l. Three calves (5 %) showed moderately successful passive transfers with values of 49 g/l to 50 g/l. Failure of passive transfer could not be detected in any of the calves fed heat-processed colostrum. The mean serum total protein concentration in this treatment group was 59 g/l. Individual values ranged from 49 to 69 g/l.

The calculated linear correlation coefficient ($R^2 = 0.01$) indicates no correlation between calf age at sampling and serum total protein concentration for calves fed heat-treated colostrum.

4.3.3 Linear Model

A general linear model was created using the mixed procedure of SAS (Statistical Analysis System, Version 9.2, SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513). The goal was to eliminate the influence of animal age at the time of blood sampling when comparing serum total protein concentrations between the two treatment groups. Hence, the total protein means for the two treatment groups were corrected for the imbalances of animal age and are shown below. According to Table 3 (see page 45), the linear equation to calculate serum total protein values for calves of any age is:

$$TP = 60.1 + TRT \times (2.5) + AGE \times (-0.1)$$

TRT for the raw treatment group is 0.

TRT for the heat-treated treatment group is 1.

Table 3: Statistical data on the adjustment of the mean total protein concentration by calf age.

Effect	Treatment	Adjusted Means (g/l)	95% Confidence Interval	Estimate	P-Value
Intercept				60.1	
TRT	Raw	56.3	54.5 – 58.1	2.5	
TRT	Heat-treated	58.8	57.3 – 60.3		0.04
AGE				- 0.1	0.05

The least squares mean of the serum total protein concentrations of calves that had received heat-treated colostrum turned out to be 58.8 g/l, while it was 56.3 g/l for calves that had been fed raw colostrum. This difference between the two treatment groups was significant ($p = 0.04$).

4.4 DISCUSSION

4.4.1 Failure of Passive Transfer – A Risk to Replacement Heifers

The National Dairy Heifer Evaluation Project rates the nationwide prevalence of failure of passive transfer (in this case defined as a serum IgG concentration of less than 10 g/l) in dairy calves to be 41 % (NDHEP, 1993). The degree of passive transfer in calves can be tested via determination of serum total protein values since a close correlation between TP and IgG in the blood of newborn calves is believed to exist until about 72 hours of age (QUIGLEY, 1998). Then, the relationship between TP and IgG changes due to the absorption of nutritional proteins other than IgG and due to the dislocation of IgG from the circulation into other body pools (QUIGLEY, 1998). Consequently, the period from 20 to 72 hours was fixed as calf age at blood sampling in this trial. This time span between the cessation of immunoglobulin uptake due to the closure of the gut barrier (BUSH and STALEY, 1980) (see 4.1.7) and the decline of the immunoglobulin content in serum due to its catabolism or transfer (STOTT et al., 1979) promises to provide maximal accuracy in refractometer measurements of total protein.

Total protein levels in cattle increase early in neonatal life. With a mean at 47 g/l, the TP concentration at birth is markedly low in colostrum-deprived calves (NAYLOR et al., 1977). Colostrum feeding may increase the TP concentration to as high as 70 g/l (JAIN, 1993). In this trial, TP concentrations of greater than 50 g/l at the time of blood sampling were regarded as proof of successful passive transfer. TP values between 47.5 and 50 g/l implied a moderately successful passive transfer, while measurements of less than 47.5 g/l were defined as failure of passive transfer (QUIGLEY, 1998).

The blood samples that were taken from the 97 study calves within the defined time frame showed wide variations in serum TP concentrations: Blood serum analysis revealed that 87 calves (90 %) had reached TP levels proving successful passive transfer, 7 neonates (7 %) showed moderately successful passive transfer, and 3 animals (3 %) had failure of passive transfer (see 4.3.1 and 4.3.2). Each of the 3 calves with TP concentrations below 47.5 g/l belonged to the treatment group that had been fed unprocessed colostrum. None of the calves within the treatment group that had received heat-treated colostrum exhibited failure of passive transfer.

Interestingly, and in contrast to previous research (GODDEN et al., 2003), calves that had received heat-treated colostrum had higher mean TP concentrations (58.8 g/l) than calves that had been fed unprocessed colostrum (56.3 g/l) (see 4.3.3). The difference between the mean serum TP values of the two treatment groups was significant ($p = 0.04$). These findings might be explained by interactions between colostral bacteria and immunoglobulins.

A pilot study in this field (JOHNSON et al., 2007) compared serum TP, IgG concentrations, and the efficiency of IgG absorption of 49 calves fed heat-treated ($n = 25$) or raw ($n = 24$) colostrum. It was found that feeding heat-treated colostrum created notably higher serum TP and IgG concentrations, as well as an enhanced efficiency of IgG absorption in these neonates. These findings were recently replicated in a much bigger study that comprised 1102 newborn calves (DONAHUE et al., 2008). The mean serum TP concentration was significantly greater for calves fed heat-treated (58.3 g/l) versus raw colostrum (57.0 g/l). The mean serum IgG concentration followed this trend and was significantly higher for calves fed heat-treated (17.0 g/l) versus raw colostrum (14.5 g/l).

4.4.2 Factors Influencing Immunoglobulin Absorption

This study's results support earlier research that documented that the presence of bacteria in colostrum, and later in the small intestine of the neonate, reduced the uptake of immunoglobulins through the gut barrier into the circulation of the calf (JAMES et al., 1981). Expectedly, the bacterial contamination of unprocessed versus heat-treated batches was greater (see Figures 1 and 3).

The competition between immunoglobulins and microbes for common intestinal receptors may be the first potential explanation for the observed reduction in the immunoglobulin resorption. *Escherichia coli*, for example, has the ability to bind to the apical microvillous membrane of the intestinal epithelium (STALEY and BUSH, 1985), thereby blocking binding sites for immunoglobulins.

Secondly, interactions between intestinal bacteria and colostral immunoglobulins may have accounted for a decrease in the number of transportable immunoglobulins (STALEY and BUSH, 1985). Some intestinal bacteria are capable of degrading glycoprotein antibodies (STALEY and BUSH, 1985) through enzymatic processes which consequently impairs the uptake of immunoglobulins.

Thirdly, microbial exposure can lead to an alteration of the absorptive surface (JAMES and POLAN, 1978; STALEY and BUSH, 1985). Immunoglobulin binding sites are simply eliminated through the exfoliation of microvilli caused by bacteria. Other viable bacteria can diminish immunoglobulin absorption by reducing the permeability of epithelial cells or by enhancing the replacement of permeable cells by cells that are incapable of macromolecular uptake (JAMES et al., 1981). It has even been suggested that intestinal bacteria or their products are likely to initiate gut closure itself (STALEY et al., 1972, cited by STALEY and BUSH, 1985).

4.4.3 Protective Value of Excess Immunoglobulins

After saturation of the blood serum with macromolecules, a large proportion of the previously absorbed IgG1 is recycled back from the circulation into the gut lumen (MAYER et al., 2002). It has been shown that after the absorption of 100 g of IgG1 from colostrum a calf would subsequently secrete 1 to 4 g back into the gut each day during the first two weeks of life. Also, it was suggested that calves with higher serum IgG1 concentrations clear larger amounts of IgG1 to the gastrointestinal tract lumen (BESSER et al., 1988). This transport, mediated by the crypt epithelial cells (NEWBY and BOURNE, 1976a), contributes to the protection of the intestinal mucosa against infections (BESSER et al., 1988). In this way, excess immunoglobulins contribute to the local mucosal immunoprotection.

The ruminant Fc receptor was suggested to play an important role in the transport of IgG and therefore, in the protection of young ruminants against intestinal infections. It selectively binds IgG1 antibodies at the basal side of the acinar epithelial cells and transports them to the luminal side (basolateral-to-apical transport), thus secreting IgG1 onto mucosal surfaces rather than absorbing it (MAYER et al., 2002; KACSKOVICS, 2004). The ruminant Fc receptor seems to favor binding to IgG1, which is more resistant to proteolysis than IgG2 (NEWBY and BOURNE, 1976b). IgG1 is therefore well-represented in mucosal fluids and in tissues like the small and large intestine.

4.4.4 Unfavorable Effects of (Pre-) Colostral Immunoglobulins

In the past various publications have stated that calves are not fully agammaglobulinemic at birth (KLAUS et al., 1969; CHIGERWE et al., 2008). Detectable amounts of serum IgG can be found in more than 50 % of neonatal calves prior to colostrum uptake (CHIGERWE et al., 2008). Mean precolostral serum values are 1.2 ± 0.5 g/l for IgG and 0.1 ± 0.0 g/l for IgM (KLAUS et al., 1969). A negative relationship between this precolostral concentration of IgG in calf serum and the amount of IgG absorption from colostrum has been suggested (VUKOTIC and MOVSESIJAN, 1976, cited by BUSH and STALEY, 1980).

Additionally, IgG antibodies transferred via colostrum appear to play a regulatory role in the development of the systemic humoral immune system of the newborn through a feedback mechanism (BUTLER, 1979). It is supposed that the amount of maternal immunoglobulins absorbed from colostrum can suppress the development rate of the calf's immune system, in particular the endogenous production of IgG1 (DEVERY-POCIUS and LARSON, 1983; LOGAN et al., 1974, cited by BURTON et al., 1989). In general, it is believed that the newborn calf has the capability for an endogenous production of IgG1 during the first three weeks of life (SASAKI et al., 1977b) at a rate of approximately 1 g of new IgG1 per day (DEVERY et al., 1979). While hypogammaglobulinaemic calves were able to synthesize serum immunoglobulin within a week of birth, calves with high serum immunoglobulin levels showed no endogenous production until they were four weeks of age (LOGAN et al., 1974, cited by BUSH and STALEY, 1980).

4.5 SUGGESTIONS FOR FUTURE RESEARCH

First, it must be admitted that the randomization of batches and calves was not perfect due to erratic worker compliance. Subsequently more batches were heat-treated and more calves received heat-treated colostrum during this study.

Additionally, there was a difference in the raw colostrum batches' bacteriological qualities between the two treatment groups. The 19 colostrum batches that were ultimately fed to calves without further processing contained considerably higher numbers of bacteria than the 29 batches that were to be heat-treated prior to feeding (see 3.3.2 and 3.3.4). The author had not been aware of this fact since the microbiological analysis was not performed until data collection had been finalized. A better approach would have been to split the colostrum batches and feed them half as raw and half as heat-treated to the particular treatment group. However, the experimental design attempted to simplify the work of employees at the dairy farm, who collected, processed, and fed colostrum to all newborn calves. The assumption that a combination of multiple individual first milkings, of diverse volumes, and of secretions from cows with different lactation numbers would even out possible variations in the individual characteristics of each batch was wrong.

