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## **Integrated Methods For Controlling Gastrointestinal Nematode Infections in Ewes and Lambs**

Sarah Tammy Nicole Keeton

*Louisiana State University and Agricultural and Mechanical College*

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INTEGRATED METHODS FOR CONTROLLING GASTROINTESTINAL  
NEMATODE INFECTIONS IN EWES AND LAMBS

A Dissertation

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

in

The Interdepartmental Program in the School of Animal Science

by  
Sarah T. Keeton  
B.S., Southeastern Louisiana University, 2007  
M.S., Louisiana State University, 2010  
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To my little sister, Dr. Mandy Orlik

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## ABSTRACT

Gastrointestinal nematode (GIN) parasites cause extensive damage to small ruminants, and *Haemonchus contortus* is a major concern to production worldwide. With the development of GIN resistance to anthelmintics, alternative strategies for control are needed. The major component of this study compared two strategies for effect on animal health and changes in GIN population. One flock of ewes and lambs (Strategy 1, S1) employed targeted selective treatment (TST) with an anthelmintic based on FAMACHA score, and one flock (Strategy 2, S2) employed sericea lespedeza (SL) supplement feeding and TST with copper oxide wire particles (COWP) based on FAMACHA score.

S1 ewes/lambs were supplement fed a concentrate ration and were dewormed with levamisole/albendazole combination when FAMACHA© was 4/5. The S2 ewes/lambs were supplemented with SL pellets and were dewormed with COWP when FAMACHA© was 4/5.

FEC for S2 ewes/lambs remained consistently lower and PVC higher than for the S1 ewes/lambs. There were fewer dewormings for S2 lambs than S1 lambs and no ewes needed to be dewormed. Weight gain for S2 lambs was consistently less than S1 lambs. It was also noted that S2 lambs had less coccidia than S1 lambs.

To follow up on a possible reason for the poorer production of S2 lambs, a trace mineral panel was done on the ewes/lambs and it was noted that S2 animals were very deficient in molybdenum (Mo). A study was done to evaluate Mo supplementation on weight gain and results indicated a slight improvement. Upon supplementation with Mo, Mn, Se and Zn serum concentrations of all increased but did not achieve normal values nor did it impact weight.

S2 lambs had fewer coccidia counts than S1 lambs. Control lambs received Purina® Honor® Show Lamb™ and treatment lambs were fed SL pellets. Lambs were inoculated with

50,000 oocyst over a 3 day period at the beginning of the study. Lambs in the treatment group had lower FEC and coccidian counts than the lambs in the control group. SL was effective in the prevention and control of coccidiosis as well as in reducing GIN infection.

## **CHAPTER 1**

### **INTRODUCTION**

Sheep play an important role in agricultural production globally. Although sheep and lamb numbers today are comparable to the numbers in the 1960's, production has increased worldwide. Sheep production on a global scale has increased from 11 billion pounds in 1965, to 18 billion pounds in 2011. The largest producers can be found in China, the European Union, Australia, and New Zealand respectively (Brester, 2012). In the United States (U.S.), the main sheep production systems are either for meat, wool production, or dual purpose. Sheep production in the U.S. has declined, as it has become economically unfeasible to raise sheep for wool. Producers in the meat production system sell lambs either for slaughter or as feeder lambs (Schoenian, 2013). Sheep also make an impact on local economies where the market focus is on “organic” meat production. In 2008, there were over 11,200 certified organic sheep and lambs, with 1.8 million acres of “organic” specified pasture and rangeland (Brester, 2012).

Gastrointestinal nematode (GIN) parasitism is a major constraint to sheep production worldwide. Across the southeastern U.S., GIN parasitism is relatively high due to advantageous environmental conditions for development and survival of the free-living GIN stages (Soli et al., 2010; Miller et al., 2011; Vatta et al., 2012). GIN have caused production losses due to cost of prophylaxis, cost of treatment, and loss in production (Urquhart et al., 2007). Heavily infected animals are less likely to do well, which results in less wool and/or meat for market. Losses due to GIN parasitism are thought to extend into millions of dollars per year. It is difficult to accurately assess these losses, because there are many factors to consider including nutrition, environmental stress, genetics, management, and concurrent disease. There is no published data obtainable in the U.S. on the estimated dollar value of annual losses, however, there is information available from other countries. For example, in Australia, the cost related to

parasitic diseases in sheep and cattle has been estimated at one billion dollars annually, according to pharmaceutical sales (Roeber et al., 2013). In Great Britain, estimated cost is approximately 84 million pounds annually, due to cost of prevention, cost of treatment, and losses in production (Niewhol and Bishop, 2005). Losses in production and anthelmintic use in New Zealand is estimated at \$300 million a year (West et al., 2002).

The GINs of concern for sheep include *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus colubriformis*, *Trichostrongylus axei*, *Nematodirus spp.*, and *Cooperia spp.* However, *H. contortus*, commonly called the barberpole worm, is considered the most pathogenic, because it feeds on blood in the abomasum leading to severe anemia and can cause death. Infected sheep can also experience mucosal pathology, which results in impaired digestive processes, and the consequent loss of protein and fluids, causing diarrhea. It is most problematic in the summer into early fall, and can also cause disease in spring when inhibited L4 larvae remerge (Menzies, 2010).

*T. circumcincta*, commonly called the brown stomach worm, is also found in the abomasum. *T. circumcincta* causes the most complications in the cooler, wetter parts of the United States (Schoenian, 2013). *T. axei*, another abomasal nematode, along with the small intestinal nematodes *T. colubriformis*, *Cooperia spp.*, and *Nematodirus spp.* are considered less pathogenic, but contribute to the overall problem.

Control of GIN in sheep is crucial in maintaining a productive and profitable operation. The most common method of control has historically been the use of anthelmintics. When anthelmintics are effective, better production and body condition can be expected, particularly in young growing animals. Anthelmintics, available in the U.S. for small ruminants, are categorized into three classes. The benzimidazoles, commonly called white drenches, are

thiabendazole (TBZ®) fenbendazole (Safeguard® and Panacur®), albendazole (Valbazen®), and oxfendazole (Synanthic®). The nicotinic antagonists (imidazothiazoles, tetrahydropyrimidines) are levamisole (Levisole®, Tramisol®, and Prohibit®), morantel tartrate (Rumatel® and Nematel®) and pyrantel tartrate (Strongid®). The macrocyclic lactones (avermectins, milbemycins), commonly called “mectins”, are ivermectin (Ivomec®), doramectin (Dectomax®), eprinomectin (Eprinex® and LongRange®), and moxidectin (Cydectin® and Quest®). All of these modern anthelmintics are considered safe, and have a wide spectrum of activity. The “mectins” are known for their “persistent-activity” that continues to kill worms for an extended period after administration (Schoenian, 2013).

The constant reliance on anthelmintics over the past several decades has led to GIN populations that have developed resistance, which has been recognized worldwide (Waller, 1994; Terrill et al., 2001; Kaplan, 2004; Burke et al., 2010; Kaplan and Vidyashankar, 2012). *H. contortus* is the most prominent GIN showing resistance (Saddigi et al., 2011; Bielek, 2014; Kaplan, 2016). As a result of repeated and frequent anthelmintic administration pressure, resistant alleles have been passed on. This results in increased pasture contamination with resistant infective larvae, and over time the susceptible larval population becomes more resistant (Papadopoulos, 2008). Resistance to all major classes of anthelmintics has been reported in South Africa and the southeastern US (Vatta and Lindberg, 2006; Howell et al., 2008).

Due to the high level of resistance, alternative methods of control are essential to maintain profitable small ruminant production. Alternative methods and integrated strategies could help to decrease reliance on anthelmintics, which can lead to more maintainable parasite management. Alternative methods include vaccines, breeding for resistance, nematode trapping fungi, copper oxide wire particles (COWP), condensed tannin containing plants, targeted

selective treatment with an anthelmintic (smart drenching), the FAMACHA System©, and pasture management.

The objective of this study was to examine the impact of 2 control strategies in a long-term 3-year experiment. It was to: 1) address possible problems of what occurs in a farm system outside of the short term period in which component research studies have reported previously and 2) examine the long term use of combinations of strategies on flock productivity, animal health, changes in GIN population, and changes in the environment. A systems approach more closely resembles what producers would encounter on farm.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Gastrointestinal Parasites

GIN are the most important group of parasites affecting ruminants worldwide. These nematodes cause substantial production losses, decrease in overall animal health, and death. These parasites include *Haemonchus spp.*, *Cooperia spp.*, *Trichostrongylus spp.*, *Teladorsagia spp.*, and *Nematodirus spp.* These GIN have a direct life cycle, meaning that no intermediate host is necessary for the completion of the life cycle. Of these parasites *H. contortus* is the most problematic.

