

2006

The effect of a condensed tannin containing forage, sericea lespedeza on existing and challenge infections of *Haemonchus contortus* in sheep

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THE EFFECT OF A CONDENSED TANNIN
CONTAINING FORAGE, SERICEA LESPEDEZA, ON
EXISTING AND CHALLENGE INFECTIONS OF
HAEMONCHUS CONTORTUS
IN SHEEP

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

Interdepartmental Program in Animal and Dairy Sciences

By

Leigh Ann Chafton
B.S., Louisiana State University, 2004
May 2006

Acknowledgements

I would like to thank the Department of Animal Science, Louisiana State University for providing me this research opportunity. I would like to especially thank Dr. James E. Miller for his guidance and support during my research. I also appreciate the invaluable help from every student worker. Also, thanks to my friend Ally who made everyday different and fun.

Special thanks to Nicholas Bourg, my rock, without you my sanity would have been lost. You were there to help with every step of the project (no one else would have done it without pay) and without you none of this would be possible.

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Abstract

Haemonchus contortus is one of the most threatening parasites to small ruminant production because of the potential economic losses that can occur. Over the last few decades, control of worms has relied on the use of chemical anthelmintics. In recent years, such control has waned because the worm population has developed resistance to most of the currently available anthelmintics. Alternative control measures are needed and are being developed. In addition, there is some consumer pressure to reduce the use of chemicals in agricultural products, thus pushing control methods to more natural and acceptable approaches for controlling this blood-sucking worm. One such alternative control measure is the feeding of condensed tannin containing forages either as fresh forage or dried products such as hay, meal, pellets, etc. This study was conducted to determine the effect of a ground meal form of sericea lespedeza (SL), a forage plant high in condensed tannins, on *H. contortus* infection in sheep. Twenty-eight mixed sex lambs with essentially zero fecal egg counts were randomly allocated to four treatment groups of seven animals each. Lambs were kept in cement floored pens. Two groups received a bolus of 5000 *H. contortus* infective larvae (L3) once and the infection was allowed to mature over five weeks (existing infection). The remaining two groups received trickle infections of 500 *H. contortus* L3 three times a week for three weeks (establishing infection). SL meal was fed over a five week period to one of the existing and establishing infection groups while the other groups were fed bermudagrass hay. All groups were fed bermudagrass hay for an additional two weeks at which time all animals were necropsied. Fecal egg count (FEC) was significantly reduced in the existing infection SL fed group than the control group over the 5 week feeding period. FEC was

lower in the establishing infection SL fed group than the control group, but the difference was not significant. After SL feeding was terminated, FEC increased in both existing and establishing infection groups which indicated an effect on female worm fecundity. At necropsy, there were fewer worms (male and female) in both SL fed groups, but the differences were not significant. This trend of fewer worm numbers suggested that there may have been an effect on reducing infection level also. These results indicate that SL meal had more of an effect on reducing FEC of existing infections than establishing infections of *H. contortus*.

Chapter 1. Introduction

Haemonchus contortus (Nematoda, *Trichostrongylidae*), is one of the most detrimental parasites affecting goats and sheep today. This nematode is also called the barber pole worm because of its red and white striped appearance in the female. The female is capable of producing over 5,000 eggs a day, which are passed through the feces onto pasture. The life cycle of the barberpole worm is 17 to 21 days, from egg to mature adult. After hatching, the eggs develop through 2 molts to infective larvae (L3). The L3 are consumed through grazing on infected pasture, and following ingestion, molt again to the L4 stage of the parasite. The L4 and adult worms are primarily found in the abomasum where they become blood feeders. This process of feeding can lead to severe anemia, edema and eventual death of the infected animal. Economical losses from infection result from reduced reproductive efficiency, decreased production of wool and meat, costs of prophylaxis, and costs of treatment of the animal. This problem, termed haemonchosis, is usually found in areas with warmer climates and can cause severe losses on individual farms. Anthelmintics have been used to combat this problem, but due to increasing parasite resistance to anthelmintics, incidence of haemonchosis is becoming more and more common. In many cases, resistance to anthelmintics is a result of overuse of a particular drug or improper usage of that drug. Another problem associated with this detrimental parasite is reduction of land available for rotational grazing. Therefore, animals are dewormed and then returned to infected pasture, only to be exposed again to the same problem. Attempts have been made in genetically breeding sheep for resistance to nematodes.

Due to the shortcomings of the traditional methods for controlling *H. contortus* infection in small ruminants, new venues have been pursued. One novel approach being studied is the use of condensed tannin containing forage, such as sericea lespedeza [SL; *Lespedeza cuneata* (Dumont) G. Don.]. This alternative to anthelmintics has proven effective in previous studies. In this study the objective was to determine the effects of SL, in a ground meal form, on parasitic infection in sheep. Two groups of sheep were given a bolus infection that was allowed to mature before feeding SL, while the other two groups received trickle infections over a three week period during SL feeding. The goal was to test efficacy of the SL meal against an already existing infection versus a developing infection.

Chapter 2. Literature Review

2.1. Ecology and Dynamics of the Parasite

Haemonchus contortus, a nematode parasite found in small ruminants, is of economic importance to the sheep industry worldwide (Troell et al., 2005). It requires no intermediate host and undergoes four very different larval stages. The female and male adult worms dwell in the abomasums of sheep, goats and other ruminant animal. The adult female is capable of depositing 5,000 to 10,000 eggs per day through the hosts' feces. In an advantageous environment, the stage I juvenile (L1) larvae will hatch from the deposited eggs. Optimal larvae development occurs at 22° C, a fairly high temperature. Stage I larvae further develop into stage II juvenile (L2) larvae. These first two stages of larvae development, feed on bacteria. Humidity, as well as high amounts of rainfall can prompt a rapid development from L2 to L3, leading to an outbreak of disease. Stage III (L3) larvae possess a protective cuticle, making this larval stage more resilient against environmental conditions, contrary to the L1 and L2 stages.