Considering this, colostrometer measurements of colostral IgG contents should have been conducted. While the colostral IgG concentration can be as high as 136 g/l in individual Holstein cows, colostrum pools from Holsteins invariably have low immunoglobulin concentrations because high volume, low concentration colostrum dilutes the concentration of the other samples in the pool. If pools are used, the diluting influence of high volume, low concentration colostrum should be limited by restricting any individual cow's contribution to the pool to 20 lbs. (9 kg) or less (RADOSTITS et al., 2000).

Next, the performed determination of serum TP concentrations via refractometer might not accurately reflect the existent immunoglobulin concentrations. It is known that refractometer measurements can be altered or biased, for example, through a possible dehydration of study calves (QUIGLEY, 1998). However, assessing serum immunoglobulin levels in a laboratory setting was intentionally rejected since the refractometer has distinguished itself as a convenient management tool for on-farm application. Previous studies support the evaluation of TP levels by refractometer (WALLACE et al., 2006) and have rated it as useful as the direct methods of measuring the serum immunoglobulin content that are represented by sodium sulfite turbidity test (TYLER et al., 1999b), zinc sulfate turbidity test or radial immunodiffusion (NAYLOR et al., 1977). The importance of sample temperature at the time of refractometry reading and the potential impact of sample hemolysis pose topics for future studies.

Finally, should precolostral immunoglobulin concentrations in the blood sera of the study calves have been measured? It is known that bovine fetuses are capable of synthesizing small quantities of immunoglobulins (TIZARD, 2004), and that a young calf produces IgG1 endogenously from birth on (SASAKI et al., 1977b). However, precolostral immunoglobulin concentrations were not measured since the values of IgG1 would have been too low to be measured accurately using the refractometer and should not have differed between both treatment groups (JASTER, 2005).

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5 ANIMAL PERFORMANCE – A COMPARISON OF CALVES FED RAW VERSUS HEAT-TREATED COLOSTRUM

The last chapter of this thesis is dedicated to the question of whether feeding heat-treated versus unprocessed colostrum has any impact on calf health, weight gain, and future performance. A previous study showed that heat treatment of bovine colostrum at 60 °C for up to 120 minutes did not affect its viscosity, IgG concentration, or immunoglobulin activity (MCMARTIN et al., 2006). However, it has not been determined yet if heat processing at this temperature alters other colostral constituents, which may be of vital importance to the calf's immune system and physical development. Therefore, the following text will initially focus on the individual components of colostrum, their functions, and their responses to heat treatment before particular attention is given to the issue of animal performance.

5.1 LITERATURE REVIEW

More than 2,000 compounds are supposed to occur in milk (TÖPEL, 2004). With a proportion of 87 – 88 %, water represents the main constituent of milk. Water occurs mainly uncombined and serves as a solvent and dispersant, while only a small proportion of it is adsorbed to milk components or bound as a hydrate. After its separation from milk through vaporization only the dry matter remains (see Table 4).

Table 4: Concentrations of major dry matter components in first milking colostrum and mature milk (FOLEY and OTTERBY, 1978).

Contents (g/l)	**Colostrum**	**Milk**
Total Protein	140	31
Lactose	27	50
Fat	67	40
Ash (Minerals)	11	7

5.1.1 The Function of Milk Proteins

The proteins of milk are probably the constituents most affected by heating (FOX and MCSWEENEY, 1998). In general, proteins play crucial roles in animal metabolism acting as enzymes, hormones, transport proteins, structural proteins or protective structures. Additionally, they contribute to the nutrition of mammals by supplying easily digestible and readily absorbable energy, as well as high levels of essential amino acids. Amino acids are crucial for the synthesis of endogenous protein and can not be assembled from other food sources; while carbohydrates can be transformed into fats, proteins can not be replaced.

Milk proteins can be classified as major (98 % of total milk proteins) and minor (2 % of total milk proteins) milk proteins. Major milk proteins comprise casein and whey proteins, while minor milk proteins include the actual minor proteins (like lactoferrin), milk fat globule membrane proteins, and all of milk's enzymes (TÖPEL, 2004).

5.1.1.1 Casein

Approximately 79.5 % of the total protein of bovine milk belongs to the group of caseins (TÖPEL, 2004). α-, β-, and κ-casein are water-insoluble phosphoproteins containing a fairly high quantity of proline. Due to their chemical composition with little secondary or tertiary structure, caseins are readily susceptible to proteolysis without prior denaturation which is an important characteristic for neonatal nutrition since this facilitates their digestibility (FOX and MCSWEENEY, 1998). Also, it has been shown that the neonatal gastrointestinal tract can digest caseins that are derived from colostrum more rapidly and efficiently than those of mature milk (YONEDA et al., 2001).

Casein, however, should not merely be considered as a nutrient, but as a source of a range of biologically active peptides (FIAT et al., 1989). In the course of its enzymatic digestion various peptides are generated (YONEDA et al., 2001), which can be used as nutraceuticals (SEVERIN and WENSHUI, 2005), for example, in the treatment of diarrhea since they minimize tissue damage through their anti-inflammatory activity (KELLEHER and LÖNNERDAL, 2001). κ-casein-derived glycomacropeptide, as another example, displays immunomodulatory and bacterial toxin binding effects (DADDAOUA et al., 2005). The peptides casecidin 15 and 17, which also show antimicrobial activity, were just recently discovered (BIRKEMO et al., 2009).

Other casein peptides (the so-called casomorphins) are endowed with opioid qualities that diminish colicky symptoms in newborns (ZIMECKI and KRUZEL, 2007) by reducing the gut motility (TÖPEL, 2004). Casein itself does show protective effects in experimental bacteremia (ZIMECKI and KRUZEL, 2007).

Casein in milk is mainly found as a suspension of particles called casein micelles, in which caseins are held together by calcium ions and hydrophobic interactions. This quaternary structure makes casein relatively heat resistant. A temperature of 140° C is needed to coagulate casein micelles at the normal pH of milk (FOX and MCSWEENEY, 1998).

5.1.1.2 Whey Proteins

About 20 % of the total protein of bovine milk belongs to a group of proteins generally referred to as whey proteins (FOX and MCSWEENEY, 1998). In normal milk whey proteins consist of approximately 56 % β-lactoglobulin, 21 % α-lactalbumin, 14 % immunoglobulins, and 7 % blood serum albumin (TÖPEL, 2004). These proteins are water-soluble, free of phosphate, and globular shaped. Due to plenty of disulfide bonds in their primary structure and their complex molecular conformation, whey proteins are heat-labile.

β-lactoglobulin

β-lactoglobulin seems to exist primarily as an alimentary source for amino acids (KONTOPIDIS et al., 2004). In normal cow's milk it is the major whey protein with concentrations of about 3.5 g/l (TÖPEL, 2004), while colostrum can contain as much as 14.3 g/l (LEVIEUX and OLLIER, 1999). This globular protein acts as a specific transporter of small hydrophobic molecules (KONTOPIDIS et al., 2004) (like steroids, fatty acids, and retinoids (GODOVAC-ZIMMERMANN et al., 1985)) and as a mild antioxidant (LIU et al., 2007). Its primary structure includes sequences of bioactive peptides that show antioxidant and antimicrobial activity, as well as opioid-like features after their release at the protein's degradation (HERNANDEZ-LEDESMA et al., 2008).

A reversible denaturation of β-lactoglobulin occurs at up to 75 °C (MOUSAVI et al., 2008). Protein aggregation at temperatures above 75 °C is irreversible since it is accompanied by the creation of new intramolecular and intermolecular disulfide bridges, and by the rearrangement of old intramolecular disulfide bonds (MOUSAVI et al., 2008).

α-lactalbumin

α-lactalbumin appears at a concentration of approximately 1 g/l in normal milk (TÖPEL, 2004), while colostrum has a mean concentration of 2 g/l (LEVIEUX and OLLIER, 1999). This whey protein plays an important role in the synthesis of lactose since it represents a subunit of the enzyme lactose synthase (FOX and MCSWEENEY, 1998). α-lactalbumin's ability to bind divalent cations (such as Ca^{2+}) may facilitate the absorption of essential minerals (LÖNNERDAL and LIEN, 2003), and turns the Ca-containing protein into the most heat resistant of all whey proteins (DEWIT and KLARENBEEK, 1984) with a denaturation temperature of 85 to 90 °C (TÖPEL, 2004). Multiple sequences of biologically active peptides are included in its primary structure (TÖPEL, 2004) and endow the protein with immunostimulatory (LÖNNERDAL and LIEN, 2003), antiviral, antitumoral, and antistress properties (ZIMECKI and KRUZEL, 2007). Diets enriched with α-lactalbumin prevent diarrhea and lead to better weight gains (ZIMECKI and KRUZEL, 2007).

Immunoglobulins

With denaturation temperatures between 65 and 70 °C (TÖPEL, 2004), immunoglobulins (IgG1, IgG2, IgM, and IgA) are very thermolabile whey proteins (DEWIT and KLARENBEEK, 1984). IgG, which accounts for 85 to 90 % of the total immunoglobulin mass in bovine colostrum (LARSON et al., 1980), exhibits two transitions when exposed to heat. The isolated Fab fragment shows a transition at 61 °C, while the Fc fragment displays a transition at 71 °C, which means that these individual transitions represent the denaturation of the IgG's Fab and Fc domains, respectively (VERMEER and NORDE, 2000).

Previous research showed that heat processing of colostrum at 65 °C for 60 minutes did not lead to a loss of IgG's antigen-binding activity (DOMINGUEZ et al., 1997). Heat treatment at 60 °C for 120 minutes (twice as long as in this trial) also did not alter the IgG concentration between pre- and post-heat-treated samples and did not seem to influence the antibody activity of IgG (MCMARTIN et al., 2006).

Blood serum albumin

Blood serum albumin is produced in the liver and crosses the blood-milk-barrier. Its concentration in milk is 0.1 to 0.4 g/l (TÖPEL, 2004), while the mean serum albumin concentration of colostrum is 1.2 g/l (LEVIEUX and OLLIER, 1999). Serum albumin serves as a carrier for molecules of low water solubility, including lipid-soluble hormones, free fatty acids, calcium, and iron. Its denaturation starts at temperatures above 70 °C (TÖPEL, 2004).

5.1.1.3 Lactoferrin

The minor milk protein lactoferrin (SEVERIN and WENSHUI, 2005) is an iron-binding multifunctional glycoprotein (TÖPEL, 2004). It is abundantly present in exocrine secretions (TALUKDER and HARADA, 2006) (normal cow's milk contains 0.1 g/l, colostrum up to 2 g of lactoferrin per liter (TÖPEL, 2004)) since it is produced by the secretory epithelium (PERSSON et al., 1992). Neonatal calves absorb lactoferrin from colostrum with the help of specific receptors located in the mucosal lining of the intestines (TALUKDER et al., 2003). Through transcytosis via brush-border membrane vesicles lactoferrin reaches the systemic circulation (TALUKDER and HARADA, 2006). During this process, the protein is able to carry metal ions like iron, manganese, and zinc, or assists with the absorption of sugars from the gut (ARTYM and ZIMECKI, 2005). Compared to other milk proteins, lactoferrin is exceptionally resistant to proteolytic degradation in the alimentary tract (ARTYM and ZIMECKI, 2005).

