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Dose titration of sericea lespedeza leaf meal on Haemonchus contortus infection in crossbred lambs

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DOSE TITRATION OF SERICEA LESPEDEZA LEAF MEAL ON *HAEMONCHUS*
CONTORTUS INFECTION IN CROSSBRED LAMBS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Interdepartmental Program in the
School of Animal Sciences

by
Dana Alicia Pollard
B.S., Southern University and A & M College, 2007
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ABSTRACT

Gastrointestinal nematode (GIN) parasitism is a problem for small ruminant producers. *Haemonchus contortus* is the most detrimental because of substantial economic losses. Over the past decades, chemical anthelmintics have been used to control GINs. Recently, GINs have developed resistance to most anthelmintics, and now reliance on anthelmintics is not possible. Alternative control methods are needed. Also, the use of fewer chemicals in agricultural products has called for alternative methods to be less synthetic and more organic. One promising alternative method is the feeding of condensed tannin (CT) containing forages as either fresh forage or dried products such as hay, ground hay, and pellets. Studies have shown that grazing or feeding sericea lespedeza (SL), a forage plant high in CTs, as whole plant resulted in some level of controlling *H. contortus* infection. The leaf has the highest CT content; so this study was conducted to determine which percentage of SL leaf meal was the most effective at controlling *H. contortus* infection in lambs and to determine any effect on reducing GIN larval development in the feces. Thirty-two cross bred lambs were randomly allocated into 4 groups with 8 animals each for a 5 week confinement trial. One group served as the control and received no SL. The other 3 groups received diets containing 25, 50, and 75% SL. Each week, feces and blood were collected to monitor infection level based on fecal egg count (FEC), blood packed cell volume (PCV), and enumeration and identification of larvae from fecal cultures. Fecal egg count decreased significantly ($P < 0.05$) over time for all 4 groups; however, there were no significant differences between groups. There was a trend on regression analysis for the control group FEC to be significantly ($P < 0.001$) greater during the trial. Reduction in FEC for the 3 treatment groups was 37.44-67.28% subsequent to week 1. There was no effect on PCV or nematode larvae population distribution and viability in feces. The antiparasitic effect of SL fed as leaf

meal was not conclusive in this trial, but there was some evidence that suggested further evaluation is warranted.

CHAPTER ONE

INTRODUCTION

Farm animals are used for many different reasons, including research, food, clothing, showing, and teaching. Sheep are an important species worldwide, and sheep breeds can be classified as meat, wool, or dual purpose. Meat sheep provide a valuable protein source for many people in both developing and developed parts of the world. Wool sheep provide fiber for making clothing and other fiber products. Because of the characteristics of wool, sometimes it is preferred over that of cotton and synthetics (Herren, 1999). Dual purpose breeds provide both meat and wool.

A major factor that negatively affects ruminant production worldwide is gastrointestinal nematode (GIN) infection. Economic evaluations have shown that major losses due to parasitism have been associated with animal production rather than mortality (Hawkins, 1993; McLeod, 1995; Perry and Randolph, 1999); therefore, GIN parasitism is usually categorized as a production disease.

Parasitism with GINs differs from that of other infectious diseases. Infected animals seem to maintain some level of infection throughout the year; whereas infectious diseases infect for a shorter duration. That may be due to the type of immune response and amount of exposure to each. Microbial infections tend to cause an aggressive and prompt immune response; whereas nematodes cause varying forms of immunological tolerance to allow them survival within the host (Dineen, 1963; Maizels and Lawrence, 1991). This means that the development of immunity to GINs is generally slower, and with parasites being ubiquitous in areas where livestock graze, they are always a constant infectious threat to the animals (Waller, 1999).

Although there are many different GINs that are capable of infecting ruminant livestock, only a few of them, including *Haemonchus*, *Ostertagia/Teladorsagia*, *Trichostrongylus*, *Nematodirus*, and *Cooperia* spp., cause problems (Waller, 2006). Generally, *Haemonchus* and *Cooperia* spp. are most important in areas that are sub-tropical and tropical. *Ostertagia* and *Nematodirus* spp. are most important in temperate regions, and species of *Trichostrongylus* can be found throughout regions that are subtropical, tropical, and temperate (Waller, 2006). The GINs of sheep are *Haemonchus contortus*, *Cooperia* spp., *Teladorsagia circumcincta*, *Trichostrongylus colubriformis*, and *Trichostrongylus axei*. They are also called the trichostrongyles of sheep. *H. contortus* is known as the barberpole worm, and it is found in the abomasum. The most common clinical sign is anemia, which can cause death. The medium or brown stomach worm is *Te. circumcincta*, and it is also found in the abomasum, and the most common clinical signs of infection are edema and diarrhea. *Cooperia* spp. and *Tr. colubriformis* are found in the small intestine (Bowman et al., 2002).

Drug (anthelmintic) therapy has been used almost exclusively over the past few decades to control infections from GINs. Approved anthelmintics for sheep are thiabendazole, levamisole, albendazole, ivermectin, and moxidectin. Thiabendazole (TBZ[®]) has been available as a drench, feed additive or bolus, but it is not currently marketed. Levamisole (Tramisol[®] and Levasol[®]) is available as a bolus or drench. Albendazole (Valbazen[®]), ivermectin (Ivomec for Sheep[®]), and moxidectin (Cydectin[®]) are available as a drench. The advantage of ivermectin and moxidectin is that they are effective against fourth-stage larvae (L₄) in the mucosa (Leaning, 1984; Williams et al., 1992).

Due to overuse of anthelmintics, GINs have adapted to the mechanism of action and resistance has become an issue. Anthelmintic resistant GINs are now an issue in the United

States and globally. This widespread occurrence of anthelmintic resistance has made alternative control methods become a necessity (Coles, 1986; Waller, 2004; Howell et al., 2008).

There are several non-chemical strategies that have been investigated. One strategy is selecting animals that are resistant to infection. Resistant individuals are retained, and susceptible ones are culled (Gasbarre and Miller, 1999; Hunt et al., 2008). Copper-oxide wire particles (COWP) have shown to be effective at controlling GINs (Knox, 2002; Burke et al., 2004; Burke and Miller, 2006). Copper oxide wire particles work by targeting *H. contortus* in the abomasum. A larvae-trapping fungus (*Duddingtonia flagrans*) has been shown to effectively control GINs (Terrill et al., 2004; Kahn et al., 2007). *Duddingtonia flagrans* trap all nematode larvae in feces preventing them from moving onto forage. There is a promising hidden gut antigen vaccine against *H. contortus*, which works well in both sheep and goats (Knox et al., 1995; Newton and Meeusen, 2003).

Condensed tannin containing plants are another option for controlling GINs (Niezen et al., 1998; Paolini et al., 2003; Iqbal et al., 2007). Condensed tannins are types of secondary compounds found in some plants and are classified based on their chemical structure. Condensed tannin-containing plants include chicory (*Cichorium intybus*), sulla (*Hedysarum coronarium*), birdsfoot trefoil (*Lotus corniculatus*), big trefoil (*Lotus pedunculatus* and *L. uliginosus*) and sericea lespedeza (SL) (*Lespedeza cuneata*). In certain regions of the United States, SL can be grown as forage. Sericea lespedeza has been at the forefront of current CT research, where grazing or feeding (dry products such as hay and pellets) has been shown to reduce fecal egg count (FEC), reduce worm burdens and, in some cases, negatively affect larval development and survival in the feces (Min et al., 2005; Lange et al., 2006; Shaik et al., 2006; Terrill et al., 2007). Sericea lespedeza will not grow in much of the United States. Some producers, where it will

grow, do not want to plant SL for grazing; therefore, supplement feeding with dry products could be considered. Additional research is needed to determine at what level SL can be used as a supplement to achieve acceptable GIN control. The objectives of this study were to: 1) Determine the effect of feeding 25, 50, and 75% SL leaf meal on reducing GIN infection (specifically *H. contortus*) in lambs and 2) to determine the effect of feeding 25, 50, and 75% SL leaf meal on development and survival of GIN immature stages in the feces.

Integrated control strategies include various combinations of all control methods to achieve the goal of controlling GINs.

CHAPTER TWO

REVIEW OF LITERATURE

2.1. *Haemonchus contortus*

Haemonchus contortus is a pathogenic gastrointestinal nematode (GIN) parasite infecting small ruminants. It is a blood-feeding, abomasal worm, and growing animals are the most susceptible to infection. Adults can also be susceptible under heavy infection conditions. Primarily affecting small ruminant production in subtropical and tropical areas, the geographic range of *H. contortus* is worldwide, and it can be found in temperate areas (Waller, 2006).

Haemonchus contortus has a direct life cycle, which begins with sexual reproduction in the abomasum. Eggs are released from the vulva of the female and are passed out of the host's body in the feces. The egg is already in the early stage of cleavage, and development continues into the first stage larvae (L₁). Under warm and moist conditions, the L₁ hatches from the egg in the feces and feeds on bacteria and other organic matter. The L₁ molts to the second stage larvae (L₂), which also feeds on bacteria and organic matter. The L₂ molts to the third stage infective larvae (L₃), which retains the cuticle of the L₂ and does not feed anymore but relies on stored nutrients to sustain further survival. The L₃ actively migrate out of the feces when a moisture medium, such as saturating rain, flooding, or heavy dew, is present. The animal becomes infected by consuming L₃ on forage during grazing. The L₃ exsheathes and loses the L₂ cuticle in the rumen and then passes to the abomasum, where it penetrates into the mucosa. While there, it will molt to the L₄ stage and then make its way back to the lumen of the abomasum. The L₄ is also a blood feeder, and after feeding, it will undergo a final molt in three days to reach adulthood (Roberts et al., 2004). The prepatent period, i.e. the time from when the L₃ is ingested

until adult females start to lay eggs, for *H. contortus* is generally 17-21 days (Morand, 1996; Foreyt, 2001).

Haemonchus contortus can infect sheep, goats, cattle, and other ruminants, but its preferred host is sheep. The parasite is protected by a cuticle made up of layers produced by the epidermis, and it is non-cellular (Roberts et al., 2004).

Haemonchus contortus uses a lancet to disrupt abomasal mucosal tissue, which allows blood to flow and be ingested. In non-fatal infections, *H. contortus* can remove up to one-tenth of circulating erythrocyte volume per day, and one-fifth of circulating erythrocyte volume may be removed per day under heavy infection, especially in lambs (Bowman et al., 2002). Anemia, edema, emaciation, and intestinal disturbances can occur with this blood loss. The loss of red blood cells results in decreasing blood packed cell volume (PCV). Anemia can be fatal in lambs, but older sheep can also succumb under conditions that are stressful.

When clinical signs are present, *H. contortus* infection is commonly called haemonchosis, which results in pallor of the mucous membranes and skin (Bowman et al., 2002). Haemonchosis is generally observed with a fecal egg count (FEC) of 10,000 eggs per gram (EPG) or higher (Bowman et al., 2002). It has been established that *H. contortus* FEC has a high correlation with PCV and worm burden (Gray and Woolaston, 1991). There is a negative correlation between FEC and PCV, whereas there is a positive correlation between FEC and worm burden. (Gray and Woolaston, 1991).

2.2. Anthelmintic Control of Helminths

An anthelmintic is a chemical substance that is used to expel parasitic GINs (helminths) from the body. This is done by killing or incapacitating the worms. The mechanism of action of anthelmintics acts to either paralyze or starve the worm to death. Worms will be eliminated if

they are paralyzed because they would lose their sense of orientation in the gut. Because worms do not store energy, they must feed continuously in order to meet their metabolic needs. Any interference with this will result in energy deficiency. Interference with feeding for 24 hours or less is enough for most adult worms to be affected (Kahn, 2005).

Although anthelmintics are marketed as many brands, there are only three chemical classes that are recognized: benzimidazoles, nicotinic antagonists, and macrocyclic lactones. Anthelmintics are classified based upon their mode of action and chemical structure. They are all classified as broad spectrum anthelmintic because they are effective against a range of GINs (Kahn, 2005).