#### 2.2 *Haemonchus contortus*

*H. contortus* belongs to the Kingdom Animalia, Phylum Nematoda, Class Secernentea, Subclass Rhabditia, Order Strongylida, and Family Trichostrongyloidea. *H. contortus* is considered the most pathogenic GIN of small ruminants in tropical and subtropical areas of the world. It can also be problematic in some temperate regions as well (Waller and Chandrawathani, 2005; Grosz et al., 2013). *H. contortus* is harmful because it feeds on blood in the abomasal mucosa and can cause severe anemia and death (Grosz et al., 2013). Symptoms depend upon severity of infection, and include anemia, emaciation, edema, poor growth, and death (Smith, 1997; Burke, 2005; Nikolaou and Gasser, 2006). Poor wool growth and weight loss are also associated with infection (Larsen et al., 1994; Hoste and Chartier, 1998).

Once clinical signs are present, small ruminants suffering with *H. contortus* infections are known to have haemonchosis, which results in paleness of the ocular mucous membranes (Bowman et al., 2002). When disease is considered subclinical, the animal's immune and erythropoietic systems are able to offset small amounts of blood loss. When the disease becomes

hyperacute, the animal has been exposed to an overwhelming number of worms over a short period of time, and are at risk of death (Schichowski et al., 2010).

Small ruminants, including sheep and goats as well as other livestock, are some of the domestic ruminant species affected by GIN. Exotic or nondomestic ruminant species, such as giraffe, roan antelope, deer, oryx, sable antelope, blackbuck, bongo, okapi, and wildebeest are species also affected by GIN infections (Fagiolini et al., 2010).

### **2.3 Life Cycle**

*H. contortus* goes through a typical trichostrongyle life cycle. Female *H. contortus* are commonly called the barberpole worm because the white uteri and ovaries are twisted around the red blood filled intestine which gives the worm a barberpole appearance (Urquhart, 2007).

Females are very fecund, and can excrete between 5,000 to 10,000 eggs per day, which pass out of the host via feces (Urquhart, 2007). Adult worms are capable of sucking blood for twelve minutes at a time causing loss of blood and subsequent anemia. Average blood loss per worm is estimated from about 0.003 mL to 0.05 mL per day (LaJambre, 1995).

The life cycle begins with the first stage larvae (L1) hatching from the eggs in the feces and developing to the second stage larvae (L2) while feeding on organic matter. Both L1 and L2 are susceptible to adverse environmental conditions and can die off quickly. They then develop into third stage larvae (L3), which do not feed and are infective to the vertebrate host. L3 are protected from adverse environmental conditions by retaining the L2 cuticle as a sheath and can remain viable for an extended period of time, up to 8-10 months. The host (ruminant) becomes infected by ingesting the L3 while grazing. The L3 exsheath in the rumen, and move to the abomasum with the ingesta. Once in the abomasum, L3 penetrate the mucosa and develop to the fourth stage larvae (L4). L4 develop a sharp lancet before becoming adults, which they use to

disrupt the mucosa and small capillaries to feed. Both the L4 and adult worms feed on blood. The life cycle continues by adults mating and the females laying their eggs. In sheep, the time from when the L3 are ingested to the time adult females begin to lay eggs, is between two and three weeks (Urquhart, 2007).

Environmental factors such as temperature and rainfall affect the viability of *H. contortus* eggs and the free living larval stages. At colder temperatures and under desiccation conditions, survivability is decreased. However, sheep infected with *H. contortus* have become more frequent in colder climates such as Sweden, Denmark, the Netherlands and Canada. In Sweden, after ingestion of L3 in late summer and fall, the L4 can go through hypobiosis, and develop the next spring (Waller et al., 2004; Grosz et al., 2013). Hypobiosis is a delay in development when environmental conditions outside the body are not conducive to survival of the free-living larval stages. The L4 remains in the mucosa for up to 3-4 months before emerging and continuing development.

## **2.4 Anthelmintic Control of GIN**

Anthelmintics are used to eliminate GIN in order to provide the opportunity for body resources to achieve as much productive potential as possible that might be due to infection. One important time for parasite control is during pregnancy and lactation, because the ewe is stressed and immunity is relaxed, thus, they are more susceptible to infection. *H. contortus* females also increased egg production at this time leading to an increase in pasture contamination for the lambs. In addition, infected ewes can lose weight, as well as decreased milk production (Thomas and Ali, 1983).

An anthelmintic is a chemical that kills and eliminates parasitic GIN. Anthelmintics work by paralyzing or starving the worm. By doing so, the worm becomes unable to function and loses its ability to maintain position in the ruminant's gut (Schoenian, 2010).

Anthelmintics are classified as broad or narrow spectrum, they either kill a variety or one to two specific parasites, respectively (Sangster and Hennessy, 2007). Anthelmintics should be easy to administer, cost effective, have a broad spectrum of activity against larval and adult stages of parasites, have a post prophylactic effect, and have little to no withdrawal period due to drug residues (Kahn, 2005).

Before the rise of modern broad spectrum anthelmintics, treatment depended on the use of other less effective drugs, which often affected the host as much as the parasite. These drugs included sodium arsenite, tetrachloroethylene, carbon tetrachloride, carbon bisulfide, copper sulfate, and nicotine sulfate (Sargison, 2011; Sargison 2012).

The three classes of modern broad spectrum anthelmintics commonly used in the US sheep industry are the benzimidazoles, imidothiazoles, and macrocyclic lactones. Two newer classes of anthelmintics are the amino-acetonitrile derivatives and the spiroindoles (Sargison, 2011).

Effective broad spectrum anthelmintics were first developed in the 1960s with the benzimidazoles. Next came the imidothiazoles-tetrahydropyrimidines (nicotinic antagonists) in the 1970s, the macrocyclic lactones (avermectins) in the 1980s (Chabala et al., 1980; Waruiru; 1997; Mitreva et al., 2007), the amino-acetonitrile derivatives (monepantel) in 2000 (Sargison, 2011; Schoenian, 2013) and the spiroindoles (derquantel) in 2010 (Sakamoto et al., 2013). However, the amino-acetonitrile derivatives are only available to sheep producers in New Zealand, Uruguay, and the United Kingdom (Schoenian, 2010). Spiroindoles are approved in

combination with ivermectin (Vercruysse and Claerebout, 2014). Neither drug is available in the U.S.

By 2000, there were many anthelmintics available for use in sheep and goats. The insoluble benzimidazoles included fenbendazole, oxfendazole, and albendazole, as well as the soluble benzimidazoles, which included thiabendazole, mebendazole, parbendazole and oxibendazole. Febantel, thiophanate, and neobimin called the pro-benzimidazoles were also available for use in small ruminants. The imidothiazoles and tetrahydropyrimidines included levamisole and morantel tartrate (Bogan and Armour, 1987). Ivermectin was the first (1984) macrocyclic lactone, followed by doramectin, moxidectin and eprinomectin.

#### **2.4.1 Benzimidazoles**

Benzimidazoles belong to the class of anthelmintics whose names end in "-azole" and are commonly referred to as the white dewormers. They were the first modern class of anthelmintic developed at the beginning of the 1960's (Sakamoto et al., 2013). They are short acting (i.e., 24-48 hrs) and effective against all the GIN, including larvae and adults, and some show effectiveness against liver flukes (Sangster and Hennessy, 2007). Benzimidazoles work by binding to the protein tubulin. Tubulin is found in worm cells, which link together to form long tubes that are important to the worm's survival. Benzimidazoles work by disrupting cellular energy metabolism (uptake of glucose) by preventing polymerization of tubulin, and the establishment of microtubules, which deprives the parasite of energy (Arundel, 1985; Beugnet et al., 1996; Melhorn, 2008). The first benzimidazole introduced was thiabendazole (Arundel, 1985; Bogan and Armour, 1987). Thiabendazole (TBZ®) and albendazole (Valbazen®) are approved anthelmintics for use in sheep by the Food and Drug Administration (FDA). However, thiabendazole is no longer available due to widespread resistance and decrease in popularity.

Fenbendazole (Safeguard®, Panacur®) and oxfendazole (Synanthic®) are commonly used “extra-label” because of present resistance issues (Rook, 2010).

