EFFECTS OF SUPPLEMENTATION WITH TROPICAL PLANTS ON THE PERFORMANCE AND PARASITE BURDEN OF GOATS

By

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To my dear family, who have always supported me regardless of the distance between us

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADG	Average daily gain
ADL	Acid detergent lignin
BG	Bahiagrass hay
BUN	Blood urea nitrogen
BW	Body weight
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
EIM	*Eimeria sp.*
FEC	Fecal egg counts
GIN	Gastrointestinal nematodes
HSP	Heat-shock proteins
IPCC	Intergovernmental Panel on Climate Change
IVDMD	In vitro dry matter digestibility
L1	Larva stage 1
L2	Larva stage 2
L3	Larva stage 3
LES	Sericea lespedeza hay
MT	Metric ton
MUC	Mucuna seeds
N	Nitrogen
NDF	Neutral detergent fiber

NDFD	Neutral detergent fiber digestibility
OM	Organic matter
PAP	Papaya seeds
PEA	Perennial peanut hay
PSM	Plant secondary metabolite
RDP	Rumen degradable protein
VFA	Volatile fatty acid

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Experiment 1 determined the effect of supplementing bahiagrass hay (*Paspalum notatum* Flügge) with perennial peanut (*Arachis glabrata* Benth.), sericea lespedeza (*Lespedeza cuneata* Dum.-Cuors. G. don), mucuna (*Mucuna pruriens* cv. Mucuna ceniza) or papaya (*Carica papaya* Linnaeus) on the intake, digestibility and nitrogen retention of goats. Thirty-eight male goats (27.4 kg) were stratified by weight and fed bahiagrass alone or supplemented with perennial peanut or sericea lespedeza hay (50% of dry matter, DM), or mucuna (10% of DM) or papaya seeds (10 g/day). Diets were fed ad libitum for 14 days of adaptation and 7 days of measurement of feed and total feces and urine output. Legume supplementation tended to increase nitrogen intake ($P = 0.07$) and perennial peanut and sericea lespedeza tended to increase DM intake ($P = 0.11$). Apparent *in vivo* digestibilities of DM and organic matter (OM) were greater ($P < 0.01$) in goats fed mucuna versus bahiagrass, whereas nitrogen digestibility was greater ($P < 0.01$) when mucuna and perennial peanut were fed. In Experiment 2, the effect of the same diets on the growth and parasite burden of goats was examined for 63 days. Goats were blocked by weight, randomly allocated to pens (3 pens/treatment, 2-3 goas/pen) and naturally infected with gastrointestinal nematode (GIN) L-3 larvae and *Eimeria* (EIM) oocysts by grazing on bahiagrass pasture for 5

hours/day and then returned to pens where diets were offered. Papaya and perennial peanut reduced (treatment x time interaction, $P < 0.05$) EIM fecal egg counts (FEC) from weeks 5 to 9. Papaya, sericea lespedeza, and perennial peanut reduced ($P < 0.05$) GIN FEC by 72, 52, and 32% (P>0.05), abomasal adult GIN counts by 78, 52, and 42%, and haptoglobin concentrations by 42, 40, and 45% relative to bahiagrass ($P < 0.01$). Dry matter intake was increased ($P < 0.05$) by sericea lespedeza, papaya and perennial peanut but animal performance was unaffected. In conclusion, mucuna increased DM, OM, and nitrogen digestibility whereas perennial peanut, sericea lespedeza, and papaya increased DM intake and decreased the parasite burden of goats.

CHAPTER 1
INTRODUCTION

In January 2012, the U.S. goat population totaled 2.86 million head of which 82% were meat goats, 10% were dairy goats, and 8% were fiber goats (USDA-NASS, 2012). The goat industry has become an important alternative livestock production enterprise in the U.S., because of increasing minority demand for goat meat as well as the relatively low cost of breeding stock, their high reproductive parameters, and the role of goats in sustaining agriculture systems in terms of nutrient cycling and soil improvement (Devendra, 1980).

Successful goat production requires proper control of gastrointestinal nematodes (GIN) and coccidian species particularly Eimeria sp. Haemonchus contortus, the main cause of ill health and low productivity in grazing goats in tropical and subtropical regions (Miller, 1996). Haemonchosis is characterized by anemia, extreme weakness, loss of condition, and eventually death (Bowman, 2009). Coccidian subclinical infections can cause diarrhea, dehydration, weakness, and death (Young et al., 2011). Economic losses arising from decreased production due to the infestation, the costs of prophylaxis and treatment, and the death of infected animals (Barger, 1982; Donald and Waller, 1982) amount to millions of dollars per year in the U.S. (Gibbs, 1986).

The effectiveness of anthelmintic treatments is a major determinant of the productivity of goats. Yet, nematode parasites of goats have become more resistant to anthelmintic drugs in recent years (Terrill et al., 2001; Mortensen et al., 2003). Anthelmintic resistance is characterized by reduced efficacy of anthelmintic compounds against a specific population of a particular nematode species compared to a normal population of the nematode (Sangster, 1999). Anthelmintic resistance cases have

become a major concern to small ruminant producers in the Southeast (Howell et al., 2008) and worldwide (Kaplan, 2004). There is also the added concern about drug residues in meat and their persistence in feces. In particular, avermectin residues in feces can kill non-target species such as insects including the beneficial dung beetle (McKellar, 1997).

Several studies have evaluated natural alternatives to anthelmintics to decrease the excessive use of drugs that may still be effective at treating gastrointestinal parasites (Waller, 1997a). These alternatives include supplementation with forages that can improve animal nutrition and enhance antiparasitic immunity (Kyriazakis et al., 1996) via the secondary metabolites (PSM) they contain. Such compounds include fatty acids (Sutherland and Scott, 2010), phenolic acids (Hartley and Akin, 1989), alkaloids (Krishna et al., 2008), terpenes (Rochfort et al., 2008), cysteine proteases (Stepek et al., 2005) and tannins (Shaik et al., 2006). However, the concentration of these metabolites in some forages may be high enough to affect adversely the health and performance of livestock (Athanasiadou et al., 2007). Consequently, nutritional experiments should be conducted in conjunction with studies aimed at evaluating the anthelmintic effects of forages containing PSM.

The objective of the current study was to investigate the effects of supplementing bahiagrass hay (Paspalum notatum Flügge) with Perennial peanut hay (Arachis glabrata Benth.), sericea lespedeza hay (Lespedeza cuneata Dum.-Cuors.), mucuna seeds (Mucuna pruriens) and papaya seeds (Carica papaya Linnaeus) on the parasite burden, health and performance of goats.

CHAPTER 2
LITERATURE REVIEW

Goat Production

Overview

Goats are one of the most widely consumed sources of protein in the world. The global goat industry is growing because of increasing recognition of the economic value of goats as efficient converters of low-quality forages into quality meat, milk, and hides (Hoste et al., 2010) as well as their ability to thrive in areas unsuitable for cropping (Glimp et al., 1986). In the United States, the size of the goat industry has increased significantly over the past 20 years, with an increment of 527% from 1987 to 2007 (Browning et al., 2011), though this increase has abated in recent years (Figure 2-1). The current US total goat population is almost 3 million head of which 82% are meat goats, 10% are dairy goats and 8% are fiber goats (USDA-NASS, 2012).

Most of the goats in the United States are produced in the South (mainly Texas), the Southeast (Tennessee, Georgia, Kentucky, North Carolina, Florida and Alabama), the Midwest (Oklahoma, Missouri) and the West (California) (Solaiman, 2007). The growth in the US goat industry is particularly evident in the Southeast because the region has many forage and browse species favored by goats (Solaiman and Shoemaker, 2009) and it is home to a high population of ethnic minorities that favor chevon consumption (Maxey, 1993; Pinkerton et al., 1994).

Goats are well adapted to the hot and humid climate of the Southeast and can become an important biological control agent for noxious plants (Glimp, 1995). Although they do not have high growth rates, they are more efficient at harvesting and utilizing certain shrub and other plants species than other domestic livestock (McDowell

and Woodward, 1982). Consequently, goats are usually managed as part of multi-species grazing systems where they reduce shrub competition and thereby maximize forage production for livestock (Glimp et al., 1995). It is estimated that at least 20 million acres in the Southeast could benefit from using goats to control undesirable browse species on pasture lands (Child et al., 1985). This area repersents 60% of the total U.S. rangelands (Warren et al., 1984) and is therefore very significant.

The demand for goat meat exceeds the supply in the U.S. and retail prices for the meat of young goats are 20 to 30% higher than beef and lamb prices (Glimp et al., 1986). Nevertheless, the US is a net importer of goat meat (FAS, 1998). Since 1989, importation of goat meat has increased linearly while exportation has decreased quadratically (Gipson, 1999). The US imported about 23 million pounds of goat meat from 718,000 goat carcasses valued at $37 million in 2007, with an average weight of 32 pounds per carcass (eExtension, 2010). In contrast, in 2006, the US exported (Table 2-1) a little over 11,000 live goats and about 469 MT of goat meat mainly to Caribbean countries, a trend started in 2002. The main exporters of goat meat to the United States are Australia and New Zealand with 92.5% of the contribution coming from Australia (Solaiman, 2007; Figure 2-2).

Imported goat meat prices increased sharply from 2003 to 2006 (Figure 2-3). The price paid per pound of imported goat meat in 2006 was $1.72, up 50% from $1.15 per pound in 2003 and up 62% from $1.06 per pound in 1999.

The high demand and continued importation of goat meat into the US indicates the considerable opportunity for growth of the domestic goat industry, which is still under-

developed, and fractionated. This demand is likely to grow even further as the population of minorities that cherish goat meat increases (Glimp, 1995).

Challenges to Goat Production

A recent Food and Agricultural Organization report emphasized the negative impact of livestock production on the environment (Steinfeld et al., 2006). The accuracy of the purported global anthropogenic greenhouse gas emissions from livestock in the study (18%) has been criticized (Seré, 2009; Van't Hooft, 2009; Dirix et al., 2012) based on the fact that livestock emissions differ among production systems and thus is highly influenced by the way animals are fed and raised. Nevertheless, environmentalists have succesfully used it to mount a campaign against livestock production, which has gained currency among policy makers in some parts of the world (Peacock and Sherman, 2010). For instance, from a Kyoto Protocol perspective, areas of extensive grassland used for grazing livestock production could be afforested to tackle climate change (Osterburg et al., 2007). Goat production may have been disproportionately affected by concerns about the environmental impact of livestock because of the 80 million metric tons of methane produced annually by livestock, 25 to 118 kg methane per head per annum is attributable to cattle (Johnson and Johnson, 1995) and sheep and goats account for 5 to 18 kg per head per annum (IPCC, 1995 cited by Peacock and Sherman, 2010). In addition, the vast majority of goats are kept in extensive/semi extensive low-input systems around the world, yet livestock systems with the greatest total environmental impact (including impacts on soil, air and water quality, biodiversity, and human and animal health) are intensive systems. Therefore, condemnation of all livestock systems, as environmentally adverse, is damaging to the

goat industry, which is far less developed and intensive in nature, compared to others (Peacock and Sherman, 2010).

Although goat numbers have experienced the greatest increase among the livestock species during the last 20 years, a global view of the goat industry indicates that few well organized selection programs have been developed, (Dubeuf and Boyazoglu, 2009). Selection programs have been established mainly in developed countries, whereas most goats in developing countries are randomly bred and mainly used to satisfy the immediate needs of the families. Most of the few breeding programs established in developing countries have failed partly because they focused on goat improvement rather than on educating the people who kept the animals (Aziz, 2010).

Another challenge facing goat production is the limited research emphasis on the species. Nevertheless, the number and quality of goat studies throughout the world is increasing (Sahlu and Goetsch, 2005) mainly in the developed world. The amount of goat research in developing regions does not reflect their importance in such areas because of prevailing infrastructure, equipment, and expertise limitations. (Sahlu and Goetsch, 2005). More goat-focussed research is needed to improve the level, efficiency, and profitability of production of goat meat, milk, and fiber.

As for other livestock industries, the goat industry is facing modern challenges that include globalization, integration, traceability and licensing, and intensive control of processing and quality of products (Sahlu and Goetsch, 2005). However, gastrointestinal parasite resistance to commercial anthelmintics is perhaps the most important current challenge of goat production. This global problem has been well documented around the world (Whitley et al., 2009) and, is unfortunately increasing

dramatically and causing millions of dollars in production losses in many countries (Waller, 1987; Jackson, 1993; Prichard, 1994; Githigia et al., 2001).

Biology and Control of Gastrointestinal Nematodes

Gastrointestinal Nematodes of Importance in the Tropics

Gastrointestinal nematodes cost the Australian sheep industry $369 million anually (Sackett and Holmes, 2006) and the impact is very similar in the United States (Craig, 1986; Mortensen et al., 2003). The most important gastrointestinal nematodes in goats in the tropics and temperate regions are *Haemonchus contortus, Teladosargia circumcincta, Trichostrongylus sp., Nematodirus sp.,Cooperia sp*, and *Oesophagostomun sp.* (Sutherland and Scott, 2010). Among these, the primary constraint to economic production of goats, especially in tropical areas and the southeastern US is from *Haemonchus contortus* (Miller, 1996; Shaik et al., 2006) due to its ability to overwhelm a herd quickly (Miller, 1996).

Haemonchus contortus

Haemonchus contortus, also known as Barber's pole worm, is an abomasal nematode thought to be the most important parasite of sheep and goats (Mortensen et al., 2003) but it also can be found in cattle in small numbers, some species of deer and other small ruminants (Sutherland and Scott, 2010). This nematodes' survival and dominance is because it is one of the most prolific parasites as blood-feeding adults produce up to 10,000 eggs per day per goat (Bowman 2009; Sutherland and Scott., 2010). Consequently, eggs of H. contortus usually accounts for 75 to 100% of the total fecal nematode egg counts on most sheep and goat farms in the Southern U.S (Uhlinger et al., 1992; Mortensen et al., 2003).

According to Sutherland and Scott (2010), eggs passed out in feces two or three weeks after infection (Figure 2-4) contain embryos that develop into the L1 stage (or larvae). After hatching, the fully formed L1 moults to the L2 stage. Both of these states are microbivorous, feeding on bacteria present in the feces. The second moult is incomplete, so that the cuticle of the L2 larvae forms a sheath around the L3, which prevents the infective larvae from feeding and arrests further development until a host is encountered (Sutherland and Scott, 2010). However the L3 is still active and begins to migrate from the feces into the external environment. The development from eggs to infective L3 can occur in 5 days under favorable conditions (approximately 25 ºC), whereas at colder temperatures (10 ºC or below), the life cycle slows dramatically and it takes several months to reach the L3 stage. Free-living larvae may achieve a degree of freeze-tolerance by using molecules like trehalose and glycerol as cryoprotectants (Wharton, 2002). However, thermotolerance has been reported and may be partialy mediated by classic heat-shock proteins (HSPs), which can prevent proteins against denaturalization (Wharton, 2002).