2.2.1. Benzimidazoles

Benzimidazoles were the first chemical class of modern anthelmintics to be developed and are commonly called white dewormers. A benzimidazole is a heterocyclic aromatic organic compound that consists of the fusion between benzene and imidazole. Benzimidazoles have a wide margin of safety (i.e. the difference between the usual effective dose and the dose that brings about severe or life threatening side effects). Benzimidazoles include compounds which end in “-azole.” If there is no resistance to benzimidazoles, then they are effective against all GINs, including adults and some larvae, and some are even effective against liver flukes. Benzimidazole mechanism of action is its ability to bind to the protein tubulin (Ireland et al., 1979; Friedman and Platzer, 1980). Found in worm cells, tubulin makes up long tubes, which are called microtubules, and these microtubules are responsible for functions, such as energy metabolism, which are essential to the worm’s survival. Benzimidazoles prevent these microtubules from forming. Interfering with energy metabolism, which is a more basic mode of action than the other classes of dewormers, leads to starvation of the nematode by intestinal

disruption and inhibition of their egg production, thus killing the worm (Mehlhorn and Armstrong, 2001).

The first drug to be developed in this class was thiabendazole in 1962 (Arundel, 1985a). Other benzimidazoles include mebendazole, flubendazole, fenbendazole, oxfendazole, oxibendazole, albendazole, albendazole sulfoxide, thiophanate, febantel, netobimin, and triclabendazole (Kahn, 2005). Those that are Food and Drug Administration (FDA)-approved for use in sheep are albendazole (Valbazen[®]) and thiabendazole (TBZ[®]) (Kahn, 2005).

2.2.2. Nicotinic Antagonists

Nicotinic antagonists are commonly called clear dewormers and include levamisole and morantel. Nicotinic antagonists inhibit the action of acetylcholine at nicotinic acetylcholine receptors by mimicking acetylcholine. Acetylcholine is the nerve transmitter, which initiates muscle contraction, and by mimicking acetylcholine, these drugs cause the worm's muscles to contract continuously. This leads to spastic paralysis, which ultimately causes the worms to be expelled from the host by the normal peristaltic action (Kahn, 2005).

From this class, the one that is FDA approved for use in sheep is levamisole (Prohibit[®], Levasol[®], and Tramisol[®]). When compared to other anthelmintics, levamisole has the narrowest margin of safety as it can cause transitory nervous signs (twitching, head tilt, circling, etc.) in treated animals. Levamisole and morantel act to disrupt nerve function (Arundel, 1985b). Levamisole acts as a nerve ganglion stimulant and causes rapid muscle contractions and a rapid reversible paralysis by depolarizing the neuromuscular system (Coles et al., 1974). Levamisole could further be classified as an imidazothiazole and morantel as a tetrahydropyrimidine, in which it is known as morantel tartrate. The tetrahydropyrimidines also include pyrantel pamoate

and pyrantel tartrate. Both morantel and pyrantel are effective against adult gut worms and larval stages that reside on the surface of the mucosa or in the lumen (Gibson, 1975).

2.2.3. Macrocyclic Lactones

The last class to be introduced was the macrocyclic lactones, which have a macrolide ring. A macrolide ring has one or more deoxy sugars attached; therefore they could also be called macrolides. This class is also known as the “mectins,” as they end with “-ectin.” If there is no anthelmintic resistance present, macrocyclic lactones have a wide margin of safety for livestock and are active against all GINs including normal and inhibited larvae and adults.

This group consists of two closely related chemical groups, which are avermectins and milbemycins. The first one developed, ivermectin, was introduced by Merck[™] in the early 1980’s. The avermectins that are commercially used are ivermectin, abamectin, epinomectin, selamectin, and doramectin (Kahn, 2005).

All the macrocyclic lactones have the same mechanism of action. They are developed from soil microorganisms belonging to the genus *Streptomyces* (Kahn, 2005). They are believed to enhance the release and binding of gamma aminobutyric acid (GABA) in neural synapses, thus blocking the GABA mediated neurotransmission (Wang and Pong, 1982; Campbell and Benz, 1984). This causes paralysis and expulsion of the worms (Kahn, 2005).

The drugs that are FDA approved for sheep in this group are ivermectin (Ivomec for Sheep[®]) and moxidectin (Cydectin[®]), the commercially available milbemycin (Kahn, 2005).

2.2.4. Amino-Acetonitrile Derivatives

Research on a potential new class is currently being conducted. They are the amino-acetonitrile derivatives (AADs), and they offer a new class of synthetic chemical with anthelmintic activity (Kaminsky et al., 2008).

2.2.5. Minor Classes of Anthelmintics

There are some minor classes of anthelmintics which have a narrow spectrum of activity (i.e. effective against fewer worm species). They are organophosphate compounds, salicylanilides, substituted phenols, and aromatic amides. The organophosphates work by stopping the removal of acetylcholine in muscle cells and result in continual stimulation of the nerve ending or muscle because the neurotransmitter, acetylcholine, was not degraded (Rew, 1978). The worms are then removed by the bowel through normal peristaltic action (Arundel, 1985a). Organophosphates that have been used for ruminants are coumaphos, crufomate, haloxon, and naftalofos (Kahn, 2005).

Salicylanilides, substituted phenols, and aromatic amides act by uncoupling oxidative phosphorylation that stops energy generation in worms (Arundel, 1985a). They are relatively toxic and are detoxified by binding to the plasma protein in the host, where it is available to blood sucking parasites such as *H. contortus* and *Fasciola hepatica*. The bound drug is separated from the plasma protein in the liver. Then it is excreted in the bile where it comes in contact with the liver fluke again. That is why they are used extensively against fasciolosis and haemonchosis in sheep and cattle. The members of this group include salicylanilides (brotianide, clioxanide, closantel, niclosamide, oxyclozanide, and rafoxanide), substituted phenols (bithionol, disophenol, hexachlorophene, niclofolan, menichlopholan, and nitroxynil), and the aromatic amide diafenetide (diamphenethide) (Kahn, 2005).

2.3. Anthelmintic Resistance

Anthelmintics were developed to control helminths. They are mainly used to eliminate existing infection rather than to prevent infection. Unfortunately, over the past few decades, anthelmintic overuse has led to many nematode species developing resistance to many

anthelmintics and in some cases to almost all anthelmintics available (Coles, 1986; Waller, 2004; Howell et al., 2008).

Resistance being developed by nematodes of sheep was first reported in the United States (Coles, 1986). Reports were usually made on farms attached to parasitological research institutions, where anthelmintics were intensively used, and the effects of treatment were monitored by worm counts and FEC (Arundel, 1985a).

Resistance is very common in the southern parts of the United States where *H. contortus* is predominant (Mortensen et al., 2003). Recently, a goat herd in Arkansas was documented to have established complete failure with all the 3 major classes of anthelmintics (Kaplan et al., 2005). Worldwide reports have shown that there is widespread resistance to benzimidazoles, imidazothiazoles, and avermectins in both sheep and goats (Miller et al., 1987; Sangster, 1999; Jackson and Coop, 2000). Unfortunately, once resistance to benzimidazoles has been established, that population will continue to show resistance even years later (Herd et al., 1984; McKenna, 1990).

Resistance to all of the broad spectrum anthelmintics has been reported, and multiple resistance and cross resistance between groups has also been seen (Arundel, 1985b). Multiple resistance occurs when the same nematodes express resistance to two or more anthelmintic groups (Arundel, 1985a). Cross resistance results when resistance to a compound occurs as a result of exposure by another compound with a similar mechanism of action. Due to a difference in drug metabolism in goats and sheep, goats can develop multiple resistance, especially with *H. contortus*, when treated at normal recommended doses because they metabolize the drugs quicker, thus leading to underdosing. Goats usually require a higher dose level than sheep. This higher dose requirement is often unrecognized, which leads to underdosing, and then resistance

occurs sooner than might be expected (Conder and Campbell, 1995; van Wyk, 2001). Because of the problems attributed to anthelmintic resistance, producers and veterinarians are at risk of having ineffective anthelmintics in the near future (Sangster, 1999; van Wyk, 2001; Waller, 2004).

When trying to understand anthelmintic resistance, it is important to know the difference between resistance and tolerance. Resistance is characterized as the ability to survive recommended dosages of treatments overtime, which are generally supposed to be effective. Whenever therapeutic drugs are used, the development of some resistant strains can occur (Coles, 1986). This could be reflected with the regular use of anthelmintics, in which selection of drug resistant GINS could occur. Tolerance, on the other hand, is used to describe a worm population that has not been previously exposed to an anthelmintic, and the worm population does not respond to it. With normal dosing of levamisole, *Trichuris ovis* is only slightly affected, thus *T. ovis* expresses tolerance to the drug (Coles, 1986). However, if the response of one species of a nematode population differs with graduated use of an anthelmintic, then resistance is present. Since resistance is controlled genetically, the worm's offspring can inherit this resistance as well (Coles, 1986).

With 74.0% of sheep producers in the United States indicating that one of the major disease problems on sheep operations is due to stomach or intestinal worms (USDA, 2003), the issue of anthelmintic resistance warrants solutions. In order to continue to have successful ruminant production, it is imperative that this resistance problem is understood and addressed. Another issue facing anthelmintic use is the market for organic quality agricultural goods, which strictly prohibits any synthetic chemical use in products (Hordegen et al., 2003). In order to

address these problems, alternative methods are being developed to combat GIN infection (Burke and Miller, 2006).

2.4. Alternative Methods of Control

2.4.1. Copper Oxide Wire Particles

Copper oxide wire particles (COWP) have been reported to control *H. contortus* infections in sheep and goats. Copper oxide wire particles, also referred to as needles, are usually administered orally as a bolus. In several studies, COWP were responsible for a reduction in worm burden and FEC. After being administered, these particles work by flowing with the ingesta from the rumen and lodging in the folds of the abomasum, where free copper is then released in the acidic environment. This increases concentrations of copper in the abomasal digesta (Dewey, 1977; Bang et al., 1990). This treatment seems to have an adverse affect against abomasal species of nematodes (Knox, 2002), primarily *H. contortus*, by changing the abomasal environment they are accustomed to and causing them to be ejected from their host (Chartier et al., 2000). It appears to work better in sheep than other ruminants, but since sheep are highly susceptible to copper toxicity, precautions should be taken when using COWP, since copper can be absorbed and stored in the liver, which can lead to toxicity. Toxicity is generally reached when copper is present at a high amount for an extended period of time, and toxicity has been seen in sheep with concentrations as low as 10 parts per million (ppm) (Church and Pond, 1988). Clinical signs with toxicity include anemia, jaundice, and red urine. However, it has been shown that multiple low doses (0.5 and 1.0 g) of COWP worked just as well as levamisole at controlling *H. contortus* without risk of copper toxicity (Burke and Miller, 2006).

2.4.2. Breeding for Parasite Resistance in Sheep

Breeds of sheep that are genetically resistant against GIN infection have been identified. These breeds can be bred to less resistant breeds to produce more resistant individuals. With either crossbreeding or breed substitution, anthelmintic dependence will decrease, which can result in an effective way to control GINs (Miller et al. 1998; Li et al., 2001).

Breeding for resistance could be advantageous in terms of the cost of anthelmintics and decreasing worm numbers (Dominik, 2005), but one downfall with this alternative would be that the outcome may result in unfavorable production qualities. For instance, the resistant St. Croix breed has a smaller frame than that of the susceptible Suffolk breed, and crossing these two breeds may result in a more resistant animal with a smaller frame. The cross might be more resistant but may not be acceptable to producers that want a higher weight of sheep. Another important issue that may affect the prospects of breeding for resistance is the variability within a breed. Depending on the breed, the heritability for this trait may be high, in which the resistance could be easily inherited. Every breed has susceptible and resistant individuals and selecting the more resistant ones will improve this trait. The variability of resistance between breeds and the resistance occurring within a breed may possibly be equivalent (Gray et al., 1987; Stear and Murray, 1994).

Fecal egg count is presently the most common way used to select for resistance. Gray (1997) stated that host antibody, parasite antigen assays, and DNA markers might provide acceptable methods of selection. However to date, none of these have proven to be successful. If breeding operations were able to use methods to select for resistance against infections, the sheep industry would greatly benefit (Dominik, 2005).