Ruminants become infected by ingesting the L3 form of the parasite during grazing. The L3 lose their outer sheath once ingested; after which, they pass into the abomasum and burrow into the mucosa, where they develop to the L4 which emerge back into the lumen and mature adult worms. Following this process, the mature worm feeds on blood and produces eggs. The cycle from egg to mature adult takes approximately 17 to 21 days. Due to the massive number of eggs that are deposited by each female adult worm and the short time it takes to attain maturity, this parasite is capable of rapidly increasing infectivity levels of the pasture and infection level in the host. These

gastrointestinal nematodes and the infections they cause are the major constraint in sheep production (Bricarello et al, 2005).

2.2. Approaches to the Control of Nematodes

2.2.1. Anthelmintic Resistance

Primarily, the control of parasitic infections has been based on the well implemented use of anthelmintics (Hounzangbe-Adote et al., 2005a). Several different products have been developed to combat this problem, but they have been used primarily to rid an existing infection rather than prevent infection. Strategic use of these drugs and their treatment is now being crippled by the increasing resistance to numerous anthelmintics in nematode populations (Hounzangbe-Adote et al., 2005a). Resistance to anthelmintics is exponentially growing and becoming a global problem. There are three major classes of anthelmintics used against gastrointestinal nematodes: avermectins, benzimidazoles, and imidothiazoles. A number of studies have demonstrated nematode resistance to drugs in all three classes of anthelmintics (Hembry et al., 1986; Miller and Barras, 1994; Zajac and Gipson, 2000; Terrill et al., 2001; Mortensen et al., 2003). Due to resistance of nematodes to anthelmintics, the prevention of Haemonchosis is being made vulnerable (Amarante et al., 1992; Echevarria et al., 1996). As a result of this growing resistance problem, alternate venues of controlling *H. contortus* infection are being pursued. In addition to resistance, the cost of anthelmintics in developing countries is yet another reason for different methods of control to be found (Hounzangbe-Adote et al., 2005a).

Reports out of Australia show that when producers are faced with anthelmintic resistance, using a combination of anthelmintics can be more beneficial than using a

single drug (Waller et al., 1990; Anderson et al., 1991 ab). According to one study, where a flock of Angora goats were naturally infected with a strain of ivermectin resistant *Haemonchus contortus*, a combination of anthelmintic drugs (fenbendazole and levamisole) reduced eggs per gram (EPG) by 62 % (Miller et al., 1996). Multiple doses of the same drug have also proven effective, but how long its effectiveness will last is yet to be determined. For example, benzimidazole administered twice in twelve hour intervals has shown promise (Sangster et al., 1991). Improvement with this drug can also be attained by withholding food before administering (Hennessy, 1997). Unfortunately, when benzimidazole has reached resistance in a flock, it continues to show resistance many years later (Herd et al., 1984; McKenna, 1990). Another problem facing the use of these drugs is the increasing demand for organic agriculture, which strictly prohibits the use of synthetic products (Hordegen et al., 2003).

2.2.2. Grazing Management

The pairing of pasture management and anthelmintic use has been another venue used to control nematodes, but has many shortcomings. For example, ‘dose and move’ and rotational grazing is becoming less feasible, due to the limited land size of many smallholder farmers and the communal land ownership of many pastoralists (Githiori et al., 2003). Due to the limitation and decreasing availability of land, use of ethnoveterinary medicine is on the rise (Hammond et al., 1997; Dano and Bogh, 1999). Based on the epidemiology of the parasite, producers have used drenching techniques during the beginning of the transmission season and then move the animal to clean grazing areas, which is termed ‘treat-and-move’ (Herd et al., 1984). This method has been effective at controlling nematode parasites, but “places heavy genetic selection

pressure for resistance on nematode populations” (Kaplan et al., 2004). This occurs because the drug is being administered to all animals, exposing this drug to all worms, leaving an unexposed environment of eggs and larvae called refugia (Kaplan et al., 2004).

There are many different factors affecting transmission of nematodes from grazing, such as effects due to climate and habitat destruction. Climatic issues affecting the transmission cycle are temperature, rain, humidity, barometric pressure, sunlight, cloud cover and wind (Stromberg, 1997). Habitat destruction is comprised of birds, insects, fungi, and wild mammals that have the resources to reduce number of larvae on pasture (Stromberg, 1997). Both factors vary both seasonally and annually (Stromberg, 1997). Because the variables involved in the calculation process are difficult, in terms of measuring (climate, larval development and behavior) it is hard to determine with complete accuracy the amount of larvae on pasture (Stromberg, 1997).

2.2.3. Refugia and Resistance

Refugia is the portion of the nematode population that is not exposed to treatment by anthelmintics, such as, the eggs and the larvae on pasture (Van Wyk, 2001). The size of the nematode population not exposed to treatment, is directly correlated with the degree of selection for resistance with a particular remedy (Martin et al., 1981). The importance of refugia is that it voids out the susceptible worm eggs when they mature because selection is reduced, which is invaluable to the susceptible portion of the worm population (Van Wyk, 2001). Therefore, drenching is only helpful when it is necessary; otherwise it is harmful in terms of resistance (Van Wyk, 2001). Overall, it is better to have a high “refugia” on your pasture, than to have a highly resistant progeny of worms on your pasture. It is important to know that resistant progenies are exposed to a grazing

site through different venues, such as an animal purchase or through sharing pastures with different flocks from several farms (Silvestre et al., 2002). Time of treatments using anthelmintics combined with rotational grazing play a significant role in the further development of resistance to anthelmintics (Silvestre et al, 2002). The problem associated with rotating your animals to clean pasture after anthelmintic treatment has been administered, is that the producer may possibly infect his clean pasture of susceptible worms to a progeny of resistant worms, which will result in problems with anthelmintic resistance (Martin et al., 1981; Martin, 1989).