Additionally, lactoferrin controls early postnatal intestinal development by inducing enterocyte growth and proliferation (BUCCIGROSSI et al., 2007). It supports the recovery from intestinal diseases by accelerating the regeneration of damaged mucous membranes (ARTYM and ZIMECKI, 2005). It controls the proper composition of the intestinal microflora (ARTYM and ZIMECKI, 2005) by preventing the growth of various pathogenic bacteria (PETSCHOW et

al., 1999) and by inducing the multiplication of *Lactobacillus* spp. (ARTYM and ZIMECKI, 2005) and *Bifidobacterium* spp. (PETSCHOW et al., 1999). This non-pathogenic microflora creates a low pH in the gastrointestinal tract, produces vitamins, increases the activity of natural killer cells, T lymphocytes, and macrophages, lowers the risk of allergies (ARTYM and ZIMECKI, 2005), and promotes the production of protective immunoglobulins (PRENNER et al., 2007). All these features of lactoferrin suggest its use in dietary formulas (BUCCIGROSSI et al., 2007). Lactoferrin supplemented animals show fewer days of disease and less severe diarrhea (ROBBLEE et al., 2003; PRENNER et al., 2007) which might also be explained by the inhibition of pro-inflammatory cytokines (ARTYM and ZIMECKI, 2005), by the modulation of the cyclooxygenase pathway in the gut (TALUKDER and HARADA, 2007), or by the presence of a bioactive peptide which has opioid functions (TÖPEL, 2004). Besides its beneficial effects on calf health, lactoferrin enhances calf performance since supplemented animals have been shown to consume more calf starter grain, have increased preweaning daily weight gains, and subsequently can be weaned at a younger age (JOSLIN et al., 2002).

Lactoferrin is present in secondary granules of polymorphonuclear neutrophils (MASSON et al., 1969) and thus part of the innate defense. It enhances intracellular killing and phagocytosis of Gram-positive and Gram-negative bacteria (DALMASTRI et al., 1988; SZUSTER-CIESIELSKA et al., 1995; ARTYM and ZIMECKI, 2005) by damaging the bacterial outer membrane and by altering the bacterial outer membrane permeability, respectively (ELLISON et al., 1988). At the same time, it eliminates endotoxins (DÖHLER and NEBERMANN, 2002; SEIFERT et al., 2002) and exotoxins (TÖPEL, 2004) from blood plasma. Lactoferrin can hinder viruses from attaching to cell membranes by binding to viral particles (TÖPEL, 2004), and it has antifungal and antiparasitic, as well as antitumoral properties (ZIMECKI and ARTYM, 2005).

Thermal denaturation of bovine lactoferrin depends on the iron status of the protein (about 15 to 20 % of bovine lactoferrin is iron-saturated) (TÖPEL, 2004). Native bovine lactoferrin shows two denaturation temperatures: one peak appears at 65 °C for apo-bovine lactoferrin, and a second peak exists at 92 °C for iron-saturated lactoferrin. The binding of iron apparently stabilizes the protein structure against heat. A study on heat processing of lactoferrin, however, showed that just 15 seconds at 60 °C lead to a denaturation of 6 % of native bovine lactoferrin (PAULSSON et al., 1993).

5.1.1.4 Colostral Enzymes

Enzymes are known to catalyze almost 4,000 biochemical reactions (BAIROCH, 2000). Many metabolic pathways could not exist independently of them or would not occur at significant rates. This enzymatic reaction rate can be increased through a temperature rise. Temperatures between 50° C and 90 °C, however, initiate protein denaturation and enzyme inactivation (TÖPEL, 2004). The sensitivity of enzymes to the impact of heat varies depending on their chemical structure.

To date, the activity of more than 60 enzymes in lacteal secretions has been demonstrated (TÖPEL, 2004). These enzymes are either produced by the tissues of the mammary gland (original milk enzymes), released from somatic cells or originate from microorganisms (TÖPEL, 2004). To list all colostral enzymes and their denaturation temperatures would go far beyond the scope of this trial. Therefore, the following text will only address lysozyme and lactoperoxidase, which are considered to be of particular interest (CLARE et al., 2003; ZIMECKI and KRUZEL, 2007). Both lysozyme and lactoperoxidase require cooperative action with lactoferrin in combating bacteria (ZIMECKI and ARTYM, 2005).

Lactoperoxidase

Lactoperoxidase, a glycoprotein containing a heme group, is present in excess in bovine milk (BJÖRCK et al., 1975) and - with up to 0.06 g/l - exceeds the content of any other original milk enzyme (TÖPEL, 2004). In combination with certain co-factors (KUSSENDRAGER and VAN HOOIJDONK, 2000), the enzyme forms a potent antimicrobial system (the so-called lactoperoxidase system). Lactoperoxidase's ability to reduce hydrogen peroxide and to oxidize a wide variety of suitable electron donors like thiocyanate results in compounds that inhibit the growth, oxygen uptake, and acid production of certain bacteria (HOGG and JAGO, 1970). Gram-positive bacteria such as streptococci are simply inhibited for a certain period of time (STEELE and MORRISON, 1969), while Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas* spp., and *Salmonella* spp. are killed (BJÖRCK et al., 1975; REITER et al., 1976; MARSHALL and REITER, 1980) by the lactoperoxidase system in the abomasum of the calf (REITER et al., 1980). Lactoperoxidase also exhibits antiviral activities, as well as immunomodulatory effects (SHIN et al., 2005).

The enzyme is a particularly stable protein capable of maintaining catalysis and structural integrity up to a high temperature before it undergoes irreversible unfolding with concomitant disruption of the catalytic heme pocket and activity loss at 70 °C (BOSCOLO et al. 2007).

Lysozyme

Lysozyme (1,4-β-N-acetylmuraminidase) is present in various biological fluids. While cow's milk contains an average of 0.1 mg/l (SHAHANI, 1966), the lysozyme concentration in colostrum ranges from 6 to 40 mg/l (WENDT et al., 1994).

Between 70 and 80 % of the total body lysozyme is either within or is released from leukocytes (FINCH et al., 1974, cited by WEAVER and KROGER, 1978). Therefore, lysozyme belongs to the non-specific defense mechanisms (GRÜN, 1985) of the body and plays a significant role in the inherent antibacterial activity (VAKIL et al., 1969) of lacteal secretions. Its bactericidal effects are based on its ability to catalyze hydrolysis of the β-glycosidic bond between muramic acid and glycosamine of glycopolysaccharides of the bacterial cell wall (VAKIL et al., 1969). Bovine lysozyme is capable of lysing Gram-positive, as well as certain Gram-negative bacteria (VAKIL et al., 1969; PELLEGRINI et al., 1991). Via colostrum, lysozyme is transferred into the gastrointestinal tract of the newborn calf where it sustains its antibacterial functions providing local resistance against infections.

Previous research has shown that heat treatment of milk at temperatures above 60 °C leads to a decrease in the activity of lysozyme (WEAVER and KROGER, 1978).

5.1.2 The Role of Milk Sugar

Lactose is a disaccharide that consists of β-D-galactose and α/β-D-glucose fragments bonded through a β1-4 glycosidic linkage. The synthesis of lactose takes place in the cystosol of the epithelial cells surrounding the alveoli of the mammary gland. The mean percentage of lactose in colostrum is only 2.7 %, whereas mature milk contains about 5 % lactose (FOLEY and OTTERBY, 1978) which equals 45 to 52 g/l. The monosaccharides glucose and galactose are found at concentrations of 50 to 60 mg/l and 20 mg/l, respectively (TÖPEL, 2004). Additionally, small amounts of other carbohydrates occur, partly in a free form and partly bound to proteins,

lipids, or phosphate (WONG et al., 1999). Approximately 26 % of the energy in cow's milk is represented by lactose (JACKSON, 2003), which is the principle carbohydrate in milk. Lactate, which is made out of lactose in the gut, increases the rate of calcium absorption and stabilizes the *Lactobacillus* flora in the intestinal tract.

Only when severely heated does lactose undergo changes like mutarotation, isomerization, and the formation of volatile compounds (FOX and MCSWEENEY, 1998).

5.1.3 The Purpose of Milk Fat

Fat is the main energy providing component of milk. The mean percentage of fat in colostrum is 6.7 %, compared to 4.0 % in mature milk (FOLEY and OTTERBY, 1978).

Milk fat is very easy to digest since it is emulsified with droplet sizes of 0.1 to 10 μm in diameter. Those droplets consist of fat cores, which are surrounded by fat globule membranes. Approximately 95 to 98 % of the total milk fat is in these fat cores, 0.5 to 1 % in the globule membranes, and the rest in milk serum (HUANG and KUKSIS, 1967). The fat cores mainly contain triglycerides and no phospholipids at all. Also, 56 to 79 % of the membrane lipids consist of triglycerides (HUANG and KUKSIS, 1967), while diglycerides and monoglycerides occur only in traces (see Table 5). The other 21 to 44 % of the membrane lipids are phospholipids, which mostly consist of a diglyceride, a phosphate group, and a simple organic molecule. One exception to this rule is sphingomyelin, which is derived from sphingosine instead of glycerol. The group of phospholipids comprises phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, phosphatidylinositol, phosphatidylserine, and lysophospholipids (PATTON and JENSEN, 1976, cited by WONG et al., 1999).

Cerebrosides and gangliosides belong to the group of glycolipids. They are carbohydrate-attached lipids of the cell plasma membrane and serve as markers for cellular recognition and cell-to-cell communication by extending from the phospholipid bilayer into the aqueous environment outside the cell. Steroids like cholesterol, as well as carotenoids like β-carotene are part of the group of isoprenoids. Free fatty acids act as specific protective structures (TÖPEL, 2004).

Table 5: Lipid groups in bovine milk (WALSTRA and JENNESS, 1984, cited by TÖPEL, 2004; HUI, 1993, cited by TÖPEL, 2004).

Lipid Group	**Concentration in Milk (Percent of Total Lipid)**
Triglycerides	98.3
Diglycerides	0.3
Monoglycerides	0.03
Phospholipids	0.8
Steroids	0.3
Free fatty acids	0.1
Cerebrosides	0.1
Gangliosides	0.01
Carotenoids and Vitamin A	0.002

Proteins of the milk fat globule membrane start to denature at temperatures above 70 °C. This damage to the milk fat globule membrane leads to the formation of free (non-globular) fat. However, of milk's principal constituents, lipids are the least affected by heat (FOX and MCSWEENEY, 1998).