2.4.3. Nematophagous Fungus

Nematophagous fungi use nematodes as a source of food. *Duddingtonia flagrans* has been the center of nematophagous research. The chlamydospores survive passage through the gastrointestinal tract and eventually are passed in the feces where they germinate and form trapping loop hyphae that trap L₃ (Faedo et al., 1997) Thus, L₃ are prevented from migrating onto the pasture and potentially infecting hosts. This would disrupt the free-living phase of the parasite's life cycle, when the parasite is outside the animal and developing out on pasture. The fungus chlamydospores are administered to sheep in feed and must be fed daily. Since the fungus works out on pasture, there is no immediate anthelmintic effect on the worm burden inside the animal. This form of control can be used to reduce pasture contamination in conjunction with some other form of control that affects worm burden (Githigia et al., 1997; Fontenot et al., 2003).

2.4.4. Vaccines

Vaccine development against GINs has been the most investigated form of alternative control and vaccines against *H. contortus* have led the field (Knox et al., 1995; Newton and Munn, 2003). The initial approach with vaccines ranged from oral vaccines to injection with collagenous proteins found in the cuticle or using irradiated larvae. These vaccine attempts met with limited success, but as worm biology and immunology of sheep and goats are further studied, development of vaccines seem much more complex, but promising (Urquhart et al, 1966; Mansfield et al, 1974; Boisvenue et al., 1991).

The most successful experimental vaccine candidates to date are hidden (gut) antigens and natural antigens (Newton and Meeusen, 2003). Hidden antigens (also called covert or concealed antigens) are classified as such because they are not immunologically recognized by the host during infection. This antigen works by causing a rise in circulating antibody titers once

the sheep is immunized. They are very effective against *H. contortus* since it is hematophagous. The reason for this is because “high antibody levels to the injected antigen are ingested along with the blood meal”, when the nematode feeds (Newton and Meeusen, 2003). The antibodies then bind to functional proteins on intestinal cells. This causes digestive processes to be compromised. This leads to starvation, loss of fecundity, and weakness. The nematode eventually detaches and is swept out of the body by peristalsis. From reports using the isolation of various gut protein complexes and proteins that were tested under experimental conditions, this antigen seems to be a promising effective means for controlling *H. contortus*, where there has been more than an 80% reduction in FEC with greater than 50% protection against worm numbers (Jasmer et al., 1993; Smith et al., 1993; Smith et al., 1994; Knox et al., 1995; Kabagambe et al., 2000).

Natural (conventional) antigens, on the other hand, are recognized by the host during infection. Natural antigens can be used against both blood-feeding and non-blood-feeding nematodes (Newton and Meeusen, 2003). Antigens found in worm somatic tissues and excretory-secretory products have been largely used in vaccines for non-blood-feeding nematodes (Griffiths and Prichard, 1994; Schallig and Van Leeuwen, 1997; Emery et al., 1999; Alunda et al., 2003). Protection against infection with *H. contortus* has been reported with two proteins (15 and 24 kd) of adult worm excretory-secretory products, which are serologically recognized by sheep (Schallig et al., 1997; Cornelissen and Schallig, 1998).

Natural antigens are advantageous to hidden antigens because the immune response of the animal is boosted due to the exposure by field conditions (Smith et al., 1993); whereas, immune response to challenge infections can only be boosted with multiple vaccinations with hidden antigens (Smith et al., 1993).

Vaccines, unlike anthelmintics, leave no chemical residues behind. This would make them more practical and safe. Producers are accustomed to vaccines and how they work, which would make them more acceptable. Effective recombinant vaccines have been developed for *Taenia ovis*, *T. saginata*, *T. solium* and *Echinococcus granulosus* (Lightowlers, 2006). These are all platyhelminths and are classified as cestode parasites; however, in the case of helminths (i.e. *H. contortus*), there are some practical problems associated with the use of vaccines based on native material. Most importantly, it is very difficult to attain large quantities of native antigens or worm material from most helminths. The need to control for batch differences or to obtain a commercially stable formulation of native parasite material is another problem that native vaccines face. For these reasons, commercialization will depend on the use of recombinant antigens (Smith and Zarlenga, 2006). Apart from the vaccines developed against cestodes, few other recombinants have been produced that induce enough protection to even consider commercialization. Thus vaccines against *H. contortus* have not been mass produced, but with more research and recombinant technology, the possibility remains optimistic (Smith and Zarlenga, 2006).

2.4.5. Antiparasitic Plants

Antiparasitic plants have long been used as a means for GIN control. They are often referred to as bioactive plants, and those that were originally used as anthelmintic medication were various extracts from jallop, quassia, areca nut, cloves, aloes, garlic, cucurbit seeds, castor oil, male fern, and chenopodium (Waller, 2006). Their anthelmintic effects may be credited to directly acting upon the infrapopulation (all the parasites belonging to one species in a single host) itself or by indirectly stimulating some host mediated regulatory mechanism. Interest in using this traditional type of medicine has accelerated due to anthelmintic resistance, cost of

treatments, and inadequate supply of veterinary services in underdeveloped nations throughout the world. Condensed tannin (CT) containing plants have been the focal point of recent research with antiparasitic plants.

2.5. Tannins: Hydrolyzable and Condensed

Tannins are one of the many different forms of secondary compounds that can be found in plants. Even though classified as secondary compounds, they are of primary importance to both forage and fodder crops (Robbins and Morris, 2000). Tannin molecular weight ranges from 500 to 20,000 kd. They are oligomeric compounds that have several structure units with free phenolic groups. They are usually water soluble (Haslam, 1989). One exception to this would be the tannins that possess high molecular weight structures, which usually form insoluble tannin-protein complexes as well as bind to proteins. Based on their chemical structure and properties, tannins are subcategorized as hydrolyzable and condensed tannins (Athanasiadou et al., 2001).

2.5.1. Hydrolyzable Tannins

Hydrolyzable tannins are usually found in plants in low amounts (Mueller-Harvey, 2001). These tannins are molecules comprised of a carbohydrate, usually D-glucose, as the central component. They can decompose in water, which allow them to react and form other substances. The hydroxyl groups in these carbohydrates are either totally or partially esterified with phenolic groups such as ellagic acid and gallic acid (Waghorn and McNabb, 2003).

Most ruminant animals are able to adjust to a diet with hydrolyzable tannins (Waghorn and McNabb, 2003). Although ruminants are able to adjust to a hydrolyzable tannin diet, an excessive amount of hydrolyzable tannins are possibly toxic to animals and can lead to lesions found on the kidney and liver, as well as death (Waghorn and McNabb, 2003). If death were to

occur, it usually takes place within five to ten days after the first excessive consumption. It is not known what toxic compound may account for this (Waghorn and McNabb, 2003).

2.5.2. Condensed Tannins

Of the tannins, CTs are the most extensively dispersed. Condensed tannins contain secondary metabolites called proanthocyanidins (PA), and CTs are commonly referred to as PAs (Iqbal et al., 2007). Heating PAs in acidic alcoholic solutions produces red anthocyanidins as a result of the acid catalyzed oxidation reaction (Haslam, 1982). The most common anthocyanidins produced are delphinidin (prodelphinidin) and cyanidin (procyanidin) (Reed, 1995). The colors in flowers, leaves, fruits, wines, and fruit juices as well as the astringent taste of wines and fruit are due to the pigments of the anthocyanidin.

The chemistry behind CTs is complex. The number of monomeric units in CTs varies from plant to plant (Foo et al., 1996; 1997). The complex chemistry of condensed tannins could definitely be shown with two CT-containing plants, *Lotus corniculatus* and *L. pedunculatus*, in which they are considerably different in regards to their chemical structure (Foo et al., 1996, 1997).

Plant secondary metabolites, such as CTs, are believed to have evolved over time into an important element in plant defense mechanisms to protect them against pathogenic microorganisms, insects, and herbivorous animals and in doing so, have marked anti-nutritional effects (Mabry and Gill, 1979; Harborne, 1999). Reductions in food intake, digestibility, rumen function, and increased mucosal toxicity have been accompanied by the consumption of tannins (Hagerman and Butler, 1991; Reed, 1995; Dawson et al., 1999). However, under some circumstances, plant secondary metabolites may also improve performance and be beneficial to the overall health of the animal. For example, it has been reported that CTs improved amino acid

flow and uptake and decreased gas production by reducing rumen fermentation (Waghorn et al., 1987). In addition, CT consumption has been linked to improvements in milk yield with lactating ewes and cattle (Barry and McNabb, 1999) and in wool growth (Wang et al., 1996). Due to all of the benefits and advantages of CTs for ruminant productivity and health, much research has been centered on these tannins (Waghorn and McNabb, 2003).

There are many different crop species containing CTs that are used as animal feed. Some examples of these crops are faba beans (*Vicia faba*), which are feed seeds, birdsfoot trefoil (*Lotus corniculatus*), big trefoil (*Lotus uliginosus* and *L. pedunculatus*), sainfoin (*Onobrychis viciifolia*), crown vetch (*Coronilla varia*), lespedeza (*Lepedeza cuneata*), which are all temperate forage/fodder, sorghum (*Sorghum vulgare*), desmodium (*Desmodium ovalifolium*), acacia (*Acacia cyanophylla*), leucaena (*Leucaena leucophala* and *L. pallida*), sesbania (*Sesbania seban*), flemengia (*Flemengia macrophylla*), and khejri (*Prosopis cineraria*), which are tropical forage/browse (Robbins and Morris, 2000). Some other examples of CT-containing plants are sulla (*Hedysarum coronarium*), dock (*Rumex obtusifolia*), and erect canary clover (*Dorycnium rectum*). All of these plants can do well even in average or poor soil as well as acidic soil (Waghorn and McNabb, 2003).

It has been reported that soil nematode populations were decreased with the use of CTs (Taylor and Murrant, 1966). This led to the notion that CTs may be able to affect nematodes in the gastrointestinal tract, and subsequent research with CTs has targeted an anthelmintic effect.

Since CTs are a poorly defined group of compounds, the polymers of these tannins are capable of covalently binding to protein (Waller, 2006). This protein binding capability has been linked to the anthelmintic effect that CT-containing plants have on internal parasites. It seems that CT-containing plants affect GIN numbers and animal performance in numerous ways, both

directly and indirectly, through improved protein supply (Iqbal et al., 2007). It is assumed that CTs strongly bind to protein and protect them from microbial degradation in the rumen (Iqbal et al., 2007). Due to this, non-biodegradable protein complexes are formed. These complexes make their way to the abomasum and dissociate at the low pH. This increased protein supply is released in the small intestine to be absorbed and metabolized (Waller, 2006). Improvement with resistance and resilience of sheep to GINs has been noted with an increase in digestible protein supply (van Houtert and Sykes, 1996). It has also been postulated that with the consumption of CT-containing plants, the biology of various worm species could be affected, and condensed tannins may be accountable for these effects (Niezen et al., 1998). Since CTs have the ability to bind to proteins, it is believed that they possibly could bind to the cuticle of worms/larvae, which is high in glycoprotein (Thompson and Geary, 1995) and cause their death. Also, CTs and/or metabolites in feces may directly affect the viability of the free-living stages, which is the development of the eggs to L₃ (Waller, 2006).

Research with CTs has been conducted with sheep and goats in experimental and grazing conditions; however more research has been conducted with goats than sheep in determining and evaluating levels of CT supplementation.

CT plants can be used in different forms, grazing and dry products (hay and pellets). Feeding hay has some challenges as feeding in either long form or as ground material results in wastage, difficulty of storage and transport, and difficulty in mixing with other ration components (Terrill et al., 2007). Pelleting helps to reduce these challenges by cutting down the wastage of the feed and improving the ease of storing and transporting. Most previous research investigating CTs used whole plant (both stems and leaves). The leaves of CT-containing plants

have a higher amount of CTs; thus leaves may provide better control of GIN infection (Mosjidis, J.A., personal communications).

2.6. Sericea Lespedeza

Sericea lespedeza (SL, *Lespedeza cuneata*) is a leguminous plant found throughout the southern United States. It is a type of warm season, perennial, forage that is high in tannins. It was originally used in soil restoration and conservation, but now it is used as forage for grazing and hay (Powell et al., 2003). It is native to the continent of Asia. Sericea lespedeza can be grown in various areas that include pasture, rangelands, roadsides, eroded slopes, ditches, fence rows, and prairies. Wherever it is grown, there must not be heavy shade, since it is intolerant to shade. Because it can adapt to acidic soils with low fertility, it has potential for being a very useful low-input forage (Puchala et al., 2005). It is also drought and insect tolerant as well as having a high concentration of crude protein. In spite of these positive qualities of SL, it is often classified as a weed in some states. In the Midwest it is considered a noxious weed because it is considered unpalatable to grazing beef cattle (Shaik et al., 2006).