The infective larvae ingested with pasture grasses exsheath in the rumen (Hertzberg et al., 2002) due to the equilibrium between bicarbonate (HCO3) and carbonic acid (H2CO3), the neutral pH of the ruminal fluid, and the relative high levels of CO2. Upon entering the abomasum, the L3 larvae penetrates into the pits and glands of the mucosa (Sutherland and Scott, 2010), starts feeding again once confined, and then moults into the L4 stage, which is thought to then emerge onto the mucosal surface to complete its development into the adult (Charleston, 1965). Adults attach to the surface of the mucosa and start feeding on blood from the small vessels. Daily

blood loss due to haemonchosis amounts to 0.05 mL per worm (Clark et al., 1962) and since worm burdens can range from 2,000 to 20,000 worms (Sutherland and Scott, 2010), blood losses can be considerable.

Severe hemonchosis is characterized by anemia (hematocrit less than 15%), extreme weakness, shortness of breath, loss of condition, and eventually death (Bowman, 2009). Extremely heavy worm burdens can result in sudden death due to hemorrhagic gastritis whereas small burdens cause chronic, low-level blood loss accompanied with weight loss and weakness (Gunn and Irvine, 2003). Diarrhea is not usually a clinical sign for hemonchosis, but feces might be dark in color due to the presence of blood. Loss of plasma protein results in anasarca (Bowman, 2009) frequently manifested externally as peripheral, submandibular and ventral edema (Figure 2-5) (Urquhart et al., 1996).

Eimeria sp.

Coccidiosis is a widespread disease caused by protozoan parasites from the *Eimeria* genus, which affect a variety of animals including small ruminants. Many species of *Eimeria* are involved in different ruminant hosts (bovine, ovine, caprine) but there is no cross infection due to the strict host specificity (Chartier and Paraud, 2012).Oocysts are the infective form eliminated in the feces and transmission happens directly by ingestion so infection is higher under specific environmental, management, and host immunity conditions (Rocha et al., 2012). Mortality due to outbreaks of coccidiosis has been reported in North America (Valentine et al., 2007), South America (Teixeira Filho et al., 2001), Asia (Yadav et al., 2007), and Africa (Nwosu, 1996). Kids are more susceptible and the disease occurs mostly between 1 and 4 months of age, normally after weaning.

Among *Eimeria* species (Figure 2-6), the most important causes of health problems in goats are *E. ninakohlyakimovae* and *E. arloingi* (Chartier and Paraud, 2012). The life cycle of Eimeria species requires only one host, includes an exogenous phase of maturation of the oocyst (sporogony) outside the host, and a parasitic endogenous phase within the host with an asexual followed by a sexual multiplication (Soulsby,1982).

Each ingested oocyst is able to produce 30 million oocysts in fecal matter (Gregory et al., 1987). The oocysts passed with the feces become sporulated 2–7 days later depending on the species of *Eimeria* and the environmental conditions (Chartier and Paraud, 2012). The sporulated oocysts are very resistant in the external environment and can survive for several months or even more than a year. However, extreme desiccation and direct exposure to the sun limit the survival of the oocysts and temperatures below −30 ºC or above 63 ºC are lethal (Foreyt, 1990). In the host, the oocysts undergo excystation. The sporozoites, which were inside the oocyst, penetrate into the small intestine epithelium to transform into a schizont. Two asexual multiplication cycles (schizogonies) occur in the small intestine only, or in the small then large intestines, according to the *Eimeria* species. Eventually, the schizozoites penetrate the large intestine epithelial cells (sexual phase or gamogony) that lead to the production of gamontes, gametes and then non sporulated oocysts that are released with the fecal matter (Foreyt, 1990).

Clinical signs of coccidiosis include diarrhea, dehydration, weakness, rough hair coat, and death (Young et al., 2011). Permanent damage of the colon due to coccidial infection slows growth (Foreyt, 1990) and these symptoms can lead to significant

economic losses in small ruminants (Foreyt, 1990). Coccidiosis can cause up to 15% mortality in young does (Craig, 1986) and up to 58% in kids (Jalila et al., 1998).

The incidence of coccidiosis is influenced by two factors: intensification of production, which involves higher numbers of animals per unit area and more confinement (Balicka-Ramisz, 1999) and occurrence of the 'coccidiosis carrier-state' in adult dams that contaminate the environment with oocysts that serve as a source of re-infection and new infection for young kids (Baker, 1975; Balicka-Ramisz, 1999).

Anthelmintic Resistance

The control of parasitic infections has been primarily based on well implemented use of anthelmintics (Hounzangbe-Adote et al., 2005). Responsible anthelmintic use includes dosing only when necessary to cure clinical disease or prevent the accumulation of infective larvae on pasture (Waller, 1997b). However, the conventional method of GIN control by farmers in the Southeast and other many other regions is by regular use of anthelmintics, sometimes monthly or even more frequently during the warm season (Miller, 1996). Consequently, nematode populations are becoming increasingly resistant to numerous anthelmintic drugs (Hounzangbe-Adote et al., 2005). Three broad families of anthelmintic drugs commonly used for ruminant livestock are the benzimidazoles, the levamisol/morantel group, and the macrocyclic lactones (avermectins and milbemycins) and resistance to all of these has been documented in the U.S. (Zajac and Gipson, 2000; Mortensen et al., 2003; Kaplan, 2004). Consequently, benzimidazoles, moratel tartrate and macrolyc lactones are no longer effective at reducing fecal egg counts (Table 2-2; Borgsteede, 1986, 1988; Terrill et al., 2001; Kaplan, 2004; Waghorn et al., 2006). However, it seems that moxidectin is still effective at reducing FEC in the southeastern U.S. (Terrill et al., 2001).

Anthelmintic resistance is characterized by reduced efficacy of anthelmintic compounds against a specific population of a particular nematode species compared to a normal population of the nematode (Sangster and Gill, 1999). The resistance is declared if mean fecal egg counts are reduced by less than 95% after drenching (Coles et al., 1992). Resistance of nematodes to the early anthelmintic phenotiazine was demostrated in the 1950s, less than 20 years after it was first introduced (Drudge et al., 1957). The first case of thiabendazole resistance was reported seven years later (Drudge et al., 1964) followed by reports from Australia (Hall et al., 1979), New Zealand (Kettle et al., 1982), Africa (Van Wyk and Malan, 1988; Mwamachi et al., 1995), South America (Echevarria and Trindade, 1989), and Europe (Scott et al., 1990; Maingi et al., 1996). Recent reports of resistance to multiple anthelmintics by GIN in goats in Texas (Miller and Craig, 1996), Virginia (Zajac and Gipson, 2000) and Georgia (Terrill et al., 2001) show that nematode resistance in the U.S. is growing dramatically.

In most cases, *Haemonchus contortus* was the first nematode to develop resistance against the different anthelmintics (Coles et al., 1994) and it is still one of the most resistant GIN (Figure 2-7; Howell et al., 2008). Goats particularly have a high prevalence of resistant nematodes due to their need for frequent drenching compared to other species (Kaplan, 2004), their innate susceptibility to worms, and the high level of rumen bypass and relatively short life of anthelmintics in goats compared to sheep (Jackson and Coop, 2000).

Several laboratory assays detect nematode resistance to anthelmintics including the fecal egg count reduction test, egg hatch assay, larval developmental assay, larval migration inhibition assay, and the use of molecular markers (Coles et al., 1992;

Prichard, 1994; d'Assonville et al., 1996; Gill et al., 1998; Gill and Lacey, 1998; Kwa et al., 1998). Recently, the use of lectin to easily and quickly detect eggs and or larvae of different parasite species in a post-drench fecal sample was studied (Colditz et al., 2002) but the approach was not successful. Another study involving lectin staining to identify *Haemonchus contortus* eggs showed promising results as a qualitative test since the eggs of this particular nematode are difficult to differentiate from most of the GIN (Jurasek, et al., 2010).

The future of using anthelmintic drugs to control GIN remains unclear. The expenses incurred by the drug companies from the identification of a new chemical until it is tested and marketed are can add up to $100 million (Gilleard et al., 2005). This factor and the growing resistance problem has made many pharmaceutical companies reluctant to invest in this area (Witty, 1999). Although there are many potential agents that have been identified and tested, they may not be commercially available for quite some time (Geary et al., 1999). These problems may be compounded by the increasing interest in organic agriculture, which strictly prohibits the use of synthetic medicinal products (Hordegen et al., 2003).

Alternative GIN Control Methods

An alternative to using anthelmintic drugs to control GIN infections is to adopt rotational grazing of goats on pastures (Banks et al., 1990; Cheah and Rajamanickam, 1997; Barger, 1999). However, this technique is becoming less feasible, due to the limited land area of many farms in developed countries and the communal land ownership among many pastoralists in developing countries (Githiori et al., 2006). Alternative approaches include breeding for resistance to nematodes (Gray, 1997), adopting biological control methods such as using predacious fungi or bacteria (Larsen,

2000; Kotze et al., 2005; Paraud et al., 2005; Santurio et al., 2009; Braga et al., 2010; Sagüés et al., 2011), administration of copper oxide (Burke et al., 2005; Burke and Miller, 2006), and vaccination (Bain, 1999; Newton and Meeusen, 2003). The use of the FAMACHA system can also help to avoid unnecessary treatment of healthy animals thus prevent the spread of anthelmintic resistance (Van Wyk and Bath, 2002). The FAMACHA system allows farmers to compare the color of the conjuctiva of the eyelid to a standard chart depicting five pictures of the conjunctiva of goat eyelids numbered from 1 (normal red color) to 5 (very pale color denoting severe anemia) along with an indication of which animal requires treatment (Bowman, 2009). The FAMACHA system has been validated by several studies (Kaplan et al., 2004; Burke et al., 2007; Burke and Miller, 2008; Di Loria et al., 2009; Reynecke et al., 2011) and is currently used around the world to monitor anemia and parasitism.

A further alternative to anthelmintics is the use of natural supplements to control parasite burdens, a broad topic that involves many plant sources with different anthelmintic properties.

Natural Supplements as Anthelmintics

Anthelmintic Forages

A great variety of natural products have shown promise to control gastrointestinal parasites. Examples of plant parts, extracts or metabolites used as anthelmintics include mannich bases in *Eucalyptus randis* leaves (Bennet-Jenkins and Bryant, 1996), eugenol essential oil from *Ocimun gratissimum* (Pessoa et al., 2002), leaves of *Zanthoxylum zanthoxyloides,*(Hounzangbe-Adote et al., 2005), coriander seeds (*Coriandrum sativum*), mango (*Mangifera indica*) leaves, alfalfa (*Medicago sativa*) stems and leaves (Hussain et al., 2008), and neem tree (*Azadirachta indica*) seeds

(Hördegen et al., 2003). In Africa, extracts from the African mahogany tree (*Khaya senegalensis*) (Ademola et al., 2004), tannins from leucaena (*Leucaena leucocephala*) seeds (Ademola et al., 2005), and wormgrass (*Spigelia anthelmia*) (Ademola et al., 2007) have all been reported to have some anthelmintic properties. In Asia, anthelmintic activities were reported in extracts of *Albizia lebbeck* (El Garhy and Mahmoud, 2002) and alkaloids from tobacco (*Nicotiana tabacum*) (Iqbal et al., 2006). In Europe, jasmine juice (*Jasminum fruticans*) (Honda et al., 1996), juniper berries (*Juniperus drupacea*) (Yesilada et al., 1993), and pine cones (*Pinus nigra*) (Fujita et al., 1995) have all shown anthelmintic properties. Also, anthelmintic effects of tanniferous forages such as sainfoin (*Onobrychis viciifolia* Scop, Paolini et al., 2005; Häring et al., 2008), birdsfoot trefoil (Lotus corniculatus L., Heckendorn et al., 2007) and chicory (*Cichorium intybus* L., Tzamaloukas et al., 2005) have been documented. Although many of these plants or plant extracts have demonstrated anthelmintic activity *in vitro* when parasites were exposed to relative high levels of at least a single bioactive compound, different results may be observed when such plants or their extracts are used in *in vivo* experiments where many factors (e.g. gender, age, ruminal fluid activity, diet composition, etc.) are involved (Athanasiadou et al., 2007). A potentially important plant source of anthelmintic activity that has not been adequately studied is papaya (*Carica papaya).*

Papaya

Carica papaya is widely distributed in tropical regions and hence often available for use by goat producers. Papaya has been used as a tropical ethno-veterinary remedy against nematode infestations because of its adaptability, agro-ecological considerations, and availability (Mundy and Murdiati, 1991). Previous studies have also reported anthelmintic activity in various preparations of papaya seeds *in vitro* and *in vivo*

(Krishnakumari and Majumder, 1960; Dhar et al., 1965; Lal et al., 1976). Papaya latex or seeds had anthelmintic activity against *Ascaris suum* in pigs (Satrija et al., 1994), *Heligmosomoides polygyrus* in mice (Satrija et al., 1995), *H. contortus* in ewes (Buttle et al., 2011), and in human children (Okeniyi et al., 2007). Some authors have ascribed these effects to cysteine proteases, papain and chymopapain in the seed, which damage intestinal nematodes by targeting and disrupting their cuticle resulting in the disintegration of the parasite (Behnke et al., 2008). In contrast, others have attributed the anthelmintic properties to the alkaloids carpaine, carpasemin (later identified as benzyl thiourea by Panse and Paranjpe, 1943) and benzyl isothiocyanate, which is probably the main source of anthelmintic activity in papaya seeds (Kermanshai, et al., 2001). Benzyl isothiocyanate is potentially toxic at therapeutic dosages since it is reported to be goitrogenic, carcinogenic, and mutagenic (Yamaguchi, 1980; Fenwick et al., 1983). Consequently, papaya latex and seeds may be toxic for ruminants (Satrija et al., 2001) and they have been ineffective against gastrointestinal parasites in some studies (Ronoredjo and Bastiaensen, 1995; Satrija et al., 1999).

These discrepancies emphasize the need for further studies on potential effects of *Carica papaya* on the parasite burden and performance of small ruminants. Such studies are also important because little is known about the effects of supplementation with therapeutic doses of *Carica papaya* on the intake, digestibility, and growth of small ruminants.

Legumes for Improving Animal Performance and Reducing GIN Burdens

Legumes typically have a higher nutritive value than tropical grasses (Devendra, 1995). The higher CP concentrations of legumes is mainly due to their symbiotic relationship with *Rhizobium* and *Bradyrhizobium* bacteria, which provide nitrogen to the

plant (Taiz and Zeiger, 2002) in exchange for energy from the legume (Franssen et al., 1992; Taiz and Zeiger, 2002). Due to their high N contents, legumes are valuable components in forage mixtures with grasses and in crop rotations with grain crops because they decrease dependence on fertilizer N.

Legumes contain less cellulose and hemicellulose than warm-season grasses and are more digestible (Frame, 2005). However, legumes have higher lignin concentrations than grasses due to their highly lignified stems (Van Soest, 1994; Frame, 2005). Despite their higher lignin content, legumes have greater rates of ruminal particle size reduction than grasses (Moseley, 1981) because of easy breakage of their leaves during mastication (Wilson, 1994). Thus, several studies have reported an increase in DM intake, digestibility and average daily gain (ADG) when warm-season grasses are supplemented with legumes (Said and Tolera, 1993; Goetsch et al., 1997; Weder et al., 1999; Mupwanga et al., 2000; Foster et al., 2009).

