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Use of Condensed Tannins Supplementation and Inorganic Copper as Deworming Agents in Naturally Infected Ewes and Lambs

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USE OF CONDENSED TANNIN SUPPLEMENTATION AND INORGANIC COPPER AS DEWORMING AGENTS IN NATURALLY INFECTED EWES AND LAMBS

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"You don't know how much I walked to get here

Traveled millions of miles before I went to sleep, I didn't even doze off

The most beautiful mountains I climbed and at dark cold nights I cried

Life teaches and time brings the tone to bare a song

And with daily faith I found a solution"

Tony Garrido

DEDICATION

To God, as it is written…

"…for without Me you can do nothing." (John 15:5)

To my father Ernandes and my mother Tania, whose teachings are my greatest strength and richest heritage

To my sister Mineia and my brothers Murilo and Maurilio, whose hands were always there holding and supporting me

To my aunts Eldiva and Edilva, for all the support and encouragement,

All my love.

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"For the things we have to learn before we can do them, we learn by doing them"

Aristotle

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ABSTRACT

Sheep production is an important socioeconomic activity for the small producers and it can represent their primary income source. Gastrointestinal parasites have become the principal limiting factor for this industry around the world, especially because of the anthelmintic resistance phenomenon that has been increasing worldwide. The negligent use of anthelmintics has been reported as the main factor in the development of resistance. Among the parasites that infect sheep, *H. contortus* is considered the most harmful and also the parasite responsible for the fastest development of nematode resistance in small ruminants. Due to the economic impact this parasite may bring upon producers, the search for alternative methods of control has become a necessity. The use of condensed tannin containing forages and copper oxide wired particles have been shown to produce promising results. Studies have shown that both of these control methods can reduce fecal egg counts, worm fecundity, egg hatchability and larvae development of H. contortus. The general objective of this research was to evaluate the effect of inorganic copper and condensed tannins on fecal egg counts of naturally infected animals and consisted of three trials. No significant differences were observed in the trials using condensed tannins as the main effect in parasite control ($p>0.05$). It was possible to observe that the number of *H. contortus* larvae decreased in the treated group, but the differences were not significant ($p>0.05$). A significant difference in fecal egg counts in the inorganic copper trial was observed ($p<0.05$). The copper oxide group yielded the greater reduction throughout the study ($p<0.001$). Copper sulfate was able to reduce fecal egg counts but there was no difference between this group and the control group, which did not receive any treatment ($p>0.05$). The use of condensed tannins did not show reductions in parasite load, but its use should not be discarded especially in areas where the use of anthelmintics is no longer possible. Inorganic copper has been shown to reduce GIN infection and its concomitant use with other control methods may represent a useful tool in controlling parasites.

CHAPTER 1

INTRODUCTION

Sheep production is an important socioeconomic activity, especially for small producers, whose primary income source comes from the commercialization of the meat, milk and skin of those animals (Pinheiro et al., 2000). In 2005, the sheep and lamb inventory in the United States comprised a total of 7.80 million head. Sheep, goats and their products represented three percent of total sales corresponding to 893 million dollars. However, a total of 600,300 head were reported lost during the same time frame. Approximately 37.3% of the losses were by predator causes and 62.7% by non-predator causes, where digestive problems accounted for 17.1% (NASS, 2005). Amongst the digestive causes of losses in sheep production, gastrointestinal parasites have become the principal limiting factor for this industry around the world. Such parasites are considered the main concern of small ruminant production and causes significant economic losses (Chiebao et al, 2006; Quadros and Vielmo, 2004).

The control of gastrointestinal nematodes is largely based on the use of prophylactic and therapeutic anthelmintics (Charles et al., 1989). The common anthelmintics used for nematode control are levamisole, ivermectin, albendazole and closantel, however ivermectin is the most commonly used (Matos and Bastos, 2000). Although such drugs are still the principal tool for parasite control, their use has not sustained the desired effect. Over the years, the attempt to control losses experienced due to gastrointestinal parasites has led to the indiscriminate use of multiple treatments at greater frequency and intensity. As a result, the development of new helminth strains, that are resistant to a variety of the principal components of the previously effective drugs, have been observed worldwide (Rosalinsk-Moraes et al., 2007; Sangster et al., 2001). Negligence in the appropriate use of anthelmintics has been reported by the National Animal Health Monitoring Survey as the most common reason anthelmintic failure. Only 16.9% and 10.3% used such drugs because worms were seen, or a necessity was indicated by fecal

tests results, respectively (USDA, 2001). The same survey also detected that the most commonly used anthelmintics were oral ivermectin, abendazole, fenbendazole and injectable ivermectin. These observations can explain how resistance has developed so fast.

There are many parasites that can infect sheep. However the ones considered most harmful are *Haemonchus*, *Trichostrongylus*, *Cooperia* and *Oesophagostomum* (Waller, 2006). *Haemonchus contortus* is a major cause of illness as well as the parasite responsible for the fastest development of anthelmintic resistance in small ruminants (Sangster et al., 2001). In some countries, such as South Africa, resistance due to *H. contortus* has made sheep commercialization difficult (Van Wyk, 1999). The pathogenesis of *H. contortus* in sheep, also known as haemonchosis, consists of an acute hemorrhagic anemia because of the hematophagous behavior of this parasite. Clinical symptoms include weight loss and anemia. Highly infected animals can also demonstrate bottle jaw (pendulant edema under the chin), which is a classical sign of haemonchosis (Urquhart et al, 1996).

Because of the economic impact of *H. contortus* on production systems, the search for alternative control methods has become relentless. Among alternative methods being evaluated are pasture management, FAMACHA, genetic selection of resistant animals, biological control (nematode-trapping fungus), medicinal plants (condensed tannins) and inorganic copper. The control of nematode infection is a necessity; otherwise the production system becomes economically unfeasible because of lower productivity, high animal mortality and the cost for workers and drugs to treat the animals (Amarante, 2004).

In spite of the variety of alternative methods to control gastrointestinal parasites, the present research focused on the use of inorganic copper and condensed tannin (CT), more specifically sericea lespedeza (SL) as the source of tannin, for the control of *H. contortus* in sheep. Copper is an essential trace mineral and is also very important in physiological processes. Copper oxide wire particles (COWP) consist of a central nuclei of pure copper

covered with a mix of cupric and cuprous copper. After oral administration, COWP capsules are dissolved in the rumen and the copper particles pass into the abomasum and adhere to the mucosa. Because of the acidity of the environment, copper is released over a long period of time (Nyman, 2000). The copper is suspected to have a direct effect on the cuticle of the worm and results in expulsion. Studies have shown that the use of up to 4 g of COWP per animal does not cause toxicity in sheep within an extensive pasture management system and is also helpful in the control of *H. contortus*, protecting against re-infections for up to four weeks (Gonçalves and Echevaria, 2004).

Copper sulfate ($CuSO_4$) is used in a variety of ways in small ruminant production. It can be used either as a supplement added in the feed/water or as a topical application in footbaths. CuSO4 is more soluble than COWP (Ledoux *et al.*, 1995). Due to its solubility, the protection against gastrointestinal nematodes may not last as long as COWP. Solaiman et al. (2007) found that $CuSO_4$ improved average daily gain and immune function and its use should be considered in animal supplementation. But regarding parasites, the use of dietary $CuSO_4$ has shown no beneficial effect in goats (Burke & Miller, 2008).

SL has been shown to reduce nematode infection in goats and sheep, however its mechanism of action is still not known (Coffey et al., 2007). The primary effect is on reducing fecundity of female worms and secondarily on survival and development of larvae in feces. This secondary effect may be due to prevention of bacterial growth (food source for larvae) or inhibition of larvae growth and mobilization due to binding of the tannin (Min et al, 2005). SL was chosen for this study, primarily because of its adaptability characteristics and because of the current positive evidence of effect reported in the literature and concurrent research.

The general objective of this research was to evaluate the effect of copper and condensed tannin on natural gastrointestinal nematode infection in sheep .This project

consisted of three experimental trials. The first trial evaluated the effect of SL pellet supplementation and COWP on the periparturient rise in ewe fecal egg count. The second trial evaluated the effect of SL pellet supplementation on open ewe fecal egg count during summer grazing. The third trial compared the use of COWP and $CuSO_4$ as deworming agents and toxicity in lambs.

CHAPTER 2

LITERATURE REVIEW

2.1. The Entrepreneur of Sheep Production

Sheep are considered one of the earliest animals to be domesticated for agricultural purposes with their domestication dates back 10,000 years to Central Asia. They are raised to produce fleece, meat and milk and their importance to nomads and the agricultural life of Hebrews dates back to 3,500 B.C. in the Old Testament, sheep production was referred to as being well established (Schoenian, 2010). Sheep production is man's oldest organized industry. Wool was the first commodity of sufficient value to warrant international trade. Sheep production systems are practiced throughout the world and fundamental to a variety of cultures. Their wide distribution is due to their capacity to adapt to a considerable range of climate conditions. However, sheep global distribution is not solely a product of physiological adaptation, but also consequence of historical and socioeconomic factors (Cunningham, 1999). Production of sheep is an important socioeconomic activity, especially for family agriculture production systems, whose producers primary income source comes from the commercialization of the meat, milk and skin of those animals (Pinheiro et al.., 2000).

In the United States, the sheep industry is no different and also assumes significant importance for small producers, although most of their products are limited to ethnic markets. In 2005, sheep and lamb inventory in this country totaled 7.80 million head. Sheep, goats and their products represented three percent of total sales corresponding to 893 million dollars. However, a total of 600,300 head were reported lost in the sheep industry during that same time frame. Approximately 37.3% of the losses were by predator causes and 62.7% by non-predator causes. Out of the non-predator cause, digestive problems accounted for 17.1% of the losses (NASS, 2005). Amongst the digestive causes of loss, gastrointestinal nematodes have become the principal limiting factor for this industry around the world. Nematodes are considered the

main worrisome problem of small ruminant production and the cause of significant economic losses (Chiebao et al., 2006; Quadros and Vielmo, 2004).

2.2. Ecology of Gastrointestinal Nematodes

In the environment, living beings are intimately related, establishing amongst themselves relationships in which the goal is to achieve a better life condition, such as food and shelter. Those relationships can occur between individuals of the same or different species, incurring mutual benefits or advantages for one species and disadvantages for the other. In the latter case, the relationship is denominated by parasitism. Parasitism is one of the most common relationships among eukaryotes and is defined as a unilateral association between two species: host and parasite, the latter one living at the expense of the former (Fortes, 1987; Georgi 1982; Poulin and Morand, 2004). The move towards parasite control emphasizes the need for more effective use of the existing knowledge of gastrointestinal nematode (GIN) ecology, so strategic control measures can be utilized at specific times to interrupt the parasite's life cycle.

Nematodes are considered the most abundant animals on earth. These organisms can be found in a variety of environments such as salt water, fresh water, soil, plants, insects as well as animals. The great majority of nematodes are inconspicuous and cylindrical in shape. There are some free-living species, while others are specific parasites that depend on other organisms for survival (Levine, 1968; Meglitsch and Schram, 1991). These organisms are usually bilaterally symmetrical, elongated with both extremities tapered. They have a digestive system that includes mouth and anus and their body is covered by a cuticle with only one layer of muscle. The majority of nematodes manifest sexual dimorphism with the female being usually larger than the male and oviparous. Regarding the digestive habits, such organisms feed extravagantly and wastefully. A typical nematode life cycle includes egg, 4 larval stages and adult. The third larval stage is usually the infective stage (Levine, 1968; Urquhart et al., 1996).

The nematodes of major importance for sheep production are *Haemonchus contortus*, *Teladorsargia circumcincta* and *Trichostrongylus* spp. because they are the most profuse and cause severe losses in production. *H. contortus*, is the most important species in regions with summer-dominant rainfall or tropical and subtropical areas such as the southeastern region of United States, where warm, moist pasture conditions are conducive to growth and survival of its larval stages (O'Connor et al., 2006).

Nematodes are not the only organisms present on the ground. Mites, insects, bacteria, fungi, plants and free-living nematodes together form an ecological complex where they benefit from each other and act upon the same climatic factors (Levine, 1968). Abundant rainfall and higher temperatures are climatic conditions more favorable for *H. contortus* development. The minimal mean temperature for eggs hatch is 18ºC. Below this temperature, larvae will not develop. Eggs will not survive at a mean temperature of 21ºC for more than 20 days. However, if an infected animal defecates in humid places with temperatures above 18ºC, eggs will eclode normally in 14 to 20 hours. The ideal temperature for hatching is between 20ºC to 30ºC degrees (Dinaburg, 1944). Oxygen is also important for egg hatchability. Eggs present on the surface of fecal matter tend to hatch more easily than those in depth (Reinecke, 1989). For larvae, moisture is the essential element influencing development. Under favorable humidity conditions, low luminosity and ideal temperature, *H. contortus* larvae actively migrate to the grass to be ingested by a suitable host. Major migration occurs in the mornings, which is also when massive infestation of hosts happens due to grazing behavior. Although migration patterns occur randomly, they are influenced by moisture and temperature. Larvae will die under dry conditions, but are able to survive for months in a damp environment (Corrêa, 1973; Reinecke, 1989).

Knowing the ecology of parasites is of great importance in understanding development, survival and transmission. Although ecologic measurements are more readily available nowadays, it is still not a simple task to methodically identify the ideal microenvironment needed

to establish infection. Knowledge of parasite ecology may be a critical factor in designing control programs or applying drug treatments against gastrointestinal nematodes (GIN).

2.3. The Disease and Its Characteristics

Haemonchus contortus, also known as barber pole worm, is the larger stomach worm found in the abomasum of ruminants. This parasite belongs to the Family Trichostrongylidae and has been reported worldwide, particularly being a problem in areas with a hot/moist climate. Sheep, especially lambs, are more consistently susceptible to the adverse effects of worms than mature animals, and clinical disease is more common with that species as well (MERCK, 2010).

Parasites of the genus *Haemonchus* are bloodsuckers. A fully developed adult is able to feed from capillaries in the abomasal mucosa for approximately 12 minutes at a time. When this parasite moves to another area, it is estimated that bleeding of the previous attachment area continues for about 6 minutes. The disease caused by this parasite, known as haemonchosis, is characterized by anemia due to hemorrhage originating from the feeding behavior (Dunn, 1978). A single worm is responsible for the removal of approximately 0.05 ml of blood a day. This parasite also utilizes calcium, phosphorus, cobalt, copper and vitamins for nutrients and the mobilization of iron and proteins for compensatory erythropoiesis due to anemia may lead to animal death (Reinecke, 1989; Urquhart et al., 1996).

Three clinical syndromes occur, hyper acute, acute and chronic. The acute and chronic syndromes are the most understood and most important. The acute form is associated with blood loss and the chronic form, occurring during dry season, is associated with weight loss (Dunn, 1978; Urquhart et al., 1996). Clinical signs vary from dark feces to weight loss, lethargy, anorexia, anemia, edema and death. Bottle jaw is the classical clinical sign for haemonchosis and is characterized by a submandibular edema due to hypo-proteinemia (Georgi, 1982).

Anemia can be observed around two weeks after infection and is characterized by a sudden drop in blood packed cell volume (Corrêa, 1973; Georgi, 1982; Reinecke, 1989).

The females of *H.contortus* are very prolific and able to deposit around 5,000 to 10,000 eggs per day that will be released into the environment through sheep feces, thus contaminating the pasture and propitiating the life cycle for this parasite (Urquhart et al., 2007). The prepatent period of *H. contortus* is two to three weeks and the presence of at least 500 adults in the host is enough for the animal to start showing symptoms of haemonchosis (Nemeseri and Hollo, 1961). As for infection, a fecal egg count of 10,000 eggs per gram of feces is indicative of trichostrongyle infection, which concurrently with lower packed cell volumes, characterizes the pathogeny by *H. contortus* (Mehlhorn, 2008).

Regulation of parasite populations is very complex and breed, age, stress, immunological and nutritional status of sheep are influencing factors in susceptibility to *H. contortus* infection. The same way a parasitic infection influences the nutritional status of the host, nutrition can also influence parasite establishment, already established infections and immune response. Animals that are well-fed can endure infection better than animals on a restrained diet and parasites are capable of interfering with the ability of the host in utilizing nutrients efficiently (Coop and Holmes, 1996; Coop and Kyriazakis, 2001; Knox et al., 2003). Host resistance to infection caused by *H. contortus* can be controlled genetically or by predominantly relying on immunity. Regarding immune response, a substantial degree of protective immunity may be produced against *H. contortus* infections. However, immunity against parasites in sheep is slowly built and is generally incomplete (Soulsby, 1982). Miller and Horohov (2006) cited immune exclusion and “self-cure” phenomenon as well recognized host responses. Immune exclusion is characterized by the failure of ingested larvae to establish infection in heavily infected animals (Miller et al., 1983; Jackson et al., 1988; Newlands et al., 1990). Self-cure is characterized by the expulsion of adult parasites after a massive ingestion of

larvae in a very short period of exposure (Stewart, 1950; 1955). The self-cure phenomenon occurs sporadically. Usually the reduction of fecal egg counts (FEC) follows periods of heavy rains, which leads to the ingestion by sheep of large numbers of infective larvae in a short period of time and consequently the expulsion of adult worms. Decrease in fecundity of parasites without loss from the host has also been reported (Adams, 1983; Gordon, 1967).

Physiological stress such as pregnancy, lambing and lactation can also reduce or affect immunity. Such stress allows the immunity of ewes to be overcome usually two weeks before lambing until approximately four weeks after parturition (Fleming, 2005). This temporary relaxation in resistance against gastrointestinal nematodes close to parturition and during lactation has been extensively reported in ewes. This phenomenon is referred to as periparturient rise, also known as periparturient relaxation of resistance. It is characterized by an increase of egg output in feces usually occurring when susceptible hosts are available and climatic conditions are favorable for larvae development. The periparturient rise in fecal egg counts is usually observed a few weeks before lambing with the peak being at the sixth week after parturition. It is believed that the effects of the periparturient rise in FEC can be minimized through protein supplementation. It is important to emphasize that the adult worm population established during this period promotes the exposure of lambs to infective larvae and should be considered for prevention and control purposes (Barger, 1993; Fleming, 2005; Proctor and Gibbs, 1968; Reinecke, 1989). Ng'ang'a et al. (2006), in a trial study found that the periparturient rise contributed to high pasture contamination in Kenya and was a major health problem and a constraint to improved sheep production in that region.

It is essential to understand that the biology of this parasite is of great concern in sheep production due to the economic losses it causes. For decades the control of infection has been solely based on the prophylactic and therapeutic use of chemicals known as anthelmintics (Charles et al., 1989). However, resistance to such chemicals has been reported worldwide, so

economic loss and resistance to drugs are of concern for both sheep producers and researchers.

2.4. Control Strategies

Control of parasite infection is a significant segment of a flock's health program. Control measures generally rely on the use of anthelmintic drugs aimed to minimize production losses. However, the erradication of worm population is not an easy or even possible task and keeping the infection low is the main goal in control strategies. To achieve effective control of parasite infections, integrated control approches should be considered (Williams and Loyacano, 2001). As a general strategy, the goals of parasite control must include prevention of heavy exposure of susceptible hosts, reduction of pasture contamination, minimization of the effects of parasite burdens on host and encouragement of the development of immunity when possible (Merck, 2010). A well planned program will take into consideration the minimal usage of chemicals and such a decision results in decreasing production costs due to use of dewormers, decreasing parasite resistance due to indiscriminate use of such drugs, and decreasing production losses due to parasitism since it takes into consideration not only prevention of parasite infection, but also treatment (Whittier et al., 2009).

Traditional control measures rely on the use of chemicals, also known as anthelmintics, but due to increased resistance to these chemicals, the use of alternative or complementary methods to limit parasitism have been constantly sought and the current supportive strategies can be summarized as:

1. Use of non-conventional anthelmintics, such as copper oxide wire particles, phytotherapy and nutraceuticals;

2. Minimizing the contact between susceptible hosts and the infective stage of the parasite, which could be achieved by grazing management to reduce larval density, increase larval death rate or accelerate larval mortality;

3. Improving host response against parasites or infections, this can be achieved through vaccination, genetic selection or nutritional manipulation (Torres-Acosta and Hoste, 2008).

2.4.1. Use of Anthelmintics and Anthelmintic Resistance

Anthelmintics, popularly known as vermicides or vermifuges, are drugs designed to expell helminths from the host body by either killing or stunning them, acting strictly on their biochemical processes. They can be used in a therapeutical or prophylaxis approach (Charles et al., 1989; Taylor et al., 2007; Urquhart et al., 1996). Anthelmintics are indeed the largest division of the animal pharmaceutical industry representing 28.5% of marketed products, which generates the equivalent of 5.3 billion dollars per year in profit (IFAH, 2010). The cost expended for treatment and prevention is a massive burden on animal production.

Veterinarians have used either metal or plant extracts as anthelmintic agents, which mechanically irritated the parasite or its site of predilection. Compounds such as arsenious acid, tartar emetic, benzine and kousso, were also used as anthelmintics, but those compounds were reported to cause more damaged to the animals than providing a cure for helminth infections due to their toxicity (Blane, 1826; Neumann, 1982). The major advances in veterinary anthelmintics were made between 1960 and 1980, with the discovery of thiabendazole and levamisole; and in 1981, a further step forward was taken with the addition of ivermectin to veterinary chemoterapy (Drudge, 1964; Neumann, 1982; Urquhart, 2007). Those drugs had an outstanding spectrum of activity and safety and are still in use today. Such discoveries led to research into mechanisms of action, but as soon as they were discovered and understood, resistance was also being reported (McKellar and Jackson, 2004).

There are several classes of broad spectrum and narrow spectrum anthelmintics: benzimidazoles and probenzimidazoles; imidazothiazoles and tetrahydropyrimidines; avermectins and milbemycins; salicylanilides/substituted phenols and organophosphates, that

have been approved and used in sheep (Taylor et al., 2007; Whittier et al., 2009). In general, the benzimidazoles and probenzimidazoles groups have a similar mode of action that inhibits glucose uptake, protein secretion and microtubule production leading to the parasite's death from starvation. Imidazothiazoles as well as tetrahydropyrimidines act causing a rapid, although reversible, spastic paralysis, thus expelling the worms through normal gut peristalsis. Avermectins and milbemycins are believed to block interneuronal stimulations of excitatory motor neurons leading to a flaccid paralysis. Salicylanilides and substituted phenols interfere with oxidative phopsphorylation decreasing the energy available to the parasites. Organophosphates inhibit cholinesterase, which will cause an accumulation of acetylcholine and consequently neuromuscular paralysis of parasites and their expulsion out of the host's body.

In 2009, the pharmaceutical company Novartis launched the first product of a new class of anthelmintics in almost 30 years. The commercial name of the product is Zolvix® (monepantel), and belongs to the anthelmintic class called the amino-acetonitrile derivatives (AADs). The mode of action consists of paralyzing worms by attacking a previously undiscovered receptor, HCO-MPTL-1, present only in nematodes. This product has been shown to be effective against GIN in sheep; however, this product is only available to sheep producers in New Zealand, Uruguay, and the United Kingdom (Schoenian, 2010).

Although anthelmintics are still the major nematode control measure, the use of those drugs has not delivered the desirable effect and anthelmintic resistance has already been established in many regions of the world (Williams, 1997). Resistance to anthelmintics has been associated with repeated use as an attempt to control losses leading sheep producers to use multiple and more often treatments. Factors related to negligent management, such as use of expired drugs or equipment that would not deliver the adequate dosage; incorrect dose level or inaccurate animal weight measurement could be considered as contributory causes of failure in controlling infections. These factors consequently led to higher frequency and intensity in the use of anthelmintics, which over years has resulted in the development of new helminth strains

that are resistant to a variety of the principal components of the previously effective drugs (Rosalinsk-Moraes et al., 2007; Sangster et al., 2001; Torres-Acosta and Hoste, 2008).

Since first being reported, anthelmintic resistance has progressively become a crisis in some sectors of livestock production, especially regarding small ruminant production in tropical and subtropical areas, where in some cases resistance to all anthelmintic groups has been reported (Rodrigues, 2006; Waller, 1997). *H. contortus* is considered the parasite responsible for fast development of resistance in small ruminants (Coles, 2005; Otero and Hidalgo, 2004; Sangster et al., 2001). This parasite has a great genetic variability, with a mitochondrial DNA mutation rate ten times greater than vertebrates and nucleic DNA that is extremely diverse (Melo and Bevilacqua, 2005). In some countries, such as South Africa, Australia and New Zealand, resistance of *H. contortus* has led to depopulation of sheep farms due to the scarcity of alternative control measures and low productivity of the flocks, making sheep commercialization sometimes impractical (Van Wyk, 1999).

H. contortus has been reported to be resistant to benzimidazoles (Le Jambre, 1979) and ivermectins (Conder and Campbell, 1995). Resistance to levamisol has not been reported as much and when it did occur it was observed that resistance occurred slowly (Sangster and Gill, 1999). The development of resistance can occur fast as observed with the introduction of ivermectin in South Africa. Resistance to that drug was reported five years later (Shoop, 1993). Resistance is more often observed in tropical and sub-tropical regions and even resistance to multiple chemical groups have been reported in some countries of South America (Waller, 1997). In most cases, anthelmintic resistance is determined by empirical observation of low drug efficacy. However a great hindrance found in testing anthelmintic resistance, is the absence of more sensible methods for resistance quantification (Molento, 2005). To evaluate anthelmintic efficacy, a comparison of the reduction in the number of eggs per gram of feces of a group of

animals treated with an anthelmintic agent with an untreated group (control), can be performed using the formula proposed by Coles et al. (1992):

$$\%\, Efficacy = \frac{mean\ EPG\ control\ group - mean\ EPG\ treated\ group}{mean\ EPG\ control\ group} * 100$$

Although the result of this test may be inconsistent, it is useful to indirectly determine if resistance is present when the efficacy of a specific compound is below 95% (Coles et al., 1992).