#### **2.4.2 Nicotinic antagonists**

The nicotinic antagonists are commonly known as the clear dewormers, and include levamisole, morantel tartrate, and pyrantel pamoate. They are short acting (i.e., 24-48hrs) and work by blocking the action of nicotinic acetylcholine receptors by “mimicking” acetylcholine, the nerve transmitter, thus, nicotine antagonists. Acetylcholine begins muscle contraction, and by “mimicking” acetylcholine, these drugs cause the worms’ muscles to contract, leading to paralysis and expulsion of the parasite (Kahn, 2005). Levamisole acts by affecting the nerve ganglion of the worm, which causes the worm to have rapid muscle contraction, leading to paralysis (Beugnet et al., 1996). Morantel tartrate works differently and acts by “depolarizing” the neuromuscular system (Arundel, 1985). Levamisole is also known as an imidazothiazole, and morantel tartrate is often classified as tetrahydropyrimidine, which also includes pyrantel pamoate (Schoenian, 2010). Only levamisole (Levasol® and Tramisol®) in this group of anthelmintics is FDA approved for sheep. Morantel tartrate (Rumatel®) and pyrantel pamoate (Pyrantel Pamoate Horse Dewormer®) are often used “extra-label” because of present resistance issues (Rook, 2010).

Levamisole is administered as an oral drench or by subcutaneous injection, and the efficacy is usually comparable, regardless of the method of administration. The tetrahydropyrimidine group is usually administered orally (Kahn, 2005).

#### **2.4.3 Macrocylic Lactones**

The macrocylic lactones are anthelmintics that end in “ectin”, are called avermectins, and include ivermectin, doramectin, and moxidectin. They are long acting (i.e., 10-21 days) and

work by blocking gamma aminobutyric acid (GABA) and mediated transmission of nerve ganglions, affecting the reproduction, and paralyzing the worm (Arundel, 1985). However, other work suggests that the avermectins work by opening the glutamate-dependent chloride channels of neuromuscular membranes of nematodes, inducing paralysis of the worm (Prichard; 1994). They are considered safe, and work against all life cycle stages of GIN. Ivermectin (Ivomec for Sheep®) and moxidectin (Cydectin® Oral Sheep Drench) in this group are FDA approved. Doramectin (Dectomax®) and injectable moxidectin (Cydectin®) are often used extra-label because of resistance issues (Rook, 2010).

The means of administration for the macrocyclic lactones include oral, subcutaneous, and pour on. Macrocyclic lactones are well absorbed (in cattle, but not so much in small ruminants), and are distributed throughout the body with a particular concentration in adipose tissue (Kahn, 2005).

#### **2.4.4 Amino Acetonitrile Derivatives**

The amino acetonitrile derivatives are a more recent anthelmintic developed in 2000. The drug in this class is monepantel (Zolvix®). The drug became available for use in sheep in New Zealand in 2009 (Leathwick, 2012). Monepantel is short acting (i.e., 24-48 hrs) and works by binding the receptor Hco-MPTL-1 which causes paralysis and death of the nematodes (Novartis Animal Health, 2010). Monepantel is considered to have a wide margin of safety in sheep. This newly discovered anthelmintic has been found to be effective against nematodes that are currently resistant to benzimidazoles, nicotinic agonists, and macrocyclic lactones, because of its mode of action (Stein et al., 2010). Monepantel is labeled as a ready-to-use oral solution (Novartis Animal Health, 2010). It is not available in the U.S.

### **2.4.5 Spiroindoles**

The most recent class of anthelmintics are the spiroindoles, developed in 2010. The only drug in this class is derquantel. The derquantel/abemectin combination (Startec®) is short acting (i.e., 24-48 hrs) and works by binding to acetylcholine receptors which causes paralysis and rapid death of the nematodes (Ruiz-Lancheros et al., 2011). Derquantel/abemectin offers a wide spectrum of anthelmintic activity with more than 95% efficacy, including activity against resistant nematodes (Sargison, 2012). Currently, this drug is not available in the U.S.

### **2.5 Anthelmintic Resistance**

Over the past few decades, GIN parasite control has relied heavily on the use of anthelmintics. These drugs were used in an attempt to eliminate all infection, which lead to frequent deworming and overuse of anthelmintics. So, with the constant reliance on anthelmintics, GIN have become resistant. Resistance occurs when it can be demonstrated that the efficacy (usually based on FEC) of the anthelmintic is reduced. It is now recognized that some populations of GIN are resistant to multiple anthelmintics and total failure has occurred.

The standard practice of deworming all animals in a population at the same time can no longer be promoted as beneficial, because not all animals harbor the same number of worms. The minority of animals harbor the majority of infection. Deworming all animals removes susceptible worms, and leaves resistant ones, consequently little refugia is left to compete with resistant worms for survival. This leads to increased populations of resistant worms (Terrill et al., 2012).

Resistance is widespread in tropical and sub-tropical regions where *H. contortus* is found. It was first documented in the U.S, and is now a common occurrence all over the world

(Coles, 1986; Moreno-Guzman et al., 1998; Flemming et al., 2006; Kaplan, 2012). Furthermore, resistance to GIN is found in sheep, goats, cattle, horses and swine (Prichard, 1994).

The first documented report of resistant strains of *H. contortus* was to thiabendazole in the United States (Conway, 1964) and then in Australia (Smeal et al., 1968). By the mid 1970's, resistance to benzimidazoles in sheep and horses was widespread (Kaplan, 2004). Resistance to benzimidazoles has been recognized in parts of Australia, New Zealand, South Africa, Europe, and the Americas (Prichard, 1994), to multiple species, including *Teladorsagia*, *Trichostrongylus*, *Cooperia*, and *Nematodirus* (Prichard, 1990; Kaplan, 2004; Waghorn et al., 2006). By the 1980's, resistance to nicotinic antagonists and avermectins had been reported (Kaplan, 2004). In a study performed in Louisiana, Suffolk lambs infected with *H. contortus* showed resistance to ivermectin when given in both oral and injectable forms at a dose of 0.2mg/kg -1 (Miller and Barras, 1994).

With the increased development of anthelmintic resistance, it is necessary to find other methods to control these GIN. In addition, there is an increasing demand for organic meats and other goods, which means that the producer cannot use a chemical drug to prevent and treat GIN. Alternative methods and integrated strategies are needed to help control of GIN infection in small ruminants in light of the increasing level of anthelmintic failure.

## **2.6 Alternate Parasite Control**

Developing and integrating alternate approaches has the potential to reduce morbidity and mortality of small ruminants infected with GIN. Such methods include vaccines, selecting resistant individuals for breeding, nematode trapping fungi, COWP, condensed tannin containing plants, targeted selective treatment (smart drenching), FAMACHA system ©, and pasture management.

## 2.7 Vaccines

Alternative control methods should include development of vaccines against GIN, especially a vaccine against *H. contortus* (Knox et al., 2003). When considering a vaccine one of the benefits is that they are safe and environmentally mindful, since they leave no chemical residue (Dalton and Mulcahy, 2001).

The two approaches that have been studied are natural and hidden gut antigens. Antigens recognized by the immune system when the host is infected are called natural antigens. They elicit an immune response that includes both antibody and cellular responses, and are triggered by surface and/or excretory/secretory products of the various stages of the worm (including irradiated larvae) (Smith and Zarlinga, 2006). Hidden gut antigens are not recognized by the immune system after the host becomes infected as they are located in the gut of the worm. These antigens are isolated from the worm's gut and used to stimulate an antibody response via vaccination. The circulating antibodies are ingested by the blood feeding worms and subsequently interfere with the ability of the worm's gut to function properly. Thus, the worm starves, becomes weak and is removed. The first hidden gut antigen was a 110 kDa integral membrane glycoprotein called H11. It was successful in providing protection to very young lambs. (Newton, 1995). H11 is attained from the intestinal microvilli of *H. contortus* (Knox et al., 2003). Studies have shown H11 to be effective in reducing FEC by more than 90%, and worm burden by more than 75% in *H. contortus* infection (Knox et al., 2003; Smith and Smith, 1993). Another study conducted with H11 used young lambs of a range of breeds, infected with anthelmintic resistant worms. H11 was effective in reducing FEC in anthelmintic susceptible and resistant strains of *H. contortus* (egg output by more than 99% and greater than 90% reduction in worm burden) (Newton et al., 1995).