Most of the protein in legumes is in the form of rumen degradable protein (RDP) that can be converted readily to ammonia nitrogen, an important nitrogen source for ruminal microbial protein synthesis (Broderick, 1995). Dietary intake of legumes also promotes microbial growth and may thus increase the supply of microbes to the small intestine (Foster et al., 2009). Because of these reasons and their high protein content, legumes can be used to improve livestock nutrition and productivity (Bagley et al., 1987; Ramírez-Restrepo and Barry, 2005; Sanderson et al., 2005). The improved nutritional status resulting from legume supplementation has also decreased gastrointestinal parasitism in some studies (Niezen et al., 1998; Min et al., 2004; Marley et al., 2005; Tzamaloukas et al., 2005, 2006; Sutherland and Scott, 2010). Some of the tropical

legumes that may be used to improve animal productivity and reduce parasite burdens are as follows:

Velvet Bean

Mucuna pruriens is a legume indigenous to tropical regions, especially Africa, India, and the West Indies, which was widely grown as a soil amendment in the southeastern U.S. until cheap fertilizers were introduced in the seventies. The seeds have relatively high crude protein concentrations (250-350 g/kg dry matter, DM; Siddhuraju and Becker, 2005) that can be digested and utilized readily by ruminants and they have reduced coccidian oocyst scores in lambs (Chikagwa-Malunga et al., 2009b). Furthermore, the ether-extracted oil from the seeds has paralyzed intestinal worms (Jalalpure et al., 2007). In addition, velvet bean trichomes were as effective against gastrointestinal parasites in pregnant does as the chemical fenbendazole (Conroy and Thakur, 2005). This may be because the trichomes of *Mucuna pruriens* contain a cysteine protease called mucunain (Reddy et al., 2008), which causes itching when it contacts the skin but may also damage intestinal nematodes (Buttle et al., 2011).

Mucuna contains 20-90 g/kg of DM of 3,4-dihydroxy-L-phenylalanine (L-dopa) (Szabo and Tebbet, 2002), which can adversely affect the performance of non-ruminant livestock by reducing feed intake, and body weight, and increasing mortality (Del Carmen et al., 1999). In contrast, ruminants seem to be unaffected by this compound, partially because 53% of dietary L-dopa can be digested by ruminal microbes (Chikagwa-Malunga et al., 2009a). Studies have shown that *M. pruriens* supplementation has increased nitrogen intake and retention, weight gain and milk production in ruminants (Ayala-Burgos et al., 2003; Castillo-Caamal et al., 2003a, b).

The nutritional potential and anthelmintic promise in a few experiments emphasize the need for further research to examine the validity of using *Mucuna pruriens* as an antiparasitic supplement in the rations of small ruminants.

Perennial Peanut

Perennial peanut (*Arachis glabrata*) is the main warm-season forage legume in the southeastern US (Foster et al., 2009). Over 54,360 ha of forage peanut is grown in Southern Georgia and Northern Florida (Sollenberger and Collins, 2003). This legume tolerates droughts and slightly acidic, low fertility sandy soils (French et al., 2006) and is more high yielding than many other warm-season perennial legumes (Jones, 2001; Sollenberger and Collins, 2003). After a two-year establishment period, which is the main factor limiting in its' widespread adoption, most growth occurs from April to September (Sollenberger and Collins, 2003). The two most common cultivars of perennial peanut in Florida are Arbrook and Florigraze, and Florigraze is more suited to continuous stocking and is more cold tolerant (Hernández Garay et al., 2004).

Perennial peanut has a high nutritive value, which is comparable to that of alfalfa (Prine et al., 1981; Williams et al., 1991), with 13 to 18% of CP and 60 to 70% of in vitro dry matter digestibility (IVDMD). This is due to its low NDF and ADF concentrations, which make it easily digestible by cattle (Foster et al., 2009). Consequently, cattle can gain 0.93 kg/day on perennial peanut pastures compared to 0.68 kg/day on other warm-season legumes (Sollenberger et al., 1989).

Perennial peanut contains 2 to 4% of condensed tannins (Valencia et al., 2007; Foster et al., 2009) and such levels typically result in improved forage nutrient utilization by ruminants (Min et al., 2003, 2005). In addition, perennial peanut silage can replace

70% of corn silage in diets containing 50% concentrate without affecting dairy cow performance (Staples et al., 1997).

The high nutritive value and excellent palatability of perennial peanut often increase dry matter and protein intake when it is supplemented to cattle grazing warm-season grass pastures or hay. This may result in an improved immune status and consequent reduction in parasitism (Athanasiadou and Kyriazakis, 2004) but this theory has not been validated.

Sericea Lespedeza

Sericea lespedeza (*Lespedeza cuneata*) is a tropical legume planted widely throughout the southern USA (Hoveland et al., 1990; Lange et al., 2006; Shaik et al., 2006). Lespedeza is a perennial, warm-season forage (Powell et al., 2003) originating from Asia and it is capable of growing in a variety of areas, such as pastures, roadsides, rangelands, prairies and eroded slopes (Puchala et al., 2005; Cummings et al., 2007) but, it is not tolerant of shade. It was originally used in soil restoration and conservation, but now it is used as pasture forage or as hay for ruminant livestock (Powell et al., 2003).

Lespedeza is resistant to acidic soils with low fertility (Puchala et al., 2005), drought (Ball et al., 1991) and insect damage (Hoveland et al., 1990), which makes it a useful forage species for hay or grazing or for natural deworming purposes in the southern US. Pure stands of sericea lespedeza may produce 195 to 386 kilograms of seed per acre per year with about 350,000 seeds in each pound. Seedlings are not very competitive, but once established, are long-lived (Cummings et al., 2007).

Lespedeza produces allelopathic compounds, which are toxic chemicals that negatively affect the germination and or growth of other plants. Root extracts from

lespedeza were reported to reduce germination of bermudagrass by 9 percent and forage production of bahiagrass, bermudagrass, rye, ryegrass, and tall fescue by 15, 24, 7, 11 and 15 percent, respectively (Cummings et al., 2007).

Lespedeza has high protein (14 to 19% DM basis, Terrill et al., 1989) and condensed tannin (4 to 22% of DM, Windham et al., 1988; Shaik et al., 2006) concentrations, hence it is used as a natural anthelmintic in ruminants. Several studies have reported that supplementation with this legume increased blood packed cell volume and reduced gastrointestinal nematode fecal egg counts, larval development, and adult worm counts in small ruminants (Lange et al., 2005; Min and Hart, 2003; Min et al., 2004, 2005; Shaik et al., 2004, 2006; Terrill et al., 2009). Specifically, feeding lespedeza has reduced adult worm burdens by up to 78% (Min et al., 2005) and has reduced female adult worm fecundity in the abomasum and small intestine (Shaik et al., 2006). The anthelmintic activity of sericea lespedeza is maintained even when it is fed as hay (Lange et al., 2006; Shaik et al., 2006).

Feeding sericea lespedeza increased DM intake and average daily gain (ADG) in goats when compared with feeding a bermudagrass hay basal diet (Moore et al., 2008). In addition, when goats were fed sericea lespedeza instead of alfalfa, DM intake was greater for the lespedeza but growth performance was similar (Solaiman et al., 2010). Carcass fat was also less in goats fed sericea lespedeza, which could potentially improve the health of those consuming the goat meat (Solaiman et al., 2010). However, some other reports indicate that feeding sericea lespedeza decreased DM intake and weight gains by dairy (Burns et al., 1972) and beef (Schmidt et al., 1987) cattle, sheep (Terrill et al., 1989), and goats (Turner et al., 2005). These discrepancies may be due to

variations in the tannin concentration and particle size of the sericea lespedeza used or to the nutritional status of the animals and the abundance of tannin-binding proteins in their saliva. In addition, some states in the Midwest classify sericea lespedeza as an invasive weed because of its unpalatability to grazing beef cattle (Shaik et al., 2006) and its invasive growth, which reduces the value of grazing land and rangelands (Duncan et al., 2004). Nevertheless, the high efficacy of using sericea lespedeza as a natural deworming agent in small ruminants (Lange et al., 2005; Min and Hart, 2003; Min et al., 2004, 2005; Shaik et al., 2004, 2006; Terrill et al., 2009) indicates that more studies are needed on how it can be used to consistently improve their performance.

Effects of Tannins on Gastrointestinal Parasites and Animal Performance

Most of the promising forages for improving the performance of ruminants and simultaneously reducing their parasite burdens owe the latter ability to their tannin content. Tannins are defined as water-soluble plant polyphenols that can precipitate proteins and other macromolecules at an appropriate pH and concentration (Mangan, 1988). They range anywhere from 500 to sometimes greater than 20,000 daltons in molecular weight and their key feature in animal nutrition is their ability to form stable crosslinks with proteins (Swain, 1979).

Because tannins are part of the plant defense mechanism system, they are toxic to bacteria, yeasts, and fungi (Scalbert, 1991) and protect plants against insects and animals (Swain, 1979). Tannins are divided into four groups: hydrolysable, oxytannins, β-tannins, and condensed tannins (Swain, 1979). Condensed tannins are the most widespread among the four groups and they are polymers of flavan-3-ols (Figure 2-8) and flavan-3, 4-diols or a mixture of the two (Chung, et al., 1998). Condensed tannins

are also known as proanthocyanidins (Figure 2-9) because they are degraded by acids to anthocyanidins (Santos-Buelga and Scalbert, 2000).

In general condensed tannins are present in legumes in greater amounts than in grasses (Reed, 1995). Tannins are commonly present in the leaves, bark and stems of many tropical legumes and in tropical forages, they are thought to promote plant growth by reducing the release of leaf litter into the soil (Palm and Sanchez, 1991). The effects of tannins in nutrition and animal health are variable and difficult to predict. However, there is increasing evidence that condensed tannins can be beneficial against gastrointestinal nematode parasitism and reduce the excretion of nutrients from animals when used strategically (Waghorn and McNabb, 2003).

Condensed tannins precipitate protein in the rumen making it unavailable for ruminal microbes. The presence of some cations, especially calcium and magnesium increases the protein-precipitating capacity of condensed tannins (Martin et al., 1985). However, the acidic conditions in the abomasum can break tannin-protein linkages and thus allow the protein to be available for digestion in the small intestine (Waghorn and Shelton, 1997; McMahon et al., 2000). Jones and Mangan (1977) reported that 95% of the protein that had been bound by tannins was released from the tannin complexes at pH values from 1 to 3. Inhibition of ruminal protein degradation by high dietary condensed tannin concentrations may increase the amount of amino acids supplied to the small intestine (Waghorn et al., 1987) and typically increases the ratio of essential amino acids to non-essential amino acids entering the ileum (Wang et al., 1996). Therefore, condensed tannins can reduce the degradation of protein to ammonia in the rumen and allow more dietary protein to by-pass the rumen to the small intestine (Barry

and McNabb, 1999; Min et al., 2003), where it can be more efficiently incorporated into tissues.

In general, at low dietary concentrations, dietary condensed tannins can have positive effects on live-weight gain and milk and meat production by ruminants (Min et al., 2004). Ruminant animal production is maximized at tannin concentrations of 3 to 4% (Barry, 1985; Barry et al., 1986) or 2 to 4% of DM (Min et al., 2003; 2005), which are just enough to make plant protein insoluble. However, tannin concentrations greater than 6% of DM often reduce forage intake and digestibility in many ruminant species by binding enzymes and forming indigestible complexes with cell wall carbohydrates (Barry and Manley, 1986; Terrill et al., 1989; Waghorn et al., 1994a; Athanasiadou et al., 2001; Min et al., 2003). Consequently, the potential of legumes that have high tannin concentrations as protein supplements for ruminants may be decreased by the anti-nutritional effects of the tannins. *In vitro* studies have shown inhibitory effects of phenolic compounds on rumen bacteria, including both cellulolytic and proteolytic species (Bae et al., 1993). Tannins are able to inhibit cell wall synthesis of susceptible proteolytic species (Jones et al., 1994) and reduce rumen microbial attachment to cellulose, thus reducing fiber digestion (Bento et al., 2005).

Condensed tannins generally do not alter mineral digestion though their effects on sulfur absorption in the literature have been equivocal. Some studies have shown a decrease in absorption of sulfur amino acids due to tannin ingestion (Prichard et al., 1992; Waghorn et al., 1994b), whereas others demonstrated an increase of up to 45% (Min et al., 1998; 1999).

High concentrations of condensed tannins in ingested forages also reduce the incidence of bloat (Mangan, 1988; McMahon et al., 2000) and methane production (Puchala et al., 2005) but one of the most important roles of tannins is that they act as a natural anthelmintic (Butter et al., 2000; Hoskin et al., 2000). Grazing sheep, goats, and deer on forages high in condensed tannins reduced nematode fecal egg counts due to the action of the tannins on the nematodes (Niezen et al., 1995; Athanasiadou et al., 2000; Hoskin et al., 2000; Min and Hart, 2003; Paolini et al., 2003; Min et al., 2005; Shaik et al., 2006; Terrill et al., 2009). However, the anti-parasitic efficacy of tannins is dependent upon the source of the condensed tannins as well as the amount of condensed tannins (Waghorn and McNabb 2003). Experiments with lambs grazing temperate plants with high condensed tannin concentrations including sulla (*Hedysarum coronarium*), sainfoin (*Onobrychis viciifolia*), and big trefoil (*Lotus pedunculatus)* have reported a decrease in fecal egg counts compared to lambs grazing alfalfa or ryegrass (Niezen et al., 1993, 1994, 1995; Robertson et al., 1995; Butter et al., 2000; Tavendale et al., 2005). Parasites whose egg counts decreased in those studies included *H. contortus, T. colubriformis, T. circumcincta, Nematodirus sp.* and *Cooperia sp.* In some cases worm counts were also reduced (Niezen et al., 1993, 1995; Robertson et al., 1995). Supplementation with tropical legumes with high tannin concentrations such as sericea lespedeza has reduced the parasite burden in small ruminants (Lin et al., 2003; Lange et al., 2005; Shaik et al., 2006; Terrill et al., 2009).

Given the variety of results that condensed tannins have had on animal nutrition, performance, and health, more research is needed to define the ideal tannin sources

and their optimal dietary inclusion levels for enhancing both animal performance and anti-parasitic effects.

This review has discussed the state of the US goat industry, the increasing demand for goat meat, the challenges posed by the increasing resistance to anthelmintic drugs and the use of forage-based alternatives to such drugs. In particular, the need to identify forages that can be used to enhance the performance of small ruminants while reducing their parasite burdens was emphasized.

The specific objectives of this project were:

- To determine the nutritive value of perennial peanut, sericea lespedeza, and mucuna and papaya seeds.
- To determine the feed intake, digestibility, and nitrogen balance of goats fed bahiagrass hay alone or supplemented with perennial peanut or sericea lespedeza hay or mucuna or papaya seeds.
- To determine the effects of the experimental diets mentioned above on the growth, parasite burden, and health of goats.