Combination of different anthelmintics is one way to delay the emergence of resistant parasites, but only when drugs with different mechanisms of action are combined together (Barnes et al., 1995). High costs, regulations and risk in marketing a new product have become barriers for the development of new anthelmintic drugs and although no chemical entities with novel activity have been marketed since 1981 in the United States, a continued research interest in this area has continued, especially concerning integrated methods for parasite control or innovative ideas such as vaccines or immune modulators (Mckellar and Jackson, 2004). Although Novartis has launched the first product of a new class of anthelmintics, the lack of research about this product and the limitation on its use to a few countries has not made it possible to cite it as a widespread approach to parasite control.

It is important to note that the search for alternative methods of parasite control is a necessity; otherwise, the production system becomes economically unfeasible because of lower productivity, animal mortality and higher costs with labor and drugs to treat animals (Amarante, 2004).

2.4.2. Integrated and Alternative Methods of Parasite Control

Integrated control is defined by the use of a combination of chemical and non-chemical methods of parasite control with the goal of maintaining acceptable levels of production without total elimination of the parasites. The main use of this method is to retard the increase of

parasite populations with a higher number of individuals genetically resistant to anthelmintics (Nari and Eddi, 2002; Vieira, 2003). This can be achieved through strategic use of drugs. For instance, drenching animals at specific times of the year will remove worm burdens when larval populations on pasture are low, thus decreasing the need for frequent drenching. This strategy combined with a low stocking rate and alternative methods for parasite control such as pasture management, FAMACHA® chart, biological control, genetic selection of resistant animals, vaccination and nutritional manipulation, use of non-conventional anthelmintics and fitotherapy/homeopathy may be helpful in reducing the chemical dependence for treating parasites, subsequently reducing anthelmintic resistance development and maintaining animal performance (Besier, 2003; Torres-Acosta and Hoste, 2008; Vieira, 2003).

2.4.2.1. Pasture Management

Forage is considered the cheapest and most viable nutrition alternative in small ruminant production and for that reason the majority of production systems keep their flocks on extensive pasture grazing. When parasite larvae hatch from eggs passed out in feces, they crawl up to the grass blades. While ingesting forage, animals also ingest infective larvae and parasitic infection becomes established. It is estimated that approximately 95% of the worms are on the pasture as eggs or larvae, so the availability of infective larvae on pasture is the key to understanding the complexity and severity of infection (Gastaldi, 1999; Quadros and Vieira, 2003).

Pasture management has been reported as a primary tool in parasite control and the number of larvae ingested by animals can be somewhat controlled through pasture management (Hale, 2006). Epidemiologic studies with helminthes have shown that anthelmintic drugs don't always have the expected efficacy when the animals remain on contaminated pastures, reinforcing the importance of pasture for the parasite cycle maintenance (Amarante, 2004). Forages with a high percentage of soil coverage can contribute to the formation of a humid micro-climate, which favors the development of infective larvae. In forages with a

structure that make sun ray penetration to the base of the plant possible, the number of infective larvae would probably be reduced (Santos et al., 2002). Stocking rate and forage utilization percentage directly influence pasture contamination (Gordon, 2000). Alternatives such as pasture rotation, tilling soil, alternate grazing with other animal species and application of chemicals on the soil can contribute to the reduction of the contamination of the pasture (Costa, 2003; Vieira, 2003).

A pasture is considered safe when parasite burdens of susceptible animals increase slowly, but it is not necessarily free of parasites. If a pasture has been harvested for hay, silage, small grain crops or if it has been grazed by other species for long periods, it can be considered safe and it usually takes a year or more for a pasture without animals grazing on it to become safe. In the case of a safe pasture, sheep should only be moved into the pasture after treating those with high infections to allow removal of harmful burdens as well as to protect the safe pasture from substantial contamination (Whittier et al., 2009). Keeping the length of forage longer than 3 to 4 inches is also a helpful way to prevent ingestion of infective larvae, since they sporadically migrate higher than that (Hale, 2006).

2.4.2.2. FAMACHA© Chart

The FAMACHA© system was developed in South Africa to categorize anemia status of small ruminants (Figure 1). This method identifies anemic animals in a scale ranging from one (red – optimal eye color) to five (white – pale eye color) by the examination of the lower inner-eyelid mucosa (Van Wyk et al., 1997; Malan et al., 2001). Several studies have been done in regions where *H. contortus* is the predominant parasite, and due to its blood feeding behavior, detection of anemia in those animals was possible using the FAMACHA© system, which seems to be a good indicator of haemonchosis (Ejlertsen et al., 2006; Molento et al., 2004; Reinecke et al., 2009). Based on FAMACHA score, only anemic (score of 4/5 and sometimes 3) animals receive treatment. That way, it is possible to reduce the use of deworming drugs and delay the

development of resistant parasites (Hale, 2006; Molento et al., 2004). Although some studies have shown high correlations between FAMACHA© scores and packed cell volumes (PCV), it is necessary to consider genetic differences between sheep breeds, especially in regions where the predominant trichostrongyle is not *H. contortus*, as well as different causes of anemia being present (Sissay et al., 2007; Moors and Gauly, 2009).

The FAMACHA© system is useful when combined with alternative methods to control *H. contortus*, helping the decision-making process with regards to treatment of animals, since only the sick animals get treated. It also helps to lower the costs of production, reduces the frequency of drug use, volume of chemicals that are released into the environment, and delays anthelmintic resistance (Molento et al., 2009; Vieira, 2005). The FAMACHA© system is very applicable in field conditions and can easily be performed by farmers. The system has been shown to be reliable even with consistent misclassification by operators. However, animals should be examined on a weekly basis, especially during higher worm challenge periods, and the labor required may represent a constraint (Reinecke et al., 2009; Reinecke et al., 2011). Riley & Van Wyk (2009), evaluated the genetic correlation of FAMACHA© with performance parameters and different worm challenge levels and found that it could be feasible and less expensive, especially for small producers, to use this system for genetic selection of animals resistant or resilient to worm infection. Selection and breeding of healthier animals leads to a stronger flock over time. In general, and for *H. contortus* infection more specifically, it can be inferred that a simple check of the eye mucosa using FAMACHA© could help prevent heavy infections and can safely be used as a tool for integrated treatment schemes (Scheuerle et al., 2010).

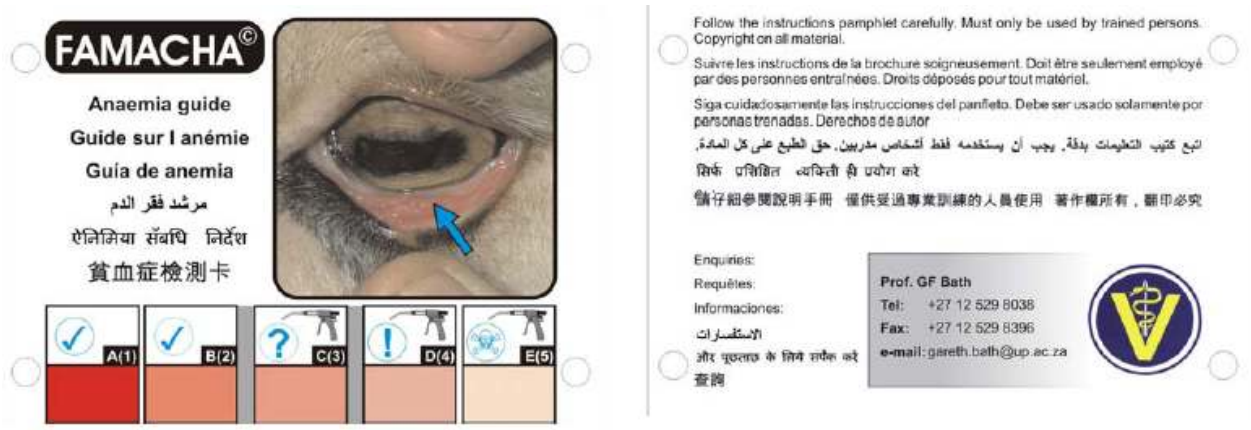

Figure 1. FAMACHA© anemia guide reduced size. Developed by Dr. Faffa Malan and distributed under the auspices of The South African Veterinary Association.

2.4.2.3. Biological Control

Biological control consists of the use of nematophagous fungus, whose spores are capable of surviving gut passage. Spores are deposited in fecal matter and germinate forming sticky loops which tray infective larvae stages are interrupt the life cycle. The main goal is to reduce the parasitic population on pasture without interfering with animal performance or welfare. The fungus used in biological control can be either predacious, trapping infective larvae, or endoparasitic, germinating spores when ingested by larvae (Jackson and Miller, 2006). Currently, the majority of work being carried out on biological control of parasites is with the nematode-trapping fungus *Duddingtonia flagrans* (Waller and Thamsborg, 2004). *D. flagrans* spores not only has the ability of surviving gut passage of livestock, but it also has the propensity to grow rapidly in freshly deposited dung and has a voracious nematophagous capacity (Larsen, 1999). Its mechanism of action consists of breaking the parasite's life cycle by capturing infective larvae before they migrate from dung to pasture, where they would then be acquired by grazing animals. Studies have shown that there is no adverse effect in the environment when using this fungus (Faedo et al., 2002). Although most of the studies on biological control have been done using *D. flagrans*, there are some species such as

Arthrobotrys spp. being successfully used and others with very close genetic similarities that have achieved similar results in different parts of the world (Melo et al., 2003).

D. flagrans has been shown to be efficient in reducing the number of infective larvae in the feces of sheep when given regularly and its continuous administration has provided an extended period of suppressed development of larvae (Chandrawathani et al., 2004; Larsen et al., 1998; Pena et al., 2002). Equivalent results were obtained when mature non-pregnant ewes were offered *D. flagrans* with supplement feed (Fontenot et al., 2003). However, implications on how to provide controlled elimination of fungus in feces for prolonged periods or the minimum required period to provide an effective parasitic control, have become an obstacle for disseminated use of biological control especially because of the lack of a commercial source of the fungus on farms where the production system is extensive. So far, none of the prototype devices available for delivery of fungus to animals have been made available for commercialization (Larsen et al., 1998; Jackson and Miller, 2006; Waller and Thamsborg, 2004).

Biological control should be perceived as a long term strategy since the fungus only preys on larvae and consequently the adult nematode population remains in the host. The use of other simultaneous control measures should be considered and have been proven to be safe and efficient (Burke et al., 2005; Jackson and Miller, 2006).

2.4.2.4. Genetic Selection of Resistant Animals

Genetic selection of resistant animals can be based on specific sheep breeds that are naturally resistant to parasitic infection or based on the selection of resistant individuals in the flock (Hale, 2006). In resistant animals, the interaction between host and parasite results in the worm's elimination. Breeding animals for parasite resistance has the advantage of lowering costs and can be a permanent solution to parasite issues. There are many breeds of sheep known to be considerably resistant to *H. contortus* infection such as Gulf Coast Native

(Amarante et al., 1999; Miller et al., 1998) and Saint Croix (Courtney et al., 1985; Gamble and Zajac, 1992). However, animals that appear to be highly resistant generally are usually not the most productive animals (Stear and Murray, 1994; Waller and Thamsborg, 2004).

Despite the benefits that genetic selection presents, factors such as long intervals of generations, an inverse relation of resistance/productive performance and the uncertainty of the evolution of parasites in relation to changes in the host (adaptation) represent barriers to the use of this method. It should be noted that there is no evidence that parasites are capable of adapting genetically to sheep selected for resistance (Waller and Thamsborg, 2004; Woolaston et al., 1992). Fecal egg count is the common way used to evaluate if an animal is resistant to parasitic infection (Dominik, 2005); however, when selecting animals for parasite resistance, breeding programs should consider that desired traits may differ according to local needs and the selection should be towards animals resistant to the effects of infection instead (Bisset and Morris, 1996).

2.4.2.5. Vaccination

Vaccination for infectious diseases is the most successful intervention method in veterinary medicine and its use has improved animal production more than any other practice as can be observed with the elimination of hog cholera (Baker et al., 1964) and brucellosis (Ragan, 2002), which could not have been possible without the use of vaccines. An ideal vaccine has to be able to induce a positive response with no harm to the individual (Molento and Pondelek, 2004).

The search for a vaccine against *H. contortus* has been continuous for over 30 years, diverging from oral vaccines to injection of irradiated larvae or proteins. Much has been learned about immunization and host immune response to GIN infection, but limited success using the vaccination approach has been achieved so far. Factors such as material availability and vaccine stability have precluded their large scale use. However, the benefits of using a vaccine

are that the protection would certainly last longer, only the vaccinated animal would be affected, and the problems of toxicity and persistence of residues would be minimized or avoided.

Some of the work done towards the search for a vaccine has been dedicated to the study of some antigens such as gut proteins (Jasmer and McGuire, 1991; Olcott, 2006; Zhao et al., 2011), glycoprotein complex H-gal-GP (Newton, 1995; Olcott, 2006), larvae antigens (Cuenca–Verde et al., 2010) and total or partial excretory/secretory products (Bakker et al., 2004; Schallig et al., 1997) with considerable success. A study done using larvae vesicular concentrate from parasites showed a partial protection against *H. contortus* establishment due to an increase of eosinophils, and other immune response cells related to prevention of worm infections (Cuenca-Verde et al., 2010). While studying the protective properties of thiol-biding fractions of excretory/secretory products of *H. contortus,* Baker et al. (2004) found that other groups of proteins were present in the fractions and could be contributing to the protective effect as well.

To date, the most protective antigen against *H. contortus* identified is an integral protein membrane, H11, from the intestinal wall of this parasite responsible for blood digestion (Yan et al., 2007). James & McGuire (1991) found that gut antigens can induce significant protection against challenge infections. They observed a FEC reduction of 95% and adult worm population reduction of 65%, which represents a substantial decrease in pasture contamination with *H. contortus*. The use of DNA vaccines encoding the same protein antigen with IL-2 as an adjuvant have induced only partial protection against *H. contortus* (Zhao et al., 2011). Olcott (2006), using a H11/H-gal-GP vaccine observed higher IgG levels in vaccinated animals and although vaccination did not prevent the establishment of infection, the development of signs of haemonchosis was slower in vaccinated animals. It was also observed that the vaccine can be administered during the course of an infection to eliminate worm burdens and it can be valuable in diminishing the use of anthelmintics.

In spite of the compound used in the development of a vaccine, it is possible to infer that vaccines against *H. contortus* do induce some level of protection and are beneficial to animals. However, future research is still needed to not only make an efficient and safe vaccine, but also a commercially available product.

2.4.2.6. Nutritional Manipulation

A balanced diet is fundamental not only for physiologic processes, but good nutrition improves the overall health of the animal and enhances immune response to parasites. Consequently, nutritional inadequacies, especially of protein, can reduce the rate of acquisition and development of immunocompetence against parasitic infection (Coop and Holmes, 1996). Therefore, nutritional manipulation can be an alternative to improve resistance and resilience of sheep to GIN infection.

The reversion of pathology as a consequence of parasitism is prioritized over physiological body functions regarding nutrient availability and allocation. So it is expected that improvements in nutrition will point to improved resilience of a host (Coop and Kyriazakis, 1999). GIN infections lead to reduction of voluntary intake and inefficient use of absorbed nutrients. Consequently, nutrient metabolites are reoriented to maintain or compensate tissue and blood homeostasis. These disturbances result in reduced growth efficiency and lower animal performance (Hoste et al., 1997). Adequate nutrition can improve the capacity of an animal to endure a parasitic challenge along with rapid acquisition and expression of immunity and affect worms' population especially if anti-parasitic compounds are present (Coop and Kyriazakis, 1999).

The most significant disturbance due to GIN is in protein metabolism and mineral absorption and retention. The extent of these disturbances is influenced by the size of the larvae challenge and the number of parasite species present (Coop and Kyriazakis, 2001; Parkins and Holmes, 1989; Van Houtert and Sykes, 1996).

Sheep are more likely to benefit from targeted nutritional manipulation than other livestock species, especially lambs as they are undergoing the development of an immune response, and ewes during early lactation, when immunological competence is temporarily suspended (Besier, 2003). Protein-enriched diets have been shown to be beneficial in resistance to GIN and production in sheep (Kahn et al., 2003). Effects of higher levels of protein in the diet have been reported to improve immunity and reduce *T. circumcincta* FEC in lactating ewes (Houdijk et al., 2003). It was also observed that lambs on a high protein diet became more resilient and resistant to *H. contortus* (Bricarello et al., 2005). While supplementing grazing kids with soybean and sorghum meal, Torres-Acosta et al. (2004), found that those animals had increased resilience against helminthes. Hoste et al. (2005) have found that in browsing goats the energy/protein balance is as important as the protein in the diet itself. They also noticed that when fed high protein diet, goats' response to GIN was more evident towards improvement of resilience than host resistance. In a later study, Hoste et al. (2008) inferred that goats seem to invest fewer nutrients towards an immune response against GIN and more nutrients towards resilience in order to face the negative effects of parasitism.

The work done concerning diet manipulation and the effects on parasites has demonstrated its use as a potential tool to assist the control of GIN; however, difficulties in applying such a method under farm conditions and keeping its costs to a minimum remains an obstacle when measuring the extent of nutritional disturbances in animals and adequate adjustment of compensations (Torres-Acosta and Hoste, 2008).

2.4.2.7. Use of Medicinal Plants in Parasite Control

The use of medicinal plants to combat illness has been reported since 3000 years before Christ. In ancient Egypt, manuscripts were found with records of over five hundred medicinal plants such as mint, rosemary, chamomile, wormwood, and aloe vera, which are still used today (Athayde et al., 2004; Athanasiadou et al., 2007). A medicinal plant is defined as a plant that

contains one or more active compounds, which confer therapeutic activity (Assis, 2000). A variety of plants initially utilized in traditional medicine, have been used for parasite control in various areas of the world in people and animals. The utilization of plants as anthelmintic agents has been reported in a variety of studies for different parasites such as *Ascaridia galli* (Akhtar and Riffat, 1985); *Fasciola hepatica* (Julien et al., 1985); *Ascaris suum* (Satrija et al., 1994) and *H. contortus* (Pessoa, 2001). Costa et al. (2002) reported 95.66% inhibition of *H. contortus* egg hatchability in a dose depend manner when using extract from seeds of *Mangifera indica*. Dose dependent ovicidal and larvicidal activity of crude extracts of *Maesa lanceolata* and *Plectranthus punctatus* against *H. contortus* was also observed for both aqueous and hydro-alcoholic extracts (Tadesse et al., 2009). It is believed that the utilization of such plants can cause a slowing or even a delay in the development of anthelmintic resistance with the advantage of normally acting over target species. Plants are biodegradable, causing minimal or no pollution in the environment and drastically reducing the problem with residual drugs (Chagas, 2004).

Mali & Mehta (2008) reviewed some plants that have been claimed to possess anthelmintic activity, reinforcing not only the originality of where major drug components come from, but also how the use of such plants can become a complement or even replacement for therapies currently in use. The majority of evidence about anti-parasitic activity of plants has been traditionally based on incidental observations, despite some screening and evaluation that has been performed to confirm such claims (Athanasiadou et al., 2007; Eguale et al., 2007; Tadesse et al., 2009). Foster et al. (2009), however, found that individual compounds of sesquiterpene lactone from chicory leaf extract differ in anthelmintic activity and the compound lactucin contributed little to inhibition of egg hatchability of *H. contortus*.

Scientific validation of fitotherapy is the first step in the correct utilization of medicinal plants or active compounds, especially for commercial purposes (Costa et al., 2002). Experimental studies designed to verify, validate and quantify anthelmintic activity are based on two distinct methods. The first method consists of subjecting plants or their parts to naturally or

experimentally infected animals and measuring the effects after consumption. The effects of consumption were observed in studies in which the methodologies included offering either fresh; conserved; dried or parts of plants to parasitized animals, such as cassava hay (Bunyeth and Preston, 2006); *Artemisia brevifolia* leaf meal (Iqbal et al., 2004); and fresh consumption, leaf meal or hay of sericea lespedeza (Shaik et al., 2004; Pollard, 2009). The second method is based on the testing of plant extractions using *in vitro* and *in vivo* systems (Athanasiadou et al., 2007). The majority of the studies done used *in vitro* tests, which has been considered the best way of screening for anthelmintic activity of plants (Asase et al., 2005; Foster et al., 2010; Tadesse et al., 2009; Wabo et al., 2011). Factors such as seasonal and environmental variability; the way it may affect anti-parasitic activity; identification of active components; mechanisms of action; and target specie are not yet fully established. Moreover, information about the effect over the efficiency of the plant, when interacting with secondary metabolites and nutrients; host physiology; and action on parasitic site of infection, is still needed (Chagas, 2004; Hoste et al., 2006). Athanasiadou et al. (2007) emphasized the necessity of incorporating additional evaluation methods in ongoing research studies such as immunity indicators and behavior observations, when considering the potentiality of such plants.

2.4.2.7.1. Condensed Tannins (CT)

Plants rich in tannins have been commonly used in traditional medicine for wound treatment, burns, inflammation (Haslam, 1996), control of insects, fungus, bacteria (Aertes et al., 1999) and gastrointestinal parasites (Fleming et al., 2006). Tannins are known for being associated with the natural defense of plants (Brownlee et al., 1990; Mueller-Harvey, 1999). Tannins are secondary phenolic composites with high molecular weight that are frequently found in fruits, trees, forages and corn commonly used in the alimentation of livestock (Butter et al., 1999; Otero and Hidalgo, 2004). Studies have shown that condensed tannins have direct and indirect effects against parasitism once they enhance the animal's overall condition due to

optimization of protein in the diet (Barry and McNabb, 1999). The direct effect of CT consists of the reduction of parasite loads since it acts on the parasite itself. Incrementing digestible protein indirectly affects parasites by boosting the host's immune system consequently improving resilience and resistance against nematodes (Iqbal et al., 2007). Butter et al. (1998) investigated the use of two commercial quebracho extracts on *Nippostrongylus brasiliensis* and *Trichinella spiralis* found that the tannins may reduce nematode infection through a direct toxic effect on the worms.

The consumption of plants with high levels of tannins can interfere in the digestive process affecting eating, growth and availability of nutrients and through this, affects the biology of different worm species. Studies have shown that CT, besides being effective against gastrointestinal parasites, can reduce the risk of bloat, increase the uptake of essential amino acids and proteins and enhance the production of milk and wool (Aerts et al., 1999; Athanasiadou et al., 2001; Min et al., 1999; Niezen et al., 2002; Paolini et al., 2004).

Experimental studies *in vivo* using quebracho extracts, a highly enriched source of CT, confirmed the hypothesis that plants with CT can affect the biology of different worm species (Athanasiadou et al., 2000; Butter et al., 2000). The effectiveness of CT is believed to be related to the reduction of egg elimination and fecundity of female worms. Such effects on *H. contortus* have been observed in both sheep and goat species (Niezen et al., 2002; Paolini et al., 2003). Iqbal et al. (2007) had concluded that anthelmintic activity of CT is beneficial in the reduction of pasture contamination through the reduction of hatching of nematode eggs and egg fecal count in sheep, but their work indicated that the effect of CT seemed to be limited to developing stages of the parasite only.

Cenci et al. (2007), found that sheep fed condensed tannins had a lighter infection and shed fewer eggs. When compared to commercial anthelmintics the effects of CT were not very high. A similar trend was observed by Githiori et al. (2004), when comparing three commercial anthelmintics and seven plant preparations. Ivermectin, levamisol and albendazole were found

to be highly efficient in reducing FEC and worm counts in lambs, but none of the plant preparations (*Hagenia abyssinica*, *Dodonea angustifolia*, *Olea europaea* var. *africana*, *Ananas comsus*, *Anmona squamosa*, *Hildebrandtia sepalosa* and *Azadirachta indica*) resulted in any significant reduction in FEC. Tannin drench was shown to be effective against *H. contortus* in sheep. Fecal egg count of drenched sheep was significantly reduced after 24 hours of the first dose and remained low for 43 days. As for worm burdens, drenched sheep showed a reduction of 87% in *Haemonchus* and 28% in *Oesophagostomun* populations (Max et al., 2004).

Condensed tannins have a higher capacity of interaction with other molecules and feeding animals with those compounds can have negative and positive effects on ruminant production (Butter et al., 1998; Otero and Hidalgo, 2004). Tannins are rarely toxic to animals, but they do act in a concentration-dependent manner to reduce digestibility of hay, which could adversely affect the animal's health (Min et al., 2003). It is believed that the beneficial effects observed after the consumption of CT are due to their protein-binding capacity, conferring protection of dietary protein from rumen degradation thus increasing protein availability in the lower digestive tract. However, the binding capacity has also been considered responsible for causing the adverse effects observed on animal production (Min et al., 2003; Waghorn and McNabb, 2003).

Minho et al. (2005) demonstrated that CT extracts of *Acacia molissima* had a direct effect on sheep experimentally infected with *H. contortus*. In a later experiment, they found that sheep drenched with acacia extracts had lower FEC, larvae and adult worms of *H. contortus* and *T. colubriforms,* but there was a difference in body weight and blood parameters of both drenched and undrenched animals. This may indicate that there is no detrimental effect on nutrition caused by some CT (Minho et al., 2008).

Although numerous studies have been performed to evaluate the anti-parasitic activity of medicinal plants, little is known about their exact mechanism of action. Different studies have

demonstrated the effectiveness of condensed tannins in attaining parasite control. Efficient use of such plants still requires detailed studies regarding active compounds. The ideal dose/concentration also needs to be identified and their actions better understood.