Another integral gut membrane glycoprotein is Haemonchus galactose-containing glycoprotein complex (H-gal-GP). Studies have shown H-galGP to be effective in reducing FEC by 93% and worm burden by 72% in *H. contortus* infection (Smith et al., 1999; Knox et al., 2003). In another study, a combination vaccine (H11 and H-gal-GP) was studied in lambs under conditions a commercial farmer would encounter. Results of that trial showed that vaccinating with H11 and H-gal-GP reduced the occurrence of haemonchosis in lambs grazing *H. contortus* infected pastures, as well as reduced the number of anthelmintic treatments (LeJambre et al., 2008).

However, since grazing lambs become infected with various parasites throughout a grazing season, unpredictability in infection within each animal and response to vaccination is probable. Furthermore, there are problems related to producing hidden gut vaccines. For example, recovering worm guts to extract the antigens for large scale vaccine production can be time consuming and unfeasible. For that reason, research efforts have been focused on the development of recombinant antigens for commercial use. Nonetheless, recombinant antigens have not produced acceptable results to be considered for production of a vaccine on a large scale against *H. contortus*. Still, with furthering the understanding of parasitism, and advancement in technology, the possibility of a future vaccine is promising (Smith and Zarlenga, 2006).

## **2.8 Breeding for Resistance**

Using breeds of sheep that have shown resistance, incorporating resistant breeds into a crossbreeding program, and/or selecting resistance sheep within a breed are potential methods to increase natural animal resistance to GIN infection.

Breeds of sheep that are genetically resistant to GIN infection have been identified. In a study comparing exotic and conventional breeds, St. Croix and Florida Native sheep were shown to be parasite resistance when compared to the Suffolk and Rambouillet breeds, respectively (Bradley et al., 1973). Further studies showed that Florida Native, St. Croix, and the Barbados Blackbelly sheep, had lower worm burden, lower FEC, and higher PCV, than Dorset/Rambouillet crosses (Courtney et al., 1985; Zajac et al., 1990). Studies conducted over an eight-year period demonstrated that Gulf Coast Native sheep were much more resistance to infection than Suffolk sheep (Bahirathan et al., 1996; Miller et al., 1998).

Incorporating resistant breeds in a crossbreeding program can be favorable. Heterosis of GIN parasitism was studied in Suffolk, Gulf Coast Native, and Suffolk/Gulf Coast Native cross sheep over a 2 year period (Li et al., 2001). The Suffolk lambs exhibited the highest FEC/weight gain and the lowest PCV, the Gulf Coast Native lambs had the lowest FEC/weight gain and highest PCV and the F<sub>1</sub> lamb were in between. The heterosis assessment demonstrated that FEC, PCV, and weight gain of the F<sub>1</sub> lambs were more like the Gulf Coast Native lambs. The results indicate that crossbreeding Suffolk to Native sheep may be an appropriate way to breed for resistance against GIN, but at the cost of a smaller framed sheep. Another study had similar results where Rhoe (susceptible), Merino Land (resistant), and a cross between Rhoe and Merino Land sheep (F<sub>1</sub>) were evaluated (Hielscher et al., 2006). The Merino Land sheep had the best weight gain and carcass performance, the Rhoe sheep had the least weight gain and carcass performance, and the F<sub>1</sub> was in between both groups. Heterosis favored the Merino Land and; therefore, in this case, more resistant with better production.

Another study compared infection levels in hair and wool breed sheep (Notter et al., 2003). Wool sheep were a crossbreed (50% Dorset, 25% Rambouillet, and 25% Finnish

Landrace), and the hair sheep were crossbred Barbados Blackbelly and St. Croix. The hair sheep had lower FEC and higher PCV than the wool sheep, thus indicating that hair sheep may be important to incorporate into crossbreeding programs

It is also important to consider breeding for resistance within a breed. It is known that within a population of animals the majority of infection is in a minority of the animals. Therefore, selecting the more resistant ones is a logical approach to improve resistance. In one study, two lines (high and low responders to infection) of Merino sheep were developed within a population and compared. A high level of heritability was detected, and thus, selecting sheep for high or low responsiveness to nematode infection can improve resistance (Sreter et al., 1994). However, the benefit to crossbreeding in resistance selection is faster than breeding for resistance within a breed (Li et al., 2001).

## **2.9 Nematode Trapping Fungi**

Another alternative control method that can help decrease the reliance on anthelmintics is implementing biological control. A biological control agent works by keeping a “pest” (GIN) under control by reducing the “pest” population’s development (Larsen, 2000). One such control method is using micro-fungi that are able to trap and kill larval stages of parasitic nematodes in the environment (Larsen et al., 1992; Larsen et al., 1997). Nematode trapping fungi are found worldwide and there are two types, one that produces structures that are able to trap by means of mycelium, and the other produces spores that cohere to the nematode’s cuticle, or are ingested by the nematode (Larsen, 2000). To be effective in the feces, these fungal spores need to be fed and survive passage through the gastrointestinal tract. In vivo and in vitro studies conducted in sheep demonstrated that 3, out of hundreds of such fungus species tested, survived passage and germinated in the feces (Waller et. al, 1994).

After evaluating these 3 species, *Duddingtonia flagrans* was shown to be the most promising as a biological control agent (Larsen et al., 1994; Larsen, 2000). *D. flagrans* forms thick-walled chlamydospores that are able to withstand the environmental condition of the gastrointestinal tract, and thus, are able to successfully grow in feces, producing hyphal loops that trap developing larvae (Terrill et al., 2004; Larsen, 2006). The survival ability of an Australian field isolate of *D. flagrans* was evaluated in different gut compartments of surgically adapted and normal sheep. Results indicated *D. flagrans* spores survived the gastrointestinal tract with no restrictions in any stomach compartments. Efficacy testing of single and continuous dosing of chlamydospores resulted in a greater than 80% reduction of L3 in the feces (Larsen et al., 1998; Pena et al., 2002). The chlamydospores mixed with a feed supplement to grazing mature dry ewes on pasture resulted in long term reduction of fecal L3 and lower forage larval levels (Fontenot et al., 2003). Pen studies conducted in Malaysia showed that giving chlamydospores in a feed supplement, or incorporated into supplement feed blocks, resulted in a continued reduction of L3 in the feces (Chandrawathani et al., 2003). This was followed by a field trial where chlamydospores were given in both feed supplement formulations with similar results (Chandrawathani et al., 2003). Commercial farms in Malaysia that had relied mainly on anthelmintic use have incorporated pasture rotation and feeding *D. flagrans* chlamydospores at night, when sheep were housed, as a beneficial manageable scheme for large scale sheep operations (Chandrawathani et al., 2004).

The use of COWP (see 2.10) in conjunction with feeding *D. flagrans* might be advantageous, but copper is also fungicidal. The interaction of *D. flagrans* with COWP was examined and the results suggested that COWP did not have a negative effect on the ability of *D. flagrans* to reduce L3 in the feces (Burke et al., 2005).

Further studies with sheep have shown that utilizing *D. flagrans* during critical periods in addition to anthelmintics is beneficial (Waller, 2003). These times include when ewes are lactating (peri-parturient rise in FEC), and when lambs are weaned. Employing a *D. flagrans* treatment program during the peri-parturient period can potentially decrease pasture contamination, during which time infected ewes are responsible for incoming larvae on pasture. When lambs are weaned, they become most susceptible to GIN infections as acquired immunity is slow to develop. Providing *D. flagrans* to control L3 in the feces can help ensure pasture infectivity remains low (Waller, 2003). In support of this, it has been reported that *D. flagrans* chlamydospores fed to sheep during the critical infection period also reduced overwintering larvae populations and subsequent pasture infectivity (Gomez-Rincon et al., 2006).

## **2.10 Copper Oxide Wire Particles**

Another method to control GIN are COWP. Previous information indicates a direct association between COWP and decreasing FEC and numbers of the GIN, *H. contortus* in small ruminants (Bang et al., 1990; Knox, 2002; Burke et al., 2004; Burke and Miller, 2006; Soli et al., 2010). COWP were originally developed to treat copper deficiency in sheep. It was noted that COWP had anthelmintic effect on GIN. After ingestion, COWP adhere to the gastrointestinal tract mucosa, and can remain there for roughly 4-5 weeks (Dewey, 1977). COWP release free copper in the abomasum, which is slowly absorbed over time, and consequently stored in the liver (Bang et al., 1990). The copper creates an environment that affects the worms' ability to remain established and are expelled (Chartier et al., 2000).