CHAPTER 3
EFFECTS OF SUPPLEMENTATION WITH TROPICAL PLANTS ON THE PERFORMANCE AND PARASITE BURDEN OF GOATS

The goat industry has become an important livestock production enterprise in the United States, mainly because of its relative low cost, the high minority ethnic demand for goat meat, and to a lesser degree goat milk (Maxey, 1993; Pinkerton et al., 1994), their high reproductive rate (Terrill et al., 2001), and their ability to thrive on native pasture or brushland that is unsuitable for cropping (Devendra et al., 1980; Glimp et al., 1986). Successful goat production requires proper control of gastrointestinal nematodes (GIN) and coccidia (*Eimeria sp.*). *Haemonchus contortus* is the major cause of ill health and low productivity in grazing goats in tropical and subtropical regions (Miller et al., 1996). Haemonchosis is characterized by anemia, extreme weakness, loss of condition, and eventually death (Bowman et al., 2009). Coccidian subclinical infections can also cause production losses for the small ruminant producer (Young et al., 2010) due to enteric disease that results in diarrhea, low weight gains, and occasionally death (Foreyt, 1990).

As the numbers of goats and sheep in the southeastern United States have grown, so have issues with drug treatments for parasitism (Howell et al., 2008). Many control programs based on anthelmintics failed to treat and control GIN because of the increased prevalence of GIN that are resistant to anthelmintics (Waller, 1994; Pomroy et al., 2002). In most cases, *Haemonchus contortus* was the first nematode to develop such resistance (Coles et al., 1994). Increasing resistance to anthelmintic drugs is now a major concern for small ruminant producers worldwide (Kaplan, 2004) and the scale of the problem has increased dramatically in goats (Terrill et al., 2001; Mortensen et al., 2003). Consequently, millions of dollars per year are lost due to decreased production,

the costs of prophylaxis and treatment, and the death of infected animals (Barger, 1982; Donald and Waller, 1982; Gibbs and Herd, 1986).

Natural supplements have been successfully used as alternative methods of treating and controlling GIN. These include feeding tannin-containing plants such as sericea lespedeza (*Lespedeza cuneata* Dum.-Cours. G. Don, Shaik et al., 2006), leucaena (*Leucaena leucocephala* Lam*,* Nguyen et al., 2005), sainfoin (*Onobrychis viciifolia* Scop, Paolini et al., 2005; Häring et al., 2008), and cassava (*Manihot esculenta* Crantz*,* Lin et al., 2003). Alternative approaches include feeding plant extracts that increase anti-parasite immunity (Kyriazakis et al., 1996) or increase the nutritional status of the animal, or supply plant secondary metabolites that directly inhibit the growth of GIN. Examples of such metabolites include fatty acids (Sutherland and Scott, 2010), phenolic acids (Hartley and Akin, 1989), alkaloids (Krishna et al., 2008), terpenes (Rochfort et al., 2008), cysteine proteases (Stepek et al., 2005). Ingestion of high concentrations of some of these metabolites can have adverse effects on livestock (Athanasiadou et al., 2007); therefore, studies on their anthelmintic potential should also examine their effects on animal nutrition and productivity.

Few studies have simultaneously compared various natural anthelmintics, particularly those derived from tropical forages. Therefore, the objective of this study was to investigate the effects of supplementing bahiagrass hay (*Paspalum notatum* Flügge) with hays of perennial peanut (*Arachis glabrata* Benth.) or Sericea lespedeza *(Lespedeza cuneata*) or seeds of velvet bean (*Mucuna pruriens*) or papaya (*Carica papaya* Linnaeus) on feed intake, digestibility, nitrogen (N) balance, parasite burden, health, and performance of goats.

Materials and Methods

Forages and Seeds

A 6-week regrowth of bahiagrass hay (BG, *Paspalum notatum* Flügge) and a primary growth of perennial peanut (PEA, cv. Florigraze) were harvested from established stands at the University of Florida Beef Research Unit, Gainesville, FL. Sericea lespedeza (cv. AU Grazer) hay, velvet bean seeds (cv. *Mucuna ceniza*) and papaya seeds (PAP, *Carica papaya*) were purchased or acquired from producers.

Animals and Deworming Protocols

All animal procedures were approved by the University of Florida Institutional Animal Care and Use Committee. The experiments were conducted at the Department of Animal Sciences, University of Florida, Gainesville, FL from December 2011 to April 2012. Thirty-eight male Boer × Spanish × Kiko cross goats weighing 27.4 ± 5.7 kg were treated for coccidiosis with amprolium (Corid, 5 mg/kg BW) and dewormed with albendazole (Valbazen, 10 mg/kg BW) and moxidectin (Cydectin, 0.4 mg/kg BW). Goats were weighed for two consecutive days and then stratified by weight and randomly assigned to 5 treatments.

Experiment 1

Housing and feeding

In the first of two consecutive experiments, the intake, digestibility and nitrogen (N) retention of the diets was measured. Goats were housed in metabolism cages (100 × 40 × 80 cm) adapted for urine collection and fed for 14 days to adapt them to the different diets followed by 7 days of measurements. Goats were fitted with canvas feces collection bags and fed for *ad libitum* consumption (110% of the previous day's intake). Diets consisted of bahiagrass hay alone or bahiagrass hay supplemented with perennial

peanut or sericea lespedeza hay (50% of diet DM) or with velvet bean seeds (10% of the diet DM) or papaya seeds (10 g/day). The inclusion level of velvet beans was aimed at matching the average CP concentration of the legume-hay supplemented diets and that of the papaya seeds was based on recommended levels for controlling GIN without adversely affecting animal health (Roig y Mesa, 1974; Chinoy et al., 1994). The bahiagrass and respective supplements were provided separately at 0800 and 1600 h daily and the papaya was orally dosed by gavage to ensure complete consumption. Water was provided *ad libitum* and 18 g of a vitamin-mineral premix (United Salt Corp., Ranch House Trace Mineralized Salt, Houston, TX) was added to the diet of each goat daily. The mineral mix contained 88% NaCl, 2.5% Ca, 1% S, 1500 mg/kg Fe, 3000 mg/kg Mn, 2500 mg/kg Zn, 25 mg/kg Co, 150 mg/kg Cu, 90 mg/kg, and 10 mg/kg Se.

Sample collection

Samples of each feed were taken daily during the 7-d measurement period and daily refusals were weighed and stored. Total fecal and urine output were collected daily from each goat, weighed, and a 10% subsample was stored (-20ºC) for further analysis. Goats were weighed and blood sampled by jugular venipuncture on day 0 and 21. A Vacutainer tube (BD, Franklin Lakes, NJ) containing sodium heparin anticoagulant was used to collect approximately 20 mL of whole blood from each goat and the tubes were stored on ice. The blood was centrifuged at 1920 × *g* for 20 min at 4ºC to separate the plasma, which was stored at -20ºC until analyzed. Ruminal fluid was collected from 30 randomly selected goats (6 per treatment) on d 21 by aspiration from orally-inserted stomach tubes 3 h after the morning feeding. A representative (100 mL) sample was analyzed immediately for pH (Accumet, model XL 25, Fischer Scientific, Pittsburg, PA)

and acidified with concentrated H_2SO_4, centrifuged for 30 min at 4ºC and 2795 × *g*, and frozen (-20ºC) for subsequent analysis.

Experiment 2

Housing and feeding

Experiment 2 was designed to measure treatment effects on the growth, parasite burden and health of goats. It was started on d 22 and it lasted for 63 d. Goats were housed in pens (20 m^2, 3 pens per treatment, 2-3 goats per pen) with concrete floors and water troughs. All goats were naturally infested with parasites by allowing them to graze bahiagrass pastures infested with L-3 stage larvae of gastrointestinal nematodes (GIN) and coccidia infective oocysts from 0800 to 1500 h daily and then returned to the pens where the same diets as in Experiment 1 were offered for ad libitum consumption (110% of the previous days intake). The bahiagrass and respective supplements were hand-mixed and offered in the same feeder at 0800 and 1600 and supplemented with 150 g/head of a corn and soybean meal supplement containing 15.4% CP and 84.5% total digestible nutrients. Water was provided *ad libitum* and 18 g/head/day of a mineral premix (United Salt Corp., Ranch House Trace Mineralized Salt, Houston, TX) was fed.

Sample collection

Samples of each feed, jugular blood, and feces were taken weekly during the 63 d experiment and stored for further analysis. Approximately 3 mL of blood plasma were collected, processed, and stored as described in Experiment 1. Fecal samples, (approximately 4 grams), were collected directly from the rectum and immediately analyzed for GIN and *Eimeria* fecal egg counts. Body weights were measured for two consecutive days at the beginning and end of the experiment and weekly in the

intervening period. Goats were monitored for evidence of parasite infection using the FAMACHA eye chart (Van Wyk and Bath, 2002). The FAMACHA Chart is a color-coded chart showing five pictures of the conjunctiva of goat eyelids numbered from 1 (normal red color) to 5 (very pale color denoting severe anemia). On d 63, goats were slaughtered at a USDA approved abattoir and abomasums were recovered and stored on ice during transport to the laboratory for counting of adult nematodes.

Analytical Methods

In Experiment 1, samples of feed, orts and feces were dried at 60ºC for 48 hours in a forced air oven and ground to pass through a 1-mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA). Samples were analyzed for ash by combustion in a muffle furnace at 600ºC overnight. Total N concentration was determined by the Dumas combustion method (AOAC, 2000) using a Vario MAX CN Macro Elementar Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) and used to calculate crude protein concentration (CP = N x 6.25). Neutral and acid detergent fiber (NDF and ADF) (Van Soest et al., 1991) were analyzed using an ANKOM 200 Fiber Analyzer (Ankom, Macedon, NY). Samples were also analyzed for acid detergent lignin (ADL) according to Van Soest et al. (1991). Amylase was used for NDF analysis but sodium sulfite was not and the results were expressed inclusive of residual ash. Apparent digestibilities of DM, OM, NDF and ADF were calculated.

Volatile fatty acids in ruminal fluid were measured using the method of Canale et al. (1984) and a High Performance Liquid Chromatography system (Hitachi LaChrom Elite, Tokyo, Japan) coupled to a UV detector (Hitachi L-2400) set at 210 nm. The column was a Bio-Rad Aminex HPX-87H (Bio-Rad laboratories, Hercules, CA) with 0.015 M H_2SO_4 mobile phase and a flow rate of 0.7 mL/min at 45ºC. Ruminal fluid

ammonia-N concentration was determined by an ALPKEM auto analyzer (ALKPEM Corporation, Clackamas, OR) and an adaptation of the Noel and Hambleton (1976) procedure that involved colorimetric quantification of N. Plasma glucose (PGluc) and urea nitrogen (PUN) concentration were measured using adaptations for a Technicon Autoanalyzer II (Bran-Luebbe, Elinsford, NY) of methods of Gochman and Schmitz (1972), and Coulombe and Favreau (1963).

In Experiment 2, feed samples were analyzed for chemical composition as in Experiment 1. Plasma haptoglobin concentrations were determined by measuring haptoglobin/hemoglobin complexing based on differences in peroxidase activity (Makimura and Suzuki, 1982). Gastrointestinal (GIN) and *Eimeria* sp. (EIM) fecal egg counts (FEC) were determined with a modified McMaster procedure (Whitlock, 1948).

Abomasum samples were dissected and adult worms were recovered and counted using a dissecting microscope (Hansen and Perry, 1994) and preserved in a 70% ethanol solution.

Statistical Analyses

Experiment 1 had a randomized complete block design with 5 treatments and 8 replicate goats in all treatments except PAP, which had 6 goats. Forage chemical composition and animal measurements were analyzed with the MIXED statement of SAS v 9.3 (2012, SAS Institute Inc®, Cary, NC). The model for analyzing the forage data included treatment and replicate, whereas, that for animal measurements included treatment, block, and goat (random) effects. The PDIFF statement of SAS (2012, SAS Institute Inc®, Cary, NC) was used to compare Least Square means.

Experiment 2 had a randomized complete block design with 5 treatments and three experimental units (pens) per treatment. The model included treatment, block

(random term), time (repeated measure) and treatment x time effects. The covariance structure with the least Akaike information criterion was chosen for each analysis performed. Data were analyzed with the MIXED procedure of SAS (2012, SAS Institute Inc®, Cary, NC) and the PDIFF statement was used to compare Least Squares means. The slice command was used to detect differences between treatments at specific time points. For FEC data, the distribution of residuals was examined using the normal probability, quantile-quantile, and predicted mean plots and the data was log transformed if appropriate. Significance was declared at $P < 0.05$, and only significance interactions effects are discussed. Tendencies were declared at $P > 0.05$ and ≤ 0.11.

Results and Discussion

Chemical Composition of Diets

Among the hays, PEA had slightly lower ($P < 0.01$) DM and OM concentration than BG and LES but CP concentrations were greater in the legumes. In contrast, NDF concentration was greater in BG ($P < 0.01$), followed by LES. Among the seeds, PAP had greater dry matter (DM), crude protein (CP), NDF, ash, and ADL concentrations ($P < 0.01$) and lower OM concentration compared to MUC.

The DM, OM and NDF values of BG hay were similar to those in other studies on tropical grasses (Kostenbauder et al., 2007; Foster et al., 2009; McCornick et al., 2011). However, the CP concentration was higher than those in the latter studies but similar to those reported by Shaik et al. (2006), Inyang et al. (2010), and McCormick et al. (2011). The relatively high CP value for BG hay is attributable to the fact that it was harvested in the fall as values for summer bahiagrass harvested in the summer at a similar regrowth interval (6 week) are typically lower (Kostenbauder et al., 2007; Foster et al., 2009). The chemical composition of the PEA (Romero et al., 1987; Valencia et al., 2001; Mislevy et

al., 2007; Foster et al., 2009; Myer et al., 2010), LES (Terrill et al., 1989; Animut et al., 2008; Puchala et al., 2012), MUC (Siddhuraju et al., 1996; Vadivel and Janardhanan, 2000; Chikagwa-Malunga et al., 2009a) and PAP (Passera and Spettoli, 1981; Marfo et al., 1986; Puangsri et al., 2005) were similar to those in the literature.

Experiment 1

Intake, digestibility and nitrogen balance

Intakes of DM tended (P = 0.11) to be greater in goats fed PEA and LES than those fed BG but intakes of OM and NDF did not differ among treatments (Table 3-2). Nitrogen intake tended (P = 0.07) to be greater in goats fed PEA, LES, and MUC than in those fed BG alone. Legume supplementation tended (P = 0.07) to increase N intake due to the greater CP concentrations of PEA, LES, and MUC versus that for bahiagrass. These results were consistent with previous reports on legume supplementation of grass-based diets (Nyambati et al., 2003 and Foster et al., 2009). Despite having the greatest CP concentration, N intake was not increased by PAP due to the small amount of PAP supplemented (10 g/day). Acid detergent lignin (ADL) intake was greater in goats (P < 0.01) fed LES and PEA than the other treatments due to the relatively high concentrations of ADL in these hays.