2.2.4. Nematophagous Fungus

Another method being used to control parasitism of sheep is nematophagous fungus *Duddingtonia flagrans*. This fungus uses nematodes as a food source. There are one in three ways of grouping these fungi: nematode-trapping fungi, endoparasitic fungi and egg-parasitic fungi. Nematode-trapping fungi being of most scientific importance, chiefly *Duddingtonia flagrans* as a fungus has been the core interest for research purposes. This fungus specifically traps the larvae found on the pasture, preventing the L3 larvae from migrating onto the pasture and potentially infecting future hosts. This fungus is administered to the sheep via feed and is ultimately passed through the feces to complete its purpose on pasture. Survival of the fungus in the gastrointestinal tract is attained using a chlamydospore that the fungus produces (Faedo et al., 2002). This form of control is better used as an additive to other control measures.

2.2.5 Nutrition

The nutritional level of the animal/host can be influential on its ability to maintain productivity and limit establishment of a parasitic infection (Albers et al., 1987). Recent

studies show that by making improvements to the metabolizable protein content of the diet, disease related changes affiliated with Haemonchosis in genetically resistant and susceptible animals can be influenced (Wallace et al., 1995, 1996, 1999; Abbott et al., 1985).

2.2.6. Copper Oxide Wire Particles

Copper oxide wire particles (COWP) are another control method being used against *Haemonchus contortus* infections in sheep and goats (Burke et al., 2004). In several studies, COWP is responsible for reducing worm burdens in growing lambs (Bang et al., 1990; Knox, 2002) and decreasing worm burden and fecal egg counts (FEC) in mature goats (Chartier et al., 2000). COWP are held in the abomasum for at least 32 days after being passed from the rumen (Dewey, 1977). In addition, COWP are responsible for releasing free copper in the abomasum, increasing concentrations of copper in the abomasal digesta, followed by storage of copper in the liver (Dewey, 1977; Bang et al., 1990). This treatment affects *H. contortus* by changing the environment they are accustomed to, causing them to be ejected from their host (Chartier et al., 2000). COWP have proven successful against *H. contortus* and is very promising as a control method (Burke et al., 2004).

2.2.7. Breeding for Resistance

Another control measure currently being pursued is breeding of genetically resistant sheep against gastrointestinal nematodes, which may prove to be a more effective means of control as well as, reduce dependence on anthelmintics. Breeding for resistance against gastrointestinal nematodes could be beneficial in terms of cost of anthelmintics and in reducing the number of worms toward production (Dominik, 2005).

Consequences to breeding for resistance have yet to be illustrated (Windon, 1996).

Another important factor affecting the prospect of genetically resistant animals is that the variability of resistance within breeds, such as that found in *H. contortus* (Stear et al., 1994), may possibly equal to that of the variability within breeds themselves (Gray et al., 1987). Currently, the only effective way to select for resistance to infection is through fecal egg count (FEC) and new methods of selection are being developed, such as DNA markers, host antibody (Ab) and parasite antigen assays (Gray, 1997). If traits that prove to be resistant against nematode infection were used more frequently in breeding programs, the sheep industry would benefit immensely (Dominik, 2005).

2.2.8. Vaccines

Development of vaccines against gastrointestinal nematodes is one of the most researched control methods today. As of yet, no vaccine has been made commercially available to producers (Newton et al., 2003). There are two classes of experimental vaccines: hidden and natural antigens (Newton et al., 2003). Hidden antigens, also referred to as concealed or covert antigens are successful against *H. contortus* because it is a blood-feeding nematode. The reason hidden antigens are effective against this particular type of nematode is simply because “high levels of antibody to the injected antigen are ingested with the blood meal” (Newton et al., 2003). Contrary to hidden antigens, natural antigens can be used against both blood-feeding and non-blood-feeding nematodes (Newton et al., 2003). Natural antigens are advantageous because the immune response is boosted due to exposure by field conditions (Smith et al., 1993). Hidden antigens have also shown immune response memory to challenge infections (Smith et al., 1993).

Recently, more vaccine work has been conducted on *H. contortus* than any other nematode affecting sheep (Newton et al., 1999; Knox, 2000). Unlike anthelmintics, vaccines do not leave any chemical residues in animals, making them consumer friendly and safe (Dalton et al., 2001). In addition, the public has a familiarity with vaccines, making acceptance to this concept easier; this will make the process of marketing a potential vaccine more expedient (Dalton et al., 2001).

Due to the success found with vaccines against *Taenia ovis* in 1990 and *Boophilus microphilus* in 1992, researchers remain positive about the possibility of a commercial vaccine against nematodes, such as *H. contortus*, *Teladorsagia*, and *Trichostrongylus* spp., taeniid cestodes, *Echinococcus granulosus*, and *Fasciola hepatica* (Emery, 1996).

2.2.9. Antiparasitic Plants

Antiparasitic plants have long been used as a method to control gastrointestinal nematodes. Renewed interest in the use of these traditional medicines has stemmed from the cost of drugs, resistance to anthelmintics, and the poor availability of veterinary services in underdeveloped countries, such as Kenya (Anon., 1996; Monteiro et al., 1998; Wanyama, 1997ab; Wanyangu et al., 1996). Recently, there has been much interest in feeding plants containing condensed tannins (CT) to animals to reduce the effects of infection with gastrointestinal nematodes.

2.3. Tannins: Hydrolyzable and Condensed

Tannins are oligomeric compounds with multiple structure units that have free phenolic groups. They range anywhere from 500 to sometimes greater than 20,000 in molecular weight. Tannins are usually soluble in water (Haslam, 1989) except for some with high molecular weight structures. They are also capable of binding proteins and

forming soluble and insoluble tannin-protein complexes. Tannins are usually divided into two groups, hydrolyzable tannins (HT) and CT (proanthocyanidins), based on their chemical structure and properties (Athanasiadou et al., 2001).