5.1.4 The Value of Macrominerals and Micronutrients

The mineral status of newborn calves depends not only on the placental mineral transfer from the dam to the calf during gestation, but on the intake of minerals from colostrum. Blood values of phosphorus, calcium, magnesium, potassium, sodium, and zinc are equally high in newborn calves and their dams (STEINHARDT et al., 1993). The transplacental passage of some trace elements like copper (STEINHARDT et al., 1993), selenium (HIDIROGLOU, 1980), or iron (TENNANT et al., 1975), however, is reduced which results in low tissue and blood concentrations in the offspring. This creates a prenatal iron deficiency, leads to a considerable drop in the plasma iron concentration during the first few hours of life (BOSTEDT et al., 1990), and subsequently causes anemia in neonatal dairy calves (TENNANT et al., 1975).

The rapid growth of calves during their first weeks of life requires additional selenium. Since parenteral administration of selenium does not significantly affect blood selenium concentrations in the calf until 10 weeks of age (KINCAID and HODGSON, 1989), colostrum and milk are the only selenium sources in the early postnatal period (SLAVIK et al., 2008).

Table 6: Concentrations of macrominerals in mature milk (TÖPEL, 2004) and colostrum (KEHOE et al., 2007).

Macromineral	Milk (g/kg)	Colostrum (g/kg)
Sodium	0.5	1.1
Potassium	1.5	2.8
Calcium	1.2	4.7
Magnesium	0.1	0.7
Chloride	1.1	not reported
Phosphate	2.1	not reported

Colostral concentrations of calcium, phosphorus, magnesium, sodium, iron, zinc, copper, and manganese are highest at parturition and decrease rapidly by 24 hours postpartum (KUME and TANABE, 1993). Macrominerals and micronutrients in colostrum occur at two- to almost twentyfold of the levels of normal milk (see Table 6 and Table 7).

Table 7: Concentrations of certain trace elements in mature milk (TÖPEL, 2004) and colostrum (KEHOE et al., 2007; SLAVIK et al., 2008).

Trace Element	Milk (mg/l)	Colostrum (mg/kg)
Zinc	4	38
Manganese	0.03	0.1
Iron	0.3	5.3
Copper	0.1	0.3
Selenium	0.01	0.03

5.1.5 The Importance of Vitamins

The neonatal calf has no reserves of vitamins A, D, and E since they do not cross the placenta in considerable amounts (QUIGLEY and DREWRY, 1998). While vitamin K is synthesized by microorganisms in the gastrointestinal tract (TÖPEL, 2004), the neonate is dependent on colostrum to obtain retinol, calciferol, and α-tocopherol postpartum (QUIGLEY and DREWRY, 1998). With its elevated fat content, bovine colostrum contains relatively high levels of these fat-soluble vitamins (HENRY and KON, 1937; SCHOTTSTEDT et al., 2005; KEHOE et al., 2007). Colostrum intake within the first 24 hours of life, however, is required for an adequate retinol and α-tocopherol status since the efficiency of the absorption of fat-soluble vitamins is reduced after the first day of life (BLUM et al., 1997). Since vitamins A, D, E, and K are practically not altered through heat treatment (OTTAWAY, 2002; AGROSCOPE), the following text will focus on water-soluble vitamins.

Vitamin B 2 (TÖPEL, 2004), pantothenic acid, biotin, and niacin are relatively heat-stable (OTTAWAY, 2002; AGROSCOPE), whereas losses for vitamins B 1, B 6, B 12, folic acid, and vitamin C are known to occur (OTTAWAY, 2002) through heat treatment. While at a later stage of life, the ruminal microflora synthesizes vitamins B 1, B 6, B 12, and folic acid (TÖPEL, 2004), and after 4 months of age dairy calves also produce endogenous vitamin C (WEGGER and MOUSTGAARD, 1982, cited by CUMMINS and BRUNNER, 1989), colostrum and milk are the only exogenous sources for these vitamins in early neonatal life.

Table 8: Percental vitamin losses in milk due to boiling or pasteurization (VITAMINE, 2002).

Vitamin	Pasteurization	Boiling
B 1	5 %	5 – 10 %
B 6	0 %	< 5 %
Folic Acid	< 5 %	5 %
B 12	0 – 10 %	5 – 20 %
C	5 – 25 %	5 – 50 %

While vitamin losses caused by boiling and pasteurization of milk have been published (see Table 8), no data exists yet on the time-temperature combination of 60 °C for 60 minutes. Gentle heat processing, however, should lead to a minor vitamin reduction only.

5.1.5.1 Vitamin-binding Proteins

Milk contains several proteins that bind vitamins, especially vitamin A, D, B 2, B 12, and folic acid (TÖPEL, 2004). Folate-binding protein, for example, normally binds half of the body's folic acid. This protects folate from folate-consuming bacteria, which need the vitamin in its free form. Since bacteria can not synthezise the vitamin, bacterial growth is hence hampered. The result is a bacteriostatic effect (TÖPEL, 2004). This phenomenon is revoked through heat processing of milk since the concentration of folate-binding protein is reduced, and the folate-binding capacity altered (WIGERTZ et al., 1996). Therefore, heat treatment, even at the time-temperature combination used in this trial, might negatively influence the bioavailability of milk folates and other vitamins.

5.1.6 The Relevance of Hormones

Based on their chemical composition, hormones can be divided into three classes: peptide hormones, amine-derived hormones, and steroid hormones. After their synthesis in various endocrine glands, all endogenous hormones are released into the circulation and can subsequently be found in lacteal secretions due to the penetrability of the blood-milk-barrier (TÖPEL, 2004).

While amine-derived hormones (like thyroid hormones) and steroid hormones (like estrogen, glucocorticoids or mineralocorticoids) are effective after oral administion, peptide hormones are mostly enzymatically decomposed by peptidases in the gastrointestinal tract before they can induce their typical effects.

The exposure to heat as during colostral heat treatment may result in constitutional changes and a decrease in biological activities of especially peptide hormones (like insulin, prolactin, and somatotropin) and growth factors. Further trials would be needed to show which hormones exactly are altered through the time-temperature combination used in this study.

5.1.7 The Significance of Maternal Leukocytes in Colostrum

Aside from antibodies, maternal immunity is transferred to the offspring through vital colostral leukocytes. These maternal cells represent a major part of the neonatal health protection during the first weeks of life (DAVIS and DRACKLEY, 1998) by passively shielding the neonate against ubiquitous disease causing agents to which the mother has previously been exposed (KELLEHER and LÖNNERDAL, 2001). It has been shown, for example, that colostral leukocytes drastically lessen fecal shedding of enteropathogenic *Escherichia coli* and shorten the period of pathogen detectability (RIEDEL-CASPARI, 1993). Additionally, maternal leukocytes seem to modulate the neonatal immune response by reducing the reactivity of neonatal cells to maternal alloantigen (an antigen existing in alternative (allelic) forms in a species, thus inducing an immune response when one form is transferred to members of the species who lack it) (REBER et al., 2005).

In general, leukocytes can be differentiated into two categories: The group of agranulocytes or mononuclear leukocytes, which includes lymphocytes, monocytes, and macrophages, and the group of granulocytes or polymorphonuclear leukocytes, which comprises neutrophils, eosinophils, and basophils. While normal bovine milk contains 61 % macrophages, 20 to 30 % lymphocytes, 5 to 20 % polymorphonuclear leukocytes, and very low levels of epithelial cells (BOUTINAUD and JAMMES, 2002, cited by SCHAEREN, 2008), data on the exact cell distribution in bovine colostrum are hard to find. Precise information exists only on the colostral composition of the mononuclear leukocyte subpopulation, which consists of 11 % B lymphocytes, about 16 % T lymphocytes, and about 69 % macrophages (PARK et al., 1992). Polymorphonuclear leukocytes pose the prevailing cell type in bovine colostrum (LEE et al., 1980) though.

The latter cells are phagocytes, which internalize and kill microbes (PAAPE et al., 2000). To perform their particular task, cell surface receptors on these leukocytes bind bacteria that have been opsonized by immunoglobulins or complement factors (PAAPE et al., 2000). The foreign particle is then surrounded by pseudopods and pulled into the interior of the cell. This creates a phagosome into which reactive oxygen species and hydrolytic enzymes are secreted. The damage to the body's own tissue through the release of these granular contents is minimized by macrophages, which quickly phagocytose apoptotic polymorphonuclear leukocytes (PAAPE et

al., 2003). In contrast to polymorphonuclear leukocytes, which only live for hours or a few days, the life span of macrophages can be several months.

Macrophages are involved in the non-specific defense through their ability to engulf and digest pathogens and in the specific defense through stimulating lymphocytes to respond to a pathogen. After digestion of a foreign particle, macrophages present the antigen by integrating it into their cell membrane. This stimulates helper T cells to proliferate and causes B lymphocytes to differentiate into plasmacytes that produce specific antibodies. While B lymphocytes elicit humoral immune response, T lymphocytes trigger cellular immune responses within the mammary gland and in its secretions (LARSON et al., 1980). After absorption from colostrum these macrophages may sustain their biological functions in the circulation of the neonate.

Leukocytes in their entirety and some epithelial cells from the mammary ducts (TSENKOVA et al., 2001) contribute to the somatic cell count. Bovine colostrum has an average somatic cell count of 2.46 million cells per ml (ANDREW, 2001), which declines rapidly after parturition. By five days postpartum the somatic cell count should be below 300,000 cells/ml (DEYOUNG, 1982, cited by RENEAU, 1986) and eventually, in normal milk, it should range from 12,000 to 151,000 cells/ml (LEITNER et al., 2000).

Studies have proven that the uptake of intact colostral leukocytes through the intestinal barrier into the circulation of the newborn calf is possible (RIEDEL-CASPARI and SCHMIDT, 1990), and that it occurs preferentially through the follicle-associated epithelium of jejunal and ileal Peyer's patches (LIEBLER-TENORIO et al., 2002). This transfer prevents the drop of lymphocyte counts in the neonate's blood (RIEDEL-CASPARI and SCHMIDT, 1991a) and increases the percentages of phagocytizing polymorphonuclear leukocytes and monocytes (MENGE et al., 1999).

Additionally, the ingestion of maternal leukocytes improves the activation of neonatal lymphocytes and - through the elevation of the proliferation rate of mononuclear cells (RIEDEL-CASPARI and SCHMIDT, 1991a) - accelerates their formation (REBER et al., 2008). This is caused by an increase in the expression of the cellular markers CD 25 (a receptor for interleukin-2, which is needed for T lymphocyte growth) and CD 26 (a membrane-bound protease and T cell costimulatory molecule) on the surface of neonatal lymphocytes (REBER et al., 2008), which occurs after the uptake of vital maternal lymphocytes.