Apparent digestibilities of DM and OM were greater (P < 0.01) in goats fed MUC than BG (Table 3-3) perhaps reflecting greater supply of fermentable carbohydrates for microbial growth by the starch in MUC (Chikagwa-Malunga et al., 2009b; Loyra-Tzab et al., 2011). The NDF digestibility tended (P = 0.07) to be less in LES than in MUC and BG. This is partly attributable to the greater ADL intake LES as high forage lignin concentrations limit digestibility (Wilson, 1994). Nitrogen digestibility was greater (P < 0.01) in MUC and PEA diets than LES, BG or PAP. Studies have shown that *M.*

pruriens supplementation has increased N intake and retention, weight gain and milk production in ruminants (Burgos et al., 2002; Castillo-Caamal et al., 2003a, b). Supplementation with PEA hay has also increased N digestibility and retention in lambs (Foster et al., 2009). Tannins in LES may have contributed to the low NDF and N digestibility of the LES diet as they form indigestible complexes with protein and carbohydrates that reduce digestibility (Waghorn et al., 1994a; Athanasiadou et al., 2001; Min et al., 2003). No differences in N retention were detected among treatments because of the relatively high CP concentration of BG, the small differences in N intake among treatments and the similar N losses in urine and feces (Table 3-4).

Ruminal fluid fermentation indices

Goats fed MUC had a lower ($P < 0.01$) ruminal pH than all other goats (Table 3-5). Ruminal ammonia nitrogen concentration was greatest ($P < 0.01$) in goats fed MUC, followed by PEA, LES and goats fed BG had the least value. Proportions of individual VFA, total VFA concentration, and acetate:propionate ratio were not different among dietary treatments.

The total VFA concentrations were at the lower end of the normal range (100 to 120 mM/L) in forage fed ruminants (Bergman, 1990) partly due to the relatively low digestibility of the diets. Ruminal pH was more acidic in goats fed MUC likely reflecting the greater starch ingestion and fermentation on the MUC diet as starch concentrations of MUC range from 27 to 31% (Siddhuraju and Becker, 2005; Chikagwa-Malunga et al., 2009a), whereas they are typically less than 10% in the other ingredients that were fed (McKell et al., 1969; Forde et al., 1976; Egli et al., 1980). Though lower than others, the ruminal pH of goats fed MUC was within the range (6.2-7.2) required to maintain normal cellulolytic activity in the rumen (Van Soest, 1994).

Legume supplementation resulted in greater ruminal ammonia-N concentrations because it tended to increase N intake compared to BG and PAP and most of the protein present in legumes is rumen-degradable (Broderick, 1995). For maximum microbial N production in the rumen, an ammonia concentration of at least 5 mg/dL is recommended, though the limiting concentration is approximately 2 mg/dL (Satter and Slyter, 1974). Feeding MUC provided sufficient ammonia for optimizing microbial N synthesis and feeding the other supplements ensured the concentrations were not limiting. Feeding BG alone resulted in insufficient ammonia-N for optimizing microbial N synthesis.

Blood urea nitrogen and Plasma glucose

There were no differences in blood urea nitrogen (BUN) or plasma glucose concentration among the treatments (Table 3-5) but the respective values were within the normal physiological ranges (8 to 20 and 50 to 80 mg/dL; Kenako, 1989). That supplementation with PEA, LES and MUC increased ammonia-N but not BUN suggests that fermentable energy supply from these diets was adequate in relation to the effective ruminally degradable protein supplied (Macrae et al., 2006).

Experiment 2

Fecal egg and abomasal worm counts

As usual, FEC values started to increase after approximately 3 weeks of exposure to GIN (Sutherland and Scott, 2010; Figure 3-1). After 3 weeks, goats fed BG and MUC had higher GIN FEC than the other treatments (treatment x time interaction, $P < 0.01$). Goats fed PAP consistently had the least values, though values for PAP and LES were similar except in weeks 5, 6, 8 and 9. Goats fed PEA had lower GIN FEC than those fed BG in weeks 3, 4, 5, 8 and 9. Goats fed MUC had higher EIM FEC than those fed BG

from weeks 1 to 7 (treatment x time interaction, $P < 0.01$; Figure 3-2) but those fed PAP and PEA, had lower values than BG from weeks 3 to 9. Goats fed LES only had lower EIM FEC than those fed BG in weeks 8 and 9. Averaged across the experimental period, goats fed PAP and LES reduced GIN FEC ($P < 0.01$) by 72 and 55%, respectively relative to those fed BG, whereas those fed PEA had intermediate values (Table 3-6). Furthermore, goats fed PAP, PEA and LES had fewer ($P = 0.02$) EIM FEC than those fed MUC, but similar values to those fed BG. Feeding MUC did not affect adult worm counts but feeding PAP, LES or PEA ($P < 0.05$) reduced the number of adult GIN in the abomasum by 78, 52, and 41%, respectively compared to feeding BG.

Relative to values for BG, the higher CP concentrations of LES (Gamble et al., 1996; Min et al., 2005) and PEA (Mislevy et al., 2007; Foster et al., 2009) would have increased their nutritional status and made the goats more resilient to the parasites. Furthermore, the presence of condensed tannins in LES (4 to 22% of DM, Windham et al., 1988) and PEA (1.9 to 3.8% of DM, Valencia et al., 2007; Foster et al., 2009) may explain their anthelmintic effects. Several studies have reported that LES intake reduced GIN FEC and larval development in small ruminants (Min and Hart, 2003; Min et al., 2004, 2005; Lange et al., 2005; Shaik et al., 2006; Terrill et al., 2009). Intake of LES has also reduced the GIN worm burden by up to 78% (Min et al., 2005) and decreased adult female fecundity in the abomasum and small intestine of goats (Shaik et al., 2006). In contrast, no published studies showing anthelmintic effects of PEA were found in the literature. A companion study conducted just before this experiment by our research group also showed that PEA supplementation decreased GIN FEC in goats

(Hamie, 2012, unpublished). More research is required to support these findings and to determine the optimal level of PEA supplementation for controlling GIN in goats.

The most effective treatment against FEC and abomasal nematode counts in this study was PAP. Previous studies demonstrated the anthelmintic efficacy of papaya extracts or seeds against GIN in *in vitro* experiments *(*Krishnakumari and Majumder, 1960; Dhar, et al. 1965), children (Okeniyi et al., 2007), mice (Satrija et al., 1995), and sheep (Ameen et al., 2010; Buttle et al., 2011). This effect has been attributed to compounds in PAP such as alkaloids carpaine (Krishna et al., 2008) and carpasemin (later identified as benzyl thiourea by Panse and Paranjpe, 1943) or to cysteine proteases (Stepek et al., 2005) or benzyl isothiocyanate (Krishnakumari and Majumder, 1960; Tang, 1971). Based on chromatographic quantification of these compounds and anthelmintic tests, benzyl isothiocyanate was reported to be the major source of the anthelmintic activity in papaya seeds (Kermanshai, et al., 2001). Because benzyl isothiocyanate is potentially toxic, goitrogenic, carcinogenic, and mutagenic (Yamaguchi, 1980; Fenwick et al., 1983), great care is needed to prevent an overdose with papaya seeds in ruminants. Previous reports in animals and humans suggest that non-lethal amounts should be between 1 to 6.2 g of seeds/d or 56–112 mg/kg of body weight according to the species (Roig y Mesa, 1974; Chinoy et al., 1994). However, doses of up to 15g/head/d are typically used in some parts of South America (Fernando Carcelén, personal communication). In this study, the dose of 10 g/head/day was used to maximize the anthelmintic potential and no adverse effects of this dose on voluntary intake, growth, or health of the goats was observed.

Very few studies have dealt with the effect of the supplements used in this study on coccidian parasites infections. Hur et al. (2005) reported a decrease in fecal coccidian oocyst counts in Korean native goats fed pine needles and oak leaves due to their high condensed tannins concentrations. Lin et al. (2003) and Nguyen et al. (2005) also reported that coccidian oocysts counts in goats were reduced by feeding leucaena (*Leucaena leucocephala*) and cassava (*Manihot esculenta*) leaves but while leucaena leaves contain low concentrations of condensed tannins (0.51 to 1.60% of DM; Garcia et al., 2006), cassava leaves contain moderate concentrations (3.25 to 4.15% of DM; Sokerya and Preston, 2003). Lespedeza and perennial peanut contain moderate condensed tannin concentrations (Valencia et al., 2007; Windham et al., 1988; Foster et al., 2009) and this may explain why supplementation with these forages decreased FEC and abomasal nematode counts in this study.

Previous demonstrations of the anthelmintic effects of papaya against coccidian infections in goats used papaya leaves (Kabirizi et al., 2004; Oyebanji and Afeni, 2009; Adiwimarta et al., 2010). This study indicates that papaya seeds can also be strategically used to decrease coccidian FEC in goats. Future research should confirm these findings and examine the optimal dietary inclusion rate of the seeds.

Although mucuna seeds have relatively high CP concentrations (250-350 g/kg of DM; Siddhuraju and Becker, 2005) and feeding MUC at 24% of the diet DM has reduced coccidian oocysts scores in lambs (Chikagwa-Malunga et al., 2009b), MUC did not reduce FEC in this study. This may be because of differences in the variety and inclusion rates of MUC in both studies. The reason why feeding mucuna increased GIN and EIM REC at certain periods in the trial is unknown but it may be related to

antinutrients in mucuna such as the 3,4-dihydroxy-l-phenylalanine (L-dopa) it contains. Though up to 53% of mucuna L-dopa can be ruminally degraded (Chikagwa-Malunga et al., 2009b), residual amounts may be sufficient to compromise the immune response as in non-ruminants (Del Carmen et al., 1999; Siddhuraju and Becker, 2001) and facilitate the growth of GIN in parasitized ruminants. Anthelmintic properties of MUC may be evident in MUC trichomes, which contain mucunaine, a cysteine protease (Reddy et al., 2008) that can damage intestinal nematodes (Conroy and Thakur, 2005) and thus control GIN infections (Buttle, 2011). Future studies should examine the effects of feeding MUC trichomes and seeds at different dietary inclusion levels on GIN and coccidian parasite burdens.

Measures of anemia and the acute phase immune response

There were no differences in hematocrit values and FAMACHA scores among treatments (Table 3-7), indicating that treatments did not affect anemia indices. Previous studies reported that GIN infections and the attendant anemia can generally be alleviated by feeding sufficient supplementary protein (Sutherland and Scott, 2010). Goats received 150 g of corn and soybean concentrate as well as forages that contained at least 12% CP daily during the evaluation period. Therefore, the high protein concentration of the diets likely prevented anemia.

Haptoglobin is a hemoglobin-binding protein, which can be used to monitor activation of the acute phase response during an infective disease (Colditz, 2002) or to assess the health status of cattle (Parra et al., 2005) or to identify immunocompromized animals (Heegaard et al., 2000; Arthington et al., 2003). Goats fed BG had the greatest haptoglobin concentrations on average, whereas those fed PEA and PAP had the lowest values. However, effects of treatment on haptoglobin concentration varied with

time (P<0.01). Mean haptoglobin concentrations were similar from weeks 0 to 3 and 5, after which those of goats fed BG increased dramatically and remained greater than those of other goats till the end of the trial (Figure 3-3). Goats fed MUC also had greater haptoglobin concentrations than others in the last 3 weeks of the trial. In this study, goats fed BG and MUC, which had the greatest FEC counts, also had greater haptoglobin concentrations, indicating that greater levels of parasitism in the goats compromised their health and elicited an inflammatory response against the infection. In contrast, due to the anthelmintic effect of PEA, PAP, and LES no inflammatory response was elicited when they were fed and consequently they did not increase haptoglobin concentrations.

Animal performance

Goats fed PAP, PEA and LES had greater DMI (P < 0.01) than those fed BG and those fed MUC had intermediate values. Therefore, goats fed BG and MUC had higher FEC and abomasal worm counts and lower DMI than others. This is supported by the statement that the major factor affecting animal performance in parasitized animals is the associated anorexia (McKellar, 1993; Fox, 1997; Greer, 2008).

There were no differences among treatments in initial or final BW, average daily gain (ADG) or gain to feed ratio among treatments. The absence of treatment differences among BW measures may be related to the supply of sufficient CP for growth in all dietary treatments as well as the relatively low level of GIN infection in the study. The experiment was conducted from January to April, when GIN parasite infestation levels are less than those in the summer and fall (Kates, 1950; Williams et al., 1987; Miller et al., 1998). Future experiments should examine the anthelmintic

effects of the supplements tested in this study against severe cases of GIN parasitosis in the summer.

Table 2-1. Trends in exportation of live goats and goat meat (Reprinted with permission from Solaiman, S. G. 2007. Assessment of the meat goat industry and future outlook for U.S. small farms. Report. Tuskegee University. Tuskegee, AL)

	2002	2003	2004	2005	2006
Live goats	26,081	29,579	3,775	3,976	11,075
Goat meat (MT)	61.5	54.8	84.4	883.2	469.0

Table 2-2. Fecal egg count (FEC) and FEC reduction (%) of Spanish goats treated with various anthelmintics (Reprinted with permission from Terrill, T. H. et al., 2001. Anthelmintic resistance on goat farms in Georgia: efficacy of anthelmintics against gastrointestinal nematodes in two selected goat herds)

Treatment	Mean FEC 2 weeks post-treatment, eggs/gram	FEC reduction%
Control	3827	
Albendazole	1450	62
Doramectin	917	76
Fenbendazole	3460	10
Ivermectin	844	78
Levamisole	335	91
Morantel tartrate	1990	48
Moxidectin	0 (<50)	100
Albendazole + ivermectin	445	88

Table 3-1. Chemical composition of the bahiagrass (BG), perennial peanut (PEA), and sericea lespedeza (LES) hays and mucuna (MUC) and papaya (PAP) seeds fed to goats.

Component,	BG	PEA	LES	MUC	PAP	SEM	*P* value
Dry matter (DM), %	90.2[b]	89.1[a]	89.9[b]	90.4[b]	92.9[c]	0.3	<0.01
Organic matter, % of DM	94.6[c]	91.8[b]	95.4[d]	96.2[e]	90.8[a]	0.1	<0.01
Neutral detergent fiber, % of DM	72.8[e]	51.9[c]	60.9[d]	13.5[a]	32.3[b]	0.4	<0.01
Crude protein, % of DM	12.4[a]	14.3[b]	14.6[b]	28.5[c]	30.0[d]	0.1	<0.01
Nitrogen, % of DM	1.9[a]	2.2[b]	2.3[b]	4.4[c]	4.7[d]	0.01	<0.01
Ash, % of DM	5.4[c]	8.2[d]	4.6[b]	3.8[a]	9.2[e]	0.1	<0.01
Acid detergent lignin, % of DM	5.1[c]	9.4[d]	17.6[e]	0.3[a]	1.9[b]	0.3	<0.01

[a, b, c, d, e] Means within a row with different superscripts differ ($P < 0.05$)

Table 3-2. Effects on intake of dry matter (DMI), organic matter (OMI), neutral detergent fiber (NDFI), nitrogen (NI) and acid detergent lignin (ADLI) in goats resulting from supplementing bahiagrass (BG) hay with perennial peanut (PEA) or lespedeza (LES) hay or mucuna (MUC) or papaya seeds (PAP).