In an early study, a 5 g dose of COWP was used in young lambs on 3 main GIN. It was noted COWP was most efficacious against *H. contortus* (96% reduction), less effective against *T. circumcincta* (56% reduction), and had no effect on *T. colubriformis* (Bang et al., 1990).

Excessive copper accumulates in the liver of sheep, therefore, toxicity is a concern with longer term use. A dose titration study was conducted using 0, 2, 4 and 6 g COWP given orally in gelatin capsules to determine the minimum effective dose (Burke et al., 2004). All 3 doses effectively controlled infection with the 4 and 6 g doses being slightly better than the 2 g dose. Copper in the liver was the lowest, for 2 g, and the greatest for 6 g, however, liver copper levels were within normal limits for all three doses (Burke et al., 2004). Another study used even lower doses (Burke and Miller, 2006). In this study multiple low doses (0.5 and 1 g in gelatin capsules) were given to lambs, and showed to be effective in reducing infection in lambs. It was noted that FEC started to increase at 3-4 weeks after each treatment (Burke and Miller, 2006). This indicated that the effect of the copper was probably no more than 3-5 days (similar to a short acting anthelmintic), even though the particles can remain in the gastrointestinal tract for about 4-5 weeks. Liver enzyme levels were alike for both COWP and untreated animals, however, liver copper levels were higher in COWP treated animals, but within normal limits. The conclusion was that multiple low doses of COWP could be used repeatedly without inducing copper toxicity (Burke and Miller, 2006).

The mechanism of action of COWP is unknown. However, one study showed that worms from COWP treated lambs had worms with cuticle damage which may disrupt the worms' ability to sustain metabolic function (Moscona et al., 2008).

The effectiveness of COWP in pregnant ewes and how it might affect their offspring has also been reported. In that study, ewes were given 0, 2 or 4 g of Copasure® COWP orally in gelatin capsules 30 days prior to parturition (Burke et al., 2005). The study concluded both doses effectively reduced infection. However, at birth, lambs from Copasure® COWP treated ewes had higher aspartate aminotransferase serum levels, and weighed less than lambs from

untreated ewes. Conversely, by 30 days of age, aspartate aminotransferase serum levels decreased, and were similar to control ewes' lambs, and by four months of age there was no difference in weight between control and treated ewes' lambs. This study demonstrates that a 2 g dose of Copasure® COWP appeared to be safe for production (Burke et al., 2005).

The effectiveness of COWP incorporated in feed pellets and sericea lespedeza feed pellets fed to ewes and does during the peri-parturient rise was reported by Burke et al., 2010. In Arkansas, does were fed 2.0 g each of COWP in sericea lespedeza pellets, and ewes were fed COWP in feed pellets supplemented with sericea lespedeza hay, and in Louisiana ewes were fed 4.0 g each of COWP incorporated in feed pellets. Results indicated, a reduction in FEC in both does and ewes fed COWP in feed pellets with or without sericea lespedeza. The benefit of incorporating COWP in feed pellets is even delivery of COWP, and reducing the risk of ewes/does not receiving enough, or receiving too much copper (Burke et al., 2010).

In another study, the effectiveness of 2.0 g of COWP in lambs and kids grazing the same pasture and exposed to the same GIN was compared. The study concluded that COWP was equally effective against *H. contortus* infection in lambs and kids (Soli et al., 2010).

## **2.11 Tannins: Hydrolyzable and Condensed**

The term “tannin” refers to “tanning” or preservation of skins to create leather by the use of plant extracts. Tannins also add to the acidity of many drinks such tea and wine. Similarly, they are found in fruits such as grapes, persimmon, and, blueberries, in chocolate, grasses, legume forages and trees. In plants, tannins are one of many different forms of secondary compounds. Tannins are believed to have evolved over time in plant defense mechanisms to protect them against microorganisms, insects, and herbivorous animals and in doing so, have marked anti-nutritional effects (Cannan, 2015).

Tannins consist of oligomeric compounds that have several structure units with free phenolic groups. They are usually water soluble, however, some high molecular weight structures are insoluble (Cannan, 2015). Based on their chemical structure and properties, tannins are categorized as hydrolyzable and condensed tannins (Proanthocyanidins).

Hydrolyzable tannins are made of a carbohydrate, usually D-glucose, as the central component. The hydroxyl groups in these carbohydrates are either totally or partly esterified with phenolic groups such as ellagic acid and gallic acid (Frutos et al., 2004). They can be toxic to ruminants when fed in excess, however ruminants are able to adjust to diets with hydrolyzable tannins (Waghorn, 2008). This toxicity ensues when animals are fed oak and certain tropical tree legumes. Animals may suffer from hemorrhagic gastroenteritis, necrosis of the liver, kidney damage, and death (Cannan, 2015).

Condensed tannins are non-branched polymers of flavonoids units. Their molecular weight is 1000-20000 Da which is frequently higher than hydrolyzable tannins, whose molecular weight is in between 500-3000 Da (Mueller-Harvey, 1999). Condensed tannins can be divided into two groups, prodelphinidins and procyanidins. Prodelphinidins bind protein more tightly than procyanidins and therefore may have a higher anthelmintic effect (Gea et al., 2011; Lorenz et al., 2013). Tannins can have positive or negative effects on animal performance depending on the type and amount of tannin consumed and the species of animal consuming it. Additionally, the molecular weight and chemical structure also affect how well an animal does (Hagerman and Butler, 1991). The negative effects of tannins are that they may affect feed intake (due to palatability) and digestibility (fullness due to undigested feed). Tannins can also be harmful to numerous rumen microorganisms by affecting their morphology (Mueller-Harvey and McAllen, 1992; Cannan, 2015). However, under some circumstances, condensed tannins may be

beneficial to the overall health of the animal. Tannins are relevant to ruminants in two ways; one they aid in the prevention of bloat, and two they inhibit GIN (MacAdam et al., 2013).

Condensed tannins have the ability to attach to soluble proteins and bypass the rumen, therefore, there is more protein available for digestion (Barry et al, 2001; Kariuki and Norton, 2001).

Plants that contain a substantial amount of tannins are birdsfoot trefoil (*Lotus corniculatus*), sainfoin (*Onobrychis viciifolia*), and sericea lespedeza (*Lespedeza cuneata*) (MacAdam et al., 2013). Sulla (*Hedysarum coronarium*), another condensed tannin containing plant has been shown to reduce GIN as well. Niezen et al., 1995 reported that sulla and sericea lespedeza, when grazed by GIN infected lambs had lower FEC and worm burden when compared to lambs just grazing lucerne (*Medicago sativa*).

#### **2.11.1 Sericea Lespedeza**

Sericea lespedeza (SL, *Lespedeza cuneata*) is a leguminous plant found throughout the southern United States. It is a variety of warm season, perennial forage that is high in condensed tannins. It was originally used in soil restoration and conservation, but it is also used as forage for grazing and hay (Powell et al., 2003). SL has shown to have anthelmintic effects on GIN with reduced FEC and development of larvae. (Min et al., 2004; Min et al., 2005). In addition, SL is well adapted to infertile, acidic soil, is easily grown, and can be a cost effective way of controlling parasitic infections. In one study, SL fed as hay to goats, had a direct effect on adult worms, egg production, and larval development (Shaik et al., 2006). In a similar study, SL hay was fed to sheep. FEC was reduced by 67-98% and worm burden by 67.2% (Lange et al., 2006). However, feeding of SL as hay to small ruminants could be a problem, as the leaf is very delicate, and can go to waste, thus not all available to be consumed by the animal. In another study, SL was fed to goats in a pelleted formulation which were effective in controlling GIN

indicating there was no loss of effectiveness of SL in the pelleting process (Terrill et al., 2007). In an additional study, lambs were either allowed to graze SL, or a mixed grass pasture, in early and late summer (Burke et al., 2012). Lambs exhibiting anemia were either treated with COWP or an anthelmintic. The results indicated that lambs grazing SL needed fewer treatments (Burke et al., 2012). Goats grazing perennial, warm season grass, during late summer and fall, were supplemented with SL pellets (Gujja et al., 2013). The study concluded that that feeding SL pellets improved animal performance. Goats fed the 95% SL pellets had a 93% FEC reduction in *H. contortus* infection while the 75% SL pellets had an 84% FEC reduction when compared to commercial feed (Gujja et al., 2013).

Research also suggests that lambs prefer condensed tannin rich plants when infected with GIN, compared to non-parasitized lambs (Juhnke et al., 2012). Parasitized lambs in this study sought out feed supplemented with condensed tannins. This behavior diminished when animals were given an anthelmintic (Juhnke et al., 2012).