Then, the development of the neonatal immune system is stimulated through the transfer of colostral maternal leukocytes (REBER et al., 2005). The antigen-presenting capacity is enhanced (REBER et al., 2005) through the expression of higher levels of major histocompatibility complex class I (REBER et al., 2008) and II (MENGE et al., 1999) molecules on the neonate's lymphocyte surfaces. These proteins are responsible for the antigen presentation at the cell surface. Their subsequent recognition by T lymphocytes (MORRIS et al., 2004) leads to an intensified T helper cell-dependent formation of antibodies (RIEDEL-CASPARI and SCHMIDT, 1991a).

Moreover, colostral leukocytes enhance the passive immunity of newborn calves by elevating the early postnatal blood concentrations of IgG, IgM, and IgA antibodies (RIEDEL-CASPARI and SCHMIDT, 1991b). This finding supports the hypothesis that phagocytic cells may be involved in the transportation of certain immunoglobulins into the neonate (RIEDEL-CASPARI and SCHMIDT, 1990).

Finally, it has been shown that colostrum activates the phagocytic activity of polymorphonuclear leukocytes and thereby influences the development of the non-specific immune system of newborn calves (SUGISAWA et al., 2001). Bacteriocidal activity of whole blood was significantly higher in calves that received maternal leukocytes via colostrum (RIEDEL-CASPARI et al., 1991) which compensates the fact that some blood phagocyte functions are evidently not fully developed at birth (MENGE et al., 1998).

It is commonly expected that processing of colostrum, like heat treatment (JOHNSON et al., 2007) or freezing (GOLDMAN, 1977), kills living leukocytes or has at least serious effects on their biological activities (LAKRITZ et al., 2000). A current study, however, showed that at the age of 24 hours there was no significant difference in total leukocyte counts, neutrophil or lymphocyte counts between calves that had previously received heat-treated versus raw colostrum (JOHNSON et al., 2007). Heat treatment of colostrum at 60° C for 60 minutes did apparently neither alter the absorption of colostral cells nor their biological functions (JOHNSON et al., 2007).

5.2 MATERIALS AND METHODS

5.2.1 Monitoring of Calf Health, Weight Measuring Methods, Determination of Animal Performance, and Record Keeping

During the study period, which ranged from January to June 2007, the health of the study animals was monitored daily by personnel responsible for the calf barn. Treatments that were performed on sick animals among the 97 study calves were recorded. Health data in this study included information on the nature of the disease, the date of the initial treatment, the number of overall treatments, the medication used, and the result of the treatment (recovered or died).

At housing change, which occurred at an average age of 55 days (ranging from 37 to 79 days depending on the need of space for younger replacement calves), the study animals were measured with a dairy calf weight tape. One animal out of each treatment group had died previous to weight taping so that data on weight gain could only be obtained for 95 study calves: 57 animals that had received heat-treated and 38 animals that had been fed raw colostrum, respectively.

Additionally, the dairy had installed a livestock scale in the midst of this study that was used to measure the exact birth weights of the remainder of the enrolled calves (n = 58), which consisted of 38 calves that were assigned to receive unprocessed and 20 calves that were designated to receive heat-treated colostrum, respectively. All obtained weights were noted in pounds, but converted into kilograms for further calculation.

On May 29, 2009 - approximately two years after the study had been conducted - additional data on the fate, health, and performance of the former study animals was taken out of the Sunnyside dairy cow file using DairyCOMP 305 (Valley Agricultural Software, 3950 South K Street, Tulare, California 93274). At this point 63 animals were still on the study farm: 24 animals that had been members of the raw colostrum group and 39 animals that had been members of the heat-treated colostrum group, respectively.

5.2.2 Data Analysis

Health Data

Morbidity and mortality rates were calculated in percent for the two treatment groups. Two-sided Fisher's exact tests were performed to determine the significance of the differences in morbidity and mortality rates between the two treatment groups, and to determine the significance of colostral heat treatment on calf health in general. Other 2-way (age/health, treatment/health, age/treatment) and 3-way (age/health/treatment) interactions were also checked with the help of the statistical program package of SAS (Statistical Analysis System, Version 9.2, SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513).

Least squares means of serum TP values were calculated using SAS to eliminate the influence of calf age at the time of blood sampling. Least squares means of the TP concentrations of healthy and sick calves within the individual treatment groups were created as well.

Unpaired two-tailed t-tests were performed to determine the significance of the difference of the least squares means of the TP concentrations between sick and healthy calves within the individual treatment groups.

Weight Data

The difference between the tape measured calf weight at housing change and the exact birth weight was divided by the particular number of days of age at tape measuring to calculate the body weight gain per day. If the exact birth weight had not been measured, the mean birth weight (of all calves that had been weighed at birth) was used for this calculation. Unpaired two-tailed t-tests were performed to analyze the statistical significance of the difference between both treatment groups. Additionally, correlation coefficients were calculated to evaluate possible linear associations between calves' blood serum total protein values and birth weights and daily weight gains, respectively.

Performance Data

Two-tailed Fisher's exact tests and unpaired two-tailed t-tests were performed to evaluate the data.

5.3 RESULTS

5.3.1 Health Data

Health records showed that 4 out of the 39 calves that had received raw colostrum, and 15 out of the 58 calves that had been fed heat-treated colostrum at first feeding became ill during the study period. All in all, a total of 20 % of the enrolled calves had to be medically treated due to respiratory problems, arthritis, or otitis. Obviously, diarrhea was not recorded by the farm personnel. Looking at the individual treatment groups, preweaning morbidity rates of 10 % (in the raw colostrum group) versus 26 % (in the heat-treated colostrum group) occured. The two-sided Fisher's exact test, however, showed a p-value of 0.07 for the effect of colostral heat treatment on calf health which is considered not significant. Other 2-way (age/health, treatment/health, age/treatment) and 3-way (age/health/treatment) interactions also proved not to be significant and were taken out of the analysis.

The previously measured serum total protein concentrations (4.3.1) of the 4 calves that became sick within the raw treatment group showed that 3 of these calves had actually reached TP values characterizing successful passive immune transfer, while the serum TP concentration of just 1 calf indicated failure of passive transfer (see Table 9). Yet, the only animal in this treatment group that died during the study period had had a TP concentration of 56 g/l at the time of blood sampling which actually represents successful passive immune transfer.

Table 9: Detailed information on the 4 sick calves within the raw treatment group.

Calf ID No.	Initially Fed with Raw Colostrum Batch No.	Observed Health Problem and Outcome	Previously Measured TP Concentration (g/l)
11857	II	Pneumonia; Died	56
11861	II	Pneumonia; Recovered	60
11869	III	Otitis; Recovered	43
11999	X	Pneumonia; Recovered	66

Least squares means of the total protein concentrations of healthy versus sick calves within the raw colostrum group were 56.3 g/l versus 56.6 g/l. The unpaired two-tailed t-test categorized this difference as not significant (p = 0.97).

Table 10: Detailed information on the 15 sick calves within the heat-treated treatment group.

Calf ID No.	Initially Fed with Heat-Treated Colostrum Batch No.	Observed Health Problem and Outcome	Previously Measured TP Concentration (g/l)
11671	9	Otitis, BVD; Died	49
11673	10	Pneumonia; Recovered	51
11679	11	Pneumonia, BVD; Recovered	63
11680	11	Pneumonia; Recovered	61
11697	13	Pneumonia; Recovered	56
11726	16	Otitis; Recovered	54
11729	16	Otitis; Recovered	49
11730	16	Arthritis; Died	58
11742	17	Pneumonia; Recovered	64
11743	17	Pneumonia; Recovered	56
11823	25	Pneumonia; Recovered	63
11827	25	Pneumonia; Recovered	62
11876	26	Otitis; Died	56
11877	26	Health Problem not recorded; Recovered	52
11878	26	Otitis; Recovered	60

The 15 calves out of the heat-treated colostrum group that faced health problems showed serum total protein concentrations ranging from 49 to 64 g/l (see Table 10). TP values of 13 of these calves were within the range of successful passive transfer, while 2 animals had TP concentrations of 49 g/l each, accounting for moderately successful passive transfer.

A total of 3 diseased calves out of this treatment group died during the study period: the first calf died because of otitis, the second calf was euthanized since it had a severe otitis and was confirmed to be a BVD PI animal, and the third calf was euthanized due to an incurable necrotic toe infection. Serum TP levels at the time of blood collection had been 56, 49, and 58 g/l for these animals (see Table 10) indicating at least moderately successful passive immune transfer.

Least squares means of the serum total protein concentrations of healthy versus sick calves within the heat-treated colostrum group were 59.7 g/l versus 56.4 g/l. The unpaired two-tailed t-test, however, revealed that this difference was not significant ($p = 0.09$), but a numeric trend only.

During the study period the mortality rate was 3 % (1 out of 39 calves) for the raw colostrum group and 5 % (3 out of 58 calves) for the heat-treated colostrum group, respectively. According to the two-sided Fisher's exact test this difference was not significant ($p = 0.65$).

The mean age at first medical treatment was 28 days for calves within the raw treatment group versus 30 days for calves within the heat-treated treatment group. While in the latter group 2 calves (ID nos. 11729 and 11730) had to be treated three times for the same problem, all 4 sick calves in the raw treatment group were only treated once.

5.3.2 Weight Data

Exact birth weights could be obtained through the use of an electronic scale for 20 of the 58 calves within the heat-treated colostrum group and for 37 of the 39 calves within the raw colostrum group. The obtained birth weights were 42.8 ± 5.3 kg (mean ± SD) for calves within the heat-treated and 42.7 ± 6.7 kg for calves within the raw group, respectively. An unpaired two-tailed t-test confirmed that the difference between the mean initial live weights of calves of the two treatment groups was not significant ($p = 0.95$). Neither was there a significant difference ($p = 0.96$) between the mean birth weights of the 34 healthy (42.7 ± 6.8 kg) versus the 3 sick (42.5 ± 7.0 kg) calves within the raw group, nor a significant difference betweeen the mean birth weights of the 15 healthy (43.2 ± 5.7 kg) versus the 5 sick (41.9 ± 4.1 kg) calves within the heat-treated group ($p = 0.65$). Also, no difference existed between the mean birth weights of all

calves that either stayed healthy (42.8 ± 6.4 kg) or became sick (42.1 ± 4.9 kg) in the course of this study ($p = 0.76$).

The subsequent calculation of the mean daily weight gains for these members of the two treatment groups produced the following results: Calves fed raw colostrum at first feeding had an average weight gain of 0.79 ± 0.12 kg (mean ± SD) per day, while calves fed heat-treated colostrum gained 0.87 ± 0.12 kg per day. An unpaired two-tailed t-test showed that the difference in the average daily weight gains between the two treatment groups was significant ($p = 0.02$). However, mean days of age at tape measuring were 53 for calves within the raw versus 63 for calves within the heat-treated colostrum group – a huge difference which does not allow for such a comparison.