	BG	PEA	LES	MUC	PAP	SEM	*P* value
DMI, g	612	757	745	627	627	89	0.11
OMI, g	580	710	702	596	593	89	0.15
NDFI, g	458	506	523	408	472	66	0.18
NI, g	12	16	15	15	12	2	0.07
ADLI, g	32[a]	50[b]	65[c]	28[a]	32[a]	7	<0.01

[a, b, c] Means within a row with different superscripts differ ($P < 0.05$)

Table 3-3. Effects on *in vivo* apparent digestibility of dry matter (DMD), organic matter (OMD), neutral detergent fiber (NDFD) and nitrogen (ND) in goats resulting from supplementing bahiagrass (BG) hay with perennial peanut (PEA) or lespedeza (LES) hay or mucuna (MUC) or papaya seeds (PAP).

	BG	PEA	LES	MUC	PAP	SEM	*P* value
DMD, %	54.9[ab]	56.5[bc]	53.1[ab]	59.5[c]	51.1[a]	1.8	<0.01
OMD, %	56.0[ab]	57.9[bc]	53.8[ab]	60.8[c]	52.1[a]	1.7	<0.01
NDFD, %	62.8	59.4	56.4	61.5	59.8	1.8	0.07
ND, %	50.5[a]	58.9[b]	49.0[a]	61.2[b]	49.7[a]	1.8	<0.01

[a, b, c] Means within a row with different superscripts differ ($P < 0.05$)

Table 3-4. Effects on nitrogen (N) balance in goats resulting from supplementing bahiagrass (BG) hay with perennial peanut (PEA) or lespedeza (LES) hay or mucuna (MUC) or papaya seeds (PAP).

	BG	PEA	LES	MUC	PAP	SEM	P value
N intake g/d	12.4	15.5	15.2	14.6	12.4	2.0	0.07
Fecal N output, g/d	5.6	6.2	7.3	5.5	5.8	0.8	0.07
Urinary N output, g/d	2.8	5.0	3.4	4.4	3.1	1.0	0.12
Retained N, g/d	4.2	4.2	4.6	4.7	3.7	0.9	0.87

Table 3-5. Effects on ruminal fermentation indices, blood urea nitrogen (BUN) and plasma glucose concentrations in goats resulting from supplementing bahiagrass (BG) hay with perennial peanut (PEA) or lespedeza (LES) hay or mucuna (MUC) or papaya seeds (PAP).

	BG	PEA	LES	MUC	PAP	SEM	*P* value
Ruminal pH	7.0[b]	6.7[b]	6.7[b]	6.3[a]	6.8[b]	0.1	<0.01
Ammonia N, mg/dL	1.2[a]	3.5[b]	3.5[b]	6.3[c]	1.7[ab]	0.8	<0.01
Total VFA, mM	89.2	100.1	88.0	95.2	98.4	11.7	0.92
Acetate, mM	63.8	68.1	61.6	66.0	70.1	8.2	0.95
Propionate, mM	19.2	22.7	18.9	19.3	20.2	3.4	0.94
Iso-butyrate, mM	0.4	0.3	0.6	0.9	0.4	0.2	0.13
Butyrate, mM	5.0	7.6	5.9	7.1	5.7	1.2	0.49
Iso-valerate, mM	0.5	1.6	1.3	1.0	1.2	0.6	0.70
Valerate, mM	0.1	0.2	0.1	0.2	0.3	0.1	0.50
Acetate:propionate	3.6	3.5	3.7	3.6	3.6	0.5	0.99
BUN, mg/dL	13.2	15.8	13.1	14.6	12.9	1.2	0.31
Plasma glucose, mg/dL	70.0	61.6	62.9	65.6	63.5	2.1	0.64

[a, b, c] Means within a row with different superscripts differ ($P < 0.05$)

Table 3-6. Effects on gastrointestinal (GIN) and *Eimeria* sp. (EIM) fecal egg (FEC) and abomasal adult worm counts in goats resulting from supplementing bahiagrass (BG) hay with perennial peanut (PEA) or lespedeza (LES) hay or mucuna (MUC) or papaya seeds (PAP).

							P values		
	BG	PEA	LES	MUC	PAP	SEM	Treatment	Week	Treatment x week
GIN FEC, epg	541[bc]	370[ab]	244[a]	739[c]	152[a]	83.3	<0.01	<0.01	<0.01
EIM FEC, epg	1235[ab]	725[a]	1031[a]	2120[b]	497[a]	315.9	0.02	<0.01	0.02
Adult worm counts	3066[c]	1798[b]	1462[b]	2934[c]	690[a]	269	<0.01	NA[1]	NA[1]

[a, b, c] Means within a row with different superscripts differ ($P < 0.05$)

[1] Not applicable

Table 3-7. Effects on hematocrit, FAMACHA scores and haptoglobin contents in goats resulting from supplementing bahiagrass (BG) hay with perennial peanut (PEA) or lespedeza (LES) hay or mucuna (MUC) or papaya seeds (PAP).

							P values		
	BG	PEA	LES	MUC	PAP	SEM	Treatment	Week	Treatment x week
Hematocrit, %	25.4	24.6	24.9	23.8	26.1	1.3	0.56	0.14	0.87
FAMACHA score	1.36	1.42	1.34	1.31	1.06	0.18	0.65	0.53	0.79
Haptoglobin, arbitrary units	0.055[c]	0.030[a]	0.033[ab]	0.040[b]	0.032[a]	0.003	<0.01	<0.01	<0.01

[a, b, c] Means within a row with different superscripts differ ($P < 0.05$)

Table 3-8. Effects on the performance of goats resulting from supplementing bahiagrass (BG) hay with perennial peanut (PEA) or lespedeza (LES) hay or mucuna (MUC) or papaya seeds (PAP).

							P values		
	BG	PEA	LES	MUC	PAP	SEM	Treatment	week	Treatment x week
DMI, g per goat	617[a]	730[bc]	750[c]	661[ab]	775[c]	52	<0.01	<0.01	0.11
DMI as BW%	2.26[a]	2.72[b]	2.78[b]	2.37[a]	2.62[b]	0.20	< 0.01	< 0.01	0.51
Initial BW, kg	27.2	26.8	26.2	26.8	27.7	3.69	0.74	NA[1]	NA[1]
Final BW, kg	30.2	29.9	30.8	30.9	32.5	3.60	0.54	NA[1]	NA[1]
Body Weight, kg	27.8	27.4	27.6	27.9	30.0	3.67	0.31	NA[1]	NA[1]
ADG, g/day	47	50	73	66	69	11	0.23	NA[1]	NA[1]
Gain:Feed ratio	0.55	0.47	0.68	0.70	0.69	0.12	0.39	NA[1]	NA[1]

[a, b, c] Means within a row with different superscripts differ ($P < 0.05$)

[1] Not applicable

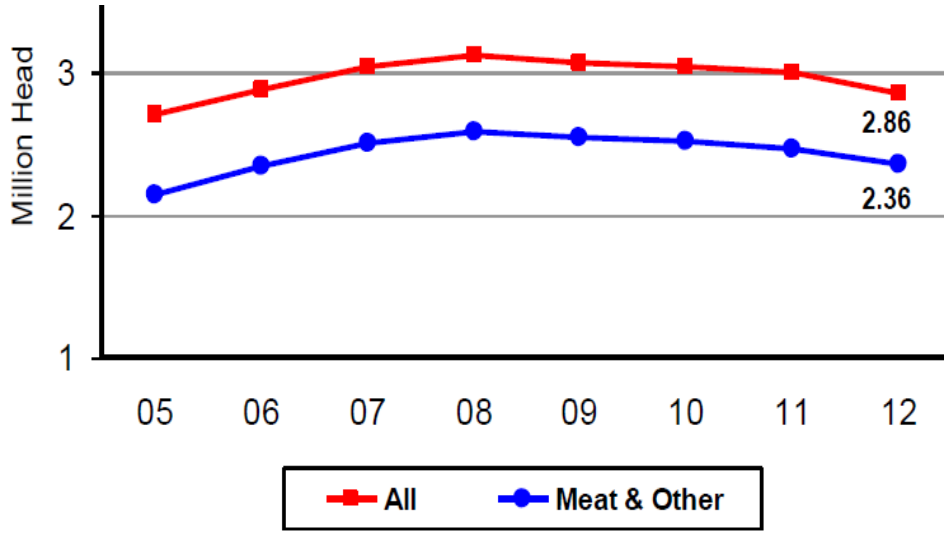


Figure 2-1. United States goat population trends (Reproduced from USDA, 2012. Sheep and goats inventory)

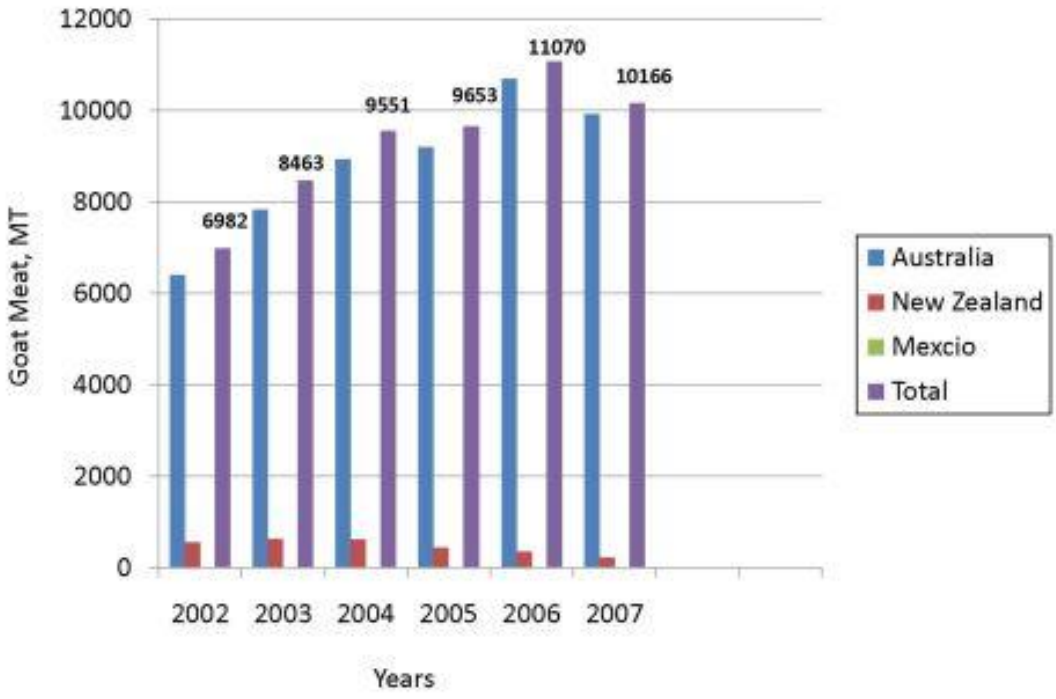


Figure 2-2. Changes in the amount of goat meat imported to the United States from 2002 to 2007 (Reprinted with permission from Solaiman, S. G. Assessment of the meat goat industry and future outlook for U.S. small farms. Report. Tuskegee University. Tuskegee, AL. 2007)

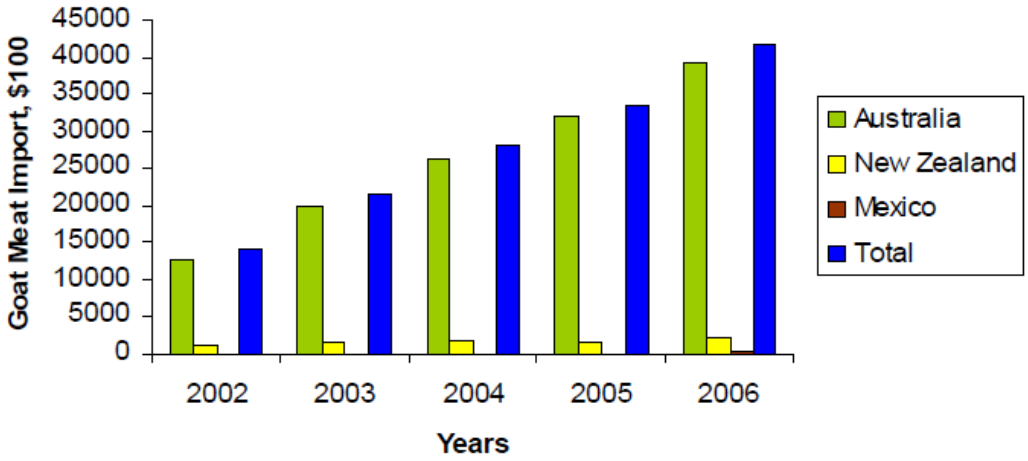


Figure 2-3. Value of goat meat imported to the United States (Reprinted with permission from Solaiman, S. G. Assessment of the meat goat industry and future outlook for U.S. small farms. Report. Tuskegee University. Tuskegee, AL. 2007)

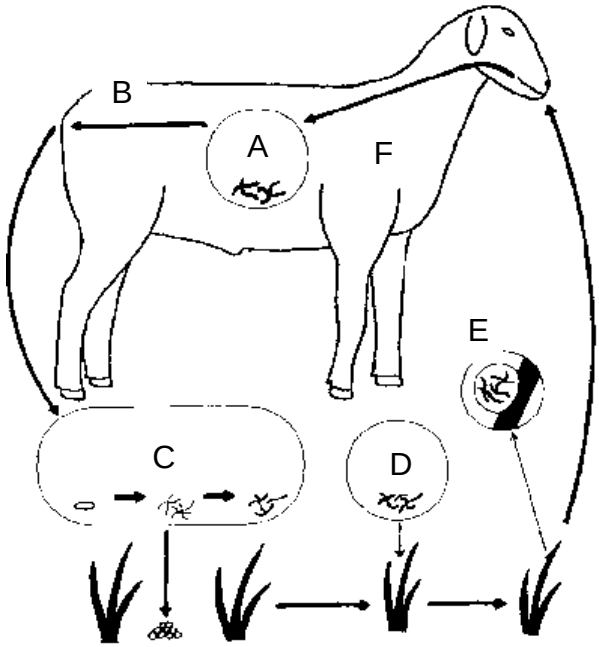


Figure 2-4. Life cycle of *Haemonchus contortus.* A) Adult worms in abomasum. B) Eggs released incorporated into feces. C) Egg to L1 to L2 larvae. D) L3 larvae in grass. E) L3 larvae suspended in dew drop. F) Swallowed larvae develop into adult. (Reprinted with permission from Machen, R. F. et al., 1998. A *Haemonchus contortus* management plan for sheep and goats in Texas. Pamphlet L-5095. Texas A & M University, AgriLife Extension Serivice, TX).