2.3.1. Hydrolyzable Tannins

Hydrolyzable tannins are molecules with a carbohydrate, generally D-glucose as a central core. The hydroxyl groups of these carbohydrates are partially or totally esterified with phenolic groups like gallic acid (gallotannins) or ellagic acid (ellagitannins) (Waghorn and McNabb, 2003). Hydrolyzable tannins are usually present in low amounts in plants (Mueller-Harvey, 2001). These tannins are found in oak (*Quercus* spp.), Acacia, Eucalypts and a variety of browse and tree leaves (Waghorn and McNabb, 2003). The browse that contain these leaves and apices can contain anywhere from 200g per kg of dry matter (DM) and in some species they can contain phenolic compounds that can exceed 500g per kg of dry matter (Reed, 1995; Lowry et al., 1996). Hydrolyzable tannins are potentially toxic to animals, but most ruminants can adjust to a diet of these tannins (Waghorn and McNabb, 2003). Ruminants are able to adjust to these toxic tannins by reducing their urinary excretion of degradation products, thus allowing them to consume these diets (Lowry et al., 1996). Although ruminants have this ability, an excessive amount of this tannin diet can lead to liver and kidney lesions, as well as death (Waghorn and McNabb, 2003). Death usually occurs five to ten days after the first excessive consumption; the toxic compound responsible is not known. Information concerning the digestion, absorption, and impact on metabolism and productivity of hydrolyzable tannins is rare.

2.3.2. Condensed Tannins

Of the tannins, condensed tannins are the most widely distributed. Condensed tannins are oligomers or polymers of flavonoid units linked by carbon-carbon bonds (Waghorn and McNabb, 2003) not susceptible to cleavage by hydrolysis (Reed, 1995). They are called condensed tannins because of their condensed chemical structure. CT, are also termed proanthocyanidins (PA), which is derived from the acid catalyzed oxidation reaction that produces red anthocyanidins through heating of PA in acidic alcohol solutions (Haslam, 1982). Cyanidin (procyanidin) and delphinidin (prodelphinidin) are the most common anthocyanidins produced (Reed, 1995). Condensed tannins can contain as little as two or greater than fifty flavonoid units. Due to the variability of flavonoid units to some substituents and because of the variable sites for interflaven bonds, condensed tannin polymers have complex structures. Condensed tannins may or may not be soluble in aqueous organic solvents, depending on their chemical structure and degree of polymerization.

Condensed tannins have a complex chemistry. The heterocyclic C-rings can be formed via 2,3-cis or 2,3-trans, which determine “how monomeric units are attached relative to one another” (Barry et al., 1999). The number of monomeric units are variable (Foo et al., 1996, 1997) making an “infinite variety of chemical structures, which in turn affect the biological properties of the condensed tannins” (Barry et al., 1999). For example, *Lotus corniculatus* and *Lotus pedunculatus* are considerably different concerning their chemical structure (Foo et al., 1996, 1997)

It is speculated that plants containing condensed tannins evolved over time to implore them as a defense mechanism, which protected them against pathogenic

microorganisms and against being consumed by insects or grazing animals (Swain, 1979). Now they are being extracted from various plants to be used in improving animal health. Extraction of these condensed tannins was once performed using acetone-water, but full extraction of the CT was not obtained with this method (Barry et al., 1999). Now condensed tannins are being detected even at trace levels using NMR spectrometry and anthocyanidin formation (Jackson et al., 1996).

Condensed tannins found in tropical forages are thought to promote plant growth by reducing the release of leaf litter into the soil (Palm et al., 1991) and reducing the release of animal feces (Waghorn and McNabb, 2003). Because of the substantial benefits of condensed tannins for ruminant health and productivity, much of research has been focused on these tannins (Waghorn and McNabb, 2003). There are many different types of foliage that contain condensed tannins: lotus, sainfoin (*Onobrychus viciifolia*), sulla (*Hedysarum coronarium*), dock (*Rumex obtusifolia*), Sericea lespedizia (*L. cuneata*) and *Dorycnium rectum*, all of which can do well even in average or poor soil as well as acidic soil (Waghorn and McNabb, 2003).

Condensed tannin containing forages have different benefits for ruminants, depending on the species of plant. For example, lotus has been proven beneficial in the prevention of bloat (Beddows, 1956). Other condensed tannins have been efficient at improving live-weight gain (Waghorn et al., 1999). In sheep, they have been shown to increase milk protein concentration (Wang et al., 1996), improve lambing percentages (Min et al., 1999), and reduce, gastrointestinal nematode infection (Niezen et al., 1995), incidence of fly strike (Leathwick et al., 1995), and methanogenesis in sheep (Waghorn et al., 2002).

In sheep (Athanasiadou et al., 2000, 2001) and deer (Hoskin et al., 2000), condensed tannins are capable of reducing parasite infection. This is dependent upon the source of the condensed tannins used as well as the amount of condensed tannins required to attain the desired effect (Waghorn et al., 2003). Although effective at reducing nematodes, this method of control is not as effective as drenching techniques, but is likely to be used as an additive to other methods of control (Waghorn et al., 2003).

In several studies, sericea lespedeza, a condensed tannin containing forage, has been used to decrease parasitism (lower total worm burdens, fecal egg counts, and larval development) in goats (Min et al., 2004, 2005; Shaik et al., 2004, 2006). Similar results were obtained using quebracho extracts except no difference in worm number was noted (Paolini, 2003). Another study using quebracho extracts exhibited positive results, such as a reduction in fecal egg counts in sheep and goats (Athanasiadou, 2001). These studies give promise to condensed tannins as a future control method against gastrointestinal nematodes.

2.4. Sericea Lespedeza

Sericea lespedeza (SL; *Lespedeza cuneata*; 15.2% CT), “a legume planted widely throughout the southern USA as a grazing, hay, soil restoration and conservation crop” is a perennial, warm season forage (Powell et al., 2003), brought here from its native Asia (Japan). Because SL is adapted to acidic soils with low fertility, it has potential of being a useful low-input forage for the southern United States (Puchala et al., 2005). Another positive aspect of sericea lespedeza is its tolerance for drought (Ball et al., 1991) and its high concentration of protein (Gamble et al., 1996). In spite of these attributes, it is often thought of as a weed and some states are trying to rid themselves of this “pest.” Its

original purpose was to fight against soil erosion, improve soil and act as a habitat and cover for wildlife, but it is now used as a forage plant as well. *Sericea lespedeza* is capable of growing in a variety of areas, such as pastures, roadsides, rangelands, prairies, and eroded slopes, but is intolerant of shade. Although there are negatives surrounding this forage, there are many positives, such as its ability to improve production and animal health.