2.4.2.7.2. Utilization of Sericea Lespedeza (SL) as a Source of Condensed Tannins in the Treatment of Gastrointestinal (GIN) Infections

Sericea lespedeza [*Lespedeza cuneata* (Dumont) G. Don.] is a legume native to Asia, recognized for being highly competitive and tolerant to a variety of conditions such as drought, acidity and low soil fertility (Ball and Mosjidis, 1991; Vermeire et al., 1998). Due to its ability to adapt to such soils, SL has been considered as potential low-input forage for the southern United States (Puchala et al., 2005). It was first introduced in the United States from Japan in 1896, as a protective cover for soil restoration and conservation sites. In the late 1940's it became a forage for livestock consumption, but due to its highly competitive and invasive nature, SL has been considered a weed in some states, especially in the midwest (Ohlenbusch et al., 2007; Shaik et al., 2006; Vermeire et al., 1998). This legume is found throughout the southern United States and has been known for its high levels of crude protein (Gamble et al., 1996). Because of the presence of high levels of tannins, around 15.2%, this forage is considered to lack palatability; however, goats and sheep select and consume SL better than bovines (Powell et al., 2003; Ohlenbusch et al., 2007).

Despite being considered a noxious weed, SL has proven to improve the health of animals by controlling GIN. SL has been scientifically shown to reduce parasitic loads in goats and sheep by the reduction of FEC; reduction in number of worms; reduction of egg hatchability; and inhibition of larvae activity. However, its mechanism of action is still not known (Coffey et al., 2007). Some hypotheses are that the tannins attach to the worm cuticle causing them distress. It is also believed that the CT present in SL feed products bind to nutrients essential for bacterial growth in the feces, disrupting the larvae's food source. They may also bind to the

larvae, inhibiting larvae growth and mobilization and reduce the female worm's fecundity, consequently reducing FEC (Coffey et al., 2007; Min et al., 2005; Shaik et al., 2006).

Sheep and goats feeding on SL forage or hay have shown a reduction of FEC (Min et al., 2004; Shaik et al., 2006). Lange et al. (2006) found lambs fed SL hay had reduced FEC during the time of feeding, however, when feeding of SL stopped, FEC increased. Such observations may reinforce the hypothesis that SL effects female fecundity. It was also observed that SL fed as hay may be more useful to remove existing worms than establishing worms.

Terrill et al. (2007), feeding goats with SL hay in ground and pellet forms, observed a reduction in FEC of 54% and 77% for the hay and pellet groups, respectively. A decrease in the number of *H. contortus* larvae and adults with SL pellets was also observed. The same authors fed goats with SL hay and found that those animals not only had a lower FEC than the control animals, but also higher PCV and less worm recovery at necropsy than the control group (Terrill et al., 2009).

Chafton (2006) demonstrated that SL meal significantly reduced FEC in animals with an existing *H. contortus* infection. The same trend in FEC reduction was observed in animals with an established infection, but no significance was observed. The number of worms recovered at necropsy was also less than the control groups, but such difference was not significant. The tendency of reduced parasite burdens may be viewed as beneficial.

The adapting characteristics of this forage allied with the amount of current available literature and research demonstrating its capability to reduce FEC, worm burden and larvae in sheep and goats make SL a potential approach to parasite control. It is necessary to emphasize though, that SL cannot be a used as a sole resource.

2.4.2.8. Inorganic Copper as Control Tool

Copper (Cu) plays an important role in the physiological and biochemical systems of animals. This microelement is an essential component in dozens of enzymatic systems and is responsible for chemical processes such as energy production, melanin and elastin formation, antibody synthesis and lymphocyte replication (Moura, 1997; Tanner et al., 1988). Moreover, Cu is also involved in hemoglobin production, bone construction, hair and wool pigmentation and heart and brain function (Baruselli, 2001; Crocker et al., 1992; Kolb, 1987).

Although Cu has been suggested as an efficient approach in parasite control in sheep and goats (Burke et al., 2004; Kallu et al., 2005), the metabolism of Cu in sheep is complex and influenced by factors such as breed, age, stage of growth, and concentration of antagonists such as sulfur and molybdenum in the diet (Angus, 2000; Radostits et al., 2007). Ruminants seem to have much higher concentrations of Cu in the blood than non-ruminants, which may be due to the higher capacity ruminants have to bind Cu in the liver and low capacity for excretion (Williams, C.C, personal notes). Improper levels of this element in the diet may lead to copper deficiency or toxicity. Copper deficiency can be due to low levels of copper or excess iron, molybdenum and sulfur in the diet. Deficiency leads to poor wool quality, low weigh gain in lambs, anemia, and susceptibility to bacterial infections, swayback, enzootic ataxia and osteoporosis. Deficiency is often considered a perennial problem while toxicity can result from a variety of sources. Toxicity is usually the result of ingestion of high levels of Cu for prolonged periods. Increasing liver Cu storage to excessive levels causes anemia, jaundice and red urine. The prognosis is grave once signs of jaundice are observed. The forms of Cu commonly used in animal feed are copper sulfate, copper oxide, copper carbonate, and tribasic copper chloride (Radostits et al., 2007; Scott, 2007).

Administration of Cu is not only an effective way of treating Cu deficiency in grazing livestock, but also an approach to GIN treatment that could reduce the need for anthelmintics by

producers. It is also inexpensive, since Cu supplementation with copper oxide needles costs approximately 0.6% of the value of a ewe (Dewey, 1977; Scott, 2007). Some studies have suggested that susceptibility to Cu varies according to parasite species. *H. contortus* appears to be the most sensitive. Copper has been shown to be an effective anthelmintic against *H. contortus* and safe doses have been suggested in various studies (Bang et al., 1990; Chartier et al., 2000; Knox, 2002).

2.4.2.8.1. Copper Oxide Wire Particles (COWP)

Copper oxide wired particles, consist of a central nuclei of pure copper recovered with a mix of cupric and cuprous copper. After oral administration, COWP capsules are dissolved in the rumen and the copper particles pass to the abomasum and adhere to the mucosa. The acidity of the environment results in copper release over time (Knox, 2002; Nyman, 2000). Copper oxide wire particles were initially developed to treat copper deficiency in sheep, but its effect on worms was promptly noticed.

The administration of COWP causes an increase in the mortality of *H. contortus* (Bremner, 1961). The changes in the abomasum environment affect *H. contortus* causing expulsion (Chartier et al., 2000). Though the mechanism of action of COWP is not yet established, one study suggested that worms exposed to Copasure® COWP had significant damage to the cuticle, which might cause a disruption on the ability of worms to maintain their metabolic function, so they become weak and die or are expelled (Moscona et al., 2008).

Watkins (2003) evaluated COWP on reduction of GIN infection of sheep under natural grazing conditions and observed that FEC and larvae numbers were reduced from the first week throughout the end of experiment. The greatest reduction occurred right after COWP administration, but significant FEC reduction appeared to last for a period of four to five weeks. In South Africa, kids experimentally infected with *H. contortus* showed an infection reduction of 93 to 95% when treated with 2 and 4 g doses of Copasure® COWP. It was also observed that

Cu levels on tissues were similar in both treated and untreated animals indicating that copper toxicity may not be an issue (Vatta et al., 2009). Bang et al. (1990) reported that oral administration of COWP in sheep significantly reduced the number of *H. contortus* recovered at necropsy by 96%. The use of COWP in ruminants not only caused mortality of this parasite, but also reduced fecundity of surviving females in the host (Bremner, 1961; Chartier et al., 2000).

Concerns about Cu toxicity have led to dose titration studies to determine the minimum effective dose. Doses of 4 and 6 g have been shown to be effective against GIN in lambs and although copper accumulation in the liver was observed, levels were within normal limits (Burke et al., 2004). Multiple low doses (0.5 and 1.0 g) of COWP have also been shown to work as effectively as levamisole in controlling *H. contortus* without risk of copper toxicity in lambs (Burke and Miller, 2006). Gonçalves & Echevarria (2004), using 3.4 g of COWP, observed that it did not cause toxicity in sheep in an extensive pasture management system and it was helpful in the control of *H. contortus*, protecting against re-infections for up to four weeks. The use of COWP in feed pellets has been reported to effectively reduce FEC in lactating ewes and it is suggested as a way to suppress the periparturient rise in FEC (Burke et al., 2009). Orlik (2010) also observed that COWP in feed pellets worked as well as the combination of levamisol/albendazole for deworming lambs on an individual basis. It is important to consider that the use of COWP in grazing animals might require repeated use.

Burke et al. (2005) demonstrated that COWP can be safe and effective enough when combined with alternative strategies. Their study reported no contrary effect in the ability of the fungus *D. flagrans* in inhibiting development of residual larvae after COWP treatment. Such results suggest a lower contamination of the pasture when both techniques are used in a sustainable control strategy.

2.4.2.8.2. Copper Sulfate ($CuSO_4$)

Copper sulfate is formed from a reaction between copper and sulphur. It can be produced industrially by treating copper metal or its oxides with sulfuric acid or by placing copper in a solution of nitric acid, sulfuric acid and water (Wiberg et al., 2001).

Copper sulfate has been used as a fungicide, bactericide and algaecide. It has been used in the past in human medicine as an emetic, but its use was discontinued due to toxicity (Olson, 2004). Copper sulfate was found to be effective in controlling the GIN in ruminants in the early 1900's and has been used in many deworming solutions since then (Corrêa, 1973; SCSRPC, 2010). Curtice (1917) concluded that a monthly drench of 1% $CuSO_4$ solution along with pasture rotation was a practical method for the control of *H. contortus* in sheep (in Logue, 1995).

For years, an oral dose of 1% solution of $CuSO_4$ was suggested as treatment of GIN in sheep, especially for *H. contortus* and *Trichostrongylus axei* (Junquera, 2010). The solution was reported as being efficient against adult stages of *H. contortus* only. Mönnig & Quin (1935) suggested the use of a 10% solution of $CuSO_4$, known to cause temporary reflex closure of esophageal groove in sheep, to help the delivery of anthelmintic compounds straight to the abomasum, so they could be more effective against GIN without passing through the other stomach compartments first. The stimulatory effect on esophageal groove, which allowed drug delivery bypassing the forestomach, was observed only in animals up to one year of age (Corrêa, 1973; Ross and Gordon, 1935). Hucker & Yong (1986) demonstrated that GIN infection significantly exacerbated an existing Cu deficiency in sheep. A study conducted to determine if copper sulfate mixed with a daily feed supplement could be used to control *H. contortus* infection in goat kids showed no differences in infection level, based on FEC, and therefore, it was concluded that $CuSO_4$ was not effective (Burke et al., 2008).

Sheep are very sensitive to $CuSO_4$, which is absorbed faster than COWP, so toxicity is more likely to occur (Schoenian, 2010). A monthly oral dose of 1 g of $CuSO_4$ has been suggested as adequate in prophylaxis of Cu deficiency. However, regular oral dosing is laborious and time-consuming and this has made $CuSO_4$ applicability no longer widely practiced. Another drawback was that it typically required 100 cc of solution in that CuSO4 is caustic and must be made up in a dilute solution (Radostits et al., 2007; SCSRPC, 2010).

With increasing levels of anthelmintic resistance and a movement towards more sustainable farming practices, there is a renewed interest in natural dewormers. Experiments are being conducted to determine the efficacy of various natural dewormers and other old-time remedies. As the interest in COWP as an alternate control method has increased, the use of $CuSO_4$ has also generated interest; however, due to limitations of the compound itself, research evaluating $CuSO_4$ as a deworming agent has not extensively been done.

CHAPTER 3

MATERIAL AND METHODS

3.1. Location

All the experiments were conducted at the Ben Hur Central Research Station Sheep Unit, Louisiana State University Agricultural Experimental Station, Baton Rouge, Louisiana.

Parasitology sample analyses were conducted in Dr. Miller's laboratory in the Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana.

3.2. Animals

The animals used in experiment 1 (Chapter 4) and experiment 2 (Chapter 5) were naturally infected 1 to 10 year old mature F1 ewes (Suffolk x Gulf Coast Native). Animals used in experiment 3 (Chapter 6) were 4 month old naturally infected Katahdin lambs acquired from the USDA Agricultural Research Service, Booneville, Arkansas.

3.3. Experimental Design

The present project consisted of three experiments. For each experiment, the animals were randomly allocated to groups based on fecal egg counts (FEC) to assure equal parasite loads in each group (treatments and control).

3.4. Techniques Employed

3.4.1. Fecal Egg Counts (FEC)

Feces were obtained directly from the rectum, placed in styrofoam cups and taken to the laboratory to be analyzed. The FEC, reported as the number of trichostrongyle type eggs per gram (EPG) of feces, was determined using a modified McMaster technique (Whitlock, 1948). Two grams of feces were weighted and broken up in a cup. Thirty ml of a saturated salt solution (737 g of iodized salt dissolved in 3000 ml of tap water) was added and the solution was mixed using an electric drink mixer (Drinkmaster® Drink Mixer, Hamilton Beach Brands, Inc., Glen

Allen, NC). As soon as the solution was thoroughly mixed a small sample was pipetted and placed into both sides of a McMaster slide chamber (Chalex Corporation, Issaquah, WA). The number of trichostrongyle type eggs counted inside of the grids of the chambers was then multiplied by 50 to obtain the FEC. FEC was used to monitor GIN infection in each animal. In addition, a FEC reduction test was used to determine the efficacy of treatments using the equation proposed for anthelmintic efficacy by Coles et al. (1992) (Equation 1).

$$FEC\ reduction = \frac{mean\ EPG\ control\ group - mean\ EPG\ treated\ group}{mean\ EPG\ control\ group} * 100$$

Equation 1: Fecal egg count percent reduction

3.4.2. Packed Cell Volume (PCV)

Blood was collected via jugular venipuncture into 7 ml EDTA containing vacutainer tubes (BD Vacutainer® Glass Whole Blood Tubes, Becton, Dickinson, and Company, Franklin Lakes, NJ). To prevent clot formation each tube was gently inverted several times as soon as it was drawn from the animal. The PCV was determined by using blood filled micro-hematocrit capillary tubes which were sealed, and centrifuged in an Autocrit centrifuge (Autocrit Ultra 3 Microhematocrit Centrifuge, Becton, Dickson and Company) for 5 minutes. The PCV was determined by direct visual comparison on a hematocrit scale.

3.4.3. Fecal Cultures

Group fecal cultures were performed weekly to allow deposited eggs to hatch and larvae to develop to the L3 stage. Feces collected from each group were weighed, crushed and mixed in a plastic container with water. Vermiculite was added in an approximately equivalent amount to that of the feces and mixed thoroughly until a moist crumbly consistency was achieved. The container was then covered with aluminum foil with several holes to facilitate air circulation and

kept at room temperature for an incubation period of 14 days. Additional water was added as necessary to prevent desiccation.

After the incubation period, a Baermann technique was used for harvesting the L3 stage larvae. The technique consists of filtering the culture in a large funnel with a 15 ml plastic test tube attached to the end. A wire mesh screen with an opening of 0.15 mm was placed in the funnel and the culture material, wrapped in cheese cloth, was placed over the wire mesh screen. Lukewarm water was added until it completely covered the culture material and cheese cloth and was left over night. Live L3 were collected in the test tube attached to the funnel. When necessary, the tubes containing the L3s were baermannized again through a kimwipe sheet in order to filter out residual culture debris. One ml of 10% formalin was added to the 15 ml test tube to preserve the larvae for further processing.

3.4.4. Larvae Identification

The sediment contents of the 15 ml test tube obtained from the fecal cultures were reduced using a pipette to either 1 or 2 ml. The liquid and sediment were mixed and a 100 µl aliquot was taken and placed on a microscope slide. The sample was stained with iodine to facilitate larval identification and two coverslips were placed over the mixture. Each aliquot taken was read in a compound microscope, and the first 100 larvae found while scanning of the slide were identified to genus according to their head and tail morphological features (Figure 2).

The remaining larvae on the microscope slide were counted and the total count was used to extrapolate the total number of larvae in the original solution. Larvae per gram of feces (LPG) were determined by the following equation (Equation 2):

$$LPG = \frac{Total\ number\ of\ larvae}{Fecal\ weight}$$

Equation 2: Determination of larvae per gram of feces

After the LPG was obtained, the percentage of larvae development was calculated following the equation (Equation 3):

$$\%\ Larvae\ development = \frac{LPG}{EPG} * 100$$

Equation 3: Percentage of larvae development

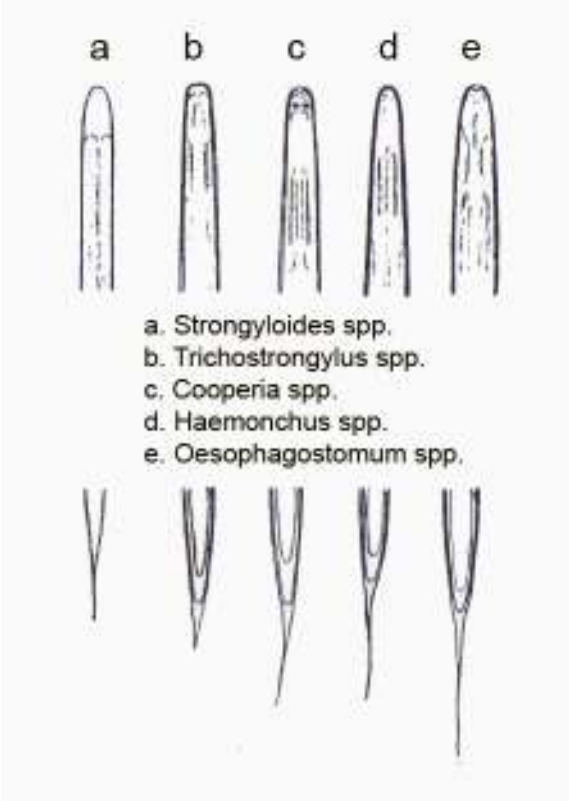

Figure 2. Anterior and posterior region of nematode infective larvae common to ruminants. Adapted from Ueno and Gonçalves (1998).

3.4.5. Necropsy

All the animals from experiment 3 were euthanized (Pentobarbital Sodium 390 mg/ml / Phenytoin Sodium 50 mg/ml (Beuthanasia-D Special, Shering, Wayne, NJ) at a dose of 6 ml per 100 lb body weight intravenously via jugular vein and necropsied at the end of the experiment (week 16).

The abdominal cavity was opened and the abomasum, small and large intestines were removed from each animal. These parts were then opened and stomach contents were emptied and then washed with tap water into a bucket with 5 liters of water. Aliquots of 500 ml (10%) were then taken and placed into plastic bottles. After 5-6 hours, 50 ml of the contents was decanted and replaced with 10% formalin as a preservative. The washed abomasum was soaked in water overnight at room temperature, rewashed and processed following the preceding technique.

3.4.6. Worm Identification

The 500 ml aliquots of each organ contents were processed 50 ml at a time. Samples were mixed and the 50 ml aliquot was filtered as follows:

- N° 200 wire sieve - (USA Standard sieve series, ASTM designation E 11, Newark wire cloth company, Newark, New Jersey 07104) – Abomasum, abomasum soak, small intestine
- N° 35 wire sieve - (US Standard sieve series, ASTM E 11, American Scientific Products, Division of American Hospital Supply Corporation, McGraw Park IL 60085) – Large intestine

The sediment containing worms was thoroughly washed in the sieve with tap water, collected in a beaker and diluted with water. A few drops of Lugol's iodine were added to the solution to stain the worms red and then the background was de-stained with a few drops of household bleach solution. The solution was stirred and small amounts were poured into a Petri dish. The first 100 worms were recovered using a tuberculin syringe needle and mounted on microscopic slides with 5 worms each under 2 cover slips in a drop of lactophenol. Additional aliquots were taken when fewer than 100 worms were recovered in the first aliquot. Once the parasites were counted and placed on microscope slides, they were identified to genus, sex and

stage of development based on morphological characteristics (Figures 3 and 4). The number of recovered worms was then extrapolated to estimate the number of parasites present per animal.

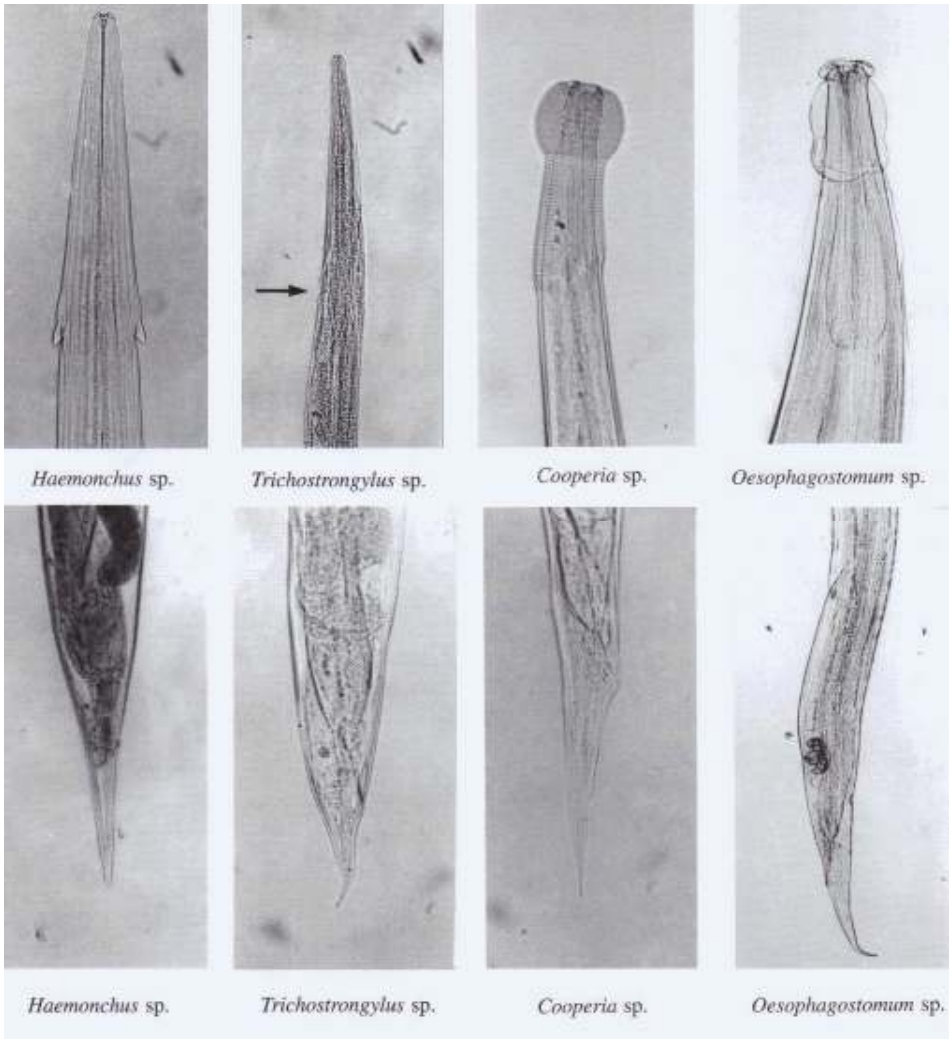

Figure 3. Morphologic characteristics of adult nematodes common in ruminants. Adapted from Ueno and Gonçalves (1998).

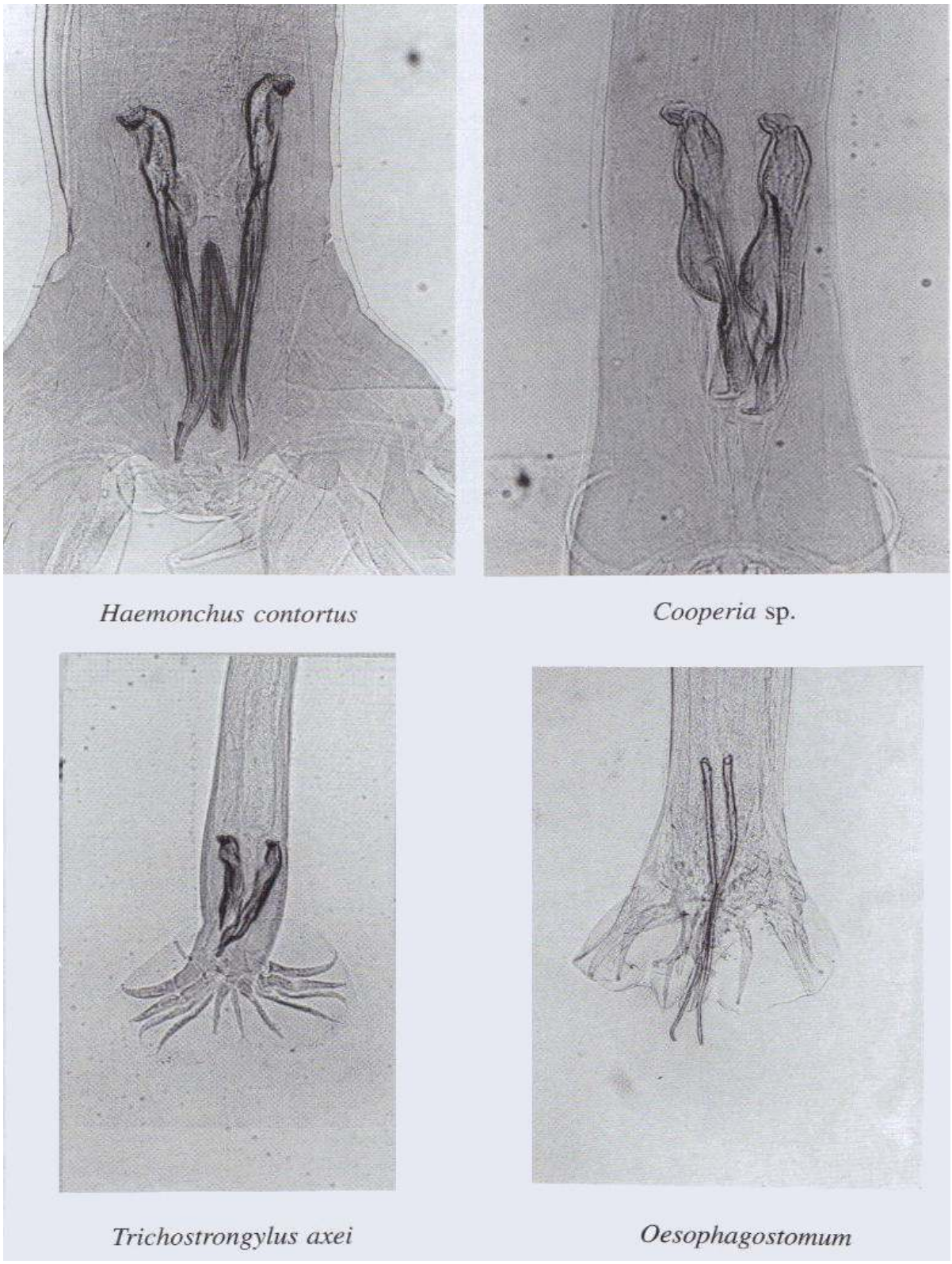

Figure 4. Morphologic characteristics of adult male nematodes common in ruminants. Adapted from Ueno and Gonçalves (1998).