SL has also been shown to be successful in preventing and controlling coccidiosis and GIN infections in lambs. Coccidiosis is often problematic in post weaned lambs, and in extreme cases, results in death of lambs (Burke et al., 2013). Furthermore, coccidiosis is difficult to treat, and there is no treatment for organically raised lambs, so SL is a viable option (Burke et al., 2013).

## **2.12 Integrated Strategies**

In view of the multi-drug anthelmintic resistance, there is a need for effective control of GIN. Current views suggest a combination of different control strategies to reduce the reliance on traditional anthelmintic-based control.

### **2.12.1 FAMACHA System©**

Integrated parasite control includes several different approaches. One approach is selective treatment (smart drenching), in conjunction with the FAMACHA System©. The FAMACHA System© is a 5 point color scale (red to white) depicted on a card, which is matched to the lower eyelid membrane color. It was developed by veterinarians and scientists in South Africa as a means to identify anemic sheep and goats infected with *H. contortus*. Treating only sheep and goats that need to be treated, improves refugia, lessens anthelmintic resistance, and reduces the cost of deworming the entire flock/herd (van Wyk, 2001; Terrill et al., 2012). In cases where resistance has developed to all existing anthelmintics, combinations of two or more may help to increase efficacy and slow anthelmintic resistance (Geary et al., 2012; Leathwick and Besier, 2014).

Besides using the FAMACHA System© with traditional anthelmintics, a study used the FAMACHA System© with COWP compared to traditional anthelmintics (Miller et al., 2011). Animals were divided into three groups. Group 1 was dewormed with levamisole (if FAMACHA© score was 4/5), group 2 with COWP (if FAMACHA© score was 4/5), and group 3 was dewormed with moxidectin followed by COWP if needed (if FAMACHA© score was 4/5). In all three groups, GIN were adequately controlled.

### **2.12.2 Other Management Strategies**

In addition to proper use of anthelmintics, pasture management should be included. This might include reducing stocking rates, reducing time animals remain on pasture, height of grass, co-grazing small ruminants with other species, or rotating grazing pastures between other species and small ruminants.

Still other integrated methods include developing host resistance and reliance by selecting sheep that are resistant /resilient and culling susceptible ones. Furthermore, keeping

animals free of other diseases is important. Animals enduring other diseases have a reduced ability to fight parasitism (Bath, 2014).

Finally, all available methods for control should be considered in some combination that fits a producer's production situation. Methods presented include, tactical use of anthelmintics (smart drenching) in conjunction with the FAMACHA System©, pasture management, genetic selection, biological control (*D. flagrans*), COWP, and condensed tannin containing plants (Hoste and Torres-Acosta, 2011). By implementing integrated means, reliance on anthelmintics will be decreased, which in turn will extend the useful life of those anthelmintics that are still successful.

## **CHAPTER 3**

### **EWE FARM STUDY**

#### **3.1 Methods and Materials**

##### **3.1.1. Location and Animals**

This study was conducted from February 2011-August 2013 at the Ben Hur Research Farm Sheep Unit of the Louisiana Agricultural Experiment Station in Baton Rouge, Louisiana.

All experimental procedures for this study and following studies (Chapter 4, Chapter 5, and Chapter 6) were approved by the Institutional Animal Care and Use Committee (IACUC).

##### **3.1.2 Experimental Design**

In this study, two integrated control strategies were compared, one strategy (S1) used a conventional anthelmintic/drug approach and the other strategy (S2) used a non-anthelmintic/drug approach. The purpose of comparing two strategies was to see whether one strategy was better than another or if both strategies were equivalent. Thirty-seven pregnant crossbred ewes (Suffolk x Gulf Coast Native) were randomly divided into the 2 groups (S1, n=13 and S2, n=14 year 1 and S1, n=15 and S2, n=16 for year 2 and 3) and then randomly assigned to graze separate pastures to acquire natural GIN infections. Randomization was accomplished by arranging the pairs of ewes by age and then tossing a coin. The average age of both the S1 and S2 ewes were 6.6 yr for yr 1 and 5.9 yr for yr 2 and yr 3. Each group had one winter and one summer pasture. They were housed separately at night, and had water and hay available at all times for the duration of the study. Lambs, after weaning, were moved to different pastures from their dams, still being maintained as S1 (n=15) and S2 (n=15) groups (See Chapter 4).

### **3.1.3. S1 Ewes**

Ewes grazed ryegrass pasture from January-May and Bermuda grass pasture from June-December of each year. Ewes were supplemented with cracked corn (8% CP and 2% CF; 0.91 kg/hd/d) from 30 days before the start of lambing to 30 days after the last lamb was born (approximately a 90 day period) to meet nutrient needs based on 2007 National Research Council requirements. Ewes lambled in late February/early March of each year, and lambs were weaned at about 90 days of age. Each year, replacement ewe lambs were added to the flock as needed. Ewes were dewormed with levamisole (LAV, Levasole®, 8 mg/kg drench) and albendazole (ABZ, Valbazen®, 10 mg/kg drench) on an individual basis when they had a FAMACHA© score of 4/5 (represents a PCV < 18). This anthelmintic combination approach was needed, because of resistance issues at the farm.

### **3.1.4 S2 Ewes**

S2 ewes were managed similarly to the S1 ewes, but were supplemented with sericea lespedeza pellets (SL, Sims Brothers Inc., Union Springs, AL) (12% CP and 20% CF; 0.91 lbs/hd/d). They were dewormed with copper oxide wire particles (COWP, Copasure®, 2 g) administered orally in gel capsules on an individual basis when they had a FAMACHA© score of 4/5 (represents a PCV < 18).

## **3.2 Techniques**

### **3.2.1 General**

Animals were naturally infected with GIN while grazing. Feces were collected for FEC and culture. Blood was collected for PCV. The interval for fecal and blood collection was every 7 (February 2011-December 2012; March 2013-August 2013) or 14 days (January 2013-February 2013). On the same schedule, feces were cultured and FAMACHA scores were

checked. Grass samples were collected every two weeks from March through September and monthly from October through February.

### **3.2.2 Fecal Egg Count**

Feces (approximately 10g) were collected directly from the rectum, and placed in labeled (animal number and date) styrofoam cups and sealed with lids. Samples were taken directly to the lab to be analyzed for FEC using a modified McMaster technique (Whitlock, 1948). Briefly, two grams of feces from each animal was broken up in a cup using a tongue depressor. Thirty mL of a saturated salt solution (737 g of iodized salt dissolved in 3000 mL of tap water) was added and the solution and mixed using an electric drink mixer (Drinkmaster® Drink Mixer, Hamilton Beach Brands, Inc., Glen Allen, NC). A sample of the mixture was promptly pipetted and transferred into one side of a McMaster chamber. The solution was mixed again and a second sample was pipetted and transferred to the other side of the McMaster slide. The total number of eggs on both sides within the outlined grid lines was counted. The number of eggs was multiplied by 50 to get an estimated EPG. The FEC was used to monitor the relative change in GIN infection.

### **3.2.3 Packed Cell Volume**

Blood was collected via jugular venipuncture into 7 mL purple top EDTA vacutainer tubes (BD Vacutainer® Glass Whole Blood Tubes, Becton, Dickinson, and Company, Franklin Lakes, NJ). These tubes contained EDTA as an anticoagulant, and each tube was inverted several times to prevent clotting. These samples were taken directly to the lab, and PCV was determined by filling micro-hematocrit capillary tubes with blood, which were sealed and centrifuged in an Autocrit centrifuge (Autocrit Ultra 3 Microhematocrit Centrifuge, Becton, Dickson and Company) for 5 min. The PCV was read directly from the centrifuge scale. PCV was used to check the level of anemia as a result of *H. contortus* blood feeding.

### **3.2.4 FAMACHA© SYSTEM**

The FAMACHA© system was used to determine when to deworm individual animals. The FAMACHA card has a five point (1-5) color scale (red to white) which is matched to the inside lower eyelid membrane color. The correlation of color score to PCV is 1 (>28), 2 (23-27), 3 (18-22), 4 (13-17) and 5 (<12). A score of 1-2 is normal and a score of 4-5 indicates severe anemia that requires treatment. This selective deworming minimizes drug use and slows the development of drug resistant GIN parasites.

### **3.2.5 Fecal Culture**

Feces were cultured and the baermann technique was used to recover L3 for determination of GIN population distribution. At each collection, bulk (all animals in a group) cultures were done with 2 replicates for each group (4 total).