Chapter 3. Materials and Methods

3.1. Location

This study was conducted in an enclosed barn at the School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.

3.2. Experimental Design

Prior to the start of this study, 35 lambs (6-7 months of age) were brought into the barn and dewormed with albendazole (Valbazen, 10 mg/kg) and levamisole (Tramisol, 8 mg/kg) for 3 days in a row to reduce infection level to the lowest possible. After 2 weeks, the 28 animals with the lowest infection level, based on fecal egg count (FEC) were randomly allocated to one of 4 treatment groups of 7 animals each. The 4 groups were maintained in separate concrete floor pens that were bedded with straw and cleaned twice a week. Based on an estimated mean weight of 34 kg, animals began being fed bermudagrass (BG) hay at 3.5% of body weight (1.2 kg/hd/d). In addition, throughout the study all animals were supplemented with a lamb growing ration (Purina Lamb Chow Ration, 16% protein at 0.23 kg/hd/d) and water was provided at all times.

The design of this study was laid out over a 12 week period in which the effect of *Sericea lespedeza* (SL) meal (ground from hay) was tested for its antiparasitic activity on controlling *H. contortus* infection. Five weeks prior to the start of the study, a bolus of 5,000 infective larvae (L3, 97% *H. contortus*) was administered to two of the four groups (groups 1 and 2, bolus infection). At the start of the study (day 0), trickle infections of 500 L3 (97% *H. contortus*) were administered 3 times per week for 3 weeks to the remaining two groups (groups 3 and 4, trickle infection). At the same time, feeding for groups 1 and 3 was switched to SL. On day 7, body weights were taken on all animals to

check and verify that the proper amount of SL meal and BG hay was being fed. SL was fed for 35 days and during the remaining 14 days, BG hay was again fed to all animals.

Fecal and blood samples were collected each week and analyzed for FEC and blood packed cell volume (PCV), respectively. Feces were collected directly from the rectum and blood was collected in 7 ml EDTA vacutainer tubes via jugular venipuncture.

At the end of the study (day 49), all animals were necropsied. During necropsy, the abomasum, small intestine, and large intestine were extracted for worm recovery and identification. This procedure required the organs to be washed in five liters of water. After thorough mixing, a 500 ml or one l sample was taken. The samples were then preserved using formalin (10%).

3.3. Techniques

3.3.1. Fecal Egg Count

Fecal samples were stored in a refrigerator until processed. The FEC was determined using a modified McMaster technique where 2 grams of feces were broken up in 30 ml of a saturated salt solution. The sample solution was then thoroughly mixed using an electric mixer. Immediately after mixing, a sample of the solution was extracted using a pipette and placed into one half of a McMaster slide. This was repeated to fill the other half of the slide. The number of eggs counted in both sides of the chamber was multiplied by 50 to estimate the total number of eggs in the sample. Results were reported as eggs per gram (epg).

3.3.2. Fecal Culture

A pooled culture of all samples per group was done every week to determine the population distribution based on L3 identification. Approximately 10-15 g of feces were mixed with water to form a slurry. The slurry was then mixed with an equivalent amount of vermiculite in a culture cup. Water was added until a moist, not wet, crumbly consistency is achieved. The cup is then covered with aluminum foil and holes (~10 to 12) are punched into the foil to allow air circulation. The cultures were incubated (room temperature) for a minimum of 14, but no longer than 21 days for optimum larval recovery. The L3 were recovered using a baermann technique.

3.3.3. Counting of Adult Nematodes

The bottles containing abomasal and small intestinal samples were processed in 100 ml aliquots. The contents of the each bottle were poured back and forth between the bottle and another container of equal size to obtain a thoroughly mixed sample. Immediately, 100 ml was poured into a 250 ml beaker which was then washed through a 200 mesh sieve to remove small debris. The remaining sample in the sieve was then washed into a petri dish, stained with a 7% iodine solution and examined under a dissecting microscope. Contents of the sample were slowly scanned for adult and larval worms that would be stained a deep red color from the iodine. Worms were removed using a 22 gauge needle, placed onto a microscope slide in a drop of lacto phenol and covered with a coverslip. Slides were labeled with animal identification number, date performed, number of worms found, and organ from which they were recovered. An additional 100 ml aliquot was processed if less than 10 worms were found in the first aliquot. The entire contents of the large intestine was processed similarly, but washed

through a 50 mesh screen. The number of worms recovered was recorded and then identified to species and sexed. Numbers were then extrapolated to estimate the number of worms in a given organ.

3.3.4. Blood Packed Cell Volume

The PCV of all samples was determined the same day on which they were collected. After thorough mixing by inversion several times, hematocrit tubes were filled to three-fourths of the volume with blood, sealed using crit-o-seal, and then centrifuged for 5 minutes in a microhematocrit centrifuge. The PCV value was determined from the hematocrit reader.

3.3.5. Feed Analysis

Analysis of SL condensed tannin content (extractible, protein-bound, and fiber-bound) and SL and BG protein content was conducted Fort Valley State University, Fort Valley, GA.

3.3.6. Statistical Analysis

SAS, a statistical computer package program (version 9.1.3), was used to analyze the data. FEC and worm count data were natural log-transformed to stabilize variance. FEC and PCV data were subjected to a repeated measures design in a split-plot arrangement of treatments with treatment group and animal within treatment group effects on the main plot and time and treatment group by time interaction effects on the subplot. Using Tukey's HSD test, pairwise comparisons of significant main effect comparisons were analyzed. Also, pairwise comparisons of least-square adjusted means were examined for significant interaction effects. For worm count, t-tests were conducted. Due to the concerns for normality with small samples, nonparametric tests

(Wilcoxon rank-sum) were generated. Differences were considered significant when $p < 0.05$.

Chapter 4. Results

4.1. FEC and PCV

4.1.1. Bolus Infections

At the start of the study (day 0), the mean FEC of group 1 (4,150 epg) was higher than that of group 2 (2,364 epg) but the difference was not significant (Figure 1).