Despite the negatives with SL, there are many positives, such as improving the health and production of animals by controlling GIN and providing good nutrition. It has been shown that SL can reduce parasite loads in sheep and goats. The mode of action of the CT is not yet known. It has been speculated that while in the abomasum, SL builds a complex around the vulva of adult *H. contortus* females (Hoste, H., personal communications). This would have an effect on the eggs being laid and passed out in the feces. This may be why consumption of SL (and other CT containing plants) has led to reduced fecundity of female worms.

Initial studies using SL in a grazing study showed that Angora does and their kids that grazed SL forage had a lower mean FEC than a group that rotationally grazed (ROT) between

the SL pasture and the control non-CT containing forage (CTF) (Min et al., 2005). The SL fed and ROT groups had substantially lower FEC than the does that grazed just CTF. The total egg output and larval development were lower for SL and ROT than CTF. Tracers (three worm-free kids) grazing on SL had lower total worm burdens than the tracers that grazed ROT and CTF. The immune response was higher for SL and ROT than for CTF at 12 hours after an injection of 250 µg phytohemagglutinin (PHA). The PCV in does was higher for SL and ROT than for CTF. Does that grazed CT-containing forage had considerably lower milk somatic cell counts than the does that grazed non-CT-containing forage.

From previous research it is believed that the CTs affect parasites either directly, indirectly, or both. *Sericea lespedeza* indirectly affects parasites by improving the protein nutrition of animals, which may boost the immune system. It has been postulated that CT-containing plants can have a direct effect on the existing worm populations in the animals and on the viability of the free-living stages in the feces. Shaik et al. (2006) found feeding SL had an effect on reducing the fecundity of adult female nematodes, and there was a trend to have an effect on directly killing the nematodes themselves. It was noted that the reductions were for both small intestinal and abomasal nematodes.

Lange et al. (2006) found that feeding SL hay to lambs reduced FEC during the time of feeding, and once the feeding of SL stopped, FEC increased. This may indicate an effect on fecundity. The decrease in FEC would have the benefit of reduced pasture contamination. Feeding the SL hay also had an effect on reducing naturally infected worm burdens. There was more of an effect on these worm burdens than on the establishment of incoming larvae. *Sericea lespedeza* fed as hay may be more useful to remove existing worms than establishing worms.

Chafton (2006) conducted a study using lambs that were either given a bolus of 5,000 *H. contortus* L₃ and time for the larvae to mature (existing infection) or given trickle infections of 500 *H. contortus* L₃ for 3 times a week for 3 weeks (establishing infection). For a 5 week confinement study, SL (whole plant) meal was fed to both an existing and establishing infection group. Two other groups served as control groups for each infection type and received bermudagrass (BG) hay. At the end of the feeding trial, all groups were fed BG for an additional 2 weeks, in which all animals were necropsied. For the 5 week feeding period, there was a significant difference in FEC for the existing infection group compared to the control, in which it was lower. The FEC for the establishing infection was lower than the control, but it was not a significant difference. The post trial FEC increased in both establishing and existing infection groups. This might have been due to an effect on fecundity. At necropsy, there were fewer worms (male and female) found in both the SL meal fed groups, but there was no significant difference. The trend of fewer worm numbers with the SL meal fed groups may imply that there may have been an effect on reducing infection level as well. This study would indicate that feeding SL meal was more effective with reducing FEC with existing infections than establishing infections of *H. contortus*.

In a dose titration study (0, 25, 50, and 75% SL hay) with goats, the SL fed groups had a lower FEC than the control animals (Terrill et al., 2009). The 75% SL-fed goats tended to have a higher PCV and had fewer abomasal worms than the control animals. It was then implied that the optimum level of SL hay in the diet for reducing worm numbers of small ruminants appeared to be 75%.

In another study with goats being fed 75% SL hay in ground and pellet forms, the SL ground and pellet hay diets had lower FEC than the control after one week, and this maintained

for the rest of the study (Terrill et al., 2007). Also during the trial, there was no effect on PCV for any of the SL fed groups, but a significant effect was observed post trial for the SL pellet group, which had a higher PCV than the control. There was also a decrease in the number of *H. contortus* larvae and adults with the SL pellet group. The data from this study suggests that SL can reduce fecundity as well as to possibly have a direct effect on killing adult nematodes, as seen with the reduction of worms in the abomasum and small intestine.

Sericea lespedeza has been evaluated for intake and performance of animals. In a study using 18 kids, total dry matter intake (DMI) was higher for the SL hay group than the alfalfa (ALF) hay group and increased with time on trial for SL but not for ALF (Turner et al., 2004). Those offered ALF had higher average daily gain (ADG) and final body weights (BW) than the SL group. The kids offered the ALF-based diet had higher plasma concentrations of blood urea nitrogen and glucose and lower creatinine compared to kids offered the SL-based diet. Moore et al. (2008) found that when SL was fed to goats, it not only reduced GIN infection levels, but it also increased performance when compared with BG hay. Therefore, SL has some nutritional qualities that can positively affect the growth rate of goats, but further studies are needed to evaluate similar effects in sheep.

2.7. Integrated Strategy

Sometimes control of GINs calls for the use of a combination of different control methods/strategies. With the advent of widespread anthelmintic resistance, this method could be used to achieve successful control of GINs.

It has become apparent that deworming all animals in a population is no longer desirable because the majority of animals do not have worm burdens high enough to warrant deworming. Deworming all animals leaves very little refugia (worms in a population still susceptible to

anthelmintics) available to dilute out anthelmintic resistant genes; therefore, the current recommendation is to deworm only those individuals that need it.

The most successful tool to assess which animals need deworming due to *H. contortus*, is the FAMACHA[®] system. The FAMACHA[®] system assesses an animal's level of anemia by the color of the inside lower eyelid conjunctiva. This is used only for animals infected with *H. contortus* since it is a primary blood-sucker. It has been shown to be an effective way of determining which animals need deworming for haemonchosis (van Wyk and Bath, 2002; Vatta et al., 2002; Kaplan et al., 2004). This method would make culling, deworming, and other methods more practical to use within a given time frame since it would eliminate having to treat all animals in a flock or herd at frequent intervals. An integrated control approach would use some combination of all the methods described above (van Wyk et al., 2003, 2006). Availability of resources may help in selecting a specific integrated control approach. In addition, some management practices such as pasture rotation (animals are out on less infective pasture), mixed livestock species grazing (cattle/horses do not share the same nematode parasites so one consumes the parasites of the other), and dry lotting (remove animals from the reinfection environment) may also be useful (Silvestre et al, 2002).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Location and Animals

With the approval from the Institutional Animal Care and Use Committee (IACUC), thirty-two (6-7 months of age) crossbred (Gulf Coast Native x Suffolk) lambs were studied over the course of a twelve week period during the months of August-November, 2008. The feeding part of the trial (5 weeks) was conducted in an enclosed barn at the Louisiana State University (LSU) School of Veterinary Medicine (SVM), Baton Rouge, Louisiana. Prior to the study, the lambs were maintained on bermudagrass (BG) pasture at the Sheep and Goat Unit located at the LSU Agricultural Center, Central Research Station, Baton Rouge, LA for 7 weeks.

3.2 Experimental Design

This trial was designed to: 1) determine which of 3 percentage levels (25, 50 or 75%) of sericea lespedeza (SL) leaf meal was most effective in reducing GIN infection (specifically *H. contortus*) in lambs and 2) to determine any effect on reducing the development of GIN larval stages in the feces. Seven weeks prior to the start of the trial, 41 lambs were dewormed with levamisole (Levasol[®], Schering-Plough, Union, NJ; 8 mg/kg) and albendazole (Valbazen[®], Pfizer, New York, NY; 7.5 mg/kg) for two consecutive days in order to reduce infections to the lowest possible level (i.e. fecal egg count [FEC] close to 0). After two weeks, the 32 animals with the lowest FEC were inoculated with a bolus of approximately 5,000 *H. contortus* infective larvae (L₃). After allowing five weeks for the infection to mature, the lambs were stratified by FEC and randomly allocated to one of four treatment groups with eight animals each. The four groups were all mixed sex. Groups 1 and 2 consisted of 5 ewes and 3 wethers each. Group 3 consisted of 3 ewes and 5 wethers. Group 4 consisted of 4 ewes and 4 wethers. Each group was

maintained in separate covered concrete pens that were bedded with wood shavings. All groups were initially fed BG hay and a lamb growing ration (Purina Lamb Chow Ration, 16% crude protein, CP) for 2 days and water was provided at all times. At the start of the trial (day 0), experimental diets were fed once daily in the morning and the BG hay part of the diet was fed in the evening (Table 1). The diets were prepared and mixed according to the guidelines of each group's diets. Subsequent to day 5, residual feed found in the feeders was weighed back to record feed intake. The small amount of feed that was spilled over onto the pen bedding was not accounted. The control group (Group 1) received a diet containing alfalfa pellets and corn with no SL leaf meal. For Groups 2, 3, and 4, SL leaf meal was 25, 50 and 75% of the diet, respectively. The amount of alfalfa pellets, soybean meal and corn varied between groups to provide a balanced ration that was approximately equivalent in CP (15%) and total digestible nutrients (TDN, 61-62%). These diets were fed for 35 days (end of trial).

Infection level was monitored throughout the study by collecting fecal and blood samples each week. Feces were collected by hand directly from the rectum, placed in a styrofoam cup, and sealed with a cup lid. Blood was collected in 7 ml EDTA-vacutainer tubes (BD Vacutainer[®] Glass Whole Blood Tubes, Becton, Dickinson, and Company, Franklin Lakes, NJ) by jugular venipuncture. Samples were processed to determine FEC and PCV, respectively. For each animal, the remaining feces each week were cultured to determine any effect on development and survival of the larvae in the feces.

3.3. Techniques

3.3.1. Infective Larval Preparation

For larval inoculation, feces from sheep with known *H. contortus* infections were collected rectally and set up as a pooled culture of samples. In a culture pan, the feces were

crushed, mixed with water, and combined with vermiculite until the mixture was moist but not wet. Aluminum foil was placed over the pan and holes were punched to allow an aerobic environment. The mixture sat at room temperature (27 °C). Fourteen days later, the mixture was

Table 1. Dietary treatment composition for crossbred lambs infected with *Haemonchus contortus*

| Ingredient | 0%SL ^a leaf meal | 25%SL leaf meal | 50%SL leaf meal | 75%SL leaf meal |
|----------------------------------|-----------------------------|-----------------|-----------------|-----------------|
| DM ^b , kg | | | | |
| SL leaf meal | — | 3.024 | 6.032 | 9.024 |
| Com | 1.200 | 0.896 | 0.600 | 0.592 |
| Alfalfa pellets | 9.000 | 5.984 | 2.984 | — |
| Soybean meal | — | 0.120 | 0.176 | 0.296 |
| Bermudagrass Hay | 1.800 | 1.976 | 2.208 | 2.080 |
| Total | 12.000 | 12.000 | 12.000 | 11.992 |
| As-fed, kg | | | | |
| SL leaf meal | — | 3.311 | 6.622 | 9.934 |
| Com | 1.315 | 0.998 | 0.680 | 0.680 |
| Alfalfa pellets | 9.979 | 6.668 | 3.311 | — |
| Soybean meal | — | 0.136 | 0.181 | 0.318 |
| Bermudagrass Hay | 1.996 | 2.177 | 2.449 | 2.313 |
| Total (without water + molasses) | 13.290 | 13.290 | 13.243 | 13.245 |
| Water + molasses | — | 5.216 | 12.566 | 16.057 |
| Total (with water + molasses) | 13.290 | 18.506 | 25.809 | 29.302 |
| Analysis, DM% | | | | |
| CP ^c | 15.075 | 15.133 | 14.963 | 15.045 |
| TDN ^d | 61.050 | 61.216 | 61.238 | 62.344 |

^aSL, sericea lespedeza

^bDM, dry matter

^cCP, crude protein

^dTDN, total digestible nutrients

filtered through cheesecloth and tissue paper to recover the L₃. The L₃ were identified microscopically to be 99% *H. contortus* based on the morphological structures (Georgi, 1980) of the first 100 larvae seen. Once the identification was finished, syringes were prepared with approximately 5,000 infective *H. contortus* L₃. In order to get 5,000 *H. contortus* L₃, four 25 µl aliquots of the mixture were taken and placed on microscope slides. The number of larvae in each aliquot was counted using a compound microscope. An average of the larvae from the four aliquots was calculated. The average was then multiplied by 4 in order to get the number of larvae in 100 µl. This number was multiplied by 10 to get the number of larvae in 1 ml. The number of larvae in 1 ml was used in calculating the volume that would contain 5,000 *H. contortus* L₃. Water was added to this volume to bring it to 5 ml, which was then administered orally to the lambs using a syringe.