Next, the healthy calves of both treatment groups were compared since their age difference was smaller. While the mean age at tape measuring had been 51 days for healthy calves within the raw group, it had been 56 days for healthy calves within the heat-treated group. The 34 healthy calves within the raw colostrum group gained 0.80 ± 0.11 kg (mean ± SD) per day on average, whereas it was 0.87 ± 0.13 kg for the 15 healthy calves of the heat-treated colostrum group. Again, this difference would have been significant ($p = 0.05$), but might have been biased by the incongruity of age at tape measuring.

The mean age at tape measuring of the sick calves allowed for a direct comparison since it was 70 days for each treatment group. The 3 sick calves in the raw colostrum group (the fourth sick animal had died before weight tape measuring) showed average daily weight gains of 0.68 ± 0.16 kg (mean ± SD), while it was 0.87 ± 0.13 kg for the 5 sick animals in the heat-treated colostrum group. This difference was not significant ($p = 0.1$) because of the fact that the number of observations was far too small.

The performed linear regression tests demonstrated that no significant relationship existed between serum total protein concentration and birth weight ($R^2 = 0.06$) or between serum total protein concentration and average daily weight gain ($R^2 = 0.04$).

5.3.3 Performance Data

At the age of 23 to 27 months, 24 of the 39 (62 %) animals of the raw colostrum group were still on the study farm. Fifteen animals were gone: 9 heifers had been sold for dairy, 3 animals had been sold because of low productivity and reproduction problems, 1 heifer had been sold for being sick, 1 animal had died of unknown cause as an adult, and 1 animal had already died as a calf during the study period. Taking only those animals into account that had been recorded dead by the dairy, the overall mortality rate for the raw colostrum group was 5 % (2 out of 39 animals) as of May 29, 2009.

Regarding the heat-treated colostrum group, 39 out of 58 (67 %) animals were still on the study farm. Nineteen animals had left the dairy: 8 had been sold because of low productivity and reproduction problems, 5 had been sold for being sick, 1 had been sold for being injured, 2 had died as adults (1 from mastitis and 1 from an injury), and 3 had already died as calves during the study period. Considering only those animals that had been recorded dead by the dairy, the overall mortality rate for the heat-treated colostrum group was 9 % (5 out of 58 animals) as of May 29, 2009.

The two-sided Fisher's exact test rated the difference between the overall mortality rates of the two treatment groups as not significant ($p = 0.7$).

Table 11: Reproduction data on the remaining animals of both treatment groups.

	Raw TRT Group	Heat-treated TRT Group	P-Value
Mean Number of Times Bred during 1st Lactation	2.1	1.8	0.63
Mean Number of Days Open	65	78	0.14
Mean Age at Freshening in Days	694	706	0.09
Mean Mature Equivalent 305 in Pounds	23,370	24,567	0.25
Mean Days in Milk	66	96	0.007

According to unpaired two-tailed t-tests, there was no significant difference in the mean number of times bred, the mean number of days open, the mean age at freshening, and the mean mature equivalent 305 (the 305-day mature equivalent term adjusts the cow's current production record for age and stage of lactation) between treatment groups (see Table 11). The number of mean days in milk significantly differed between treatment groups ($p = 0.007$) though.

Table 12: Reproductive status of the remaining animals of both treatment groups.

Number of Animals (Percent)	Raw TRT Group	Heat-treated TRT Group	P-Value
First Time Pregnant	3 (12.5 %)	2 (5.1 %)	0.36
First Time Fresh	11 (45.8 %)	12 (30.8 %)	0.29
Rebred after First Calving	9 (37.5 %)	13 (33.3 %)	0.79
Confirmed Open after Rebreeding	-	2 (5.1 %)	0.52
Confirmed Second Time Pregnant	1 (4.2 %)	10 (25.6 %)	0.04
Lactating	21 (87.5 %)	37 (94.9 %)	0.36

The percentage of heifers that was pregnant for the first time was smaller among the heat-treated group, while the percentage of cows that was confirmed pregnant for the second time was significantly higher ($p = 0.04$) among the heat-treated group. Also, a higher percentage of animals among the heat-treated group was currently lactating (see Table 12).

Table 13: Health events of the remaining animals of both treatment groups.

	Raw TRT Group	Heat-treated TRT Group	P-Value
Number (Percentage) of Cows with Mastitis	9 (37.5 %)	3 (7.7 %)	0.006
Number of Total Mastitis Events	16	3	-
Number (Percentage) of DA Events	-	1 (2.6 %)	1.0
Number (Percentage) of Metritis Events	5 (20.8 %)	10 (25.6 %)	0.77
Mean Calving Ease	1.3	1.1	0.35

Interestingly, the percentage of cows with mastitis was significantly ($p = 0.006$) higher among the raw colostrum group. Also, the once affected cows seemed to develop mastitis repeatedly. There was no difference between treatment groups in terms of the development of metritis or abomasal displacement, or the mean calving ease (see Table 13).

5.4 DISCUSSION

5.4.1 Morbidity and Mortality Rates – Nationwide and on the Study Farm

According to a 2006 research project of the National Animal Disease Center more than 40 % of neonatal calves on U.S. dairy farms are either clinically ill or die during the neonatal period (USDA, 2006): While the morbidity rate for preweaned dairy calves in the U.S. is estimated at approximately 37 %, the mortality rate ranges between 8 and 11 % (USDA, 2006). These losses and the treatments of calves cost the industry 90 to 180 million dollars and approximately 1.3 million man-hours dedicated to the management of sick calves (USDA, 2006). Considering these facts, an overall morbidity rate of 20 % and an overall mortality rate of 4 % among preweaned dairy calves seem to be acceptable results for the study farm. (It should not be forgotten, though, that the morbidity rate at the study farm would have been higher had the cases of diarrhea been recorded.) Viewed individually, the mortality rate was 3 % for preweaned calves of the raw colostrum group and 5 % for preweaned calves of the heat-treated colostrum group. The two-tailed Fisher's exact test rated this difference as not significant ($p = 0.6$).

On May 29, 2009, mortality rates had risen to 5 % for the raw colostrum group and to 9 % for the heat-treated colostrum group, respectively. (These numbers are, however, only based on deaths recorded by the dairy. Whether additional animals died after leaving the dairy is unknown.) Based on these numbers, the two-sided Fisher's exact test rated the difference between the overall mortality rates of the two treatment groups as not significant ($p = 0.7$).

Morbidity rates in the preweaning period reached 10 % within the raw colostrum group, while the heat-treated colostrum group attained 26 %. This outcome is not significant ($p = 0.07$), but shows a numeric trend. It seems as if calves that had received raw colostrum at first feeding were more resistant against infection.

5.4.2 The Association between IgG/Serum Total Protein and Calf Health

As proven previously (see 4.3.3), calves that had received heat-treated colostrum at first feeding developed higher serum total protein concentrations. At the same time, however, the rate of animals that needed medical treatments during the preweaning period in this treatment group tended to be higher. How can this be explained?

In general, it is believed that lower perinatal IgG concentrations are significantly associated with higher morbidity and mortality rates during the preweaning period (DEWELL et al. 2006). Calves classified as having inadequate IgG concentrations are at greater risk of neonatal and preweaning morbidity, as well as preweaning mortality compared to calves classified as having adequate IgG concentrations at 24 hours of age (WITTUM and PERINO, 1995). The odds to get pneumonia are twice as high for calves with low IgG levels compared to calves with higher IgG concentrations (VIRTALA et al., 1999).

Regarding the occurrences, age of onset, and severity of pneumonia and septicemia, the concentration of serum total protein proved to be a significant risk factor (DONOVAN et al., 1998). Calves classified as having inadequate plasma protein concentrations 24 hours after birth have a greater risk of respiratory tract disease and overall morbidity compared to calves classified as having adequate plasma protein concentrations (WITTUM and PERINO, 1995). Also, low serum total protein is believed to be a significant risk factor for mortality (DONOVAN et al. 1998). The increase of total protein from 40 to 50 g/l leads to a dramatic decrease in mortality, the increase from 50 to 60 g/l only triggers a small improvement, while virtually no improvement in mortality rates is seen as total protein exceeds 60 g/l (DONOVAN et al. 1998). This increase of the risk of mortality associated with low levels of serum total protein is suggested to be evident through six months of age (DONOVAN et al. 1998).

Studies, however, showed that immunoglobulin levels were neither correlated with the viability of calves (LOMBA et al., 1978), nor was low serum total protein a significant risk factor for omphalitis or diarrhea (DONOVAN et al., 1998). The latter finding may be explained by the presence of the local immunity in the gastrointestinal tract that exists besides the systemic immunity and cannot be evaluated through the mere measurement of serum total protein.

5.4.3 Sick Calves despite a Successful Passive Immune Transfer?

Failure of passive transfer is a common occurrence on U.S. dairy farms (MAUNSELL et al., 1998) and is believed to lead to an increase in morbidity and mortality rates among dairy calves. It is supposed that failure of adequate passive transfer of colostral immunoglobulins occurs in more than 40 % of dairy heifer calves (MAUNSELL et al., 1998) and that ≥ 22 % of all calf losses in the U.S. could be avoided by preventing it (NDHEP, 1993; cited by MAUNSELL et al., 1998).

Among the calves that had received heat-treated colostrum, the healthy animals showed a higher serum total protein least squares mean (59.7 g/l) than the sick animals (56.4 g/l). Among the animals of the raw colostrum group, the total protein least squares mean for sick calves (56.6 g/l) slightly exceeded that of healthy calves (56.3 g/l). Looking at all 19 sick study calves, serum total protein values implied successful passive immune transfer in 16 cases and moderately successful passive transfer in 2 cases, while failure of passive transfer occurred in only 1 case. It may be concluded that adequate serum total protein concentrations are not sufficient to grant a satisfactory immune protection.

Although the main focus has always been on immunoglobulins as the major protective components of colostrum, additional factors have been found to play significant roles in the innate immunity of neonates.

5.4.4 Maternal Leukocytes – Important Protective Colostral Compounds?

Much effort has been put into research on maternal colostral leukocytes after the discovery of their ability to stimulate the development of the calf's immune system (RIEDEL-CASPARI and SCHMIDT, 1991a/b/c). Even though it would be expected that heat treatment inactivates living leukocytes, previous studies (JOHNSON et al., 2007) suggested that processing of colostrum at 60 °C for 60 minutes does not alter the biological functions of these colostral cells. However, neither the uptake of heat-treated nor raw colostrum resulted in significant increases of leukocyte counts in neonatal calves. Generally, no to very minor changes in leukocyte counts occurred during the first 24 hours of the calves' lives (JOHNSON et al., 2007).

This phenomenon could be explained with the fact that maternal colostral leukocytes from the neonate's own dam are selectively absorbed by the neonate, whereas colostral leukocytes from an animal other than the dam may not be absorbed (TUBOLY and BERNATH, 2002). It is possible that calves in both treatment groups failed to absorb colostral leukocytes because the assembled colostrum was from a pooled source that included cows other than the dam. Therefore, further studies need to be conducted to evaluate other possible protective colostral compounds.