Fecal samples were weighed, divided into plastic containers, and broken up using tongue depressors. Sample number, date, and weights were recorded. Vermiculite and water were added to the feces and mixed until a crumbly moist consistency was attained. Aluminum foil was used to cover the containers, and holes were punched into the foil to create an aeriated environment for the larvae to hatch and develop. Cultures were incubated at approximately 27 °C for two wk. After the two wk, 15 mL tubes were attached to large mouth funnels with rubber tubing. A screen was placed in the funnel. Next, a 19-20 in<sup>2</sup> piece of cheese cloth was placed on top of the screen, ensuring it covered the screen. Feces from the culture was placed inside the cheese cloth and the cheese cloth was folded over the fecal mass. Lukewarm water was added to the rim of each funnel to saturate the fecal mass. Cultures were allowed to sit, fully submerged, for 24 hr to allow L3 to migrate out of the culture mass, and to accumulate in the bottom of the

15 mL tube. The 15 mL tubes were removed, 1 mL was decanted off and replaced with 10% formalin for preservation purposes. The tubes were then stored for further processing.

### **3.2.6 Larval Counts and Identification**

The supernatant in the 15 mL tubes was vacuumed to 1 mL. A pipette was used to mix the solution, and to draw off a 100 uL aliquot which was placed on a microscope slide. A drop of iodine (150 g potassium iodide and 100 mL water) was mixed into the aliquot, and a coverslip was placed on top of the stained aliquot. All the L3 in the aliquot were counted and the first 100, or total (if the total was less than 100) L3 were identified to genus to obtain an approximate population distribution.

### **3.2.7 Grass Collection**

Grass samples were collected to monitor pasture infectivity levels. This procedure distinguishes what GIN larvae are available on a pasture that could potentially infect the animals. Pastures were walked in a “W” pattern and 20-30 grass samples were collected along the legs by randomly throwing a hoop (a coat hanger that had been modified into a square) onto the pasture. The grass was cut to ground level inside the hoop using electric scissors. The samples were placed in individual plastic bags labeled with the pasture number, date collected, and number of the sample and taken directly to the lab to be processed.

### **3.2.8 Grass Processing**

Grass bags were weighed to obtain both bag and grass weight. Buckets were labeled with process date and sample number and filled with lukewarm water. Dish soap (1-2 drops) was added to the water to decrease the surface tension which assists in L3 release from the grass. Each grass sample was placed in the correlating bucket, and then inverted to wash off any L3 adhering to the bag. The excess water was shaken from the bags and weighed to record bag

weight. After 24 hr, a subsample was taken from each bucket and placed on a paper towel, patted dry and placed in a known weight 120 mL cup which was then weighed to get the total weight of the cup and wet grass. The subsample cups were then placed in a drying incubator (27°C) for 2 wks. The dry grass was removed from the cups and weighed. The dry weight was recorded, and using the wet weight, percent dry matter was calculated. The remaining grass in each bucket was removed and placed in a biohazard box. The grass water was poured through a tea strainer (to remove excess particulates) into another bucket and then poured back into its original bucket. The tea strainer and buckets were washed in between samples. The grass water was allowed to sediment for 24 hr, and the grass water was poured off, making sure the bottom sediment of the bucket was not disturbed, leaving less than 400 mL. The grass water sediment was mixed and transferred into 400 mL tri-corner labeled cups. Again, the grass water was allowed to sediment for 24 hr and the grass water was vacuumed down to approximately 70 mL making sure the bottom sediment was not disturbed. The sediment was mixed and transferred to 120 mL cups. A Kimwipe was placed over the cup and secured with a rubber band. Next, 250 mL cups were labeled with the correlating sample and lukewarm water (75 mL) was poured in each cup. The corresponding 120 mL cup was inverted in the 250 mL cups making sure the water came in contact with the Kimwipe and sample. The cups were maintained at room temperature (approximately 27°C) for 24 hr to allow L3 to migrate through the Kimwipe into the larger cup. The inverted 120 mL cups were pulled above the water level of the 250 mL cup and allowed to rest for 30 min at an angle on the 250 mL cup. The 120 mL cup and Kimwipe were then removed. The remaining grass water in the 250 mL cup was vacuumed down to <50 mL, transferred to a labeled 50 mL tube and capped. After 24 hr, the grass water was vacuumed

down to <15 mL, transferred to labeled 15 mL tubes and 10% formalin was added for preservation purposes.

### **3.2.9 Larvae Identification**

The supernatant was vacuumed down to 1 mL and a pipette was used to mix the solution and draw off a 100 uL aliquot. The aliquot was placed on a microscope slide and a drop of iodine was mixed into the aliquot. A coverslip was placed on top of the stained aliquot. The larvae were counted and identified to genus to obtain an estimate of population distribution.

### **3.3 Temperature and Precipitation Data Year 1-Year 3**

The free living stages of GIN are strongly affected by environmental conditions. Temperature and moisture (precipitation and relative humidity) are of major importance for the development of the parasite. When humidity and temperatures are high, larger numbers of L3 can be found higher up on vegetation (Silva et al., 2008). For year 1, the coldest temperatures of the year were from February 1<sup>st</sup> through February 17<sup>th</sup>, consisting of 17 days with colder than average low temperatures (Figure 1). The warmest month of year 1 was August with an average daily high temperature of 36° C to the average norm of 33° C. The hottest temperature was from July 30 to September 2 which comprises 35 days of above average temperatures. September had the highest amount of rainfall with 22.7 cm which was above average for the time of year. February had the longest period of dry weather with 15 days (February 10<sup>th</sup> through February 24<sup>th</sup>) with no rainfall. July had the largest fraction of days of rainfall, with 48% of days reporting some measured precipitation (WeatherSpark, 2013; Weather Underground, 2013).

For year 2, the coldest month of the year was November, with the chilliest temperatures, being from November 12<sup>th</sup> through November 26<sup>th</sup> which were colder than average temperatures. August had the highest amount of rainfall with 27.8 cm which was above average for the time of

year. May had the longest period of dry weather with 18 days (May 14<sup>th</sup> through May 31<sup>st</sup>) of no rain (WeatherSpark, 2013; Weather Underground, 2013).

For year 3, the coldest temperatures of the year were in March and December, with 71% of days in March with lower than average temperatures. The longest period of cold temperatures was from May 3<sup>rd</sup> through May 17<sup>th</sup> (15 days) of colder than average temperatures. The hottest months were August and September, with the warmest temperatures being from July 30 to September 2 which comprises 23 days of above average temperatures. September had the largest portion of hotter days, with 90% days above average temperatures. January had the highest amount of rain with a total of 37.4 cm. February 26<sup>th</sup> through March 11<sup>th</sup> had the longest period of dry weather with 14 days of no rain. March had the longest portion of no measurable rainfall with 84% of days recording no rain altogether (WeatherSpark, 2013; Weather Underground, 2013).

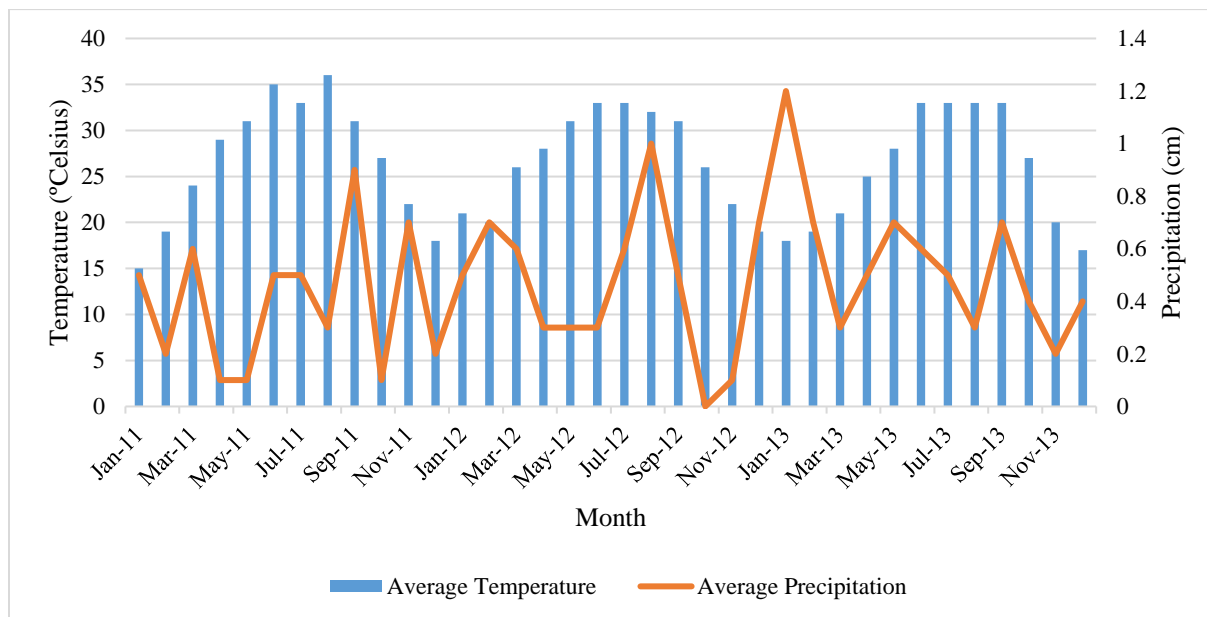


Figure 1. Average temperature and precipitation for Year 1 through Year 3.