3.5. Statistical Analysis

Data were analyzed using the statistical package SAS/STAT® (2008) version 9.2. FEC, PCV, and LPG, 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 (Devore and Peck, 2001). In order to stabilize variance, FEC data were transformed to a natural base logarithm (Winer, 1971). Pairwise comparisons were conducted with Tukey's HSD test for main effects and with t-tests of least-square means for interaction effects. Pearson correlation coefficient tests were used to analyze data sets for a linear association over time. Differences were considered significant at the 5% confidence level.

CHAPTER 4

EXPERIMENT 1

4.1. Experimental Design

Pregnant F1 (Suffolk x Gulf Coast Native) ewes, 1 to 10 years of age were selected for the study. The animals were randomly allocated to groups based on fecal egg counts (FEC) to assure equal parasite loads in both groups (treatment and control). Twenty eight animals were divided into a control and a treatment group with 14 animals in each group. The experiment was conducted over a 12 week period during the months of February to May, 2009 and each group was kept on a pasture area of 3 acres and rotated every week. The objective of this trial was to assess the impact of supplementing lactating ewes with SL pellets and COWP combination as a strategic treatment for the reduction of the periparturient rise in FEC of sheep.

The control group was supplement fed alfalfa pellets (Grainland Selection™) as 25% of their daily intake (1.5 lbs per head/day) and grazed a 3 acre pasture of Bermuda and Bahia grass. The composition of the alfalfa pellets consisted of 15% crude protein, 1.5% crude fat and 30% crude fiber. The treatment group (sericea lespedeza, SL) was supplement fed SL pellets also as 25% of daily intake (1.5 lbs per head/day) and grazed a 3 acre pasture of Bermuda and Bahia grass as well. The composition of SL pellets consisted of 95% SL leaf meal, 4% dry molasses, 1% trace mineral mix and was about 14-15% crude protein. Copper oxide wire particles (Copasure®,COWP) was incorporated into SL pellets and administered to the SL group at week zero and four (2.0g/head) and at week six and ten (4.0g/head). The two gram dose did not appear to be effective in mature ewes and the dosage was then adjusted to 4.0 g/head. Both groups were offered free choice mineral (PURINA®) and pure salt (NaCl). The animals were fed as a group in the morning before they were allowed to go on the pasture and kept in pens at night to avoid predator attacks.

Fecal egg counts (FEC), packed cell volume (PCV) and FAMACHA© scores were determined weekly from each animal in order to monitor parasite infection. Feces was obtained directly from the rectum and put into styrofoam cups labeled with animal identification number and date. Blood samples for PCV were collected in 7 ml EDTA coated vacutainer tubes (BD Vacutainer® Glass Whole Blood Tubes, Becton, Dickinson, and Company, Franklin Lakes, NJ). All blood samples were collected by jugular venipuncture. FAMACHA© scores were obtained using the FAMACHA© chart and examining the eye mucosa of each animal. The scores obtained were used to identify the necessity of spot treatments as well as to identify anemia and correlation with the PCV. Bulk fecal cultures were performed to allow eggs to hatch to L3 larvae for larval identification and to estimate the relative worm population (Figure 5).

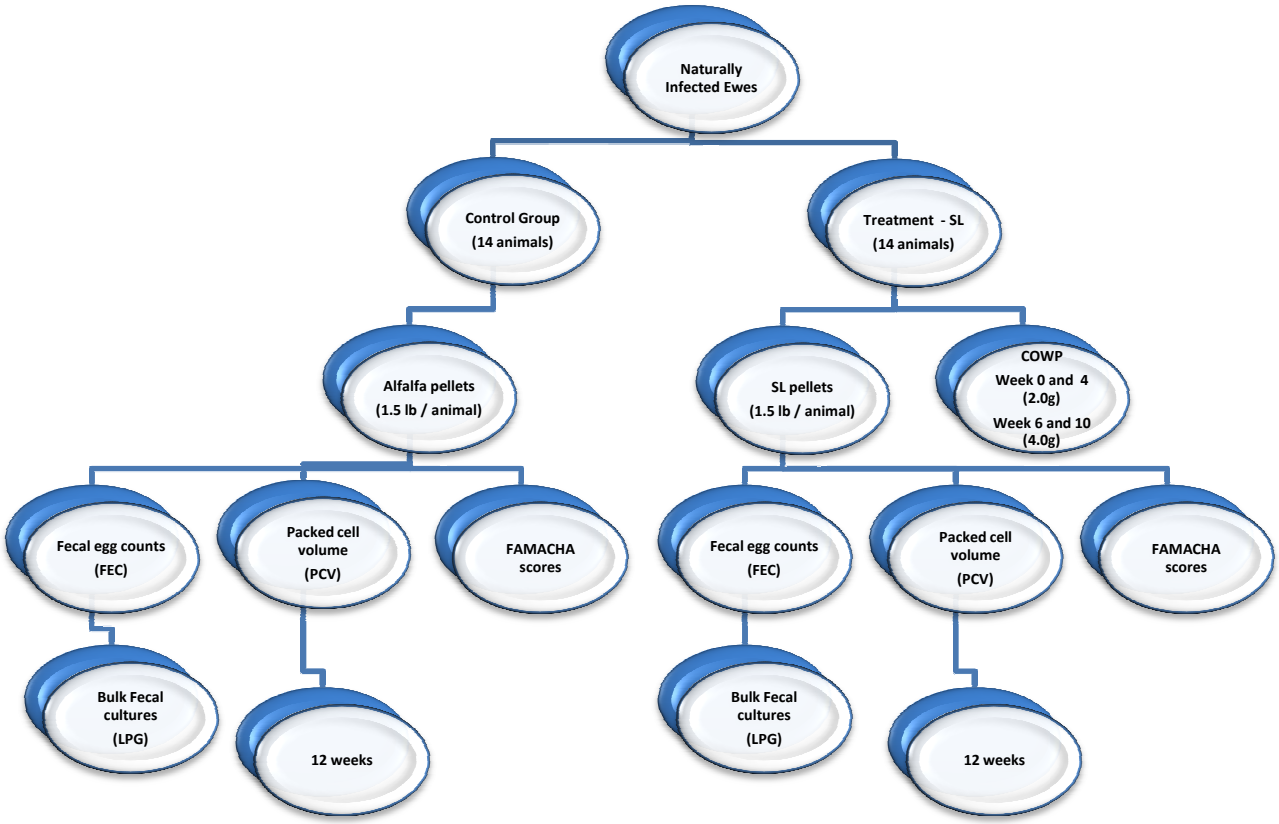

Figure 5. Flow chart of methodology for Experiment 1.

4.2. Results

During this experiment, one animal died of unknown causes (animal was found dead) and one animal was euthanized because it became debilitated. Two animals had to be removed from the experiment because they were not pregnant and did not meet the objective of the study. Since the number of animals removed from the trial was equal for both groups there was no need for re-allocation of animals.

From week 1 to week 11, FEC percentage reduction was calculated using the formula proposed by Coles et al. (1992), to assess reduction in FEC between SL fed and control animals. When the results were negative, the percent reduction was considered zero (Table 1).

Table 1. Mean fecal egg count (FEC) and FEC percent reduction comparing sericea lespedeza (SL) supplemented and control ewes.

	Fecal egg count		
Week	Control	SL	FEC Reduction (%)
0[a]	819.2	973.8	0.00
1	603.8	530.9	12.08
2	619.2	633.4	0.00
3	611.5	715.8	0.00
4[a]	1126.9	1302.9	0.00
5	1630.7	1545.4	5.23
6[b]	3103.8	2702.0	12.94
7	1415.3	939.0	33.65
8	953.8	911.6	4.42
9	526.9	554.8	0.00
10[b]	473.0	533.5	0.00
11	384.6	474.4	0.00

Copper oxide wire particle bolus incorporation into SL pellets (2 g[a] and 4 g[b]).

The first dose of COWP (2 g) administered to the SL group did not appear to be effective. Therefore, to prevent animal loss, a second dose of COWP (4 g) was administered at week six and repeated at week 10. The 4 gram dose did appear to be effective, but in spite of COWP administration, the FEC percent reduction observed was minimal or none. The greatest

FEC reduction observed during this trial (33.7%) coincided with the week after the copper dosage was adjusted, but no significant difference was observed (p=0.54) (Figure 6).

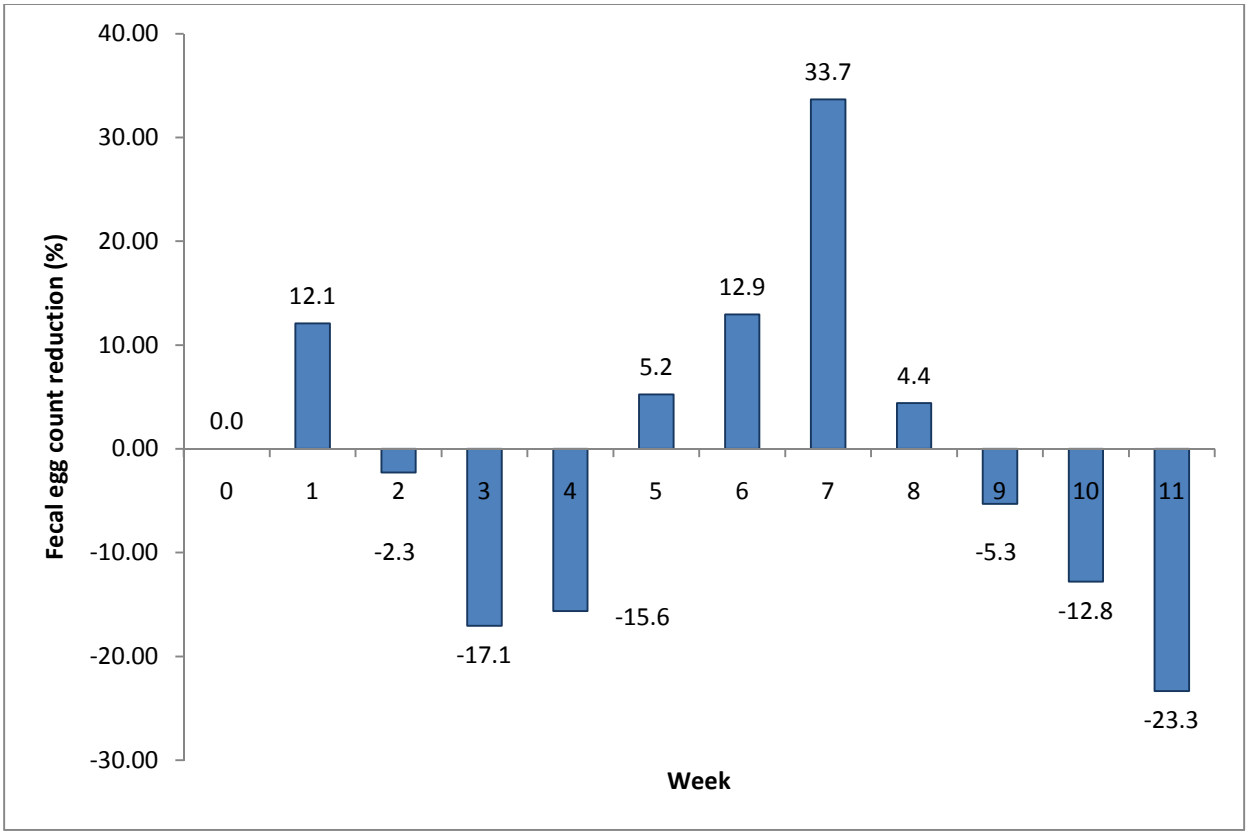

Figure 6. Percent reduction in fecal egg count (FEC). Copper oxide wire particles were administered at weeks 0 and4 (2 g) and weeks 6 and 10 (4 g). The reductions observed were not significant (p=0.54).

FECs were comparatively similar between groups throughout the study. Overall, the mean FEC output for the control group was 1022.44 EPG (±219.75) and for the SL group was 941 EPG (±158.58). No differences between groups were observed (p=0.95). Overtime, a significant difference was observed on week 7 (p=0.033) and week 8 (p=0.015) (Figure 7).

The PCV for both groups was also relatively similar with some oscillations throughout the experiment. The mean PCV for the control group throughout the study was 28.46 % (±0.42). The lowest PCV detected was 25.54% and the highest was 30.31%.

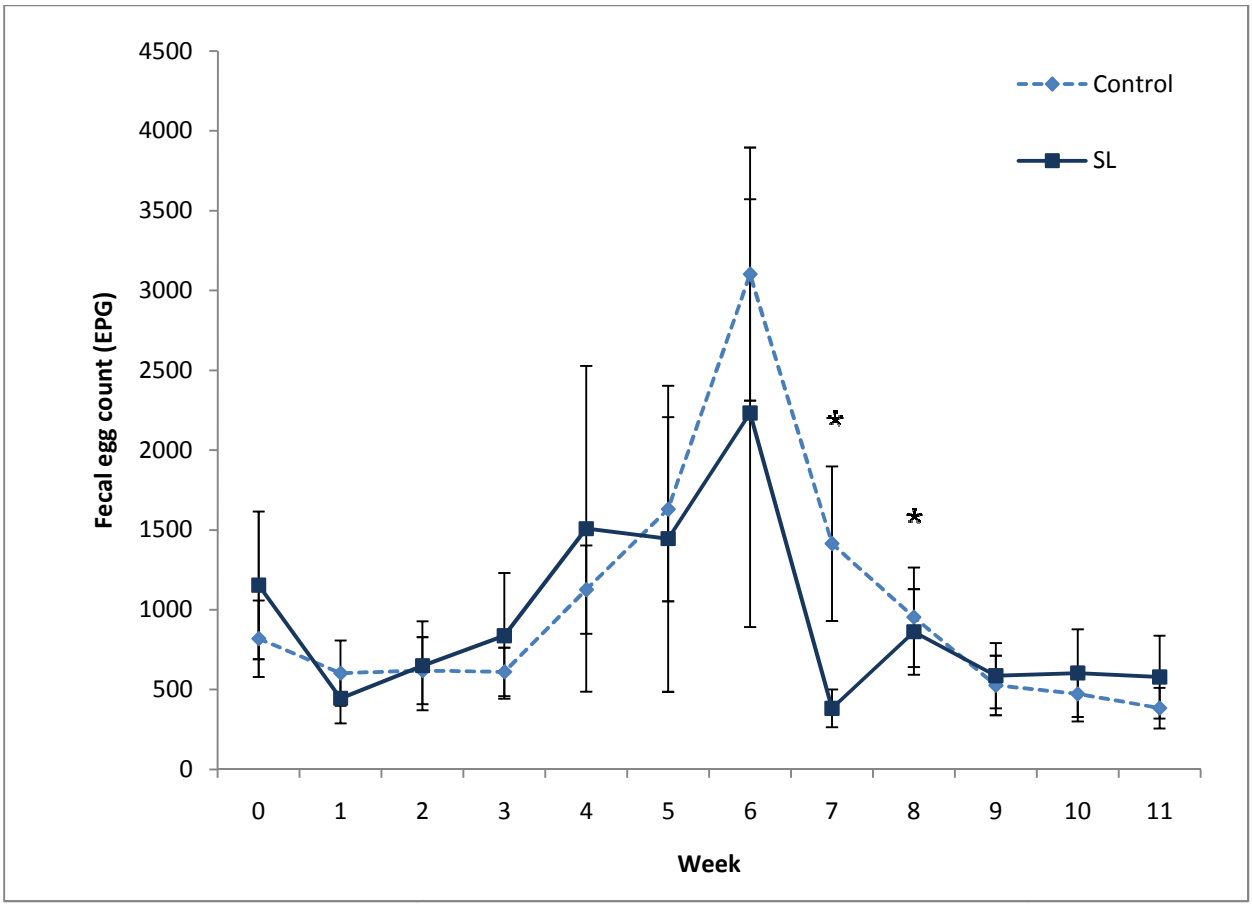

Figure 7. Mean fecal egg count for control and sericea lespedeza (SL) groups (EPG ± S.E.M.). *Significant difference was observed on week 7 and 8 (p=0.033 and p=0.015 respectively).

For the SL group the mean PCV observed during the study was 27.84% (±0.46), with lowest PCV of 25.1% and highest 30%. No significant difference between groups was observed (p=0.47). There was though a significant change in PCV from week 5 to week 6 (p=0.04) (Figure 8).

A negative correlation was observed between FEC and PCV (r^2 = -0.43, p<0.0001). It was expected that the PCV would decrease as FEC increased, since anemia tends to become more evident as the infection gets worse. Although FEC and PCV were significantly correlated, the strength of the correlation was not strong. A similar trend was also observed regarding PCV and FAMACHA© scores (FAM) (r^2 = -0.55; p<0.0001).

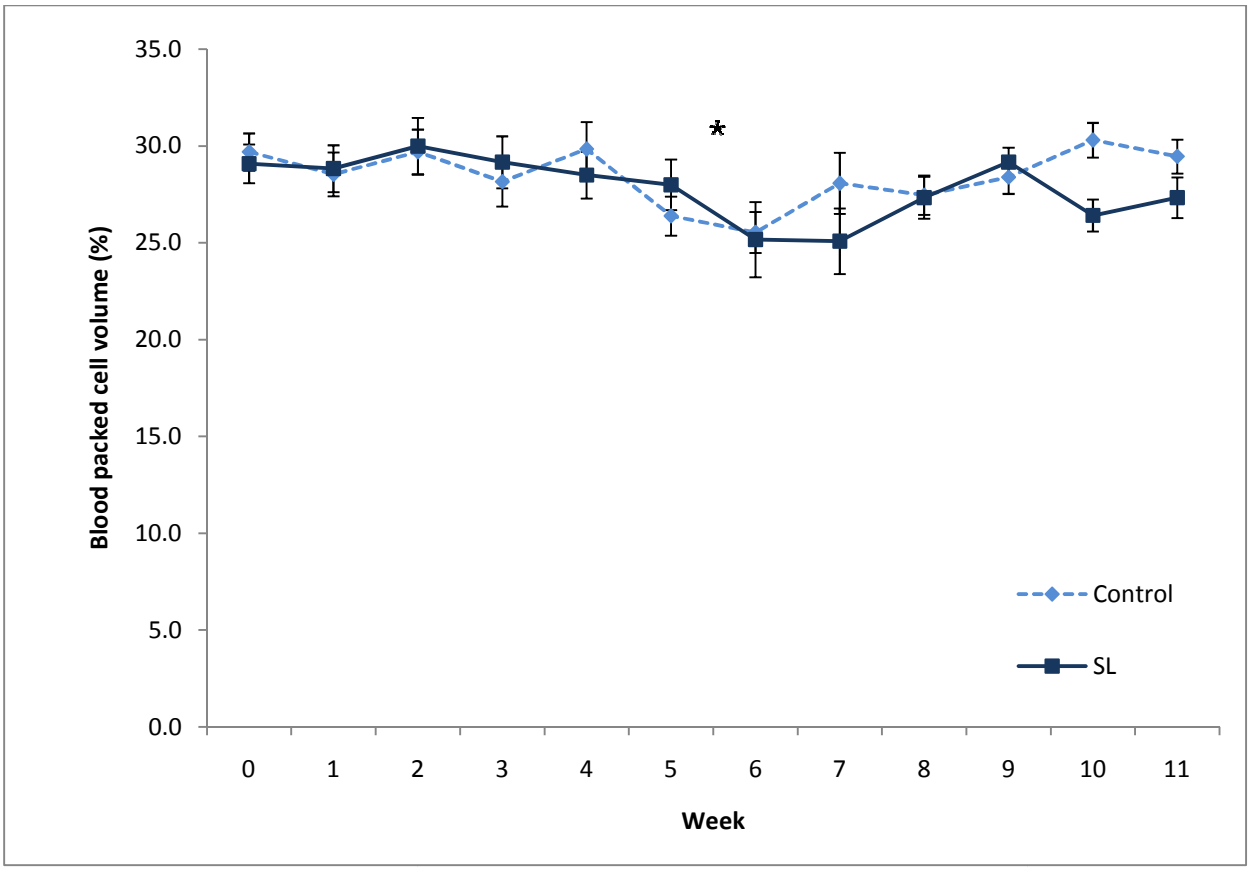

Figure 8. Mean blood packed cell volume comparing sericea lespedeza (SL) and control groups (PCV±S.E.M.). Significant difference was observed from week 5 to week 6 (p=0.04).

Such results were expected as well; generally animals with high FAM tend to have lower PCV. When FAM and FEC were analyzed, a positive correlation was observed (r^2 = 0.31, p<0.0001). The correlation indicates that higher FECs are associated with higher FAM scores (Figure 9). The majority of FAM scores observed were in categories 2 and 3 (Table 2) and they represented approximately 29% and 50% of the total observations, respectively.

Although the majority of the FAM scores observed during the study were in categories 2 and 3, only 19.3% of the PCVs in the control and 23.3% in the SL group were below the reference values for sheep (27-45%) (Duncan and Prasse, 1986).

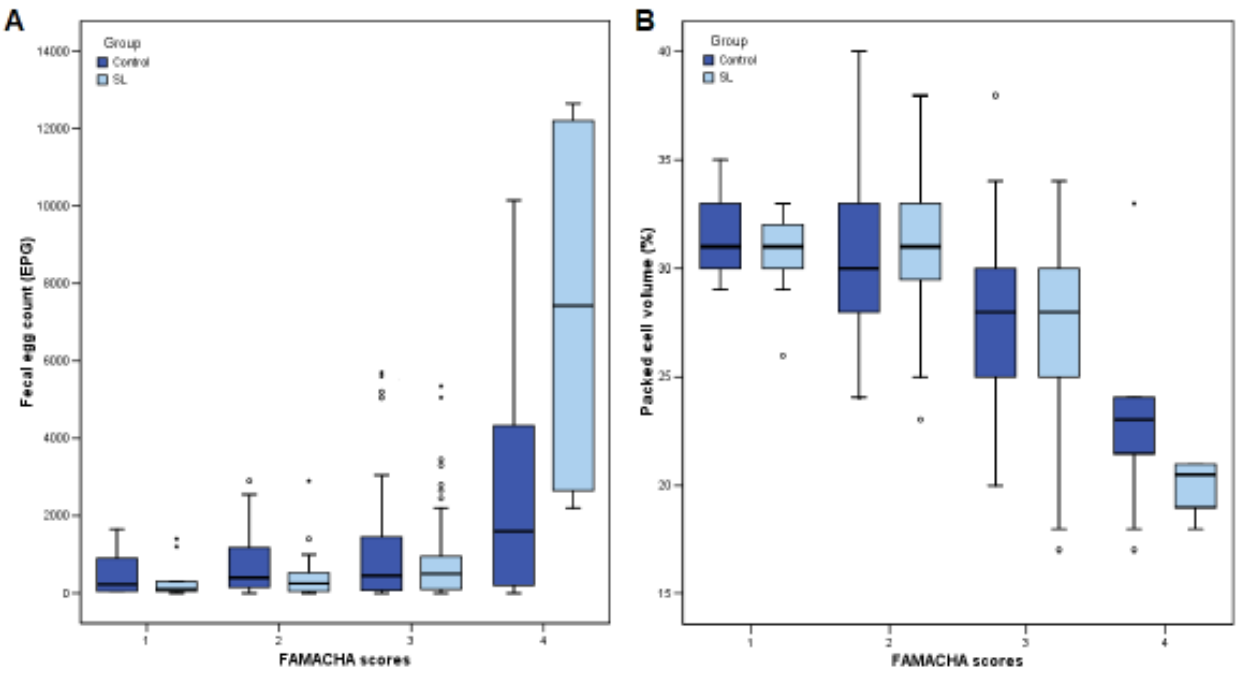

Figure 9. Box plots demonstrating the relationship between fecal egg count (FEC), packed cell volume (PCV) and FAMACHA© scores. A - Total FEC (overall) and FAMACHA© scores; B - PCV and FAMACHA© scores. Lower and upper borders of the box represent the 25th and 75th percentiles, respectively. Median (solid line) values are presented within the box. Whiskers above and below the box indicate the maximum and the minimum non-outlier observation. Circles indicate outliers.

Table 2. Mean and standard error of the mean (S.E.) of fecal egg count (FEC) and packed cell volume (PCV), respectively, for each FAMACHA© score (FAM) by group (Control and sericea lespedeza, SL).