5.4.5 The Influence of Heat Treatment on Other Colostral Components

The thermal processing of colostrum in this study should have affected neither the functions nor the contents of lactose or milk fat.

The situation is a little more complex when talking about milk proteins. It can be assumed that heat treatment did not affect the biological activities of the rather heat-labile colostral whey proteins since previous research has shown that temperatures of up to 60 °C only reversibly affect their solubility and foaming properties (DEWIT and KLARENBEEK, 1984). Casein is not expected to lose its biological activity through heat treatment at 60 °C for 60 minutes either. What happens to its peptides, however, remains to be elucidated. Colostral immunoglobulin concentrations should neither have been reduced at this time-temperature combination nor should the immunoglobulin activity have changed. It is not known, however, if heat processing altered the activity of lactoferrin, lysozyme, and other colostral enzymes.

The colostral concentrations of macrominerals (MILKFACTS, 2007) and micronutrients were not affected by heat treatment, while losses occurred for certain water-soluble vitamins (OTTAWAY, 2002) and vitamin-binding proteins (WIGERTZ et al., 1996). Furthermore, the impact of heat treatment on colostral hormones and growth factors is still unknown and requires additional research.

5.4.6 Did the Feeding of Certain Colostrum Batches lead to an Increase in Health Events?

Looking at Table 9 and Table 10, it catches one's eye that many of the diseased calves had received colostrum that came from the same batches. Therefore, the assumption that poor colostral quality or incorrect colostral heat treatment lead to an increase in morbidity rates stands to reason.

Among the batches that occur repeatedly on the list of sick calves of the heat-treated colostrum group, batches no. 17, no. 25, and no. 26 contained 20 CFU/ml each after heat processing, which were coagulase-negative staphylococci in all three cases. Batches nos. 11 and 16 showed no microbiological growth at 48 hours after plating and should therefore have been practically sterile when fed to the study calves.

The only batch that shows up twice on the list of diseased calves of the raw colostrum group was batch no. II, which contained 80 CFU/ml of environmental streptococci, 200 CFU/ml of coagulase-negative staphylococci, 100 CFU/ml of Gram-negative bacilli, and 20 CFU/ml of Gram-positive bacilli. With a total of 400 CFU/ml, batch no. II is still considered to have a low plate count (JAYARAO et al., 2004), which is unlikely to have caused health problems and - in one case - even the death of a study calf.

Since bacterial contamination cannot be responsible for the observed health events, the process of colostral heat-treating must be considered instead: Thermal processing must have altered the structure or function of biologically active colostral components or must have left other live pathogens besides these listed bacteria in colostrum.

Interestingly calves that received the same batches often developed analogous health problems. While calves that had received batches nos. II, 11, 17, and 25 had pneumonia, calves that had been fed batches nos. 16 and 26 got otitis. These findings, however, are more likely to be a consequence of housing arrangements (calves that were born around the same time were housed next to each other) and the season, or of prevailing weather conditions (most health events were recorded in February and March 2007), than of colostral processing.

5.4.7 Differences in Animal Health, Weight Gain, and Performance between Treatment Groups

Besides the fact that calves from the heat-treated colostrum group seemed to have a higher morbidity rate, health records indicated that diseased calves from the heat-treated group had to be medically treated multiple times, while sick calves from the raw treatment group had to be treated only once for their health problems.

Looking at the adult animals, however, the percentage of cows with mastitis was significantly ($p = 0.006$) higher among the raw colostrum group and the once affected cows seemed to develop mastitis repeatedly. No difference between the two treatment groups could be obtained in terms of the development of metritis or abomasal displacement (see Table 13).

Regarding animal performance, the reproductive status of animals within the heat-treated colostrum group tops that of the raw group (see Table 12). The percentage of animals that was confirmed pregnant for the second time ($p = 0.04$) was significantly higher within the heat-treated group as compared to the raw group. Also, the recorded difference in mean days in milk between the two treatment groups was significant ($p = 0.007$). These facts, however, can easily be explained by the age difference. On May 29, 2009, animals that had received heat-treated colostrum at first feeding had a mean age of 807 days, while animals that had been fed raw colostrum showed a mean age of 762 days.

The fact that the farm sold 9 heifers of the raw colostrum group versus none of the heifers of the heat-treated colostrum group for dairy doesn't seem to be random and raises the question of whether there has been a farm bias to sell study animals that received raw colostrum. The farm management, however, did not have any knowledge of the initial treatment allocation of the individual calves. Therefore, it must have been the physical development of animals that had received raw colostrum at first feeding that prompted the decision to sell them.

In terms of birth weight, exact weights did neither differ between treatment groups nor between animals that either got sick or stayed healthy in the course of this study. The study data imply that (healthy) calves fed heat-treated colostrum had significantly greater average daily

weight gains than (healthy) calves fed raw colostrum at first feeding. These results, however, might have been biased by the incongruity of age at tape measuring (see 5.3.2) and need to be re-evaluated in further studies. It could also not be reliably clarified if sick animals had lower average daily weight gains than healthy animals.

5.5 STUDY FLAWS

The collected health and weight data must be regarded with caution. The researcher had tape measured animals whenever the working schedule allowed for a visit at the study farm. The 10-day difference of mean calf age at tape measuring between the two treatment groups had not become obvious before data analysis had been performed. Tighter windows should have been created or an exact age at weight tape measuring fixed. By mistake, the researcher had assumed that through various irregular visits the age distribution would have been evened out. Regarding the assembled health data of calves, farm workers apparently did not pay attention to scouring events. Morbidity rates would probably have been much higher if enteric diseases had been recorded.

The lactation number of the dam is known to be a major factor for alterations in colostral components. To prevent fluctuations within the colostrum batches, all batches should have been analyzed for their individual contents. Even though pooled colostrum was fed in the current study, lactation numbers might have been different for batches created for the two treatment groups. It would have been better if the same basic raw material had been fed as raw or as heat-treated. If all colostral components had been quantified prior to and after heat processing, definitive statements could have been made on the eventual reduction of each component through the used time-temperature combination. Still, it would have been hard to prove if biological activities of compounds had been altered through the heat treatment of colostrum. It remains the task of other studies to cast a light on this.

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6 CONCLUSION

I conclude from the data presented herein that on-farm heat treatment of colostrum at 60 °C for 60 minutes leads to a significant reduction of bacteria counts ($p = 0.0002$) compared to the raw product (see 3.3.3). The trend towards colostral heat treatment has grown throughout the last years: While in 2002 (NAHMS, 2002) 0.4 % of small, 0.8 % of medium, and 3.6 % of large operations pasteurized colostrum, the percentages shifted to 0.2 % of small, 0.9 % of medium, and 6.4 % of large operations in 2007 (NAHMS, 2007). The overall percentage of dairy farms in the U.S. that pasteurize colostrum prior to feeding is still low, but grew from 0.6 % in 2002 to 0.8 % in 2007 (NAHMS, 2002; NAHMS, 2007). For a long time dairies have been slow to adopt this management practice due to the inherent technical issues in pasteurization (NAHMS, 2007) that led to changes in viscosity and IgG concentration. Heat treatment at the time-temperature combination of 60° C for 60 minutes, however, has shown not to affect colostral viscosity, IgG concentration, or immunoglobulin activity (MCMARTIN et al., 2006).

As a matter of fact, feeding heat-treated versus raw colostrum created a significantly higher ($p = 0.04$) mean serum total protein concentration in neonatal calves (see 4.3.3) since the immunoglobulin absorption was no longer hindered by excess bacteria in colostrum. Controversially and contradictory to other studies (DONAHUE et al. 2008), morbidity and mortality rates tended to be higher for calves that had received heat-treated versus raw colostrum at first feeding (see 5.4.1) in this study. Heat denaturation of essential nutrients and biofunctional colostral constituents other than immunoglobulins might account for these findings. Surprisingly, calf mortality cannot be predicted through the mere assessment of serum total protein concentrations (REA et al., 1996). Even though calves with total protein concentrations below 45 g/l are more likely to die, failure of passive transfer does not inevitably stand for a higher risk of mortality (REA et al., 1996). Hypogammaglobulinaemia can be a common finding amongst normal calves (FEY and MARGADANT, 1961). In general, though, failure of passive transfer is associated with an increase of morbidity and mortality rates in dairy calves (NOCEK et al., 1984).

While in the 1996 study of the National Animal Health Monitoring System the percentage of dairy heifers that died prior to weaning was as high as 10.8 %, it dropped to 8.7 % in 2002, and reached 7.8 % in 2007 (NAHMS, 1996; NAHMS, 2002; NAHMS, 2007). In this study the overall preweaning mortality rate was only 4 %. The nationwide preweaning morbidity rate is estimated to be around 37 % (USDA, 2006). The overall preweaning morbidity rate amongst the study calves was only 20 % - events of diarrhea had not been recorded for both treatment groups though. The observed differences of mortality and morbidity rates between treatment groups were not found to be significant (see 5.4.1).

Finally it is to say that even though the effect of colostral heat treatment on animal health did not prove to be significant, thermal processing of colostrum is at least a simple way to minimize colostral bacterial counts and to reduce the chances of spreading pathogens throughout the future adult herd. Pasteurization of colostrum creates a safe calf feed, paves the way for the uptake of sufficient immunoglobulins, and reduces failure of passive transfer in neonatal calves possibly by diminishing the number of colostral bacteria.

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7 SUMMARY

Tina W. Rebelein (2010)

"The Effect of Heat Treatment on Microbiological Qualities of Bovine Colostrum, Passive Immune Transfer of Neonatal Calves, and Future Animal Performance."

While the importance of timing, volume and quality of colostrum fed to neonatal calves has long been understood, the concept of improving colostrum quality via on-farm heat processing was only developed within the last few years. In this thesis, the effects of colostral heat treatment on the microbiological qualities of bovine colostrum, on the passive immune transfer, and on the health and weight gains of neonatal calves, and their future performances were investigated.

To this end, a field study was conducted at a large commercial dairy in Upstate New York in 2007. The objectives in the first part of the trial were to identify the most common bacteria in bovine colostrum, to quantify the bacterial contamination of colostrum on the study farm, and to illustrate the effect of on-farm heat treatment of colostrum on colostral bacterial counts. For this, colostrum from various fresh cows was pooled and 20 ml samples of each of the created colostrum batches ($n = 51$) were taken for a subsequent microbiological analysis. Next, the colostrum batches were either refrigerated to be fed as raw ($n = 19$) within the next 24 hours or underwent heat treatment ($n = 32$) at 60 °C for 60 minutes in a commercial on-farm batch pasteurizer (Dairy Tech, Inc., Windsor, Colorado) prior to their usage as calf feed. After heat processing, the latter batches were re-sampled to assess the differences in the microbiological characteristics of pre- and post-heat-treated colostrum. Heat treatment using this lower-temperature, longer-time approach proved to be an effective method to significantly ($p = 0.0002$) reduce, and in the cases of *Enterobacteriaceae* and Gram-positive bacilli to fully eliminate, colostral bacteria. Environmental streptococci and Gram-negative bacilli were reduced by 93 % each, coagulase-negative staphylococci by 92 %. With a reduction of 84 %, the effectiveness of the heat-treating device was lowest for species of the genus *Corynebacterium*.