Subsequently, throughout the study the group 2 FEC varied from a low of 1,514 epg on day 7 to a high of 4,836 on day 49. The mean FEC of group 1 decreased immediately to a low of 300 epg on day 7, maintained a level around 1,000 epg through day 35 and subsequently increased to 3,486 epg by day 49. Overall, the difference between groups was significant ($P = 0.0245$) and only days 0 and 49 were not different ($p > 0.05$).

For both treatment groups, mean PCV varied from 24.9% to 30.1%, being higher during the early days of the study and lower during the later days (Figure 2). No significant difference in PCV was found ($P = 0.8705$) between treatment groups throughout the study.

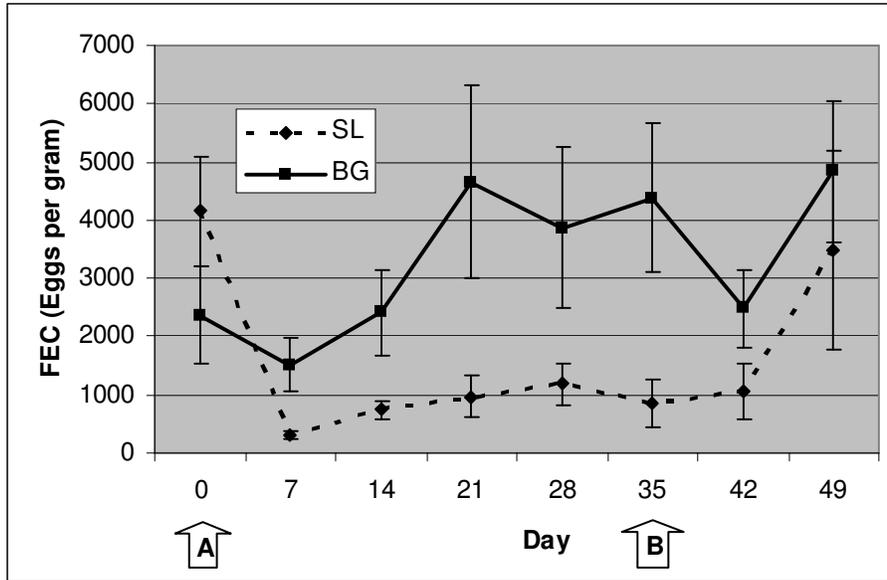


Figure 1. Fecal egg count (FEC) in one time experimental infections of *Haemonchus contortus* in sericea lespedeza (SL) meal and bermudagrass hay fed lambs. A = Start of SL feeding, B = SL feeding stopped. Overall difference between groups was significant ($P = 0.0245$).

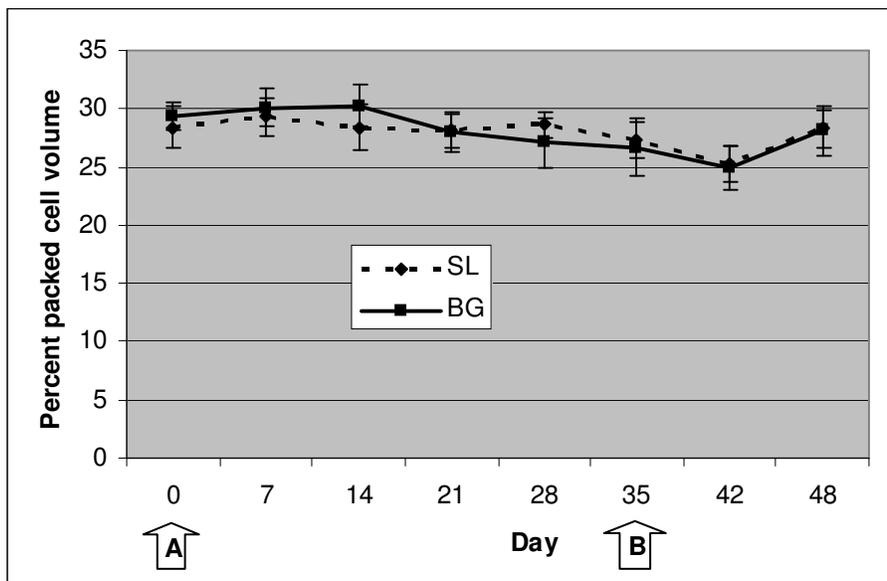


Figure 2. Percent blood packed cell volume in one time experimental infections of *Haemonchus contortus* in sericea lespedeza (SL) meal and bermudagrass hay fed lambs. A = Start of SL feeding, B = SL feeding stopped. Overall difference between groups was not significant ($P = 0.8705$).

Another way to look at effect of treatment is to determine percent reduction in FEC. Percent reduction was 80.2% on day 7 and maintained 70-80% when SL was being fed through day 35 (Figure 3). After SL feeding stopped, percent reduction was 58% and 28% on days 42 and 49, respectively.

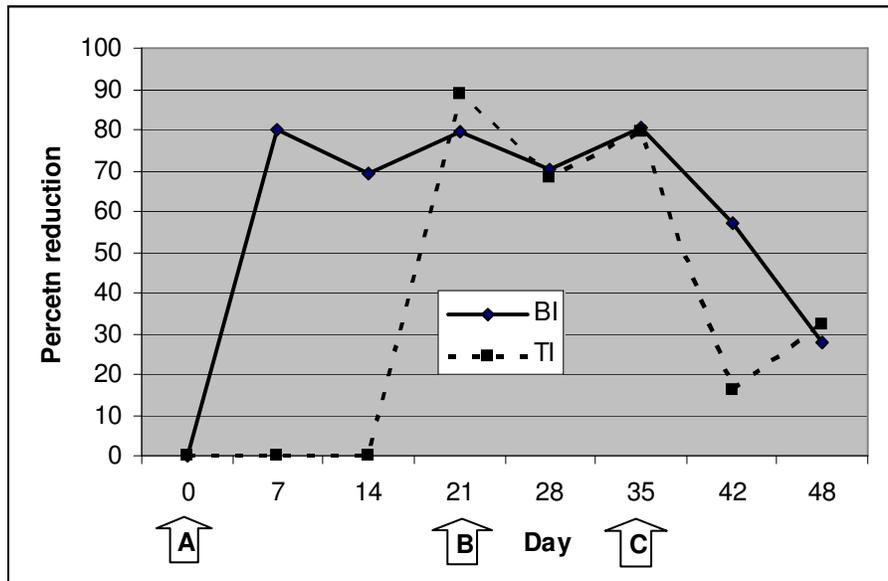


Figure 3. Percent reduction of bolus (BI) and trickle (TI) infection groups in sericea lespedeza (SL) and bermudagrass fed lambs. A = Start of SL feeding and trickle infections, B = Trickle infections stopped, C = SL feeding stopped.