3.3.2. Fecal Egg Count

Fecal samples were refrigerated until ready to process. The FEC was determined with a modified McMaster procedure (Whitlock, 1948). Two grams of feces were weighed and broken up in a cup using a tongue depressor. Thirty ml of saturated salt solution (737g of iodized salt dissolved in 3000 ml of tap water) was added to the feces and mixed by hand. This was followed by mixing with an electric paddle type mixer (DrinkMaster[®] Drink Mixer, Hamilton Beach Brands, Inc., Glen Allen, NC) to break up the feces as much as possible. Before the solution could settle, a 1 ml sample of the solution was pipetted and placed into one half of a McMaster slide chamber (Chalex Corporation, Issaquah, WA). This process was then repeated for the other half of the McMaster slide. From both sides of the chamber, the number of nematode eggs was counted under each grid and each egg represents 50 eggs per gram. The total number of eggs counted was then multiplied by 50 to get an estimate of the number of eggs per gram of feces.

The FEC reduction test (FECRT) was also used to determine the reduction in FEC for each post-treatment day compared to day 0. This was calculated as: $(\text{PreFEC} - \text{PostFEC}) / \text{PreFEC} \times 100$. Generally a FECRT is conducted with a FEC at treatment (PreFEC) and another FEC taken 7-14 days later (PostFEC). To see if any reduction continues beyond this time frame, a FEC can be taken at additional time points which will serve as the new PostFEC. The PostFECs are compared with the original PreFEC in calculating the percentage reduction for each time point using the FECRT formula.

3.3.3. Packed Cell Volume

On each collection day, blood was collected by jugular venipuncture, and the PCV was determined. The blood was collected using 7 ml tubes containing K₃EDTA 15% solution (BD Vacutainer® Glass Whole Blood Tubes, Becton, Dickinson, and Company, Franklin Lakes, NJ) and inverted several times to prevent clotting. In the lab, each tube was thoroughly mixed by inversion several times, and a sample of the blood was transferred to microhematocrit tubes. They were filled to three-fourths of the volume of the tube sealed with Critoseal® (Critoseal®, Capillary Tube Sealant, Krackeler Scientific, Inc., Albany, NY) and centrifuged (Autocrit Ultra 3 Microhematocrit Centrifuge, Becton, Dickinson and Company) for five minutes. The PCV value was determined from the microhematocrit reader located inside the centrifuge.

3.3.4. Fecal Culture

Individual cultures were set up weekly to allow deposited eggs to hatch and larvae to develop to the L₃ stage. Enumeration and identification of these larvae would establish any effect of SL on numbers and any shift in population distribution. Each fecal sample was weighed (10-12g) in a cup and mixed with water to make a slurry. Vermiculite was added in an equivalent amount to that of the slurry and mixed thoroughly. More water or vermiculite was added as

necessary to provide a moist, not wet, crumbly consistency. The cup was covered with cheesecloth and inverted into a larger cup that contained about 5 ml of water at the bottom. The larger cup was then labeled with tape that indicated the animal number and culture date. Cultures were incubated at room temperature for a minimum of 14 days but no longer than 21 days for optimum larval recovery. After the incubation period, the cultures were flooded with water, i.e. the larger cup was filled with water up to the brim of the cup. The following day, the smaller cup, along with its contents, was removed, and using rubber vacuum tubing that was connected to the vacuum line of the fume hood, the liquid in the larger cup was reduced to about 50 ml. The liquid and the labeling tape from the cup were transferred into a 50 ml tube. After settling overnight the liquid in the 50 ml tube was further reduced (using vacuum) to less than 15 ml and transferred with the tape to a 15 ml tube. One ml of 10% formalin was added as a preservative. The tube was then inverted several times and set aside until ready for processing.

3.3.5. Larval Identification and Enumeration

From the fecal cultures, the supernatant in the 15 ml tube was reduced (using vacuum) to either 1 (less than about 250 μ l of sediment) or 2 ml (more than about 250 μ l of sediment). The liquid and sediment were mixed well and a 100 μ l sample was removed and placed on a slide. The sample was stained with iodine and covered with two coverslips. Using a compound microscope, consecutive columns top to bottom across the slide were scanned, and according to their distinguishing morphological features, the first 100 larvae found were identified to genus. The remaining larvae were just counted. The count was then extrapolated back to get the total number of larvae in the 1 or 2 ml. The larvae per gram (LPG) were determined by taking the total number of larvae and dividing it by the number of grams in the original fecal sample. This

was used to calculate the percentage larval development by taking the LPG and dividing it by the EPG and multiplying this by 100.

3.3.6. Feed Analysis

The SL leaf meal was analyzed for CP at the University of Arkansas, Fayetteville, AR, and analysis for extractable, protein-bound, and fiber-bound CT content was conducted at Fort Valley State University, Fort Valley, GA as described by Terrill et al. (1992). Feed samples of all other experimental diet ingredients were sent to the Agricultural Research Service (ARS), Booneville, Arkansas to be analyzed for CP and TDN.

3.3.7. Statistical Analysis

Using SAS 9.1.3, the FEC, PCV, percent FEC reductions, and fecal culture data (percentage larval recovery, percentage *H. contortus*), were analyzed as repeated measures analysis of variance in a split-plot arrangement of treatments with treatment group and animal within treatment group effects as the main plot and time by group interaction effects on the subplot. Animal within treatment group was used as the error term for testing group effects. In order to stabilize variance, FEC and fecal culture data were natural log-transformed (Winer, 1971). A regression analysis was conducted using the log-transformed FEC. Heterogeneity of regression was used to examine treatment effect on the relationship between response variables (FEC or PCV) and day of experiment (Wilcox et al., 1990). These models were adjusted for the appropriate independent variables described previously, and the relationship was found to be linear. A Tukey's HSD test was used for analyzing pairwise comparisons of significant main effect comparisons. When the overall ANOVA indicated significant interaction effects, pairwise t-test comparisons of least-square means were conducted. Significant differences were determined using a P value of < 0.05 .

CHAPTER FOUR

RESULTS

4.1 Fecal Egg Count

At the beginning of the study, all groups had relatively the same mean fecal egg count (FEC) (Figure 1). At week 1, the mean FEC for groups 1 and 4 increased, and the mean FEC for groups 2 and 3 decreased. At this point in the trial, group 1 had attained the highest mean FEC of $6,481 \pm 1,235$ eggs per gram (EPG) out of all groups for the entire trial. This was followed by group 4 that had a mean FEC of $6,150 \pm 1,365$ EPG. During the first week it was noted that Group 4 was not consuming much of their experimental diet, and the left over feed was fed to Group 1. This error in feeding was corrected, and subsequent to week 1, the correct diet was fed. Since Group 4 was not consuming all of their experimental diet, this may have accounted for the high mean FEC. It might be assumed that if Group 4 animals had consumed 1/3 or more of their feed that they should have had a similar response to Group 2, but feed consumption was not monitored until the end of week 1 and it is not known just how much feed Group 4 actually consumed as the left over feed was given to Group 1. After week 1, all groups had a decrease in mean FEC, including Group 1. The Group 1 decrease may have resulted from being fed the leftover Group 4 feed. Subsequent to week 2, the mean FEC for Group 1 increased, and the other 3 groups remained lower than the control. However, there was an increase in Groups 4 and 3 by week 4 and 5, respectively. Based on LogFEC there was no significant difference ($P > 0.05$) between the groups at any time point during the trial; however, there was a significant ($P < 0.05$) difference over time.

Because the control group was compromised during week 1, an alternative effect of treatment can be expressed by using the FECRT, where the reductions ranged from 37.44-

67.28% for all 3 SL treatment groups at and subsequent to week 2 (Figure 2). There was no significant difference between the groups at any time point, but time points 2, 3, 4, and 5 were significantly different from time point 1 ($P < 0.05$).

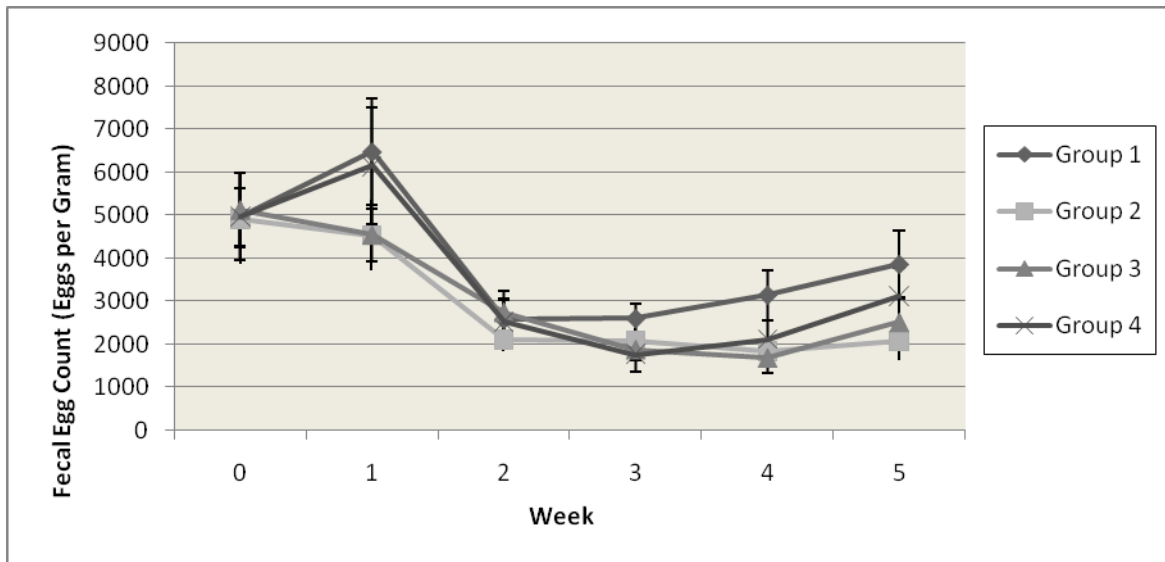


Figure 1. The effect of feeding 0% (Group 1), 25% (Group 2), 50% (Group 3), and 75% (Group 4) sericea lespedeza leaf meal on fecal egg count (FEC \pm S.E.M.) of lambs infected with *Haemonchus contortus*. There was no significant difference ($P > 0.05$) between groups.

Another way to look at the relationship of FEC between treatment groups is to conduct a regression analysis. This was used to show any relationship in FEC from the diets each particular group consumed over time (Figure 3). The regression equations (logFEC/untransformed) of FEC overtime for each treatment were SL1: $y = 8.43 - 0.232SL$ / $y = 5033 - 441SL$, $R^2=0.08$; SL2: $y = 8.28 - 0.286SL$ / $y = 4524 - 637SL$, $R^2=0.14$; SL3: $y = 8.06 - 0.180SL$ / $y = 4682 - 643SL$, $R^2=0.08$; and SL4: $y = 8.20 - 0.184SL$ / $y = 5023 - 634SL$, $R^2=0.11$; and the overall $R^2 = 0.13$.