FAM	FEC						PCV					
	SL			Control			SL			Control		
	n	Mean	S.E.	n	Mean	S.E.	n	Mean	S.E.	n	Mean	S.E.
1	9	378	178	10	515	198	9	31	0.7	10	32	0.6
2	43	376	78	118	729	94	43	31	0.4	118	30	0.5
3	75	826	124	76	1015	155	76	28	0.4	76	28	0.4
4	4	7425	2769	11	2977	1025	4	20	0.7	11	23	1.2

Larval development in fecal culture was used to verify egg hatchability, to estimate the species present in the gastrintestinal tract, and to indirectly evaluate the effects of the applied

treatment. A percent reduction in the number of infective larvae was observed on weeks 3, 7, 8 and 9. The greatest reduction observed was of 29.5 %, which was significant (p=0.05) (Table 3).

Table 3. Mean fecal egg count (FEC), larval count (LPG) and percent larval reduction in feces from ewes fed sericea lespedeza (SL) pellets (n=12) compared to feces from control ewes (n=12).

Week	FEC		L3		Percent reduction[c]
	SL	Control	SL	Control	
1[a]	833	733	72.59	24.65	0.00
2	317	483	23.87	18.19	0.00
3	417	800	5.03	5.70	11.71
4[a]	400	1167	17.60	13.24	0.00
5	1183	883	83.02	6.77	0.00
6[b]	817	1467	35.23	27.49	0.00
7	767	2817	49.59	53.18	6.73
8	584	1934	13.67	19.37	29.45*
9	550	1350	9.63	11.14	13.56
10[b]	450	717	30.22	4.52	0.00
11	517	317	42.71	12.31	0.00

[a] 2.0g COWP. [b] 4.0 g COWP.
[c] [*(*control L3 – SL L3*)/*control L3] × 100.
* Significant at 0.05 confidence level.

The bulk culture in the control group had a significantly higher FEC than the SL group (p=0.04). As for larval development, the SL group yielded a significantly higher percent larval development than the control (p=0.03). No significant differences between FEC and sample weight (p=0.35) or larvae development (p=0.43) were observed (Figure 10).

Cooperia spp. was the predominant nematode identified in the fecal cultures (44.6%), followed by *Haemonchus contortus* (39.4%). *Trichostrongylus spp* and *Oesophagostomum spp.* were also found in smaller proportions (13.8 and 2.21% respectively). However, when groups were considered separately, it was observed that in the control group, *H. contortus* was the most abundant species (45.3%) followed by *Cooperia spp.* (39.3%) and in the SL group, it was the inverse, *Cooperia spp.* being the most abundant followed by *H. contortus* (50% and 34% respectively) (Table 4) (Figure 11).

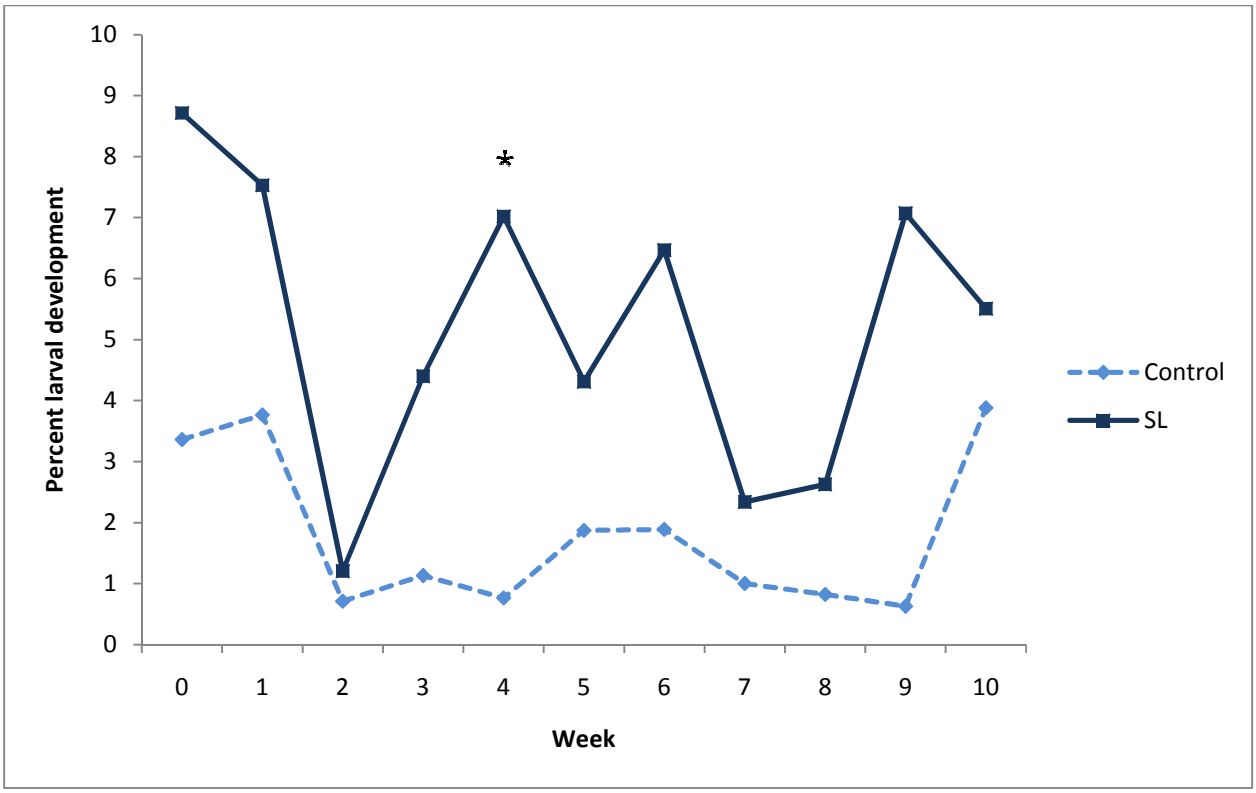

Figure 10. Percentage of larval development in fecal culture of naturally infected ewes. There was a significant difference in egg hatchability between groups (p= 0.03) and on week 4 (p=0.05).

Table 4. Mean percentage of nematode species identified from fecal cultures by group (Control and sericea lespedeza, SL).

Species	Mean percentage of nematode species in each group	
	Control	SL
Haemonchuscontortus	45.27	33.6
Trichostrongylus spp.	13.64	14
Cooperia spp.	39.27	50
Oesophagostomum spp.	1.82	2.6

Larval enumeration showed that there were no significant differences in LPG between groups throughout the study ($p<0.05$). The number of *H. contortus* larvae recovered from the SL group was almost 12% less than in the control group at the end of the study. It was possible to observe a tendency of *H. contortus* diminishing over time in the SL group and there was a shift in larvae population in the SL fed group from *H. contortus* to *Cooperia spp.* (Figure 12).

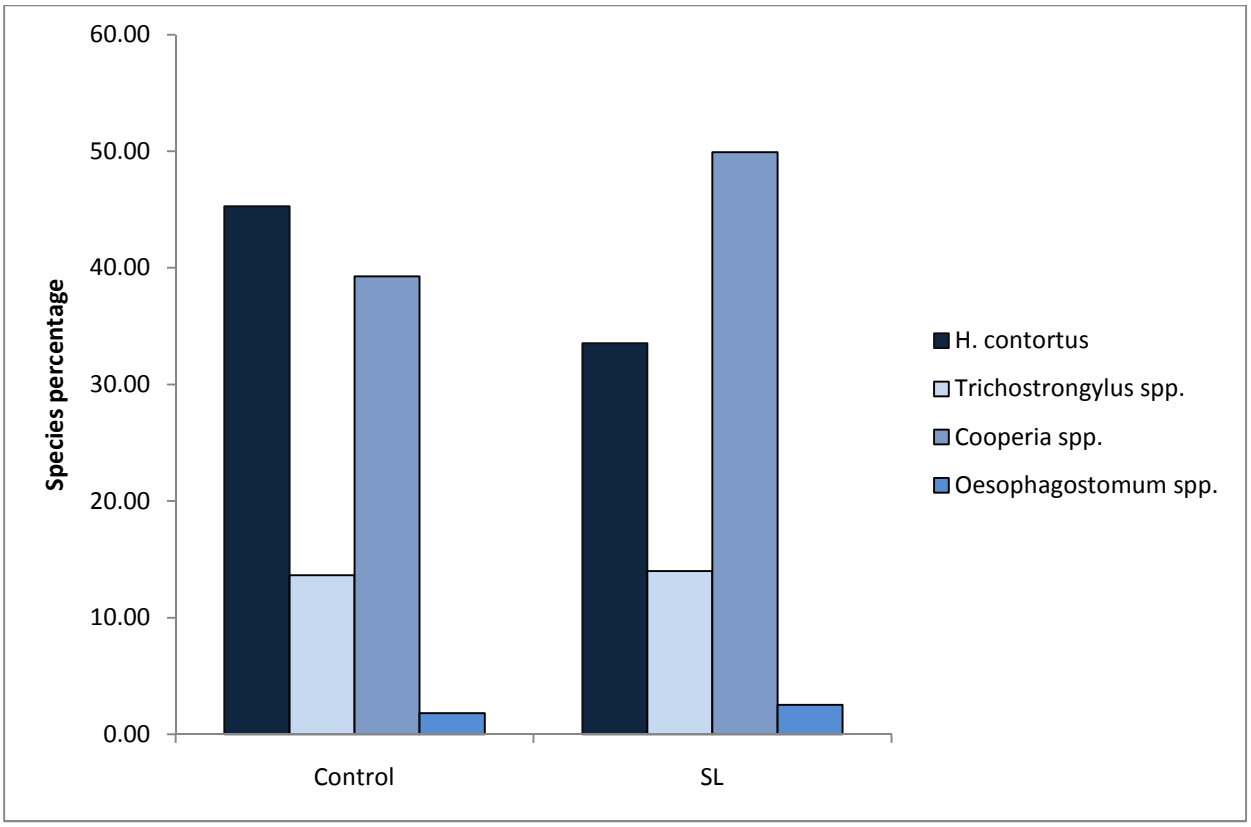

Figure 11. Percentage of infective larvae identified in control and sericea lespedeza group fecal bulk cultures of naturally infected ewes.

4.3. Discussion

A temporary relaxation of pregnant ewe immunity starting before lambing usually results in the peri-parturient rise in FEC. Such a rise was observed in this experiment. However, the increase was not as prominent as expected, and the FEC of both groups was relatively constant throughout the study (Barger, 1993; Procter and Gibbs, 1968). It has been reported that the effects of the periparturient rise can be minimized through protein supplementation and in some cases genetic aspects are involved (Kahn et al., 2003; Rocha et al., 2004). Sericea lespedeza is known for its high protein levels (14-15%) and Terrill et al. (2007) reported that SL pellets, fed as the total diet, reduced FEC in goats by 77%. SL hay, fed as the total diet, was also efficient in reducing FEC and worm burden in lambs (Lange et al., 2006).

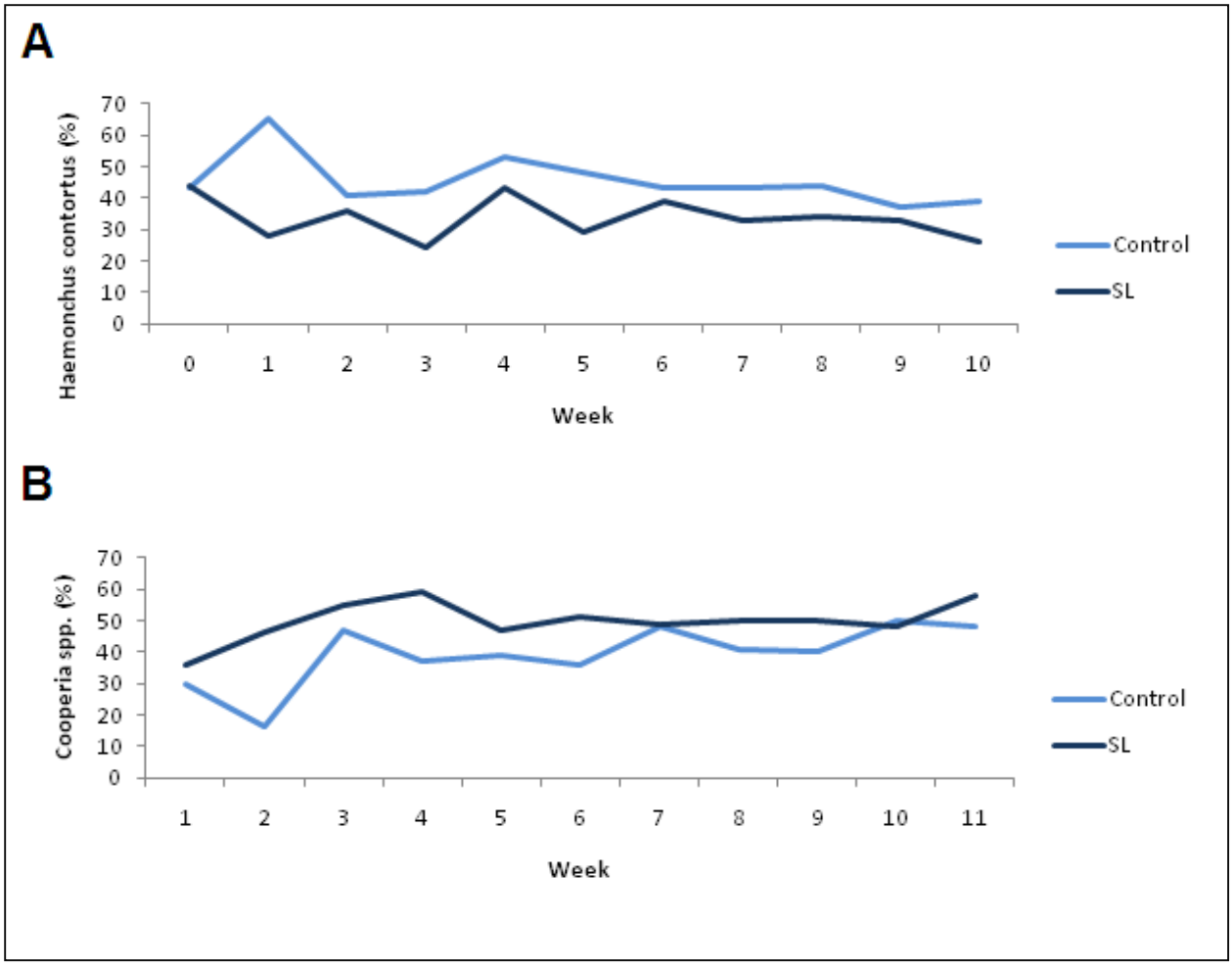

Figure 12. Percentage of predominant infective larvae identified from fecal bulk cultures of naturally infected ewes by group. A – Percentage of *Haemonchus contortus* larvae identified in the sericea lespedeza (SL) and control groups. B – Percentage of *Cooperia spp.* larvae identified in the SL and control groups.

Since both groups were fed high protein supplements at about 25% of total intake throughout the trial, this could be the reason FECs remained low. Age and breed resistance (Gulf Coast Native influence) of the animals used in this trial could also be influencing the establishment of infection, and although it was not possible to measure the extents of such influence it should be taken into consideration.

No effect of SL pellet supplementation was observed on LPG during this trial. There was though, a trend, in the SL group, to have a lower percentage of *H. contortus* in the larval

population towards the last weeks. These findings somewhat support the observations made by Anthanasiadou et al. (2001) that larval viability is adversely affected by the binding capacity of the CT. The fecal culture results are also consistent with the work done by Max et al. (2005), that showed that CT are active only against *H. contortus* and not *T. colubriformis* since it was observed that the percentage of *Trichostrongylus spp.* remained relatively constant throughout the experiment.

H. contortus feeds on blood and each worm present can be responsible for a daily removal of about 0.05 ml of blood through ingestion or leakage from lesions (Urquhart et al., 2007). With low infections the animal is able to compensate by erythropoiesis before a decrease in PCV can become apparent. In this trial, ewes treated with SL were able to maintain their PCV slightly higher than the control group and a significant change was observed overtime as well. Terril et al. (2009) reported an increase in PCV during the final stages of their trial in goats fed SL pellets. No improvement in PCV was observed in any group which could be due to the infection not being high enough to cause severe blood loss, thus improvement of blood parameters was not needed. The minor variations observed in PCV indicate a low to moderate parasitic challenge during the periparturient period and have also been observed by Chartier et al. (1998).

FAMACHA© scores have been suggested as an indirect way of evaluating anemia, more specifically *H. contortus* infection, in a flock. Malan et al. (1992), found a correlation between the coloration of eye mucosa, PCV and *H. contortus*. In the present study it was possible to verify a significant correlation between FAM scores, FEC and PCV. These results agree with the findings of Burke et al. (2007) that there was a progressive increase in mean FEC as FAM scores increased in sheep. Scheuerle et al. (2010) observed that PCV and FAM score were significantly negatively correlated and low PCV values were associated with high FEC, which was also observed in this study.

The results observed in this experiment seem to be atypical, since both SL and COWP have been proven to have a beneficial effect in treating GIN infection in sheep. The absence of such an effect has also been observed (Minho, 2006; Pollard, 2009; Watkins, 2003). Even if it was not possible to observe the effects of SL on FEC, Minho (2006) suggested that CT may reduce pasture contamination by decreasing egg viability.

The incorporation of COWP in the SL pellets was neither beneficial nor detrimental in FEC reduction or larvae development. Although Orlik (2010) showed that the use of Copasure® COWP in feed pellets decreased parturient rise in FEC of grazing ewes, the extent of the interaction between SL and COWP in GIN infection could not be measured in this study.

CHAPTER 5

EXPERIMENT 2

5.1. Experimental Design

The F1 ewes used in experiment 1 were randomly divided into two groups, control and treatment. The allocation of the animals into groups was again based on FEC to assure equal parasite loads. Twenty four animals were divided into a control group (n=11) and treatment (SL) group (n=13). This trial was conducted during a 12 week period from June to October, 2009 and each group was kept on a pasture area of 3 acres and rotated weekly. The objective of this trial was to assess the impact of supplementing ewes with SL pellets as a strategic treatment for the reduction of nematode loads in sheep. For this trial all the animals were considered, differing form the first trial were only parturient ewes were targeted. The experimental design was the same as experiment 1, with exception that, during this trial SL pellets were offered without incorporation of Copasure® COWP.

The control group was fed alfalfa pellets (Grainland Selection™) as 25% of their daily intake (1.5 lbs per head/day) and grazed Bermuda and Bahia grass *ad libitum*. The treatment group (sericea lespedeza, SL) was supplement fed SL pellets also as 25% of daily intake (1.5 lbs per head/day) and grazed Bermuda and Bahia grass *ad libitum*. The composition of the alfalfa and SL pellets was the same as previously mentioned in the Experiment 1 (Chapter 4). Both groups were offered free choice mineral (PURINA®) and pure salt (NaCl). The animals were supplement fed as group in the morning before they were allowed to go on the pasture and kept in pens at night to avoid predator attacks.

Fecal egg count (FEC), packed cell volume (PCV) and FAMACHA© scores were determined weekly from each animal in order to monitor parasite infection. Fecal and blood sample collection were obtained as described in Experimental 1 (Chapter 4). FAMACHA© scores were obtained using the same procedure as described in Experiment 1 (Chapter 4) with

the same scope of identifying anemia and the need for spot treatments. Bulk fecal cultures were performed to allow eggs to hatch to L3 larvae for larvae identification and to estimate the relative worm population (Figure 13).

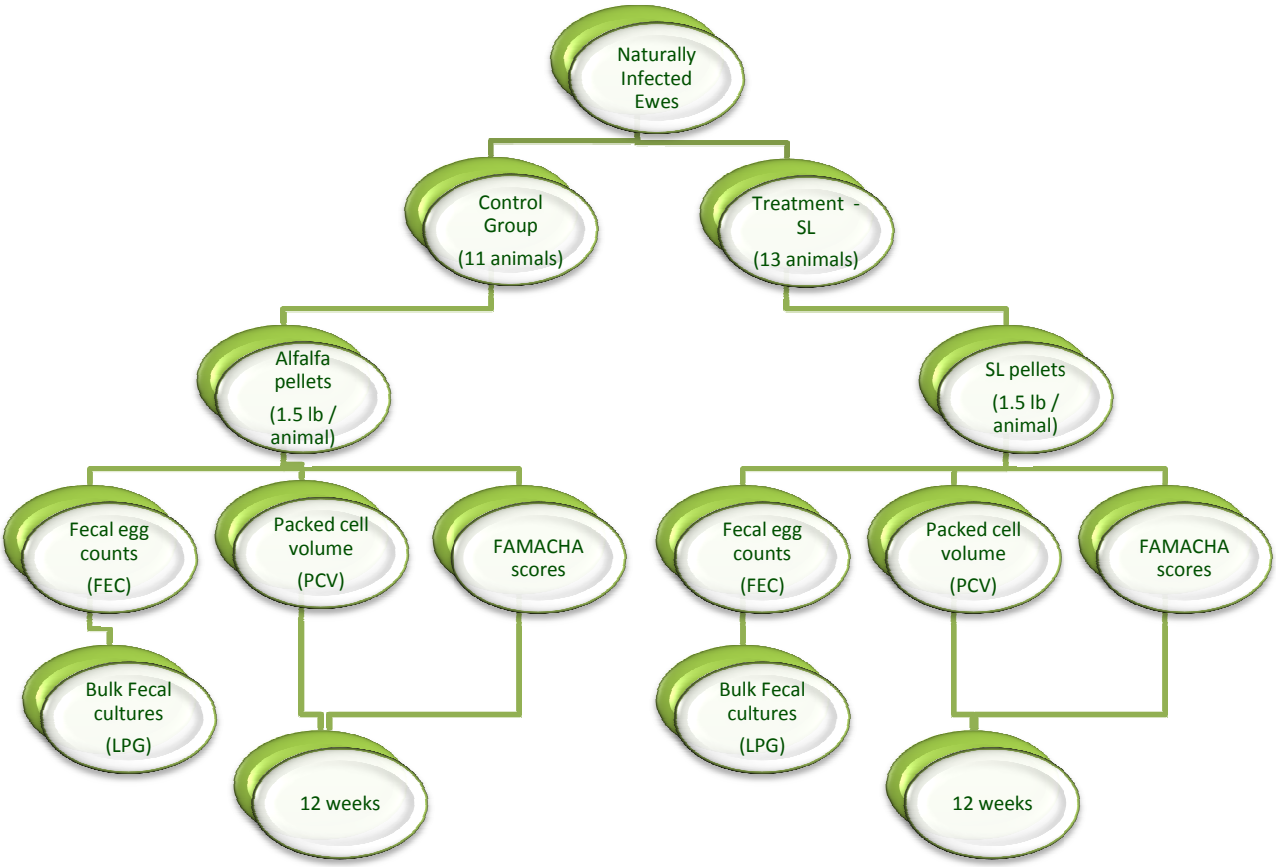

Figure 13. Flow chart of methodology for Experiment 2.

5.2. Results

Fecal egg count percentage reduction was calculated following the same procedure described in Experiment 1 (Chapter 4) (Table 5).

Animals in the SL group had higher FEC than the control group and the only percent reduction observed for this experiment was 60%, which was significantly different between groups (p=0.007) (Figure 14).

Table 5. Mean Fecal egg count (FEC) and FEC percent reduction comparing sericea lespedeza (SL) supplemented and control ewes.

Week	Group		FEC Reduction (%)
	Control	SL	
0	309.09	550.00	0.00
1	363.64	615.38	0.00
2	468.18	707.69	0.00
3	454.55	715.38	0.00
4	468.18	1119.23	0.00
5	655.00	258.33	60.56*
6	218.18	250.00	0.00
7	63.64	165.38	0.00
8	231.82	303.85	0.00
9	300.00	707.69	0.00
10	418.18	1634.62	0.00
11	730.00	1292.31	0.00
12	590.91	2542.31	0.00

* Significant at the 0/05 confidence level.

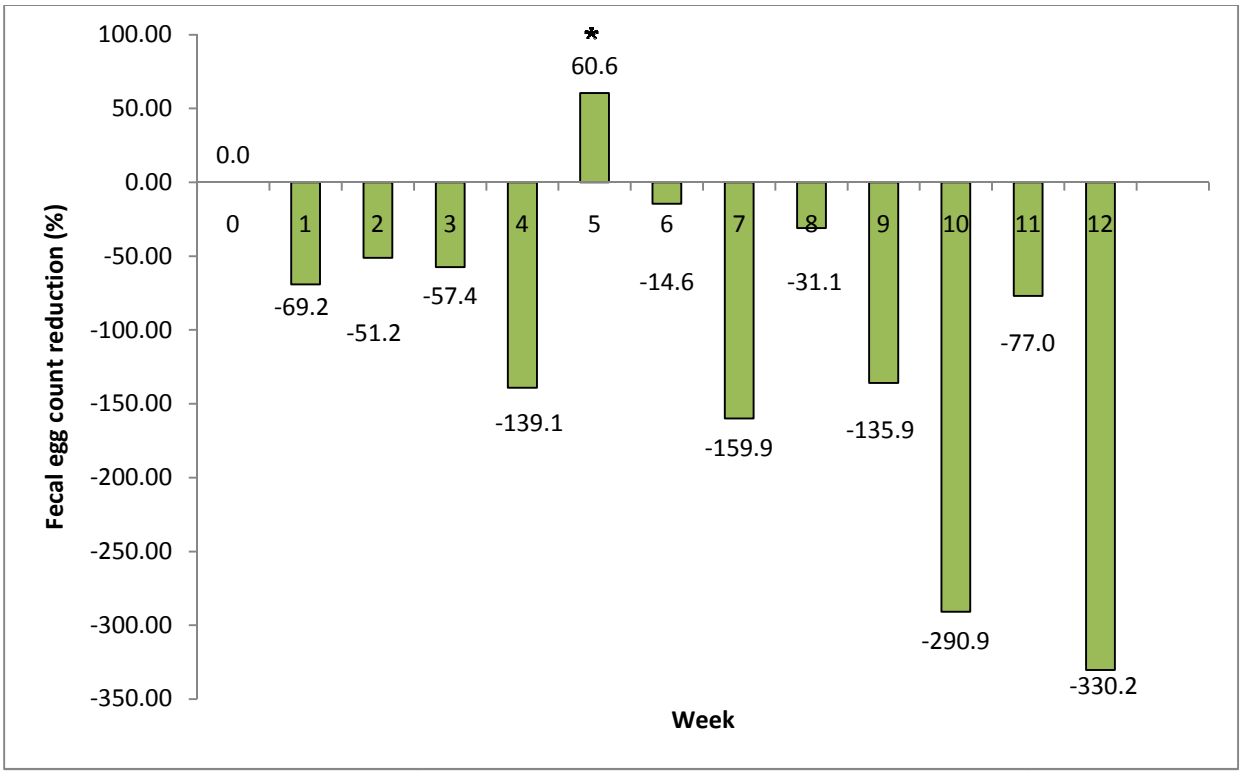

Figure 14. Percent reduction in fecal egg count comparing sericera lespedeza and control groups. *Significant at the 0.05 confidence level.