4.1.2. Trickle Infections

From day 0-14, the mean FEC of both groups varied from 0 epg to 130 epg (Figure 4). Starting on day 21, FEC increased in both groups and group 3 remained below that of group 4 throughout the rest of the study. Overall, the difference between treatment groups was not ($P = 0.0988$), but almost reached significance at day 35 ($P = 0.0523$).

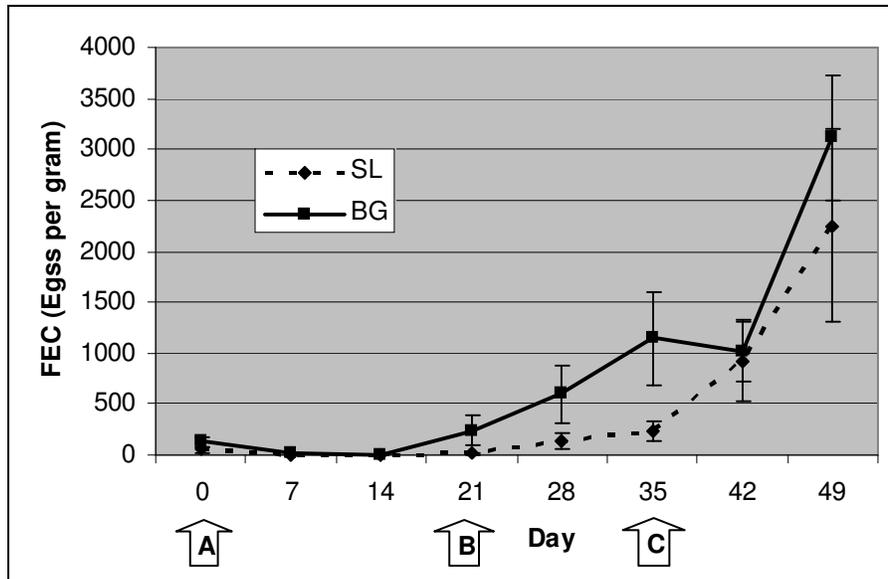


Figure 4. Fecal egg count (FEC) in trickle challenge infections of *Haemonchus contortus* in sericea lespedeza (SL) meal and bermudagrass hay fed lambs. A. = Start of SL feeding and trickle infections, B. = Trickle infections stopped C. = SL feeding stopped. Overall difference between groups was not significant ($P = 0.0988$).

The mean PCV of group 3 started the study significantly higher than that of group 4 and remained higher throughout the study (Figure 5). Both groups maintained PCV between 27% and 35%. The overall difference between groups was not significant ($P=0.7464$), however, the difference was significant on Days 0 ($P = 0.0005$), 7 ($P = 0.0182$) and 21 ($P = 0.0247$).

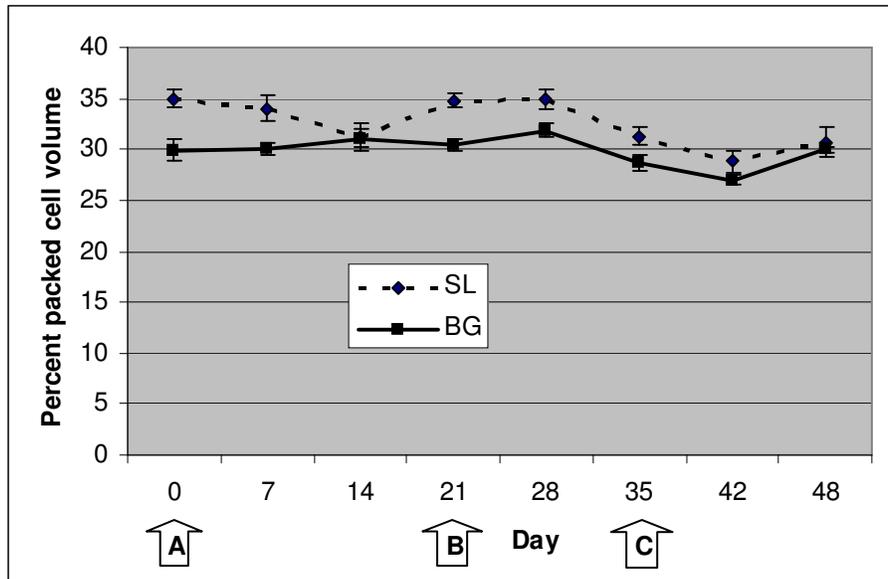


Figure 5. Percent blood packed cell volume in trickle challenge infections of *Haemonchus contortus* in sericea lespedeza (SL) meal and bermudagrass hay fed lambs. A = Start of SL feeding and trickle infections, B = Trickle infections stopped, C = SL feeding stopped. Overall difference between groups was not significant ($P = 0.7464$).