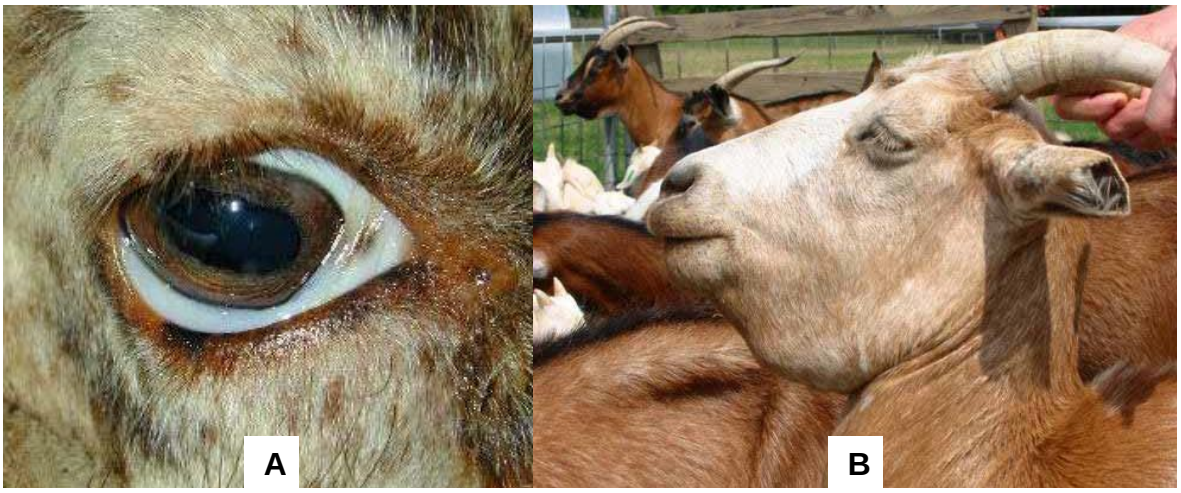
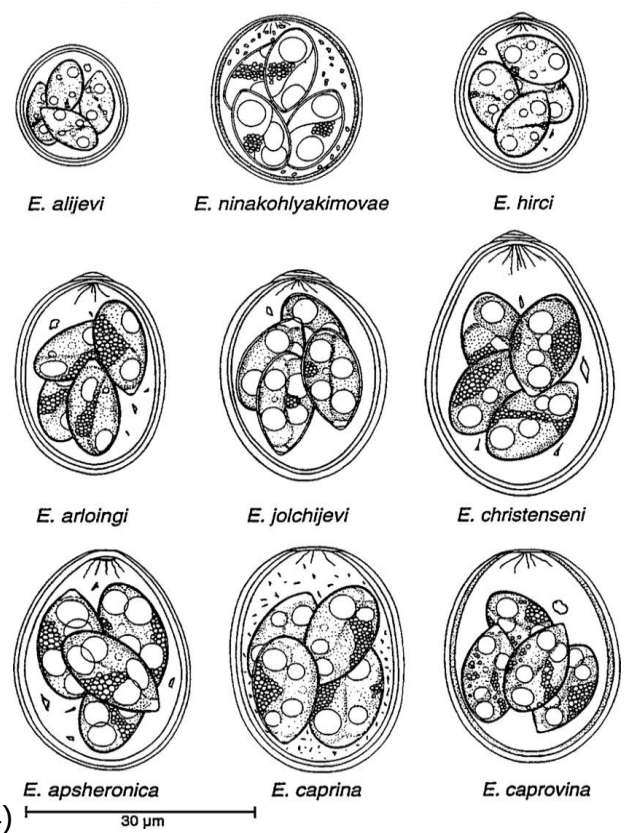


Figure 2-5. Haemonchosis symptons in goats. A) Anemia. B) Submandibular edema (Reprinted with permission from http://www.extension.org/pages/19914/goat-abomasal-worms)



4)

Figure 2-6. Sporulated oocysts of the principal species of *Eimeria* in goats (Reprinted with permission from Eckert, J. et al., 1995. Guidelines on techniques in coccidiosis research. European Commission, DGXII)

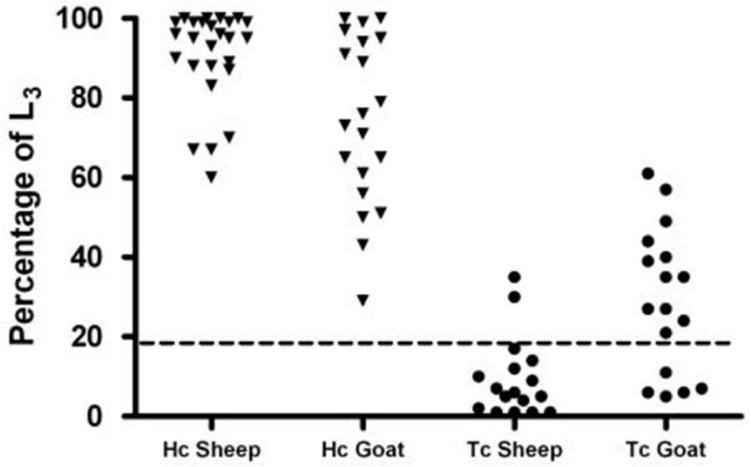


Figure 2-7. Scatterplots of nematode larvae identified as *Haemonchus contortus* (Hc) and *Trichostrongylus colubriformis* (Tc) in pooled fecal samples from 26 sheep and 20 goat farms in the southeastern United States. The horizontal dashed line represents the cut off for testing a species for anthelmintic resistance (Reprinted with permission from Howell, S. B. et al., 2008. Prevalence of anthelmintic resistance on sheep and goat farms in the southeastern United States).

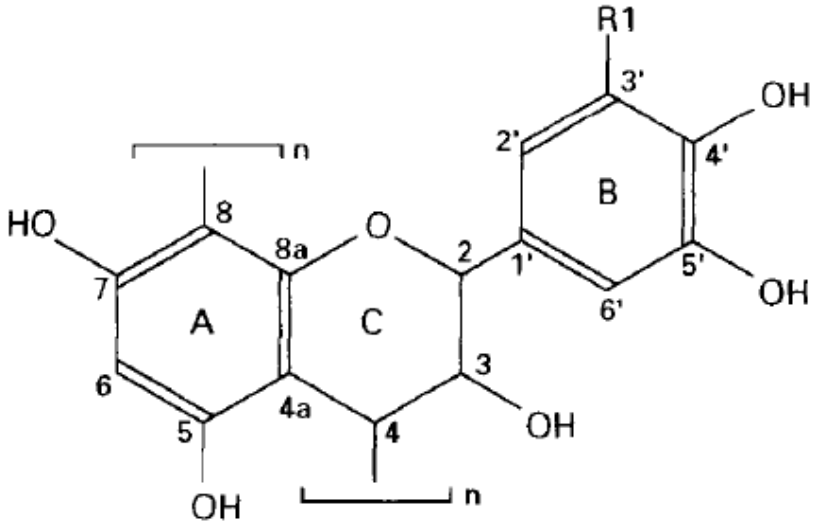


Figure 2-8. Constituent flavan-3-ols of condensed tannins (Reprinted with permission from Schofield, P. et al., 2001. Analysis of condensed tannins: A review)

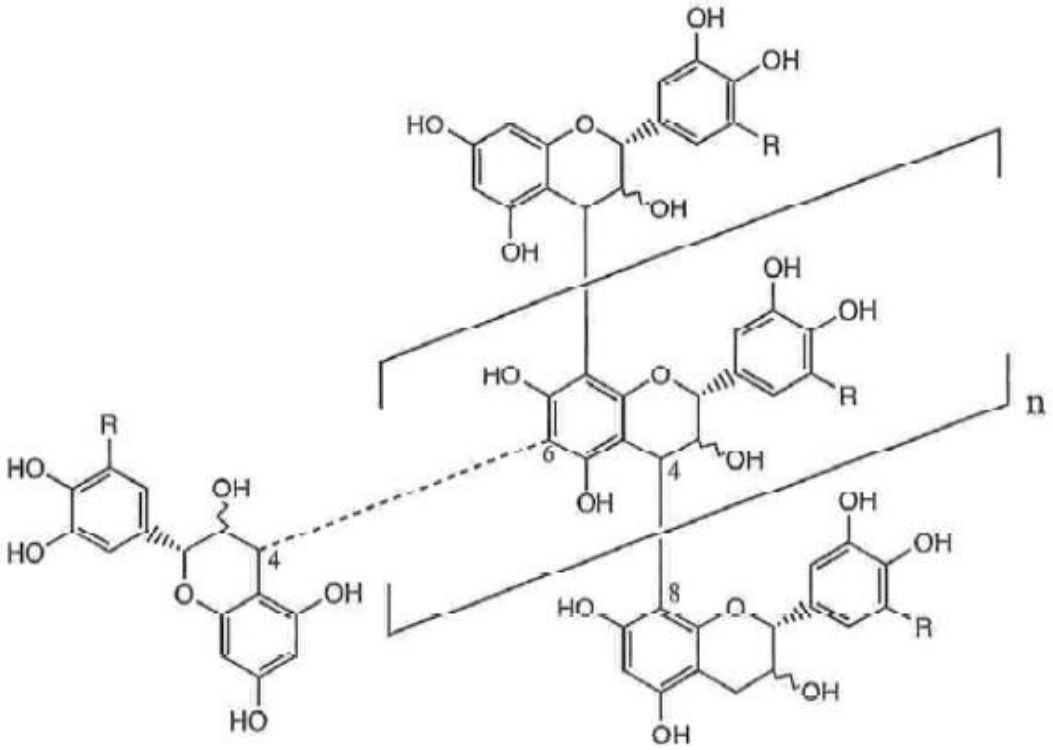


Figure 2-9. The generalized structure of proanthocyanidins. R = H in procyanidin or OH in prodelphinidin (Reprinted with permission from Schofield, P. et al., 2001. Analysis of condensed tannins: A review)

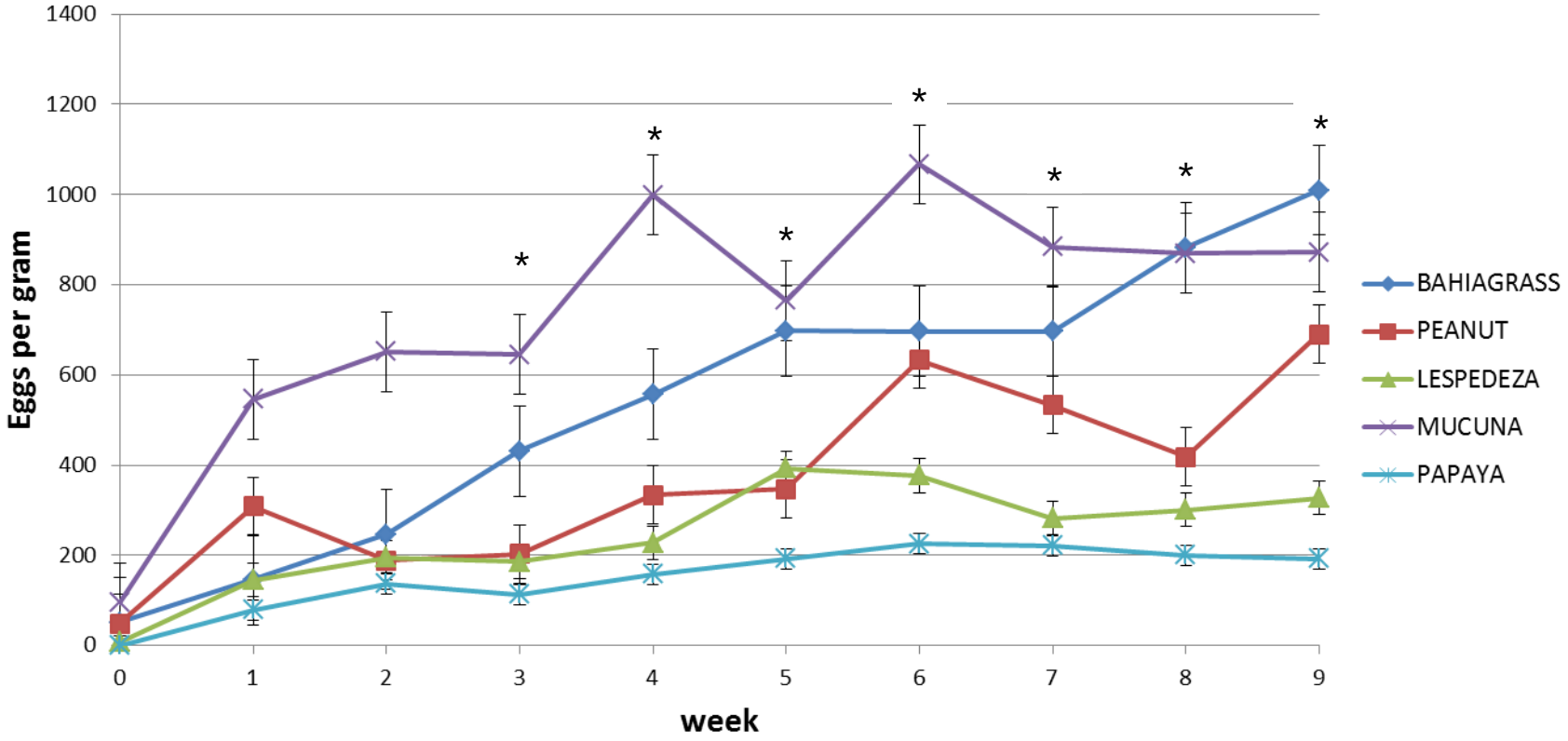


Figure 3-1. Effects of supplementing bahiagrass (BG) hay with perennial peanut (PEA) or lespedeza (LES) hay or mucuna (MUC) or papaya seeds (PAP) on gastrointestinal fecal egg counts. Treatment x time, $P < 0.05$. Error bars are standard errors. * Counts at the week specified differed ($P < 0.05$).