This indicated that 13% of the FEC reduction differences can be accounted for by SL and that the rest by other unknown (breed, sex, age, weather, etc.) factors. There was a significant difference ($P < 0.001$) in the linear regression of treatment from control group. Repression of

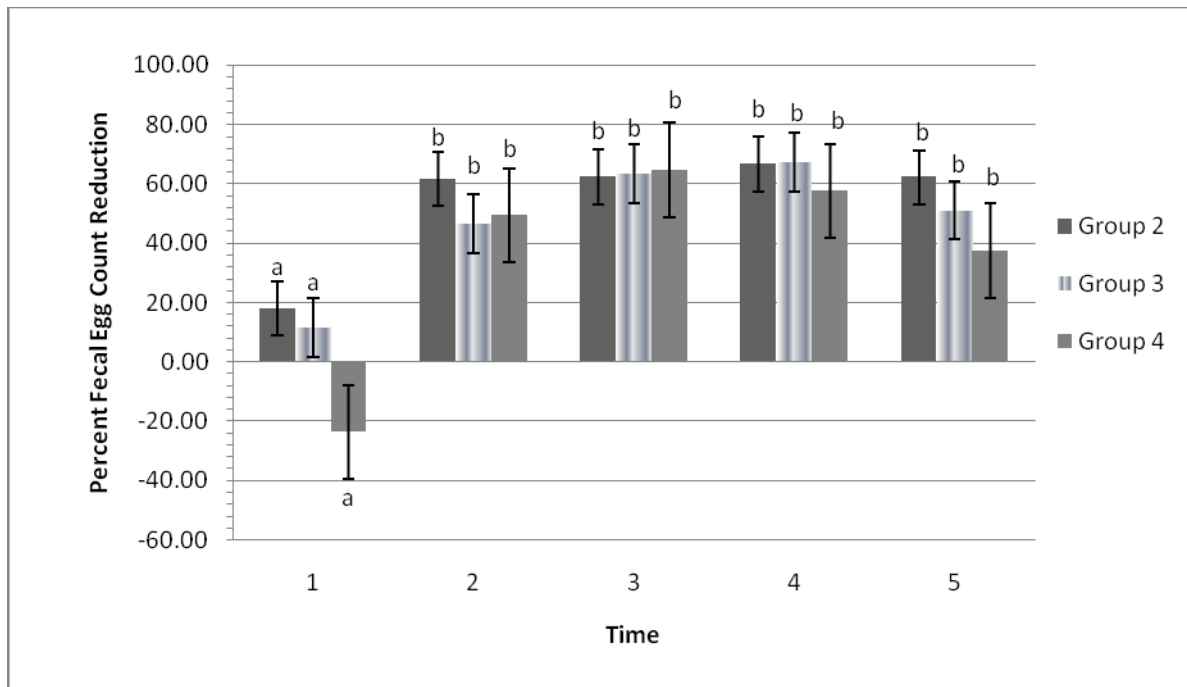


Figure 2. The effect of the treatment of 25% (Group 2), 50% (Group 3), and 75% (Group 4) sericea lespedeza leaf meal on percent fecal egg count reduction of lambs infected with *Haemonchus contortus*. All superscripts that are of different letters indicate significant difference ($P < 0.05$).

higher order equations was not significant, but there were significant differences ($P < 0.001$) for the linear regression equations of all groups. The slope of group 1 was not parallel to the other 3 groups (which were parallel) indicating that Group 1 FEC decreased at a slower rate than the other 3 groups.

4.2 Packed Cell Volume

The blood packed cell volume (PCV) was monitored weekly (Figure 4). Through week 4, Group 3 had the highest mean PCV of $29.9 \pm 0.6\%$. The PCV did change over time ($P < 0.0001$), and there were no significant differences between treatment groups ($P > 0.05$).

Normal PCVs for sheep range from 27-45% (Duncan and Prasse, 1986). During most of the experiment, all groups fell within the range.

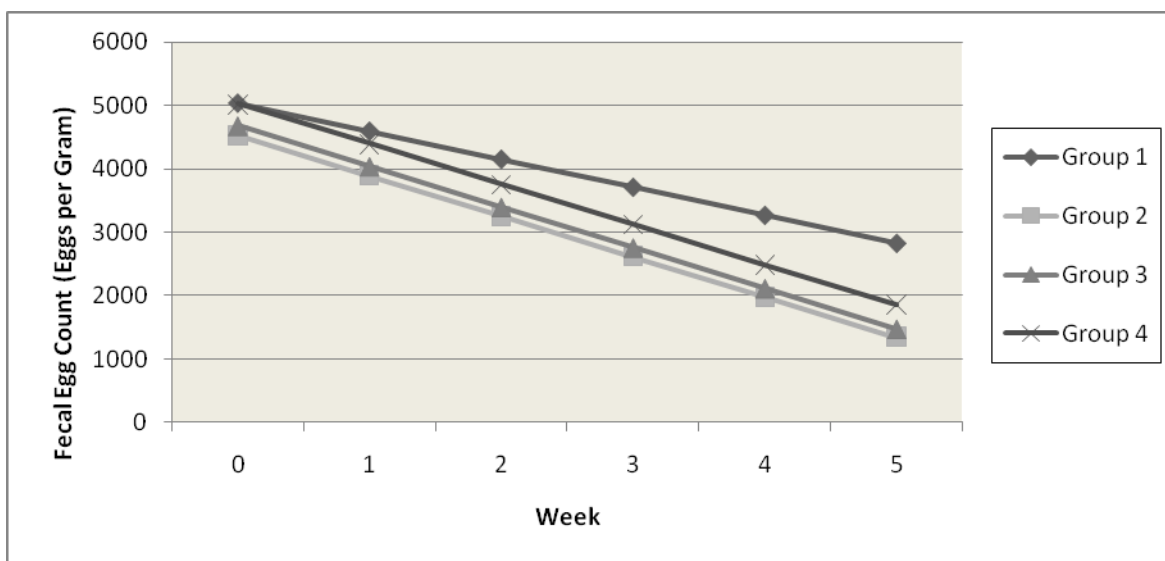


Figure 3. Regression analysis of relationship in fecal egg count (FEC) from feeding 0% (Group 1), 25% (Group 2), 50% (Group 3), and 75% (Group 4) sericea lespedeza leaf meal over time of lambs infected with *Haemonchus contortus*. There was a significant difference ($P < 0.0001$) between groups.

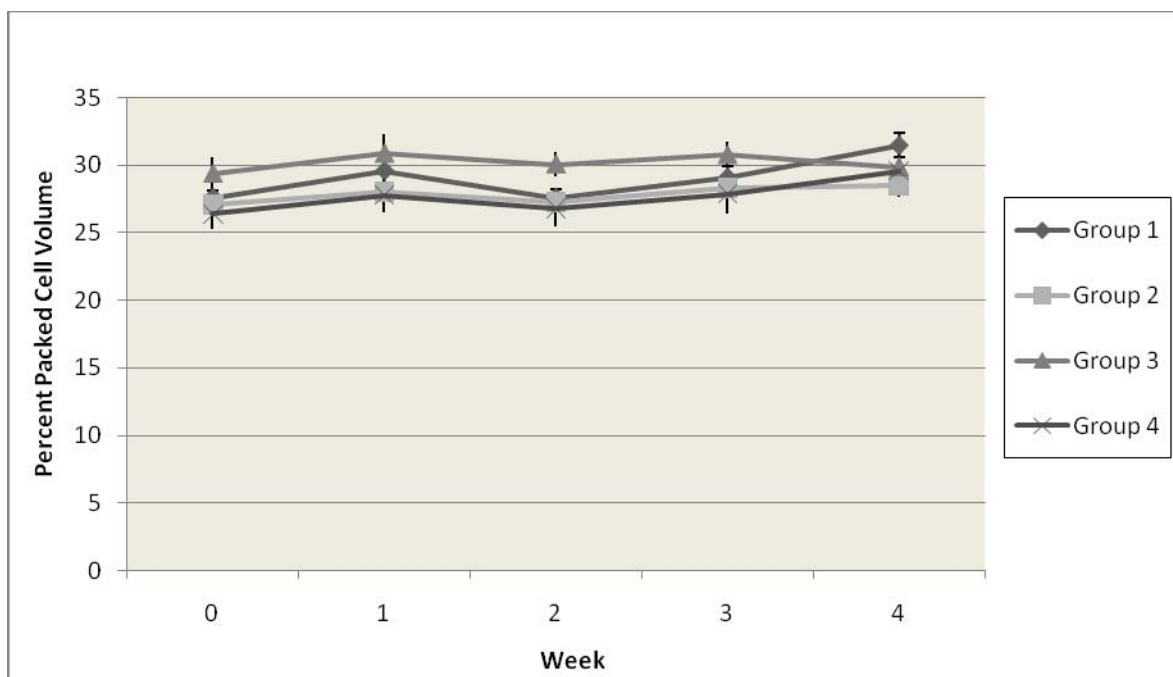


Figure 4. The effect of feeding 0% (Group 1), 25% (Group 2), 50% (Group 3), and 75% (Group 4) sericea lespedeza leaf meal on packed cell volume (PCV \pm S.E.M.) of lambs infected with *Haemonchus contortus*. There was no significant difference ($P > 0.05$) between groups.

4.3 Development and Survival of Larvae

The larval development in fecal cultures was similar between all four groups throughout the trial (Figure 5). Fecal culture data were natural log-transformed and reported as logHATCH, and this was used in determining any significant difference. There was a significant ($P < 0.05$) difference over time, but there was no significant difference ($P > 0.05$) between groups. At week 4, there was a discrepancy in the fecal culture technique, in which samples were prematurely flooded. This may account for that week's lower number of larvae identified and enumerated.

At week 5, there were lower numbers of larvae identified and enumerated. This could possibly be due to the fecal culture technique for that week as well, but this is not known.

For all 4 groups, *Haemonchus contortus* was the predominant (97-99%) nematode found. *Trichstrongylus columbriformis* was the only other larvae found (1-3%).

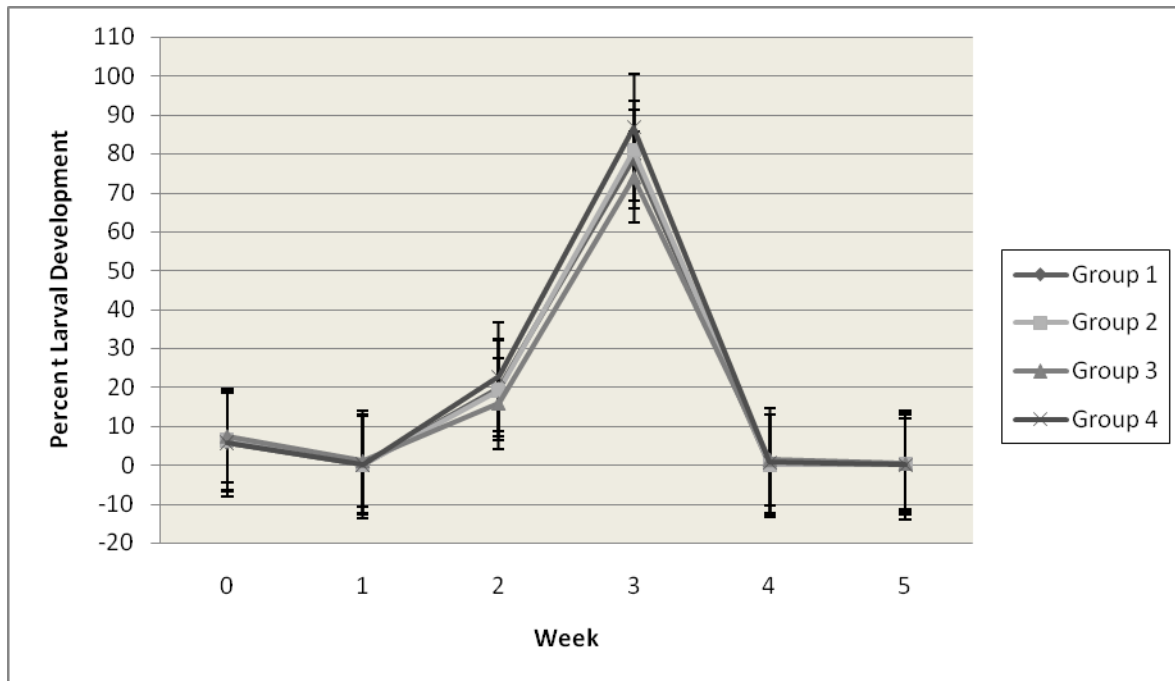


Figure 5. The effect of feeding 0% (Group 1), 25% (Group 2), 50% (Group 3), and 75% (Group 4) sericea lespedeza leaf meal on percentage of nematode infective larvae recovered from the feces of lambs infected with *Haemonchus contortus* (\pm S.E.M.). There was no significant difference ($P > 0.05$) between groups.