Once the experimental infection started to mature, percent reduction in FEC was 70-90% during the SL feeding period (Figure 3, day 21 through day 35). After SL feeding stopped, percent reduction was 17% and 32% on days 42 and 49, respectively

4.2. Worm Burden

Only *H. contortus* was found in the abomasum. The mean number in group 1 (468) was lower than that of group 2 (793), but the difference was not significant ($P = 0.5696$). Similarly, the number in group 3 (471) was lower than that of group 4 (928), but the difference was not significant ($P = 0.1707$). For groups 1 and 2, the number of female *H. contortus* was 252 and 305, respectively, but this difference was not significant ($P = 0.8820$). Similarly, for groups 3 and 4, the numbers were 242 and 438, respectively, but the difference was not significant ($P = 0.2536$). The female to male ratio for groups 1 and 3 was 1.17 and 1.48, respectively, and for groups 2 and 4, the ratio was 0.63 and 0.89,

respectively. There were a few *Cooperia* spp. found in the small intestine of 2 animals (25 each) in group 3.

4.3. Feed Analysis:

Condensed tannin (CT) content was 1.34% extractible, 7.82% protein-bound, 1.52% fiber-bound, for a total of 10.68% CT. The SL and BG protein content was 11.9% and 12.5%, respectively.

Chapter 5 – Discussion and Conclusion

Reduction in nematode FEC has been reported in goats grazing SL forage compared to grazing other perennial grass forage (Min et al., 2004). It is not always practical or economical to establish new pasture; therefore, providing SL in other forms would enhance its usefulness. Forages can be cut for hay and processed further into meal, pellets, cubes, etc. In addition, active compounds can also be extracted which may provide some effect when administered by oral drench or incorporated into feed products. Athanasiadou et al. (2001) reported that FEC and small intestinal worm burdens were reduced in sheep fed pellets containing CT extracted from quebracho trees. Confinement studies in which SL hay was fed *ad libitum* to sheep and goats with natural and experiment infections have demonstrated a reducing effect of this CT forage on FEC and worm burdens with the effects attributed primarily to reduced fecundity (Shaik et al., 2004; Lange et al., 2005).

The objective of this study was to determine if another form of processed SL hay, ground meal, would have a similar effect when fed at 3.5% of body weight rather than *ad libitum*. It was demonstrated that this feeding regimen reduced FEC of existing (bolus) infections and tended to keep FEC lower in establishing (trickle) *H. contortus* infections. This effect could be a result of reduced fecundity of female worms and/or reduction in number of adult female worms. One possible explanation for this is that CT, mixed with abomasal contents, may result in a rather sudden change in environment that makes it unpleasant for existing worms; thus, they are induced to lay fewer eggs and/or vacate. Because worms from incoming infection are entering an already existing CT

environment, they would not be expected to experience such a change in conditions and; therefore, may not be as severely affected as the existing worms.

The effect on fecundity is supported by the observation that subsequent to the reduced FEC for both existing and establishing worm burdens during the period of SL feeding, FEC increased after SL feeding was stopped. The observation that FEC increased in the SL feed group but not to pre-SL feeding levels, indicated that the effect may have also been partly due to a reduced number of female worms.

Anthelmintic effects using other dried CT containing forages have also been reported recently. Goats that were fed sainfoin hay showed reduced FEC (Paolini et al., 2003) and those fed sun-dried *Acacia karoo* foliage had a reduction in both FEC and worm counts (Kahiya et al., 2003). These studies and the current investigation have demonstrated a range of effect on nematodes, which indicate that forages may have different CT activity. The CT moieties in various forages have been shown to differ in how they are utilized in the animal. (Terrill et al., 1989; Kahiya et al., 2003). In sheep fed two different *Lotus* species, it was observed that CT degraded plant proteins differently. *Lotus pedunculatus* CT had more of an effect at protecting plant protein from degradation by rumen microorganisms than that of *Lotus corniculatus* CT, even though their CT concentrations were similar (Aerts et al., 1999). In addition, *L. pedunculatus* has a higher MW, as well as a higher ratio of prodelphinidin to procyanidin subunits than *L. corniculatus* (Foo et al., 1996; 1997). *L. pedunculatus* also has been shown to have more of an effect of decreasing nematode FEC than *L. corniculatus* (Niezen et al., 1998). SL also has a higher ratio of prodelphinidin to procyanidin subunits than most other CT-containing legumes (Burns, 1966).

Condensed tannins that are unbound or free (extractable), are more reactive than the bound form (Barry et al., 1986). It is likely that this unbound fraction that may be involved in the observed effect on worms. The amount of total CT can vary between plant types, the maturity of the plant and dried products of the same plant. Generally, mature plants have higher extractable CT content than growing plants and both have more than dried products (Terrill et al., 1992). Also, the higher the CT content, the more unbound reactive moiety is present. However, the percent of unbound CT remains relatively the same for fresh and dried forages. Knowing this, it has been questioned whether fresh forage, dried products or extractions are equally effective at providing an anthelmintic effect. In a study where goats were fed SL hay in confinement (Shaik et al., 2004), results were similar to goats that grazed SL forage (Min et al., 2003; 2004). In the current investigation, a different form of the same plant produced similar results, thus more extractable CT may not be as important as selecting the type of condensed tannin (i.e. high ratio of prodelphinidin to procyanidin subunits). However, a high level of CT can have negative effects, such as reduced intake and digestibility (Terrill et al., 1989; Barry et al., 1986). Low palatability to grazing beef cattle due to high CT content of the forage has contributed to giving SL a reputation in the Midwest as a noxious weed. Sheep fed a fresh-frozen high-tannin SL diet had a decrease in intake and digestibility compared to those fed fresh frozen or dried low-tannin forms of SL (Terrill et al., 1989). However, when the high tannin fresh-frozen forage was dried and fed in a hay form, intake and digestibility improved which indicated that dried products may be more beneficial than fresh forage, particularly with animals that do not like to graze SL (beef cattle, horses, sheep).

SL, fed as a ground meal, was shown to be effective in reducing FEC and to a lesser extent, worm numbers, in sheep. Therefore, SL could be useful in reducing pasture contamination from *H. contortus* over time, but probably should not be used as the sole strategy for controlling infection in the short term. However, because worms are resistant to most anthelmintics, it is important to develop control programs which may include CT forages and their dried products in combination with other alternative methods. The selection of such forages should consider the chemical structure of CT in the plant, its concentration, and activity.

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