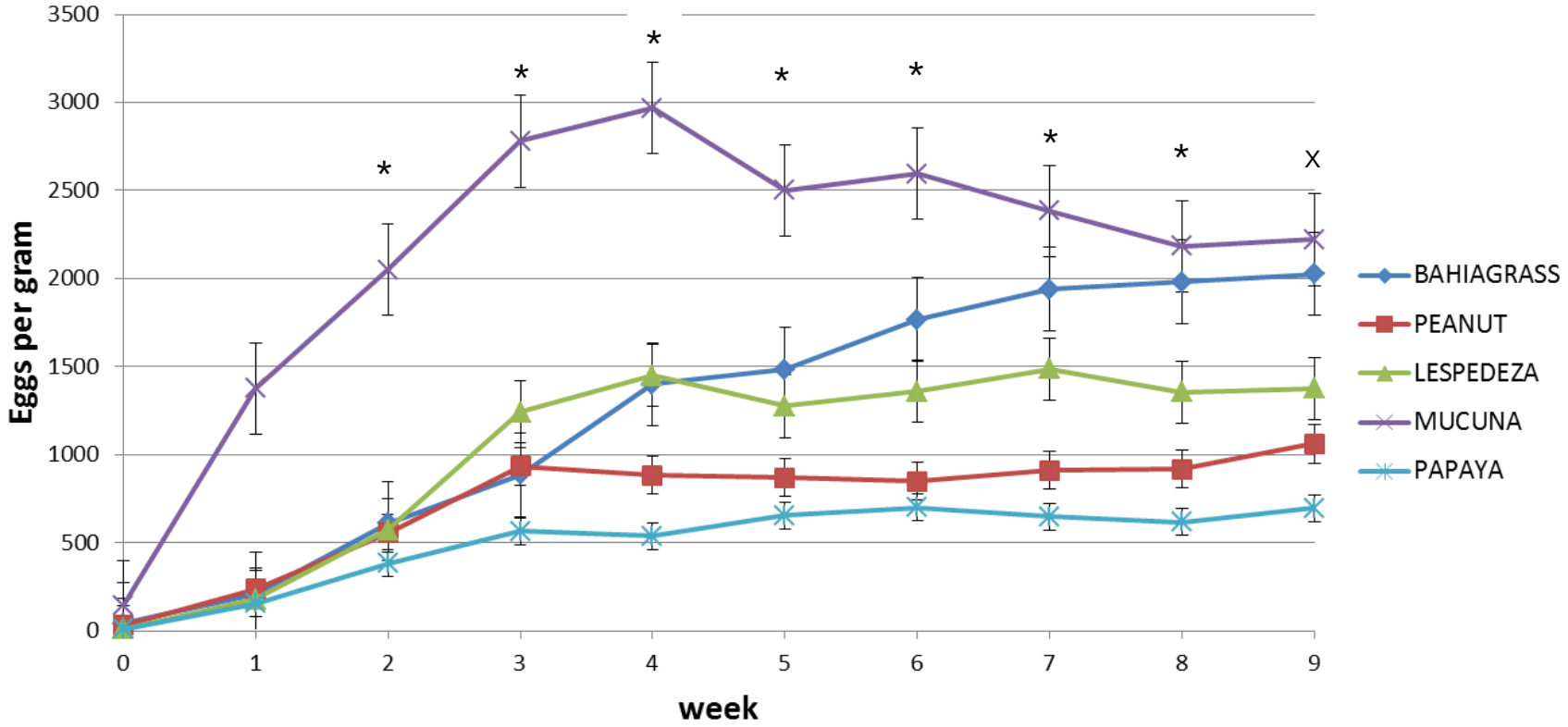


Figure 3-2. Effects of supplementing bahiagrass (BG) hay with perennial peanut (PEA) or lespedeza (LES) hay or mucuna (MUC) or papaya seeds (PAP) on *Eimeria sp.* fecal egg counts. Treatment x time, $P < 0.05$. Error bars are standard errors. * Counts at the week specified differed ($P < 0.05$); ^{X} Counts at the week specified tended to differ ($P < 0.1$).

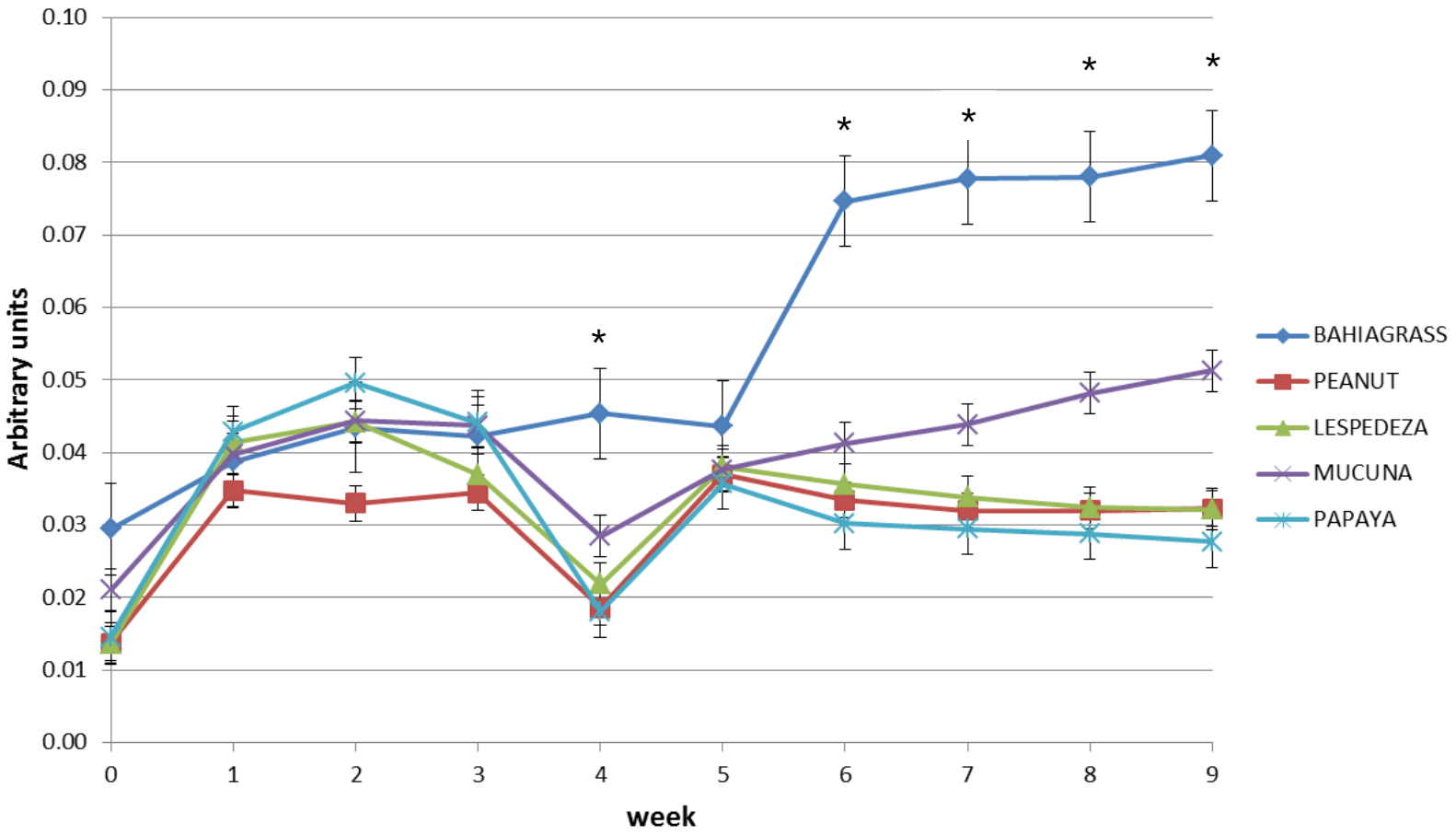


Figure 3-3. Effects of supplementing bahiagrass (BG) hay with perennial peanut (PEA) or lespedeza (LES) hay or mucuna (MUC) or papaya seeds (PAP) on plasma haptoglobin concentrations. Treatment x time, $P < 0.05$. Error bars are standard errors. * Counts at the week specified differed ($P < 0.05$).

CHAPTER 4
CONCLUSIONS

Feeding MUC increased digestibility of DM, OM, NDF and N but did not reduce GIN infection or increase the performance of goats. Feeding PAP, PEA and LES increased DMI but did not increase the performance of goats. Nevertheless, PAP, LES and PEA reduced abomasal GIN counts in goats by 78, 52, and 41%, respectively. In particular, this study confirmed that LES is an effective anthelmintic against GIN but is less effective against EIM. Perhaps for the first time, this study showed that PEA could be used to decrease the GIN and EIM burden of small ruminants. More research is needed to confirm the efficacy of using PEA as a dewormer and to determine the optimal inclusion rate in small ruminant diets. Papaya seeds were the most effective anthelmintic treatment in this study. Although papaya seeds are not available in large quantities in the southeastern US, they are readily available in tropical US territories and many tropical countries. This factor and the low dose required for efficacy indicates that papaya seeds may be a promising natural dewormer for controlling GIN and EIM infections in tropical and subtropical regions. Future research should determine the relative economic value of using PAP, PEA, and LES as anthelmintics and investigate their long-term effects on small-ruminants and other species.

APPENDIX A
ASSAY FOR DETERMINATION OF HAPTOGLOBIN

Reference: Makimura, S. and N. Suzuki. 1982. Quantitative determination of bovine serum haptoglobin and its elevation in some inflammatory diseases. Jpn. J. Vet. Sci. 44:15-21.

REAGENTS:

1) O-Dianisidine.

- 2.4 g O-Dianisidine.
- 2.0 g Na_2EDTA.
- 55.2 g $NaH_2PO_4.H_2O$.

Bring volume to 4 L, stir overnight, and filter undissolved particles under vacuum (this requires 3 passes through filter). Use Whatman paper filter # 541. Carefully bring pH to 4.1 with phosphoric acid or NaOH.

2) Methemoglobin.

30 mg of methemoglobin
100 ml of DDH_2O.

3) 156 mM H_2O_2.

RUNNING PROTOCOL

1) Label the number of tubes (13 mm x 100 mm) that you need to fill a 96 well plate considering that each tube will fill 2 wells duplicating each sample.

2) Pipette 25 µl of methemoglobin solution into each tube.

3) Pipette 5 µl of DDH_20 into tube 1, 5 µl of pooled plasma into tube 2, and 5 µl of each unknown plasma sample into each tube.

4) Pipette 7.5 ml of O-Dianisidine into each tube. Vortex.
5) Incubate at 37^0C in water bath for 45 min.

6) Pipette 100 µl of 156 mM H_2O_2 into each tube. Vortex.
7) Incubate at room temperature for 1 hour.

8) Transfer 200 µl of sample in tubes into each well of the 96 well plate (flat bottom).

9) Read at 450 nm on microplate reader.

10) Use the absolute absorbance values for the statistical analysis. Report as arbitrary units (optional it can be reported as OD x100).

APPENDIX B
ABOMASUM NEMATODE COUNTING PROCEDURE

Reference: Hansen, J. and B. Perry. 1994. The epidemiology, diagnosis and control of helminth parasites of ruminants: A handbook. Int. Livest. Res. Inst., Nairobi, Kenya.

Equipment

- A tray or bucket.
- Normal physiological saline, 0.9% or tap water.
- Sieve -#50 mesh.
- Petri dishes.
- Wash bottle.
- Two 1 L volumetric flasks.
- A 1L plastic beaker.
- A cup or dipper/ladle that will hold precisely 50 ml.
- Microscope/dissecting microscope

Procedure:

NOTE: Perform all steps in a sink with drain and faucet sprayer or spout with tubing to create a forceful stream of water for washing the rumen contents thru the sieve.

1) Place the opened and washed abomasum with the mucous membrane face down in the tray/bucket containing normal saline solution and leave the abomasum to soak overnight.

2) Remove the abomasum, rinse well with saline solution and discard.

3) Pour the saline solution left in the tray/bucket through the sieve/nylon net, which will retain the nematodes/larvae. If there is a lot of rumen content, use a forceful flow from the faucet to wash as much as you can thru the sieve leaving the nematodes as clean as possible to make counting easier.

4) Tilt the sieve and wash the nematodes to one side of the sieve in one mass and while holding the sieve at an angle over the 1L beaker back flush (spraying from the bottom side of the sieve) the worms from the sieve into the 1L beaker using the wash bottle or gentle flow of sink sprayer using as little water as needed to flush them off the sieve and into the container. Pour all of the back wash into one of the 1L volumetric flasks and bring the total volume up to 1000 ml. with water. To keep the suspension mixed and

nematodes evenly distributed, pour the contents back and forth between the two volumetric flasks several times to ensure even distribution and holding the 50ml dipper or container under the flow as you are pouring over collect the 50ml aliquot.

5) Using a dissecting microscope, examine the 25ml aliquot in a petri dish and count the nematodes or larvae. Count different species separately if more than one species is observed.

6) To identify the parasite species, transfer further sub-samples by Pasteur pipette to micro-slides for examination under the microscope.

7) The total number of larvae is calculated as follows: number in 50 ml sub-sample x 20 = total abomasal larval count.

APPENDIX C
PROTOCOL OF FECAL EGG COUNTS (MODIFIED MCMASTER TECHNIQUE)

Reference: Whitlock, H. V. 1948. Some modification of the McMaster helminth egg-counting technique and apparatus. J. Counc. Sci. Ind. Res. 21:177-180.

1) Collect 4 g of fecal material with 26 ml of sodium nitrate flotation (40%, 1.2 specific gravity) solution to yield a total volume of 30 ml. The test can also be performed with 2 g of feces and 13 ml of flotation solution when only small amounts of feces are available.

2) Homogenize using a laboratory stirrer or place in a shaker jar with about 45 8-mm diameter glass beads and shake until all the pellets have been broken up.

3) Strain through a cheesecloth if desired.

4) Immediately fill each chamber of the McMaster slide with the mixture using a Pasteur pipette or syringe. The entire chamber must be filled, not just the area under the grid. If large bubbles are present, remove the fluid and refill.

5) Allow the slide to sit for at least 5 minutes before examining to allow the flotation process to occur.

6) Look at the slide within the 10X lens. Count eggs in each line of both chambers.

7) At 10 × magnification count all the eggs under the two ruled grids (total volume 0.3 ml). Multiply the number of eggs by 25 to give the epg (eggs per gram) in the fecal sample.

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BIOGRAPHICAL SKETCH

Miguel Zárate Urbano was born in Lima, Peru. He attended Santo Tomas de Aquino high school, graduating in 1999. In 2000, he was admitted to Universidad Nacional Mayor de San Marcos, Lima, Peru to study Veterinary Medicine and became an active member of Rotaract, the youth division of Rotary International in 2003. His involvement with Rotaract, which focusses on developing young adults as leaders through community service heightened his sense of duty and desire to help people and communities in need. During his senior year, his clinical rotations took him to areas of Peru where he saw firsthand people's need for practical treatment solutions to animal and human parasitic infections. He graduated with a Bachelor of Veterinary Medicine degree from the University in 2005.

Miguel worked as a surgeon's assistant and subsequently as a surgeon from 2006 to 2008 at Hipódromo de Monterrico, the most prestigious racetrack in Peru. These positions helped him become skillful in a variety of laboratory and surgical procedures and increased his knowledge of parasitology, internal medicine, pharmacology, radiology, and nutrition. His responsibilities as a veterinary practitioner also helped him to learn the importance of conducting studies with integrity and the highest ethical standards. He also participated in the Andares-Peru program, which teaches riding skills to people with disabilities and he often volunteered to teach children about taking care of horses.

In 2008 he worked for six months as a research scholar in animal nutrition at the University of Florida in the Animal Sciences Department under the guidance of Dr. Adegbola T. Adesogan. When he returned to Peru in 2009, he continued his work as a

veterinarian and he also taught Language Arts and Science at the Canonesas de la Cruz School, in Lima, Peru.

In 2010, he received an assistantship to pursue a Master of Science degree in the Animal Sciences Department under the tutelage of Dr. Adegbola T. Adesogan at the University of Florida. After he completes his M.S. program, Miguel will continue his graduate studies at the University of Florida in the Interdisciplinary Program in Biomedical Sciences in the College of Medicine. He will seek to further his knowledge and understanding of the molecular basis of parasitic infections (in-vitro and in-vivo) and to determine and interpret complex models of parasite interactions.