In this experiment, FECs were also comparatively similar between groups. The mean FEC for the duration of the study was 405.5 EPG (±51.7) for the control and 835.6 EPG (±197.1) for the SL group. There was no difference between groups nor across time (p=0.90) (Figure 15). It was observed that the SL group had two animals that were outliers and thus affected the mean FEC. When those animals were removed from the analysis, the differences between groups were found to be significant (p=0.05) (Figure 15). For statistical purposes, the outliers were kept in their group to maintain the experimental integrity as initially proposed.

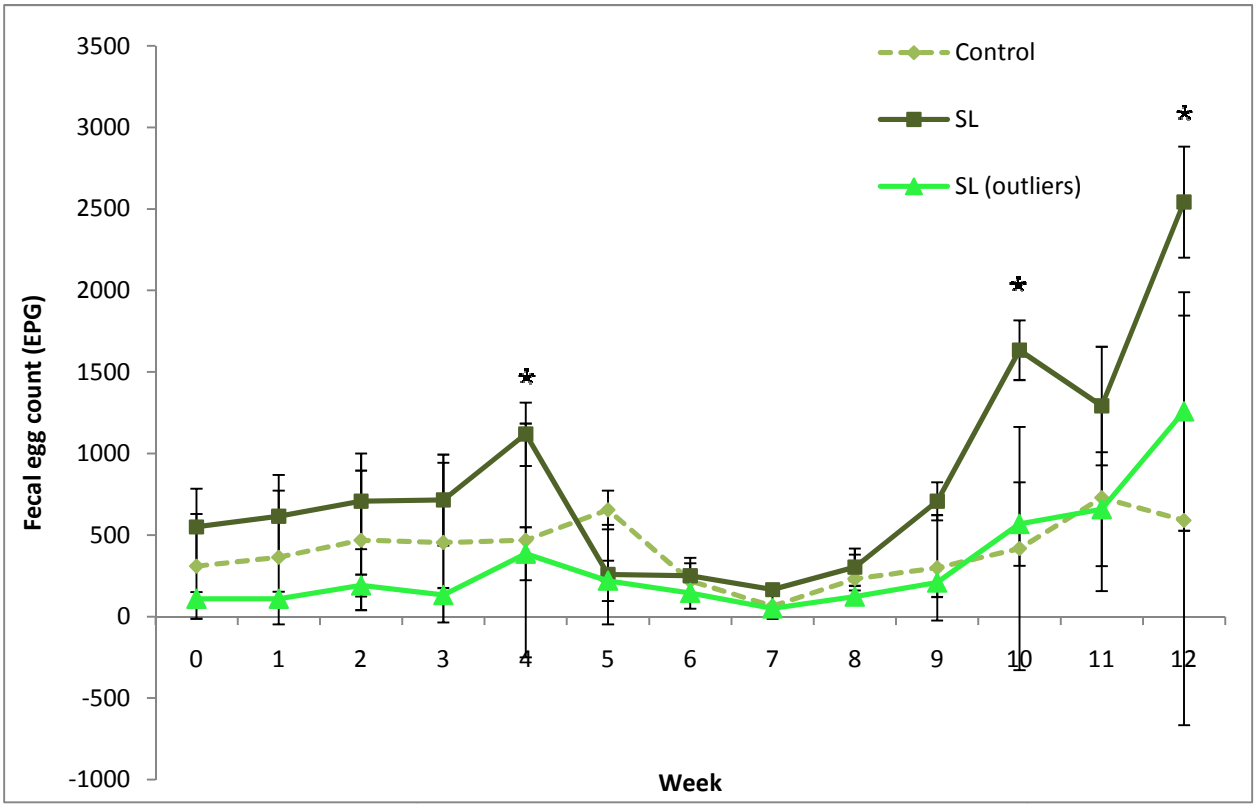

Figure 15. Mean fecal egg count for control and sericea lespedeza (SL) groups (eggs per gram (EPG) ± S.E.M.). No significant differences were observed between or within groups (p=0.90). *Significant differences at the 0.05 confidence level. SL outliers represent the two animals affecting FEC mean. Outliers were kept in their respective groups for all the analysis performed.

The PCV for both groups was also relatively similar. The overall mean PCV for the control group was 27.2 (±0.3) and for the SL group 26.3 (±0.4). The lowest PCV detected was 25.4% for control and 24% for SL. The highest PCVs were 28.5% and 28% for control and SL

groups, respectively. Again, the control group seemed to have a slightly higher PCV than SL, but no effect of treatment on PCV was observed (p=0.72) (Figure 16). One animal in the SL group had a considerably higher FEC throughout the experiment and, due to its low FAMACHA (and PCV below 20%) score, it was necessary to administer an anthelmintic drug. The dewormer used was a combination of levamisole (Levasol®, Schering-Plough, Union, NJ; 8 mg/kg; PO) and albendazole (Valbazen®, Pfizer, New York, NY; 7.5 mg/kg; PO) and it was only necessary to deworm the animal once (Week11).

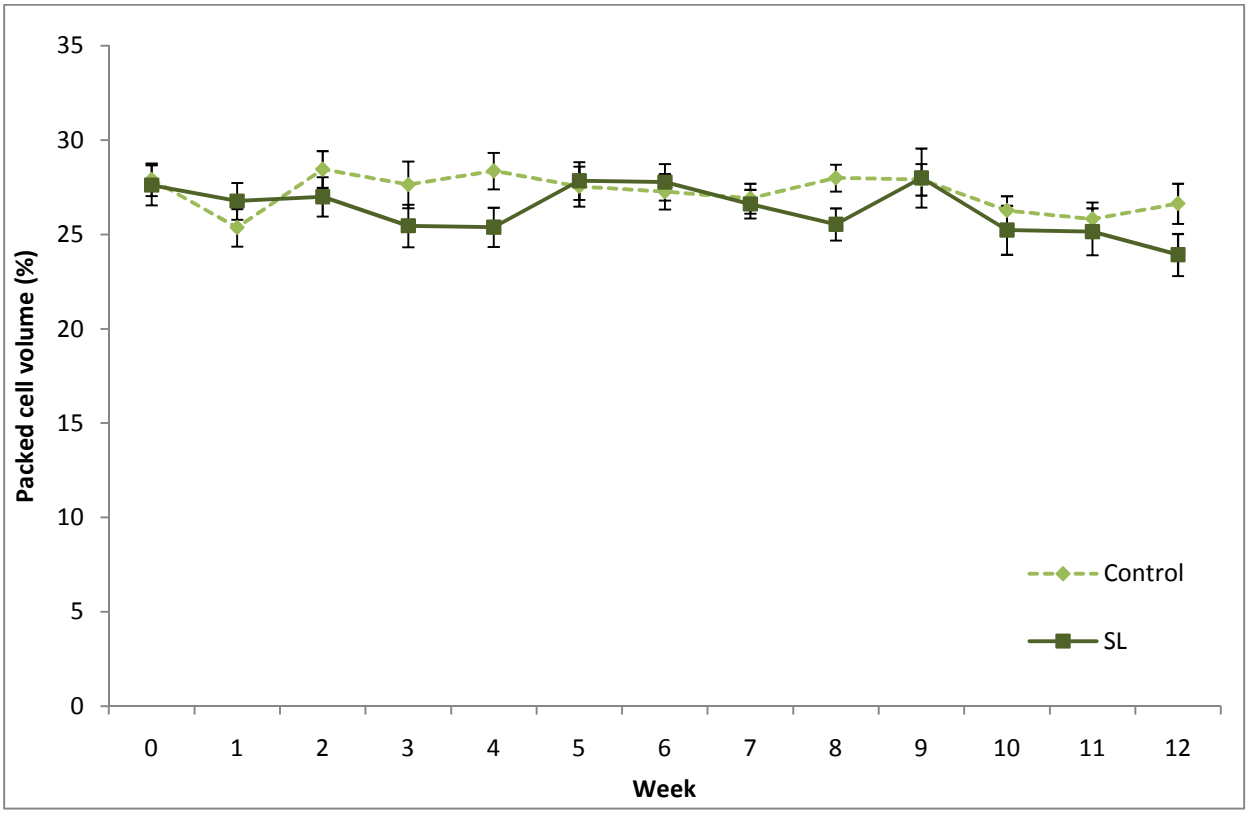

Figure 16. Mean blood packed cell volume comparing sericea lespedeza and control groups (PCV±S.E.M.). No significant differences were observed (p=0.72).

An inverse correlation was observed between FEC and PCV (r^2 = -0.36, p<0.0001), which reflected a decrease in PCV as FEC increased and is explained by the blood feeding behavior of *H. contortus*, one of the GINs of the animals used in this study. A similar correlation

was observed between FAM and PCV (r^2 = -0.48; p<0.0001), and it has been reported that FAM scores tend to increase as PCV decreases. Considering the correlation between FAM and FEC, a positive relationship was observed (r^2 = 0.12, p<0.0001) (Figure 17). The FAM tended to increase with intensification of infection, represented by the FEC.

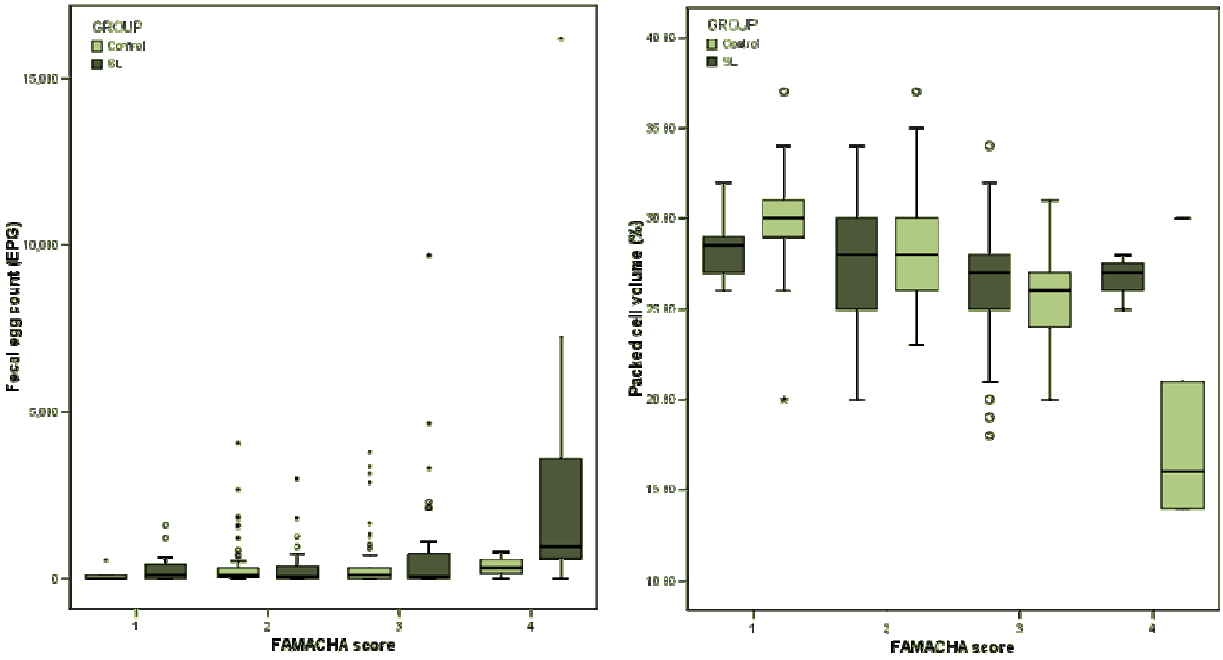

Figure 17. Box plots demonstrating the relationship between fecal egg count (FEC), blood packed cell volume (PCV) and FAMACHA© scores. A - Total FEC (overall) and FAMACHA© scores, B - PCV and FAMACHA© scores. Lower and upper borders of the box represent the 25th and 75th percentiles, respectively. Median (solid line) values are presented within the box. Whiskers above and below the box indicate the maximum and the minimum non-outlier observation. Circles indicate outliers.

The majority of the FAM scores observed were in categories 2 and 3 (Table 6). Thirty seven percent of the PCVs in the control group and 41.4% in the SL group were below the reference values.

Larval development in fecal culture was also comparatively similar between groups. A percent reduction in the number of infective larvae was observed for the SL group in the weeks 3, 7, 8 and 11.

Table 6. Mean (S.E.) fecal egg count (FEC) and packed cell volume (PCV) for each FAMACHA© score (FAM) observed by group (Control and sericea lespedeza, SL).

FAM	FEC						PCV					
	SL			Control			SL			Control		
	n	Mean	S.E.	n	Mean	S.E.	n	Mean	S.E.	n	Mean	S.E.
1	21	276.2	92.8	6	108.3	89.8	21	28	0.4	6	27.5	0.8
2	45	286	83	61	373	92.2	45	28.3	0.5	61	28	0.4
3	48	875	301	73	440	98.4	48	26	0.4	73	27	0.4
4	14	3225	1164	3	366.7	233.3	14	19	1.4	3	26.7	0.9

The greatest reduction observed in larvae development between groups was 95.62%, but the reduction was not significant (p=0.08) (Table 7). The SL group seemed to yield better larvae development than the control group, but no significant differences between groups were observed (p=0.13) (Figure 18). The sample weight of the bulk cultures in both groups were relatively similar with no significant differences (p=0.41).

Table 7. Mean fecal egg count (FEC), larval (L3) count (LPG) and percent larval reduction in fecal cultures from ewes fed sericea lespedeza (SL) pellets (n=13) compared to fecal cultures from control ewes (n=11).

Week	Fecal Egg Count		L3 count		Percent reduction[a]
	SL	Control	SL	Control	
1	117	67	10.90	10.89	0.00
2	667	50	12.78	8.77	0.00
3	150	190	6.91	11.96	42.22
4	317	284	8.60	1.19	0.00
5	334	117	14.77	0.96	0.00
6	334	534	8.97	2.28	0.00
7	84	100	0.04	0.83	95.62
8	100	167	0.78	3.44	77.49
9	84	184	1.33	1.17	0.00
10	334	84	0.68	0.09	0.00
11	84	117	13.69	15.45	11.38
12	534	267	6.51	4.06	0.00
13	217	167	17.89	10.29	0.00

[a] [(control L3 – SL L3)/control L3] × 100.

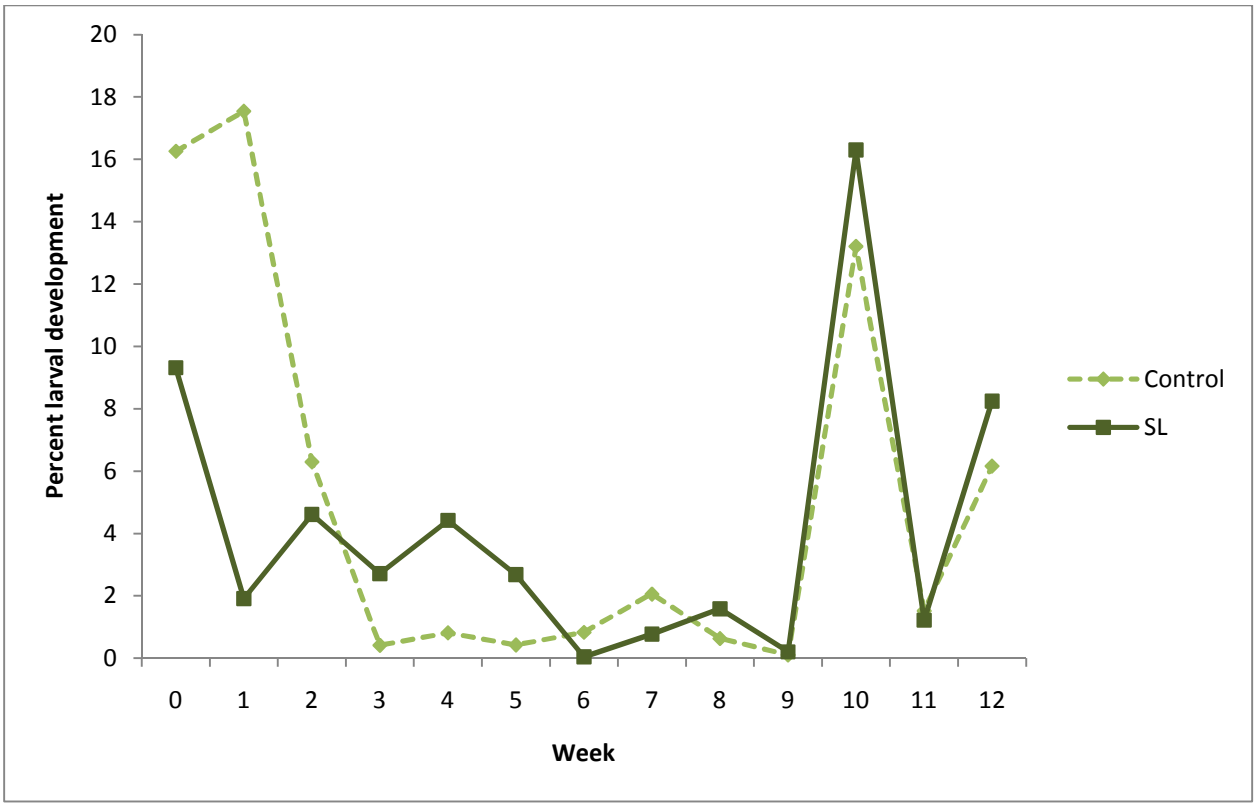

Figure 18. Percentage of larvae development in fecal culture of naturally infected ewes. No significant differences between groups were found (p=0.13).

The bulk culture in the SL group had a slightly higher FEC (258.2±51.5) than the control group (179.1±35.6), but no significant difference between groups was found (p=0.13). There were no significant differences between FEC and sample weight (p=0.14). However, a significant difference was observed between FEC and larvae development (p=0.05).

Overall, *Cooperia spp.* was the predominant nematode identified in the fecal cultures (42.1%), followed by *Haemonchus contortus* (36.2%). *Trichostrongylus spp* and *Oesophagostomum spp.* were also found in smaller proportions (13.3 and 0.3% respectively). When groups were considered separately, it was observed that in the control group, *H. contortus* was the most abundant species (43.6%) followed by *Cooperia spp.* (35.5%) and in the SL group, it was the inverse, *Cooperia spp.* being the most abundant followed by *H. contortus* (48.7 and 28.8% respectively) (Table 8) (Figure 19).

Table 8. Mean percentage of nematode species identified from fecal cultures by group.

Species	Mean percentage of GIN species in each group	
	Control	SL
Haemonchus contortus	43.6	28.8
Trichostrongylus spp.	13.5	13.1
Cooperia spp.	35.5	48.7
Oesophagostomum spp.	0.3	0.2

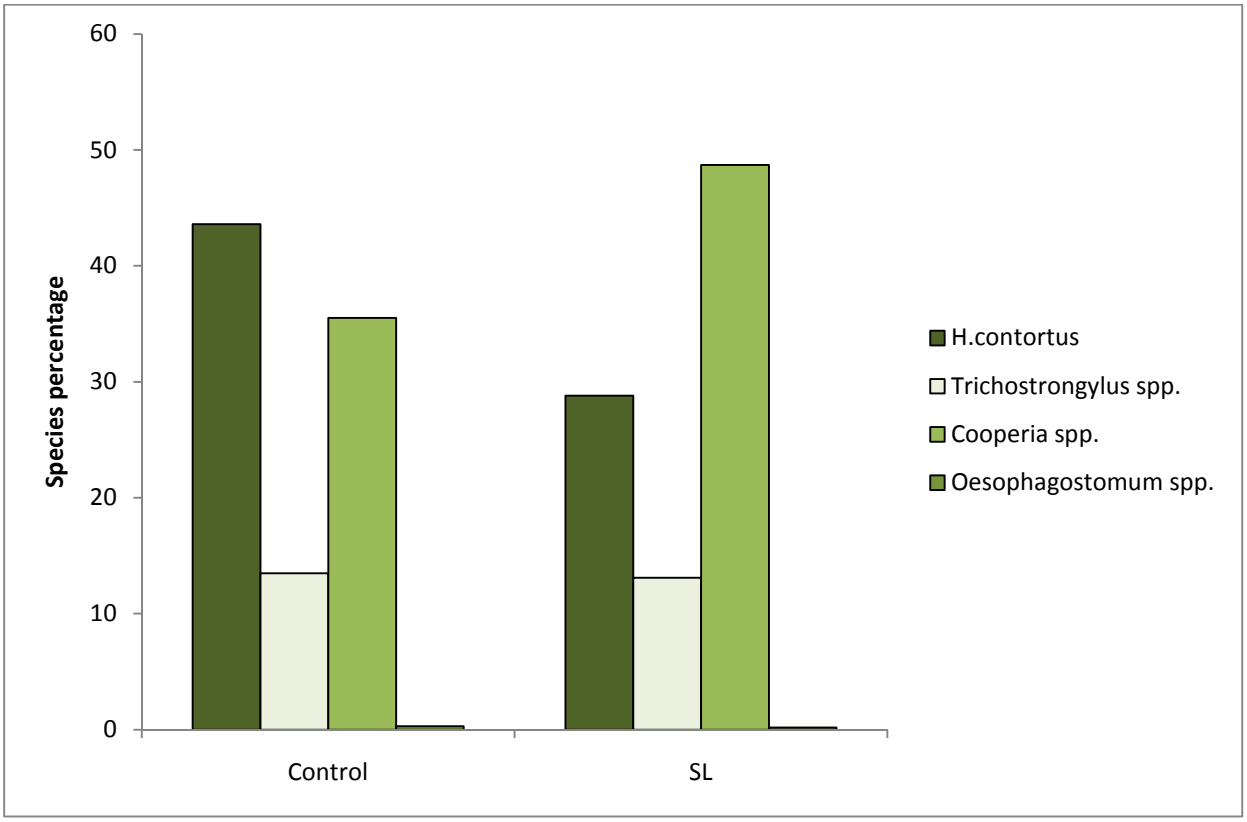

Figure 19. Percentage of infective larvae identified from bulk cultures of naturally infected ewes.

Larval identification showed that there were no significant differences in LPG between groups throughout the study ($p<0.05$). The number of *H. contortus* larvae recovered the SL group was almost 15% less than in the control group at the end of the study. It was possible to observe a shift in larval population in the SL fed group from *H. contortus* to *Cooperia spp* with oscillations in percentage of species recovery throughout the study (Figure 20).

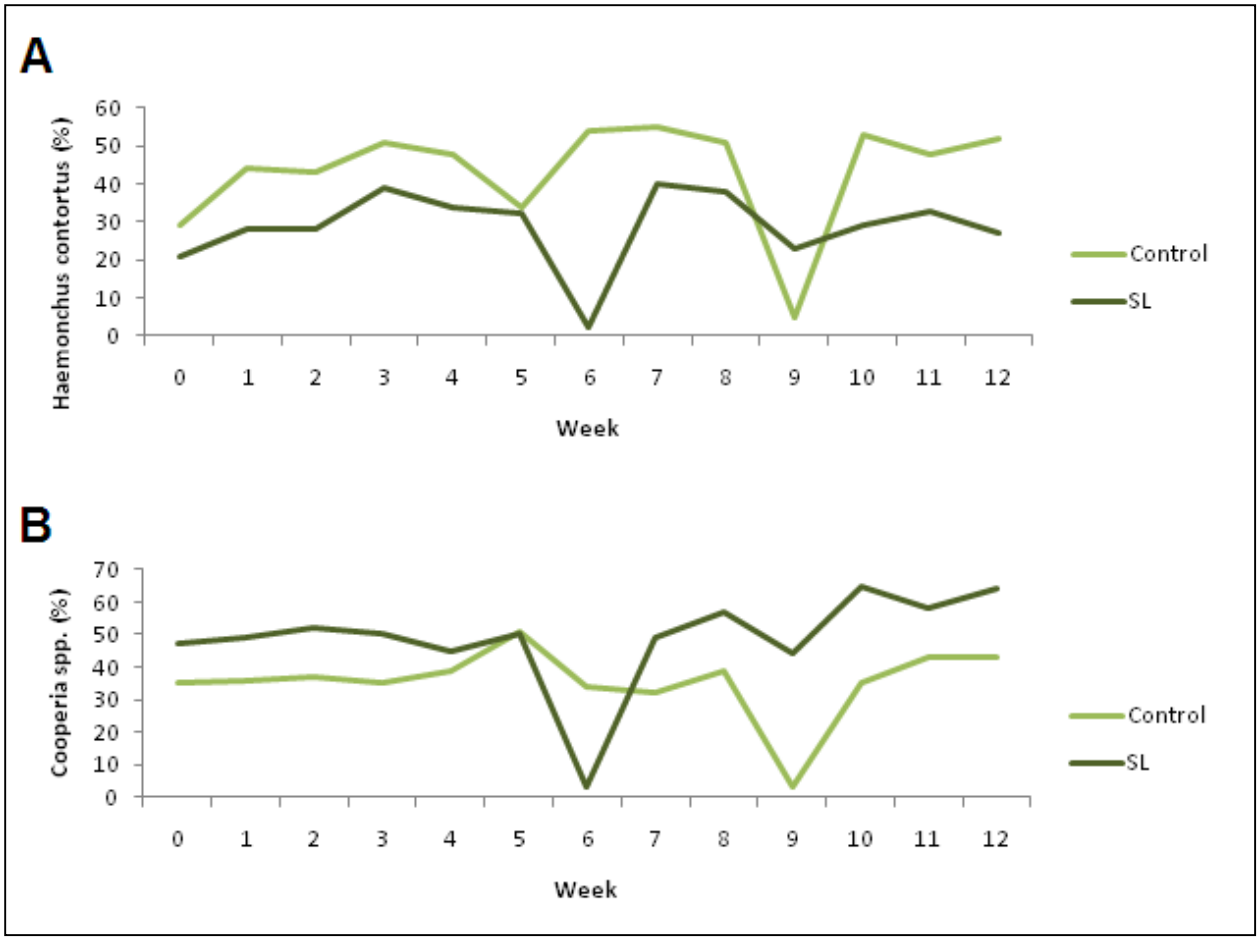

Figure 20. Percentage of infective larvae identified from fecal bulk cultures of naturally infected ewes by group. A – Percentage of *Haemonchus contortus* larvae identified in the sericea lespedeza (SL) and control groups. B – Percentage of *Cooperia spp.* larvae identified in the SL and control groups.