In the second part of this thesis the serum total protein values of the 97 study calves that had either been fed heat-treated (n = 58) or raw (n = 39) colostrum at first feeding were determined and the means compared. These neonates had been removed from their dams before suckling could have occurred and had alternately been allocated to a dietary treatment group receiving either 3.8 l of unprocessed or 3.8 l of heat-treated colostrum within 1 hour after birth. Afterwards all study calves had been housed in individual pens and had received pasteurized waste milk until weaning. Postcolostral blood samples were taken between 20 and 72 hours of age to assess the passive transfer of colostral immunoglobulins. Serum total protein concentrations of these calves were determined at room temperature using a validated hand-held refractometer (VET 360, Reichert Inc., Depew, NY). A comparison of the (for calf age at blood sampling) adjusted mean total protein concentrations between the two treatment groups revealed significantly ($p = 0.04$) greater values for calves fed heat-treated (58.8 g/l) versus raw colostrum (56.3 g/l). While failure of passive transfer did not occur among calves of the heat-treated group, it was found in 8 % of calves that had received raw colostrum. Total protein values indicating a moderately successful passive transfer were recorded in 10 % of calves that had been fed raw compared to 5 % of calves that had received heat-treated colostrum. The bacterial load of unprocessed colostrum may provide an explanation for these findings since the interactions between microbes and immunoglobulins in the gastrointestinal tract of the neonate may interfere with the resorption of immunoglobulins from colostrum.

While previous studies have proven that heat processing at 60 °C for 60 minutes alters neither immunoglobulin concentrations nor their biological functions, it is not known how the rest of the presumably 2,000 compounds of colostrum are affected. While, according to literature, components like lactose, milk fat, marominerals, micronutrients, and maternal leukocytes should not have been altered by the time-temperature combination used in this trial, heat treatment certainly causes vitamin losses and denatures milk proteins, peptides, peptide hormones, and enzymes. Thus, the third part of this trial was focused on health and performance data. With the help of DairyComp 305 data were assembled to assess if differences in calf health, weight gains, or adult performance between the two treatment groups existed. Even though preweaning morbidity (26 % versus 10 %) and mortality (5 % versus 3 %) rates tended to be higher for calves within the heat-treated versus the raw colostrum group, the effect of colostral heat treatment on calf health was not significant ($p = 0.07$). At the age of approximately 24 months, the overall

mortality rate had risen to 9 % among animals that had received heat-treated and to 5 % among animals that had received raw colostrum at first feeding. Since animals that had received heat-treated colostrum were 45 days older on average, they were already further in their lactation and reproductive status. The obtained weight data was also biased by an incongruity of calf age at weight tape measuring and should be re-evaluated in future studies.

8 ZUSAMMENFASSUNG

Tina W. Rebelein (2010)

„Der Einfluss einer Hitzebehandlung auf die mikrobiologischen Eigenschaften des Rinderkolostrums, den passiven Immuntransfer bei neugeborenen Kälbern und die zukünftige Leistung der Tiere."

Eine Feldstudie zu diesem Thema fand im Jahre 2007 auf einem Milchviehbetrieb im Bundesstaat New York statt.

Im ersten Teil des Projekts wurden die auf dem Versuchsbetrieb am häufigsten in bovinem Kolostrum vorkommenden Bakterienstämme ermittelt und die dort vorherrschende kolostrale Keimbelastung quantitativ bestimmt, um den Effekt einer Hitzebehandlung auf die mikrobiologische Eigenschaften des Kolostrums darstellen zu können. Hierzu wurde das Erstgemelk von mehreren Kühen gesammelt und den entstandenen Chargen (n = 51) je eine 20 ml Probe für eine mikrobiologische Untersuchung entnommen. Die Kolostrumchargen wurden dann entweder in einem handelsüblichen Kühlschrank gekühlt (n = 19), um innerhalb von 24 Stunden unbehandelt an die Versuchstiere weiterverfüttert zu werden, oder wurden vor ihrer Verfütterung in einem Erhitzer der Firma Dairy Tech bei 60 °C für 60 Minuten erwärmt (n = 32). Nach der Hitzebehandlung wurde den Chargen eine zweite 20 ml Probe entnommen, um die mikrobiologischen Eigenschaften des Kolostrums vor und nach Hitzeeinwirkung vergleichen zu können.

Die Wärmeeinwirkung bei dieser - im Vergleich zu handelsüblichen Pasteurisierern - relativ niedrigen Temperatur über einen längeren Zeitraum hinweg erwies sich als äußerst effektive Methode: Sie reduzierte die Zahl der Bakterien in Kolostrum signifikant ($p = 0{,}0002$) und tötete *Enterobacteriaceen* und Gram-positive Stäbchenbakterien vollständig ab. Umweltassoziierte Streptokokken und Gram-negative Stäbchenbakterien wurden um je 93 %, koagulase-negative Staphylokokken um 92 % vermindert. Die Wirkung der Hitzebehandlung gegen Vertreter der Gattung *Corynebacterium* war mit einer Reduktion um 84 % am niedrigsten.

Im zweiten Teil dieser Arbeit wurden die Gesamtproteinspiegel im Serum von Kälbern, die bei der ersten Fütterung wärmebehandeltes bzw. unbehandeltes Kolostrum erhalten hatten, bestimmt und miteinander verglichen. Hierfür wurden 97 neugeborene Kälber von ihren Müttern getrennt, bevor sie selbständig Biestmilch aufnehmen konnten, und sogleich abwechselnd Behandlungsgruppen zugeteilt, in denen sie innerhalb der ersten Stunde post natum entweder 3,8 l unbehandeltes (n = 39) oder 3,8 l wärmebehandeltes (n = 58) Kolostrum per Schlundsonde bekamen. In einem Zeitrahmen von 20 bis 72 Stunden post natum wurden den Kälbern Blutproben entnommen, um die Resorption kolostraler Antikörper zu bewerten. Die Gesamtproteinspiegel wurden im Labor der Ambulatory Clinic bei Raumtemperatur mit Hilfe eines validierten Refraktometers (VET 360, Reichert Inc., Depew, NY) aus dem Serum der Versuchskälber bestimmt.

Nachdem die Mittelwerte der Gesamtproteinkonzentrationen der beiden Versuchsgruppen mit Hilfe geeigneter Software an das Alter der Kälber zum Zeitpunkt der Blutentnahme angepasst worden waren, zeigten sich signifikant ($p = 0,04$) größere Gesamtproteinwerte bei Kälbern, die hitzebehandeltes (58.8 g/l) an Stelle von unbehandeltem (56.3 g/l) Kolostrum bekommen hatten. Während 8 % der Kälber, die unbehandeltes Kolostrum erhalten hatten, einen ungenügenden Immunglobulintransfer aufwiesen, konnte dies unter den Kälbern, die hitzebehandeltes Kolostrum bekommen hatten, in keinem einzigen Fall festgestellt werden. Gesamtproteinspiegel, die lediglich einen moderaten Immunglobulintransfer kennzeichnen, wurden bei 10 % der Kälber, die unbehandeltes Kolostrum erhalten hatten, und bei 5 % der Kälber, die hitzebehandeltes Kolostrum erhalten hatten, beobachtet. Die Erklärung hierfür dürfte in der Keimbelastung von unbehandeltem Kolostrum liegen, da Wechselwirkungen zwischen Bakterien und Immunglobulinen im Gastrointestinaltrakt der Neugeborenen die Resorption von kolostralen Immunglobulinen beeinträchtigen.

Im letzten Teil dieser Arbeit wurde versucht herauszufinden, ob die Verfütterung von hitzebehandeltem Kolostrum Auswirkungen auf die Gesundheit und die Gewichtszunahmen neugeborener Kälber und auf deren zukünftige Leistungen als Milchkuh haben kann. Obwohl sich die Konstellation einer 60-minütigen Erhitzung bei 60 °C in vorhergehenden Studien als besonders günstig herausgestellt hatte, da diese die Konzentrationen und biologischen Aktivitäten der Immunglobuline unangetastet lässt, ist nicht bekannt, in welcher Form andere der rund 2000

Bestandteile der Kolostralmilch beeinflusst werden. Während Laktose, Milchfett, Mineralstoffe, Spurenelemente und maternale Leukozyten nicht von der in der Studie verwendeten Zeit-Temperatur-Kombination verändert werden sollten, führt eine Hitzebehandlung zu Verlusten an Vitaminen und zu einer Denaturierung von Milchproteinen, Peptiden, Peptidhormonen und Enzymen.

Um herauszufinden, ob Unterschiede bezüglich Gesundheit, Zunahmen und anderen Leistungsmerkmalen zwischen Tieren der beiden Versuchsgruppen bestanden, wurden notwendige medikamentelle Behandlungen, gemessene Gewichte und weitere Leistungsdaten der Tiere (mit Hilfe von DairyComp 305) aufgezeichnet und anschließend analysiert. Obwohl die Morbiditäts- (26 % versus 10 %) und Mortalitätsrate (5 % versus 3 %) für Kälber in der Phase vor dem Absetzen in der Versuchsgruppe, die hitzebehandeltes Kolostrum bekommen hatte, tendenziell höher zu sein schienen, entpuppte sich der Effekt der Hitzebehandlung von Kolostrum auf die Kälbergesundheit als nicht signifikant ($p = 0,07$). Im Alter von 24 Monaten war die Mortalitätsrate unter den Tieren der hitzebehandelten Versuchsgruppe auf 9 % gestiegen, während sie bei den Tieren, die unbehandeltes Kolostrum bei der ersten Fütterung erhalten hatten, bei 5 % lag. Da die Tiere, die hitzebehandeltes Kolostrum erhalten hatten, auf Grund eines Fehlers in der Studienplanung im Schnitt 45 Tage älter waren als jene, die unbehandeltes Kolostrum erhalten hatten, waren sie auch weiter in der Laktation und Reproduktion fortgeschritten. Die ermittelten Daten hinsichtlich der täglichen Zunahmen sollten in zukünftigen Studien ebenfalls erneut evaluiert werden, da auch sie durch das unterschiedliche Durchschnittsalter der Kälber der beiden Versuchsgruppen beeinflusst wurden.

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"A man travels the world over in search of what he needs and returns home to find it."

George Moore (1852-1933)