4.4 Feed Analysis

On a dry matter (DM) basis, the SL leaf meal CP content was 14.4%. The CT content was 3.95% extractable, 13.61% protein-bound, 0.48% fiber-bound, for a total of 18.04% CT.

4.5 Feed Consumption

During the trial, groups 1, 2, 3, and 4 were fed the same amount (approximately 11 kg, DM) of feed each morning followed by being fed (approximately 2 kg, DM) of bermudagrass (BG) hay each evening. The feed that remained in the feeders, subsequent to day 5, were recovered the following day and weighed to measure how much feed was actually being consumed (Figures 6, 7, 8, 9, and 10). For most of the trial, the feed for each group was consumed. Group 4 consistently consumed the least amount throughout the trial.

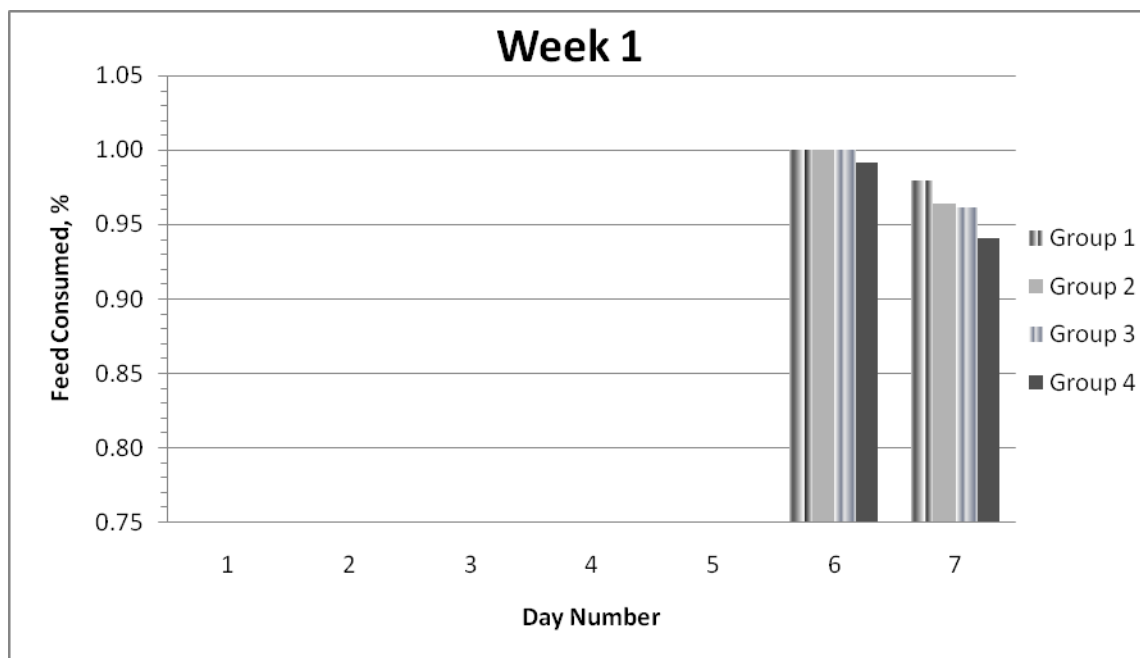


Figure 6. Daily feed consumption percentages for lambs fed 0% (Group 1), 25% (Group 2), 50% (Group 3), and 75% (Group 4) SL leaf meal during week 1 of the trial.

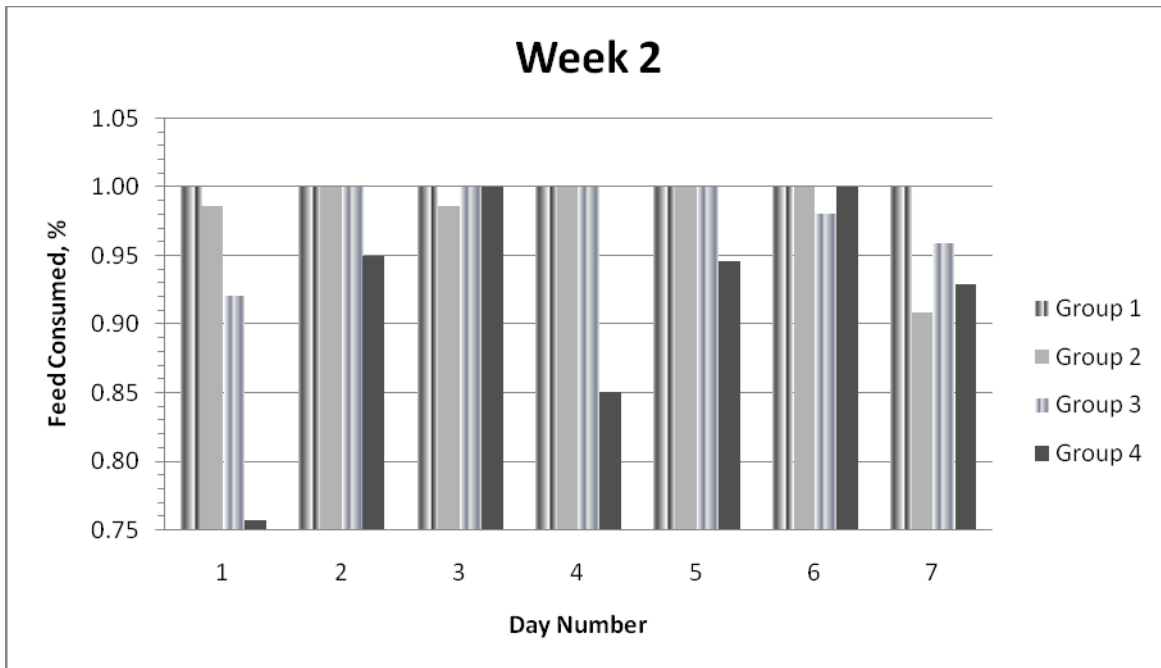


Figure 7. Daily feed consumption percentages for lambs fed 0% (Group 1), 25% (Group 2), 50% (Group 3), and 75% (Group 4) SL leaf meal during week 2 of the trial.

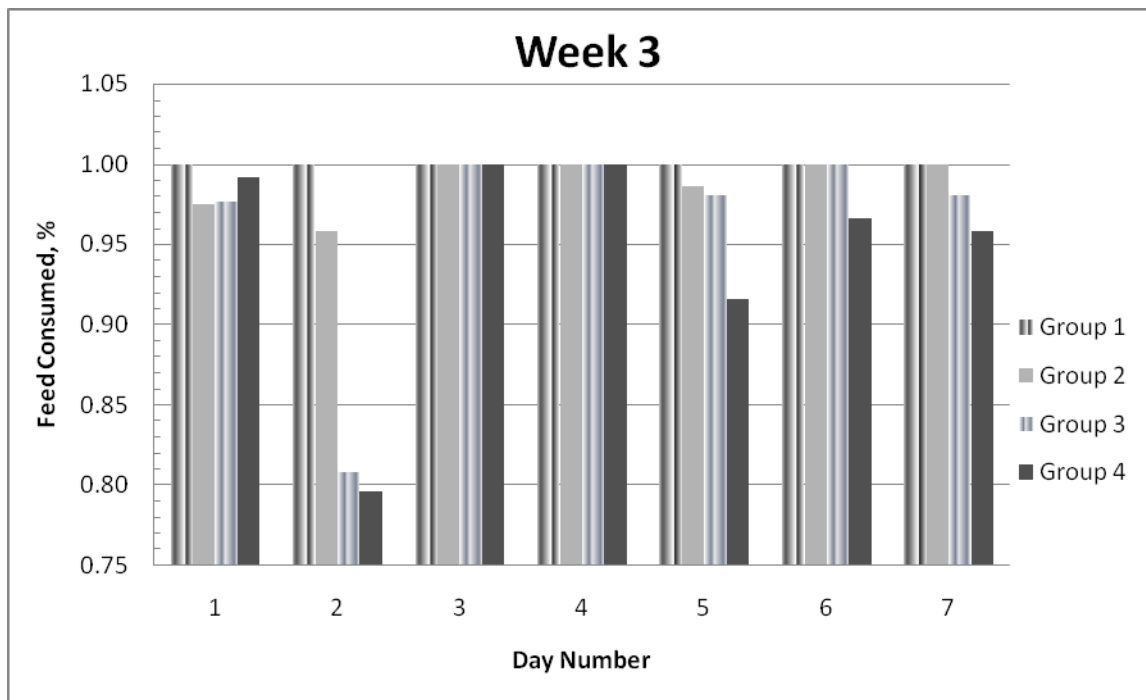


Figure 8. Daily feed consumption percentages for lambs fed 0% (Group 1), 25% (Group 2), 50% (Group 3), and 75% (Group 4) SL leaf meal during week 3 of the trial.

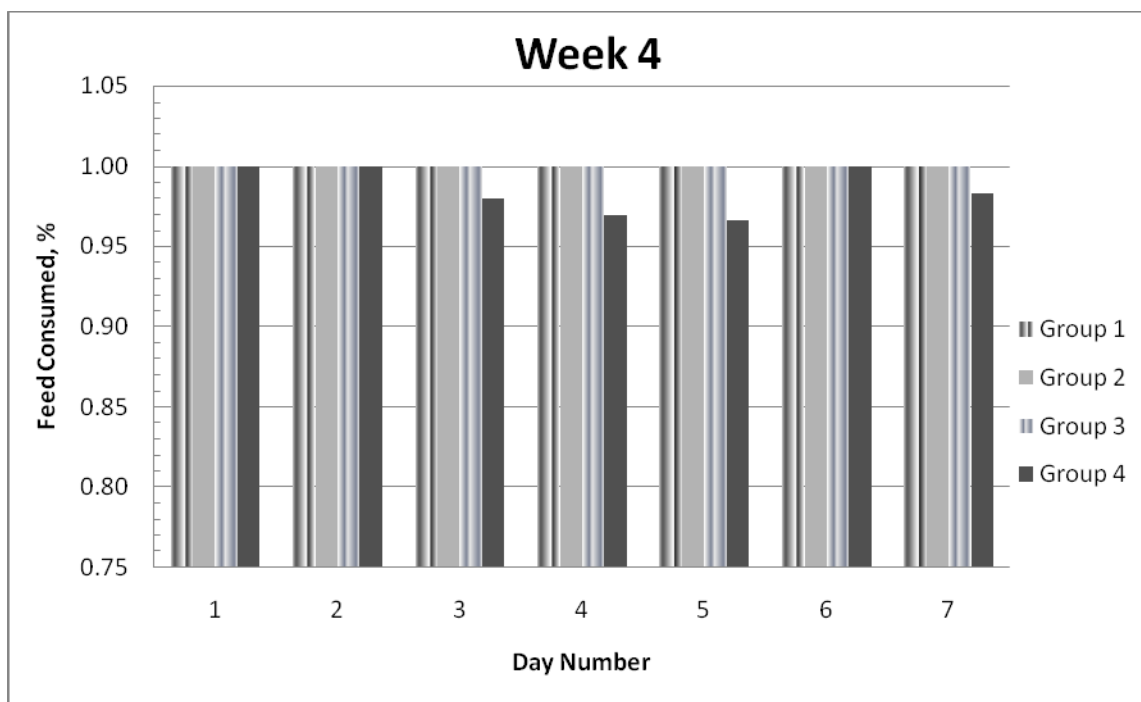


Figure 9. Daily feed consumption percentages for lambs fed 0% (Group 1), 25% (Group 2), 50% (Group 3), and 75% (Group 4) SL leaf meal during week 4 of the trial.