5.3. Discussion

Nutritional manipulation has been suggested as an alternative to improve the resistance and resilience of sheep to GIN infection. Depletion of nutrients, especially protein, can reduce the rate of acquisition and development of immunocompetence against parasitic infection (Coop and Holmes, 1996). The animals participating in this trial were supplemented throughout the study with SL pellets, which is known for its high protein levels (14-15%). As it was observed in the Experiment 1, such supplementation could be the reason behind the lower FECs in that study. However, no differences between SL supplemented and control ewes were observed in

this study, which could be explained by the fact that the alfalfa pellet supplement had equivalent protein level as SL (15%), so the protein effect could be eliminated as interfering with the results and the focus would be directed to the effect of CT on GIN infection. Protein-enriched diets have been shown to be beneficial in resistance to GIN and production in sheep (Kahn et al., 2003) and although this was not the objective of this trial such observation should not be disregarded. Amarante et al. (1998) reported that helminthes are not homogeneously distributed in the flock. In some cases, just a few animals host the majority of GIN and this was clearly observed in this experiment, with the identification of outliers which were affecting the mean FEC.

No significant differences were observed in larval development between groups, which contradict the findings of Minho (2006) that larvae development in fecal culture of sheep treated with CT tend to be 50% less than non-treated sheep. There was however, a tendency of the SL group in having smaller percentages of *H. contortus* in the larvae population throughout the length of the trial.

No significant differences in PCV were observed between SL and control groups and similar findings were reported by Louvandini et al. (2006). They did not find large variations in blood parameters of animals supplemented with or without CT. Similar observations regarding variations in blood parameters and serum constituents of sheep were also reported by Cenci et al. (2007).

The correlations observed between FAM scores and FEC and PCV agree with the current literature about the validation of the FAMACHA© chart as a support tool in alternative parasite control and more importantly, the identification of isolated animals that needed treatment (Ejlertsen et al., 2006; Malan et al., 1992; Molento et al., 2004; Reinecke et al., 2009). In this experiment only one animal required a conventional anthelmintic intervention. This

observation agrees with the report of Sotomaior et al. (2003) that it is possible to reduce the number of animals that need to be dewormed by about 86% by using the FAMACHA© chart.

As it was observed in Experiment1, the results of this trial also seem to be atypical, since SL has been shown to have a beneficial effect in treating GIN infection in sheep. Increasing the number of animals used in the trial or even increasing the length of the experiment would be a way of minimizing the variability observed and perhapes yeild more conclusive results.

CHAPTER 6

EXPERIMENT 3

6.1. Experimental Design

Fifteen Katahdin lambs, 4 months of age, body weight of 67.4 (± 4.1) kg, were randomly divided into three groups as follows: control group (n=5); copper oxide wire particle (COWP) group (n=5), which received a bolus containing 2.0 g of COWP (Copasure®) and $CuSO_4$ group (n=5), which received 4% copper sulfate solution (5.0 ml/kg orally). The objective of this study was to assess the effect of COWP and $CuSO_4$ in reducing *Haemonchus contortus* infection and the potential toxicity in lambs. This trial was conducted during a 16 week period from June to October, 2009. All the lambs in this trial were kept on the same 2.71 acre pasture of Bermuda and Bahia grass and kept at pens at night to avoid the attack of predators. The copper treatments were administered every four weeks.

Fecal egg count (FEC) and blood packed cell volume (PCV) were determined at weekly intervals following the same procedures as described in Chapter 3. A PCV below 19% was used as a cutoff point for strategic chemical deworming. Bulk fecal cultures were performed and larvae were identified as described in Chapter 3. Blood samples were collected a 4 weel intervals for serum separation using 10 ml vacutainer tubes (No Additive, Becton, Dickinson, & Co.) to measure activity of the liver enzyme, aspartate aminotransferase (AST). Animals were also weighed at 4 week intervals to evaluate weight gain.

At the end of the experiment all animals were euthanized and necropsied to collect liver samples and contents from the abomasum, and small and large intestines. The abomasum, small and large intestines were opened and washed individually in separate buckets and wash samples were collected for worm recovery, identification and enumeration. The abomasum was also soaked in water overnight and soaked samples were used for worm recovery and identification. Recovered worms were then identified by genus, gender and stage of

development. The liver samples were submitted to the Louisiana State University School of Veterinary Medicine Toxicology Laboratory to determine copper concentration.

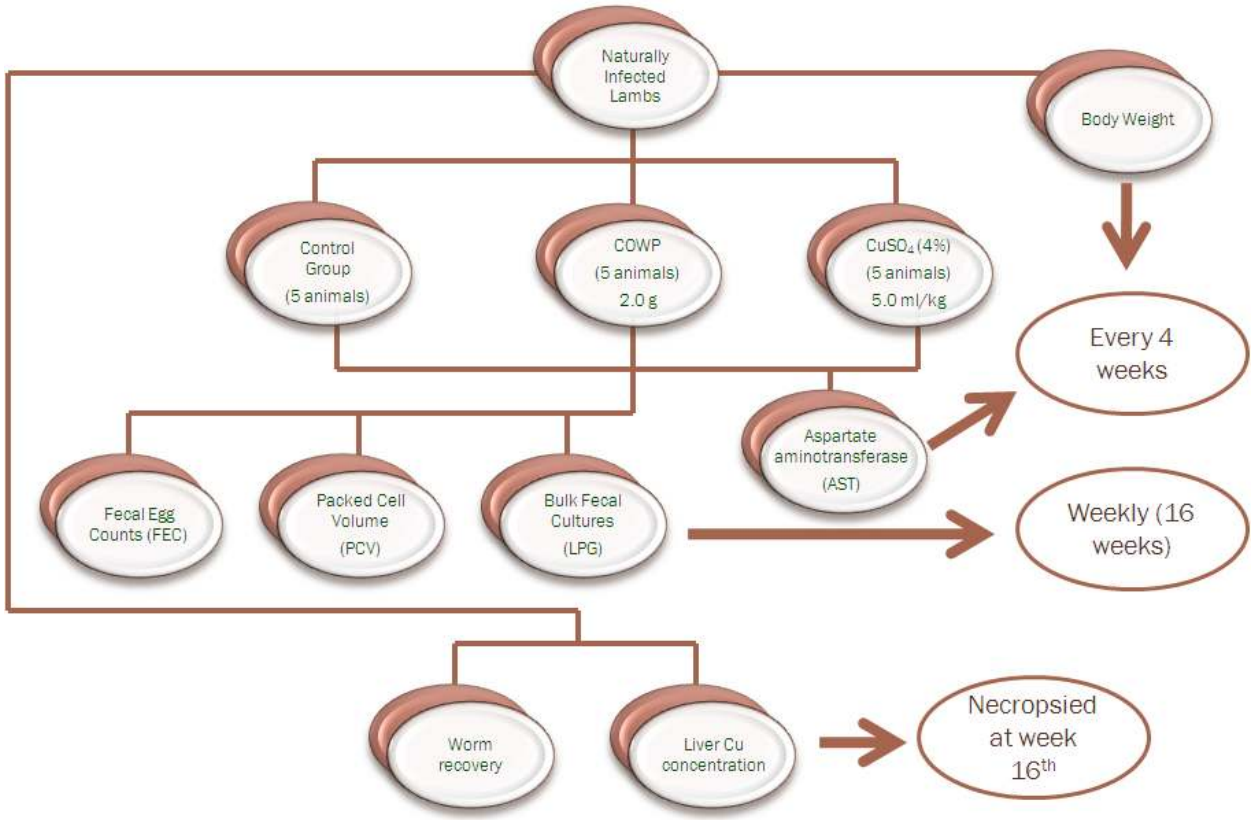

Figure 21. Flow chart of methodology for Experiment 3.

6.2. Results

At the beginning of the experiment, all the animals had relatively similar mean FEC (week 0). From week 1 to the last week (week 16), FEC percentage reduction was calculated using the same procedure as the previous experiments (Chapters 3 and 4, Table 9). At week one of the experiment all the animals showed a very high parasite load and low PCV. To maintain animal welfare and prevent losses they were all treated with pyrantel (Strongid®, Schering-Plough, Union, NJ) at a dose of 8 mg/kg orally and albendazole (Valbazen®, Pfizer, New York, NY) at a dose of 7.5 mg/kg orally. However, even after being treated, one animal from the COWP group died as a result of haemonchosis.

Table 9. Mean fecal egg count (FEC) and FEC percent reduction comparing Control, CuSO4 and copper oxide wire particle (COWP) treatments in naturally infected lambs.

Week	Control	COWP	FEC Reduction (%)	Control	$CuSO_4$	FEC Reduction (%)
0[a]	5190	5140	0	5190	5160	0
1[b]	9600	5090	46.98	9600	6310	34.27
2	690	62.5	90.94	690	700	0
3	2790	387.5	86.11	2790	2670	4.3
4	2890	112.5	96.11*	2890	2860	1.04
5	2450	150	93.88	2450	3480	0
6	4470	287.5	93.57	4470	3250	27.29
7	5090	412.5	91.9	5090	1200	76.42
8	1860	1662.5	10.62	1860	7530	0
9	3700	3125	15.54	3700	9130	0
10	4500	1637.5	63.61	4500	3340	25.78
11	7890	950	87.96	7890	6710	14.96
12	10030	1287.5	87.16	10030	1610	83.95
13[a]	7790	600	92.3	7790	7180	7.83
14	6180	1125	81.8	6180	5080	17.8
15	7420	825	88.88	7420	5070	31.67
16	8130	1175	85.55	8130	1175	85.55*

a Cu treatment administration. COWP group (2.0 g of COWP Copasure®) CuSO4 group (4% of CuSO4 solution). b Dewormed with the combination of PYR/ABZ. * Significant at the 0.05 confidence level.

The COWP group yielded better FEC reductions than the $CuSO_4$ group. The greatest FEC reduction observed between COWP and control was almost 96% (p=0.001), while between the $CuSO_4$ and control, the greatest reduction observed was 85.5% (p=0.001) (Figure 22).

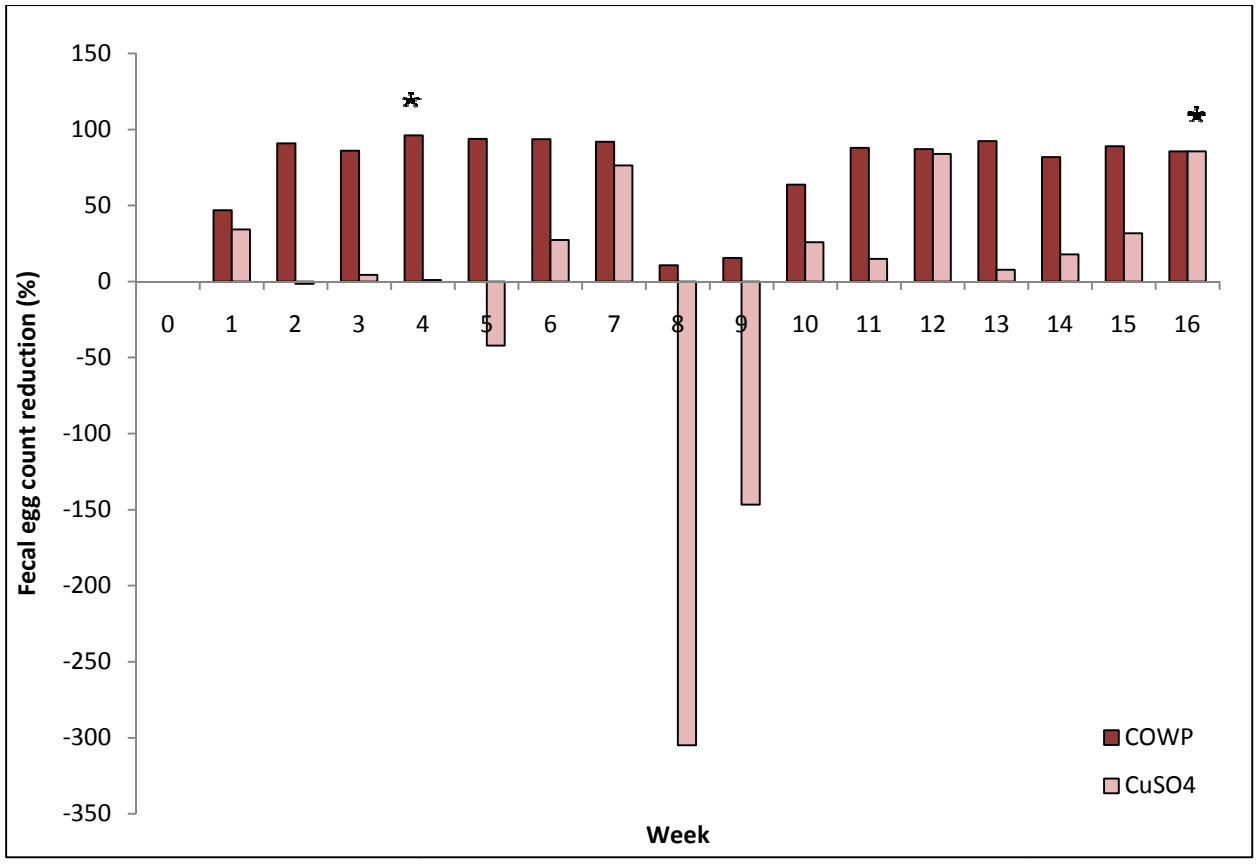

Figure 22. Percent reduction in fecal egg count (FEC) comparing copper oxide wire particles (COWP), $CuSO_4$ and control groups. COWP was administered at weeks 0, 5, 9 and 13. *Significant at the 0.001 confidence level (p<0.001).

A significant difference in FEC was observed between the control and COWP groups (p<0.05) and between COWP and $CuSO_4$ (p<0.05). There was no difference between $CuSO_4$ and control groups (p>0.05). The COWP group maintained relatively lower FEC throughout the study and the differences observed were significant (p<0.05). Overtime, a significant difference between groups was observed on weeks 9, 10, 12 and 14 (p<0.05) (Figure 23).

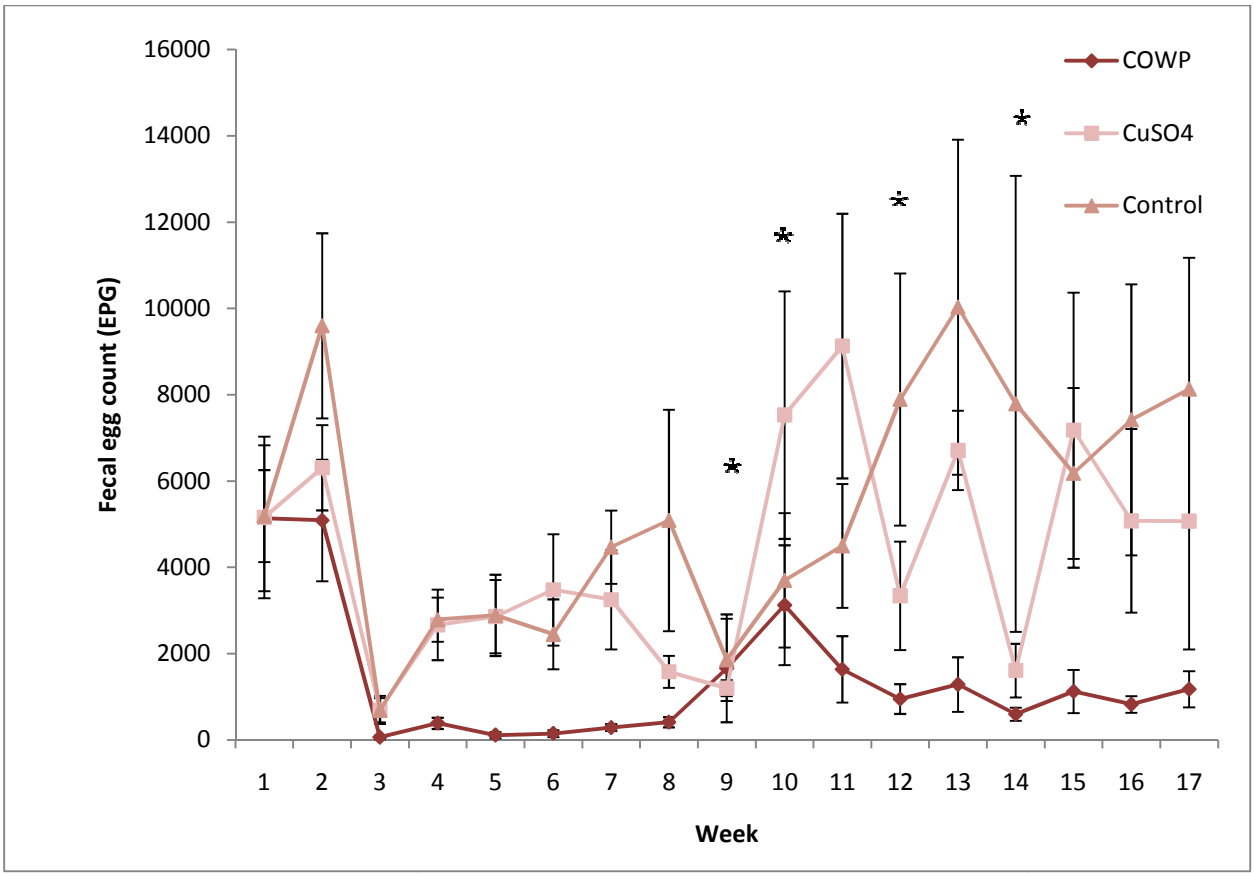

Figure 23. Mean fecal egg count (FEC) for control, copper oxide wire particle (COWP) and $CuSO_4$ groups (EPG ± S.E.M.). *Significant at the 0.05 confidence level ($p<0.05$).

The PCV for the COWP group was higher than all the other groups and the $CuSO_4$ had the lowest PCVs throughout the trial (Figure 24). The mean PCV for the control group was 19.7% (±0.8) with 15.4% and 25.6% being the lowest and highest, respectively. For the COWP group, the mean PCV was 22.8% (±1.0) with 16.6% and 29.5% being the lowest and higher, respectively. For the $CuSO_4$ group, the mean PCV was 17.7% (±0.8) with 12.2% and 22.8% being the lowest and highest, respectively. No significant difference between groups were observed ($p=0.47$) (Figure 24).

All the PCVs during the trial were below the reference limits of ????, Based on the value used to determine if a strategic chemical dewormer was necessary (19%), some animals in the control and $CuSO_4$ groups needed to be treated.

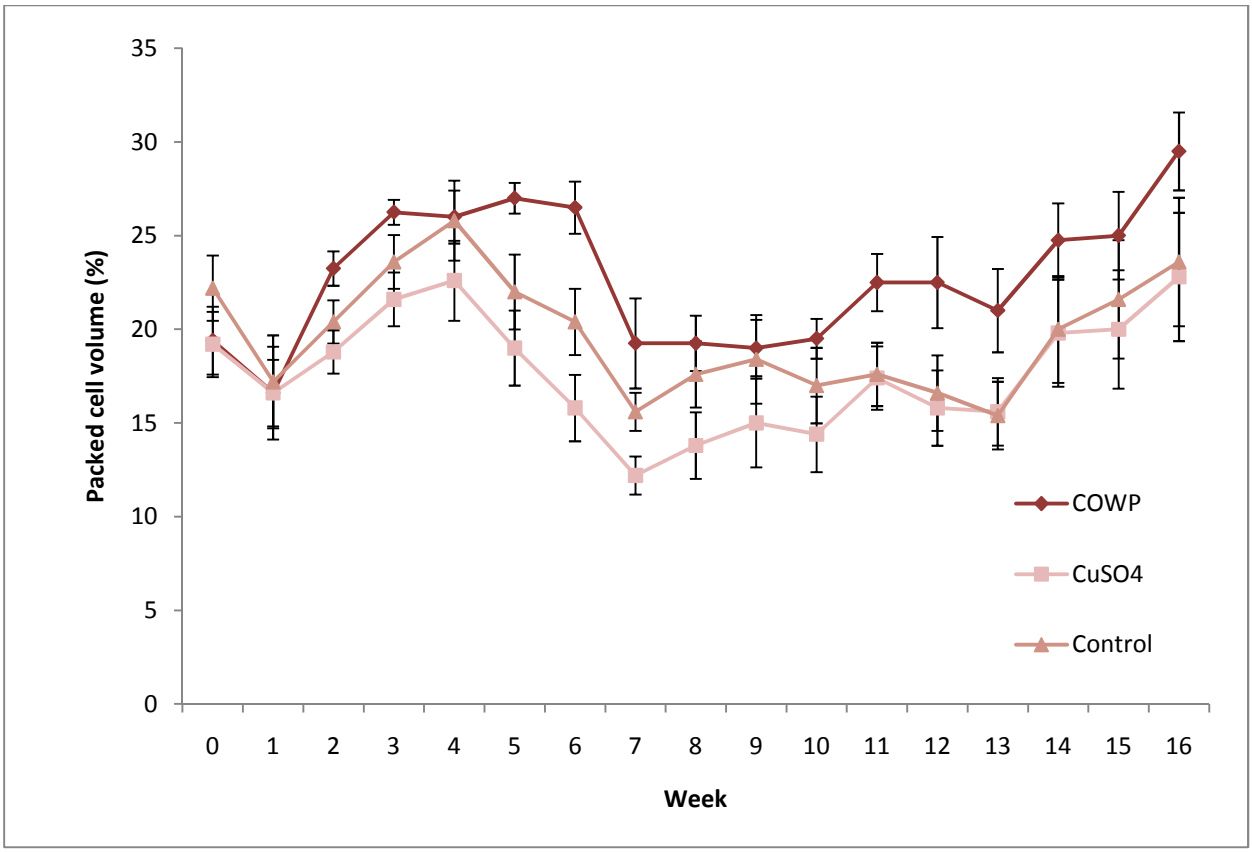

Figure 24. Mean blood packed cell volume (PCV) for control, copper oxide wire particle (COWP) and $CuSO_4$ groups (PCV ± S.E.M.). No significant differences were observed overtime (p=0.47).

A combination of pyrantel (Strongid®, Schering-Plough, Union, NJ; 8 mg/kg PO) and albendazole (Valbazen®, Pfizer, New York, NY; 7.5 mg/kg PO) (PYR/ABZ) was used. A negative correlation was observed between FEC and PCV (r^2 = -0.53, p<0.0001). Although this was a significant correlation, the strength of the correlation was not strong.

With the exception of one animal, all the AST levels were within the normal reference values for sheep (49-123 u/L) (MERCK, 2010). The animal that had a high AST (155 u/L) was in the $CuSO_4$ group, but no differences were observed in AST levels between groups or within animals (p>0.05).

Weight gain for control, COWP and $CuSO_4$ groups was 12.4, 18 and 12.4 kg, respectively. Differences in weight among groups were not significant ($p>0.05$) (Figure 25).

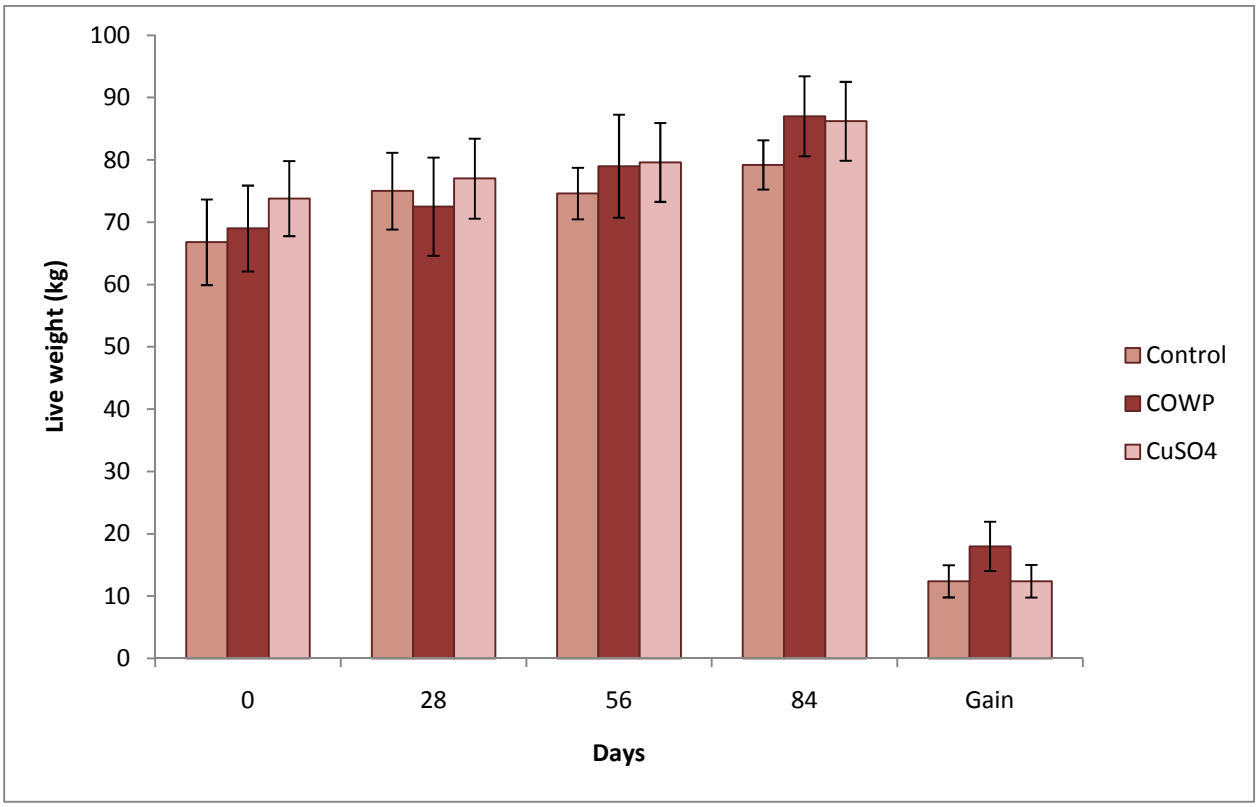

Figure 25. Weight at days 0, 28, 56, 84 and weight gain for control (n=5), copper oxide wire particle (COWP) (n=4) and $CuSO_4$ (n=5) groups. Differences were not significant ($p > 0.05$).

A significant difference in larval development was observed among groups ($p=0.02$). The $CuSO_4$ group yielded a higher percent larval development (11%) than the COWP (0.8%) and control (1%) groups (Figure 26).

Overall, *Cooperia spp.* was the predominant nematode identified in the fecal cultures (43%), followed by *Haemonchus contortus* (30.2%) (Table 10) (Figure 27). *Trichostrongylus spp* and *Oesophagostomum spp.* were also found in smaller proportions (5.2 and 1% respectively). However, when groups were considered separately, it was observed that in the control group, *Cooperia spp.* was the most abundant species (36.5%), followed by *H. contortus* (33.1%).

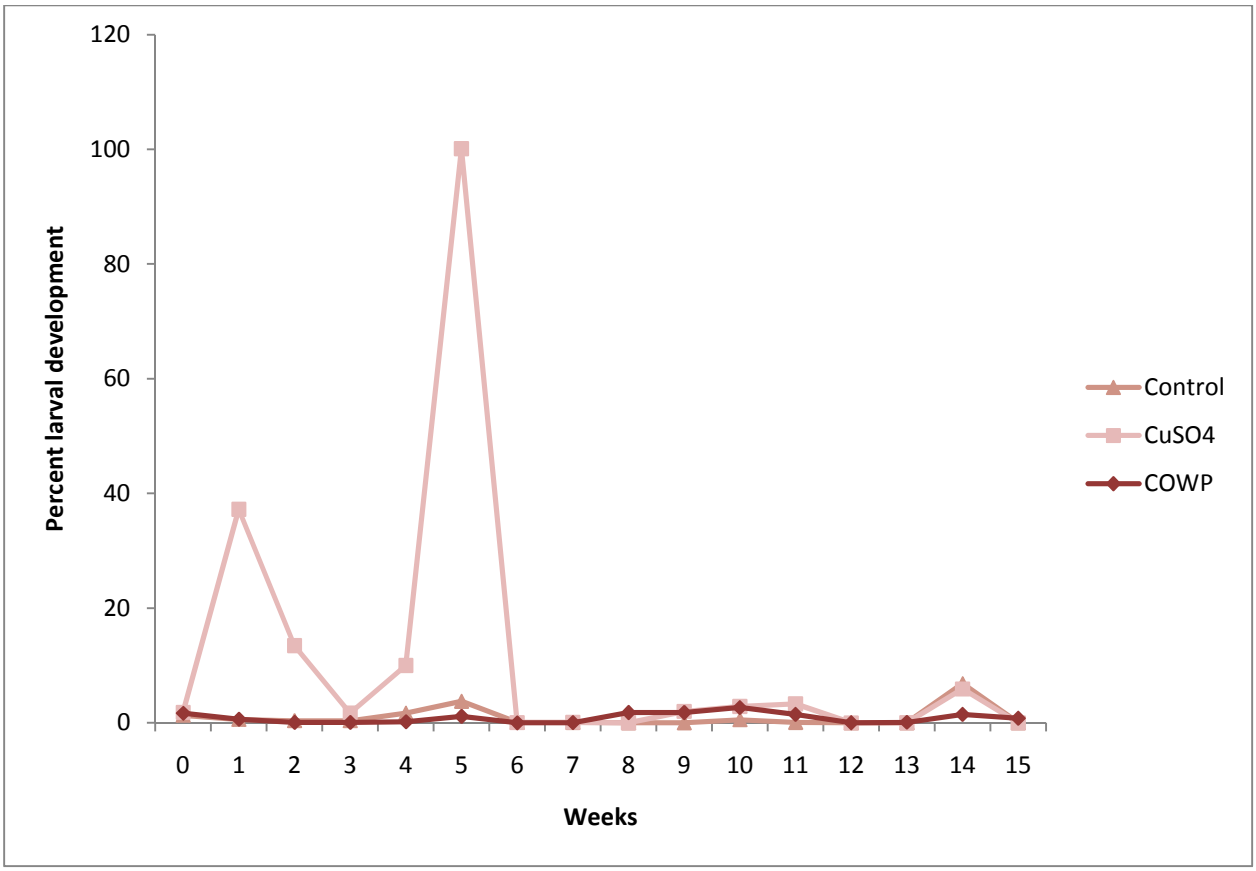

Figure 26. Percentage of larvae development in fecal culture of control, copper oxide wire particle (COWP) and $CuSO_4$ groups. The difference among groups was significant (p=0.02).

In the COWP group *Cooperia spp.* was the most abundant (57%) followed by *H. contortus* (21%) and in the $CuSO_4$ group, C*ooperia spp.* was also the most abundant followed by *H. contortus* (39.6% and 31.6% respectively) (Table 10) (Figure 27).

Table 10. Mean percentage of nematode species identified from fecal cultures of control, copper oxide wire particle (COWP) and $CuSO_4$ groups.

Species	Mean percentage of nematode species		
	Control	$CuSO_4$	COWP
H.contortus	33.1	31.6	21
Trichostrongylus spp.	6.4	4.6	9
Cooperia spp.	36.5	39.6	57
Oesophagostomum spp.	1.1	0.1	13

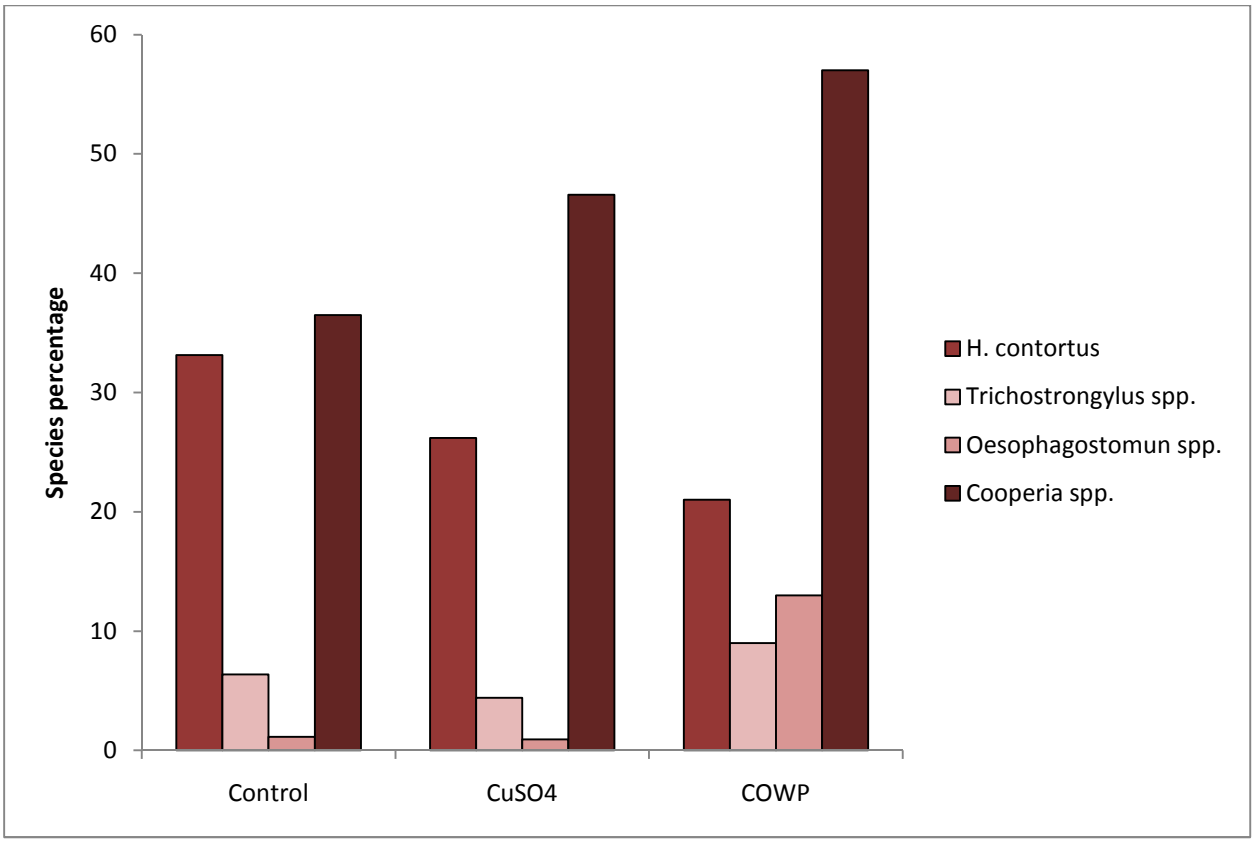

Figure 27. Percentage of infective larvae identified from control, copper oxide wire particle (COWP) and $CuSO_4$ group fecal cultures of naturally infected lambs.

Concentrations of copper in the liver of all lambs were within the normal range (25–500 mg/kg dry matter) (Whitelaw et al., 1982) (Table 11). The COWP group had significantly higher concentration of copper in the liver ($p=0.025$) but the lambs were still within the normal range. Two animals in the control group had very low copper concentration (6.5 and 10.8 mg/kg), which could be considered as a sign of Cu deficiency.

H. contortus was the predominant species recovered from the abomasum (99.6%) and *Cooperia spp.* was the predominant species found in the small intestine (59%) (Table 12). However, other species (*Trichostrongylus spp.*; *Oesophagostomum spp.* and *Trichuris spp.*) were also recovered (Figure 28). There was a significant difference in the total number of worms recovered as well as the number of *H. contortus* ($p<0.005$).

Table 11. Liver copper concentration for control, copper oxide wire particle (COWP) and $CuSO_4$ lambs.

Liver copper concentration					
COWP		$CuSO_4$		Control	
Animal ID	Cu	Animal ID	Cu	Animal ID	Cu
9184	108	9163	38.6	9179	96.5
9178	252	9172	41	9182	6.5
9165	340	9174	43.4	9183	35
9167	273	9176	28.9	9162	56.9
		9177	51.4	9186	10.8

The percentage of *H. contortus* in the control, COWP and $CuSO_4$ groups was 26.8%, 17.5% and 25%, respectively. The highest percent of adults were recovered from the $CuSO_4$ group. The COWP group had the least number of worms (Figure 29). The number of males recovered where higher than the females but there was no significant differences between gender or life stage.

6.3. Discussion

Inorganic copper was originally used for the treatment of copper deficiency in cattle and sheep. In addition it was found to also be an inexpensive approach for nematode treatment (Dewey, 1977; Scott, 2007). $CuSO_4$ was used in the early 1900's and is still used by some today. The percent reduction for $CuSO_4$ was not consistent and seemed to increase slowly after administration, which may indicate that this treatment only provides limited protection. FEC was also the highest for $CuSO_4$. In contrast, COWP treatment resulted in lower and relatively consistent high reduction in FEC. There was essentially very little difference between control and $CuSO_4$ FEC throughout the study and the COWP FEC remained the lowest of the three. Hucker and Yong (1986), demonstrated that sheep supplemented with $CuSO_4$ daily on their feed had a slow rise in FEC throughout the trial. The use of $CuSO_4$ mixed with a daily feed supplement to control *H. contortus* infection in goat kids was not effective (Burke et al., 2008). Therefore, $CuSO_4$ treatment has not demonstrated very efficient control.

Table 12. Nematode recovery from control, copper oxide wire particle (COWP) and $CuSO_4$ groups.

	H. contortus					
Group	Adult m	Adult f	L5m	L5 f	L4 m	L4 f
Control	131	83	47	97	65	131
COWP	102	62	35	86	14	63
$CuSO_4$	167	118	22	83	51	68

	Trichostrongylus spp.					
Group	Adult m	Adult f	L5 m	L5 f	L4 m	L4 f
Control	13	16	0	0	0	0
COWP	9	3	0	0	0	2
$CuSO_4$	24	18	0	1	0	0

	Cooperia spp.					
Group	Adult m	Adult f	L5 m	L5 f	L4 m	L4 f
Control	29	22	5	6	2	5
COWP	28	32	1	6	0	0
$CuSO_4$	38	45	3	9	0	2

	Oesophagostomum spp.	
Group	Adult m	Adult f
Control	0	0
COWP	0	6
$CuSO_4$	0	1

	Trichuris spp.	
Group	Adult m	Adult f
Control	1	5
COWP	5	3
$CuSO_4$	0	0

m = male; f = female;
L3 recovered (Control = 136; COWP = 31; $CuSO_4$ = 159).

The decreased larval development in cultured feces and fewer number of worms recovered from the COWP group suggests that COWP is a good choice in the treatment of GIN infections, especially if *H. contortus* is the main parasite present. Copper sulfate may also be somewhat beneficial if COWP are not available.

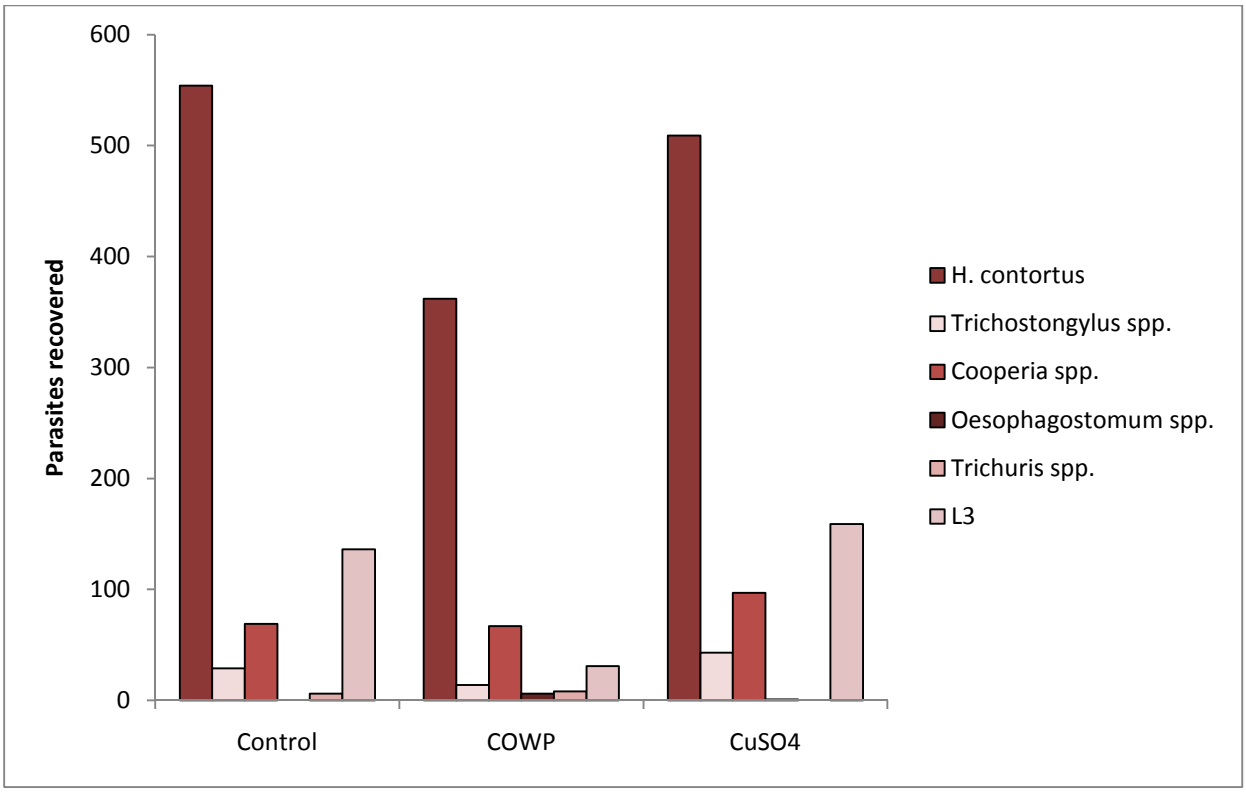

Figure 28. Total number of nematodes recovered from control, copper oxide wire particle (COWP) and $CuSO_4$ groups by species.

In this trial COWP and $CuSO_4$ reduced the infection level of *H. contortus* (more so for COWP) as evidenced by reduced FEC, and also reducing larvae development in cultured feces. One of the advantages of COWP over $CuSO_4$ is that it is usually administered in gelatin capsules, so COWP is delivered to the rumen and the released particles are transported to the abomasum where they adhere to the mucosa. The acidic environment eludes Cu ions which directly affect and to reduce *H. contortus* infection based on FEC by up to 96% (Bang et al., 1990). Studies using doses as low as 0.5 g or as high as 10 g showed reduction in the infection caused by *H. contortus* without causing toxicity in sheep and lambs (Burke at al., 2004; Burke and Miller, 2006; Burke et al., 2007; Burke et al., 2010; Knox, 2002). The use of inorganic copper has also been shown to be effective when combined with other alternative strategies, for example the nematode-trapping fungus, as demonstrated by Burke et al (2005).

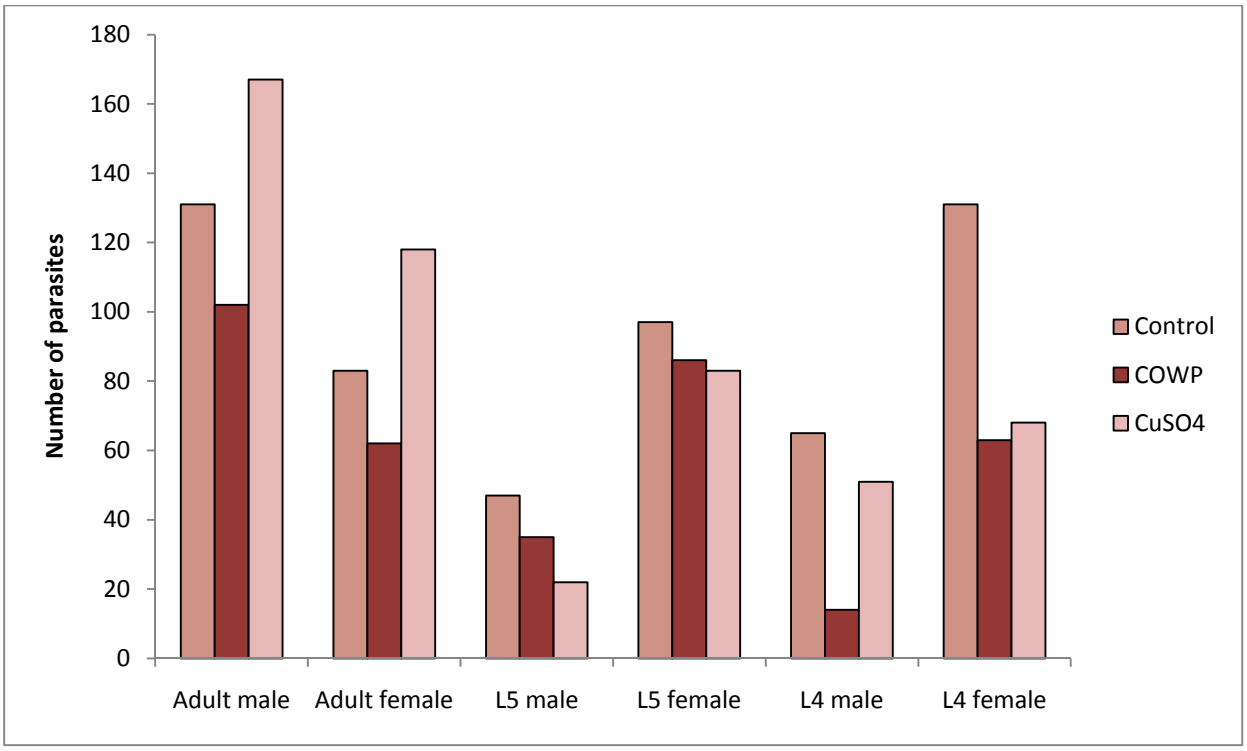

Figure 29. Number of *H. contortus* recovered by stage and gender from control, copper oxide wire particle (COWP and $CuSO_4$ groups.

Studies have measured the reduction of *H. contortus* burden by parasite recovery at necropsy and found an average percent reduction of 66% in adult worms (Bang et al., 1990; Chartier et al., 2000; Knox, 2002). In this study COWP had the lowest number of recovered worms recovered and the percentage of *H. contortus* was also significantly less than the other groups. There were also fewer numbers of female worms compared with the number of males with significant differences between groups.

Due to sheep sensitivity to Cu toxicity, it is necessary to determine the effect of repeated doses on the overall health of sheep. Aspartate aminotransferase in serum was used to measure changes in liver function that may be due to Cu accumulation. There was no significant difference in AST activity among groups and all were within normal limits. Similar findings were reported by Burke et al. (2007). Other studies have shown that doses of 4 g for adult sheep and 2 g for lambs were effective and repeated doses at 4 week intervals did not result in Cu levels

out of normal range (Burke and Miller, 2006; Burke et al., 2007). In one study, no differences were observed in liver Cu concentration among groups, but animals treated with COWP had greater concentrations that remained within the normal limits (Burke et al., 2006). Burke & Miller (2005) also reported a negative effect in the offspring (reduced weaning weights) of ewes treated with COWP.

In this study, the COWP lambs weighed more at the end and gained more, but overall there were no differences among groups. This observation agrees with that observed by Burke et al. (2007) where there was no difference in weight of lambs treated with 0.5 or 1 g of COWP. Monitoring production is important for these kinds of studies. It is expected that the effect is positive or at least a neutral and not negative.

In conclusion, there was an advantage of using inorganic copper, especially COWP, to treat lambs with natural GIN infection and the dosage used under the conditions of this study was safe regarding toxicity.

CHAPTER SEVEN

SUMMARY AND CONCLUSIONS

7.1. Summary

A balanced diet enhances an animal's immunity and overall health. When affected by parasites, the most significant nutritional disturbance occurs in protein metabolism and mineral absorption and retention. Supplementation of animals has been reported as a way to minimize the effects of GIN infections. Although there was no significant differences in FEC between SL supplemented and control animals, there was a tendency for SL supplemented ewes to have lower FEC. The supplementation with SL pellets could be a reason for the lower FECs observed in the two trials. . There were also no significant differences in larval development, but it there was a trend of *H. contortus* diminishing towards the last weeks of the experiment. A shift in parasite population from *H. contortus* to *Cooperia spp.* was also observed and such findings may be indicative of SL action against *H. contortus*. The shift in the population may also contribute in reducing pasture contamination with *H. contortus*.

The results observed using SL as supplement are not what was expected, considering the amount of literature that shows a benefit to using this forage as an alternative approach in controlling GIN in sheep. But, there is also similar results reported as well. So, it appears that the use of SL supplement may have limited value.

The trial using inorganic copper showed more promising results with not only a reduction in FEC, but also in larval development and worms recovered at necropsy. Copper sulfate had the smallest reduction in parasite load and the reductions seemed to happen after administration of copper. The group treated with COWP had the greatest and consistent parasite load reduction throughout the study. Both forms of copper (COWP more so than CuSO4) appear to be beneficial and safe in reducing parasite burden. Sheep are known to be

more susceptible to copper toxicity than other species, but in this trial no signs of copper toxicity or deficiency were observed.

The use of COWP was the most effective approach to control GIN in that infection was kept low, and the effect was prolonged for at least four weeks, when the next treatment was administered. The practicability of this approach should be considered.

7.2. Conclusions

The use of CT in the control of endoparasitic infections is still controversial due to the lack of information about its specific mode of action. But the use of CT in the control of GIN should not be discarded especially in areas where the use of anthelmintics is no longer possible.

Inorganic copper has been shown to reduce GIN infection and its concomitant use with other control methods may represent a positively useful implement for producers in controlling parasites as well as reducing the dependency on conventional anthelmintics. Resistance has been often reported and the use of copper products should be contemplated as an effective and cheap way to control GIN infection until more efficient commercial products become available.

The practical strategy to control anthelmintic resistance relies in diminishing the utilization of chemical drugs, which have been shown to develop resistance at some point. It is necessary to search and utilize non-chemical approaches in the treatment of GIN infections, but if the use of anthelmintic drugs is necessary, its utilization should be done in a non-indiscriminant manner.

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