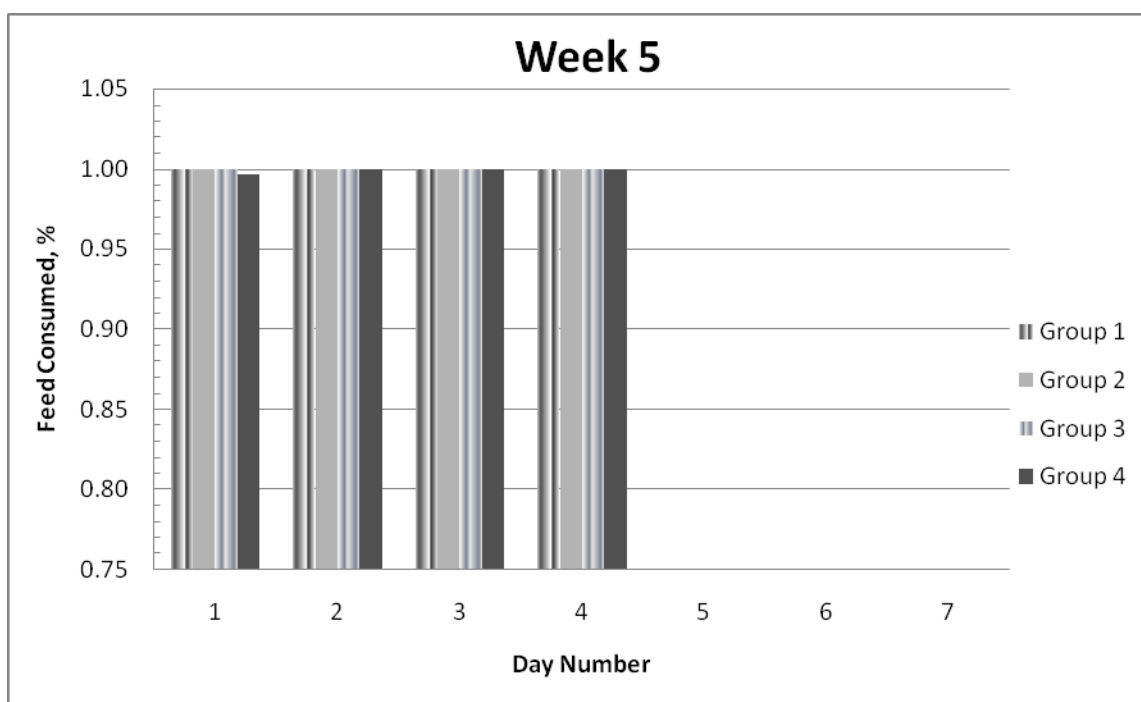


Figure 10. Daily feed consumption percentages for lambs fed 0% (Group 1), 25% (Group 2), 50% (Group 3), and 75% (Group 4) SL leaf meal during week 5 of the trial.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

Grazing and feeding SL as hay has been shown to be effective against *H. contortus* infection in goats. Shaik et al. (2006), reported that there was a decrease in the number of worms from the abomasum and small intestine in goats that were fed a SL hay diet. Sericea lespedeza hay also has been shown to be effective in controlling *H. contortus* infection in sheep where Lange et al. (2006) reported a reduction in fecal egg count (FEC) and worm burden. However, hay is not always the most economical or convenient way to feed animals. Feeding hay can contribute to feed wastage, and storage and transportation could be both difficult and expensive (Terrill et al., 2007).

Sericea lespedeza (SL) in different forms could be more flexible and provide more practicality. It might be possible to extract CT from SL and other CT containing plants and administer it via drench or integrate it into feed. Athanasiadou et al. (2001) reported that there was a decrease in small intestinal worm burdens and FEC in sheep fed pellets containing CTs that were extracted from quebracho trees. Sericea lespedeza hay could also be processed into ground hay (meal) and pellets. Terrill et al. (2007) reported that SL whole plant ground hay and pellets reduced FEC in goats by 54 and 77%, respectively. This reduction was consistent and maintained throughout the trial. It was also found that the blood packed cell volume (PCV) increased during the final stages for the SL-pellet fed goats.

Leaves of SL have a higher CT content than the whole plant. In this study leaves were 18.4% compared to 11-12% for the whole plant in other studies (Lange et al., 2006; Terrill et al., 2009). It has been shown that feeding 75% SL leaf meal reduced FEC in goats by 71% (T.H.

Terrill, unpublished observations). This is comparable to the 71.7% reduction in FEC reported by Min et al. (2004) in goats grazing SL pastures.

The purpose for this study was to determine the effects of SL leaf meal fed at varying percentages of the diet on FEC (specifically *H. contortus*) in lambs. In addition, the effectiveness of SL on reducing the development of larval stages in the feces was also determined. The direct comparison between control and treatment groups was not significant. This may be due to the control group being compromised during Week 1 which resulted in a similar pattern to that of the treatment groups. Therefore, the FECRT, which is another measure of comparison over time, indicated that there was a 37.44-67.28% reduction in FEC for all 3 SL groups subsequent to week 1. Previous studies in goats have shown that SL reduced development of larvae in feces (Min et al., 2004; Terrill et al., 2009), but that was not observed in this trial.

Even though there was no significant difference in FEC between groups, there was a trend of the control to have a higher FEC than all the other groups by regression analysis. This represents a different observation on the relationship between the FEC of the treatment groups. All groups had a negative linear correlation in FEC (Figure 3), and the slope difference between the 4 groups was significant. The slopes of the 3 treatment groups were parallel, and the control was shallower, which indicated the control FEC decreased at a lesser rate than the 3 treatment groups.

Condensed tannins are not readily absorbed in the digestive tract (Terrill et al., 1994) and are more concentrated in the feces. The concept of more bound protein in the abomasum could presumably cause a rather sudden change in the environment of the abomasum, which might make it undesirable for the existing worms residing there. There are reports that feeding CTs reduced fecundity of female worms and also reduced worm numbers to an extent. Incoming

worms that enter this CT environment of the abomasum may not be as severely affected as the existing worms because they would not be subjected to such a sudden change in conditions. It is also possible that CTs in feces could have an effect on the hatchability of eggs or development of the larvae. After being exposed to various concentrations (0-10%) of quebracho tannins, the viability of larvae was adversely affected (Anthanasiadou et al., 2001). Anthanasiadou et al. (2001) assumed that the protein binding capacity of the CT in the quebracho extract may have decreased nutrient availability and led to the starvation and death of the larvae.

Anthelmintic effects using other dried CT containing forages have also been reported. Goats fed sainfoin hay had reduced FEC (Paolini et al., 2003), goats provided sun-dried *Acacia karoo* foliage had a reduction in FEC and worm counts (Kahiya et al., 2003), and sheep fed *Lotus pedunculatus* had a reduction in FEC (Neizen et al., 1998). These plants have relatively high amounts of CTs (Terrill et al., 1989; Kahiya et al., 2003), but the reactivity of CT among these plants may differ. In sheep that were fed two *Lotus* species of plants, the plant proteins degraded differently in the presence of the CTs in each species. Despite having similar concentrations of CTs, *L. pedunculatus* CT was more effective at protecting plant protein from degradation by microorganisms in the rumen than that of *L. corniculatus* CT (Aerts et al., 1999). The CT in *L. pedunculatus* has a higher molecular weight (MW) and has a higher prodelphinidin to procyanidin subunit ratio than *L. corniculatus* (Foo et al., 1996; 1997). These differences may account for *L. pedunculatus* being effective on decreasing nematode FEC than *L. corniculatus* (Niezen et al., 1998). The prodelphinidin to procyanidin subunit ratio is higher in SL than most other CT-containing legumes (Burns, 1966). It appears that not only protein degradation, but the concentration and chemical structure of CT needs to be considered in studies involving the controlling of GINs.

Barry and Manley (1986) stated that free (extractable) or unbound CT may be more reactive than the bound form. The unbound or “free” CT is more capable of making protein complexes and complexes with other macromolecules than bound forms of CT. This unbound CT might be the active agent giving SL its anthelmintic properties. The total CT concentration can be different between the stages of the plant, plant types, and dried products of the same plant. Generally, more mature plants have a higher amount of extractable CT than that of growing plants, and both of these have more extractable CT content than dried products (Terrill et al., 1992). However, the percentage of CT in unbound forms remains relatively the same for dried and fresh forages. It has been questioned whether fresh forage or dried products are equally effective at providing an anthelmintic effect. The anthelmintic effects observed from studies where goats were fed SL hay, ground hay or pellets in confinement (Shaik et al., 2004; Lange et al., 2006; Terrill et al., 2007) were similar to reports with goats from grazing studies (Min and Hart, 2003; Min et al., 2004). Therefore, consumption of fresh SL forage or dried products produces similar results. Sheep that were fed a fresh-frozen high tannin SL diet had a lower digestibility and intake than sheep fed fresh frozen or dried low-tannin forms of SL (Terrill et al., 1989); however when the high tannin fresh-frozen forage was dried as hay and fed, it improved the digestibility and intake, which indicated that dried products could be more beneficial than fresh forage.

It should be mentioned that some issues may have contributed to minor problems during the trial. Due to facility expenses, timing for the study became an issue, and there was minimal adjustment period for the animals to get acclimated to their new surroundings. Also during week 1, some of the leftover 75% SL diet was fed to the control group. After correcting this mistake, all the groups from that point on were fed the correct diets. Another problem occurred when the

set of cultures for week 4 were prematurely flooded. Because of this, the flooded liquid was recovered, and the cultures were left to sit until the correct flood date. Then the liquid from both floodings were combined and used in reading the cultures. This may have contributed to the low numbers of larvae counted and identified for that week.

Since as early as the 1940's, SL has been grown for grazing and for hay in the southeastern United States, and its agronomic benefits, which include drought tolerance, resistance to insect damage, and very productive growth on acidic and infertile soils, are well acknowledged (Hoveland et al., 1990). *Sericea lespedeza* is a forage that is well suited to the southern region of the United States. Grazing or feeding it as a dried product (i.e. a natural non-chemical deworming agent) may be a cost-effective, environmentally friendly substitute or addition to the extensive use and reliance on chemical anthelmintics by small ruminant producers in this region.

The results of this trial are not conclusive that SL leaf meal was effective in reducing FEC as the control group was compromised. Additional trials are needed to further evaluate whether SL fed as leaf meal is effective in reducing FEC in lambs. Regardless, SL has been shown in other trials to effectively control infection. Using SL should not be relied on solely, and with anthelmintic resistance on the rise, developing an integrated control program which may include CT forages like SL and their dried products with other alternative methods is important.

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APPENDIX

The GLM Procedure

Dependent Variable: logEPG

logEPG

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|--------------------|-----|-------------|-------------|---------|--------|
| GROUP | 3 | 2.52894732 | 0.84298244 | 0.17 | 0.9154 |
| ANID(GROUP) | 28 | 138.4811724 | 4.9457562 | 7.27 | <.0001 |
| TIME | 5 | 33.8814870 | 6.7762974 | 9.96 | <.0001 |
| GROUP*TIME | 15 | 7.8571628 | 0.5238109 | 0.77 | 0.7090 |
| ERROR | 140 | 95.2770130 | 0.6805501 | | |

Dependent Variable: PCV

PCV

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|--------------------|-----|-------------|-------------|---------|--------|
| GROUP | 3 | 235.187500 | 78.395833 | 1.24 | 0.3129 |
| ANID(GROUP) | 28 | 1766.291667 | 63.081845 | 18.37 | <.0001 |
| TIME | 5 | 133.666667 | 26.733333 | 7.79 | <.0001 |
| GROUP*TIME | 15 | 56.625000 | 3.775000 | 1.10 | 0.3624 |
| ERROR | 140 | 480.70833 | 3.433631 | | |

Dependent Variable: logHATCH

logHATCH

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|--------------------|-----|-------------|-------------|---------|--------|
| GROUP | 3 | 0.23800897 | 0.07933632 | 0.24 | 0.8678 |
| ANID(GROUP) | 28 | 9.2591497 | 0.3306839 | 0.92 | 0.5916 |
| TIME | 5 | 437.2827922 | 87.4565584 | 242.16 | <.0001 |
| GROUP*TIME | 15 | 2.9256817 | 0.1950454 | 0.54 | 0.9139 |
| ERROR | 138 | 49.8395691 | 0.3611563 | | |

VITA

Dana Alicia Pollard was born to Mr. and Mrs. Grover Pollard, Jr. in February, 1985. She attended elementary school and middle school in the Assumption Parish School District and graduated with honors from Edward Douglas White Catholic High School in May, 2003 in Thibodaux, Louisiana. In the fall of 2003, she entered Southern University and A & M College in Baton Rouge, Louisiana. While enrolled at Southern University, she studied animal science in which to pursue her degree of Bachelor of Science. After four years, she graduated as the Student Marshall for the College of Agricultural, Family, and Consumer Sciences. Then she applied and got accepted into the Louisiana State University Graduate School in order to pursue her master's degree through the School of Animal Sciences while concentrating in ruminant parasitology. Upon graduation, she will enter the workforce to gain more field experience and see where this will motivate her to next.