### 3.4 Statistical Analysis

Data were analyzed using SAS® (version 9.4, SAS Institute, Cary, NC) as a repeated measures analysis of variance using Proc Mixed. Fixed effects included Group, Week and the Group\*Week interaction. The random effect was Animal (Group). The response variables were EPG, logepg, PCV, and FAMACHA. Differences were considered significant when  $p \leq 0.05$ .

### 3.5 Results

At the beginning of yr 1 (February, 2011), ewes in both groups had similar ( $p > 0.05$ ) mean FEC through wk 4 (Figure 2). From wk 5 through wk 12 the FEC were similar, however, the FEC increased for S1 ewes, while S2 ewes remained low. On wk 12, supplement feeding for both groups was stopped. The FEC for the S2 ewes was significantly ( $p < 0.05$ ) higher than S1 ewes and continued to be significantly higher for wk 13-16 and wk 18 and 19. From wk 20 through the end of the year the FEC continued to remained low and similar ( $p > 0.05$ ) for both S1 and S2 ewes.

The PCV values for both groups were similar ( $p > 0.05$ ) at the beginning of the study through wk 13 (Figure 3). For wk 14, 16, and 29 the PCV for the S2 ewes was significantly ( $p < 0.05$ ) higher than the PCV for the S1 ewes. The PCV stayed similar for the remainder of the year.

The FAMCHA© scores for both groups were similar ( $p > 0.05$ ) at the beginning of the study through wk 13 (Figure 4). For wk 14-16 The FAMCHA© scores were significantly ( $p < 0.05$ ) higher for the S2 ewes than the S1 ewes. They remained similar for the rest of the year.

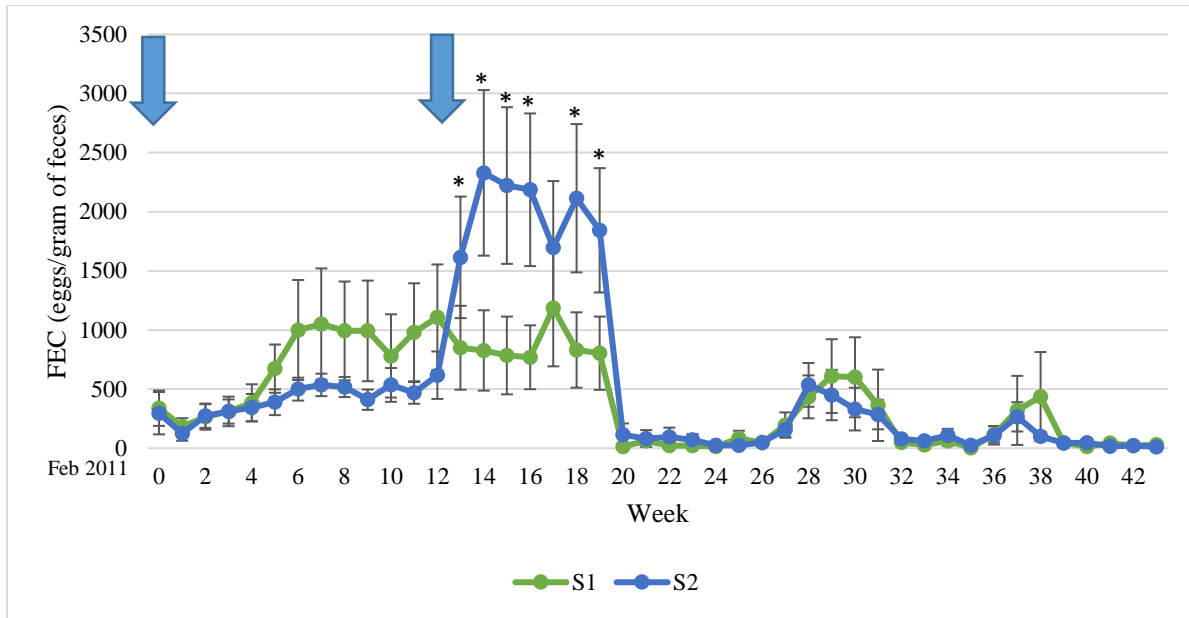


Figure 2. Year 1 mean ( $\pm$ SEM) fecal egg count (FEC) for strategy 1 (S1) ewes (n=13, supplemented with 0.91 kg/hd/d cracked corn) and strategy 2 (S2) ewes (n=14, supplemented with 0.91 kg/hd/d sericea lespedeza pellets). Arrows indicate start and end of supplemental feeding. \*Significant ( $p < 0.05$ ) difference. No dewormings were necessary.

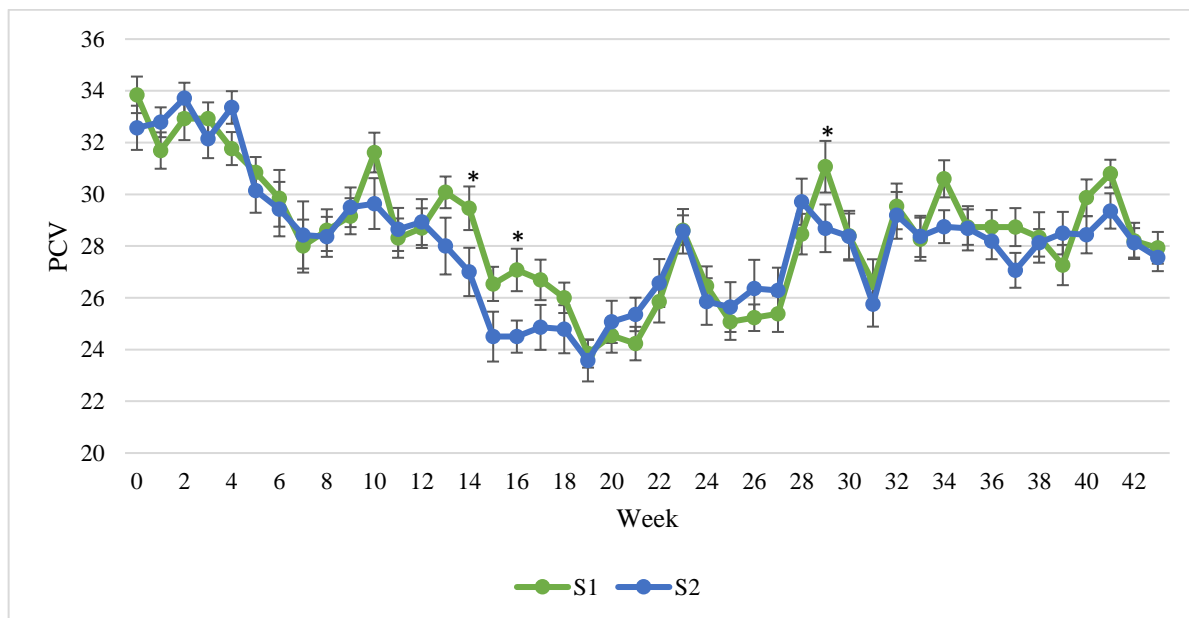


Figure 3. Year 1 mean ( $\pm$ SEM) blood packed cell volume (PCV) for strategy 1 (S1) ewes (n=13, supplemented with 0.91 kg/hd/d cracked corn) and strategy 2 (S2) ewes (n=14, supplemented with 0.91 kg/hd/d sericea lespedeza pellets). \*Significant ( $p < 0.05$ ) difference. No dewormings were necessary.

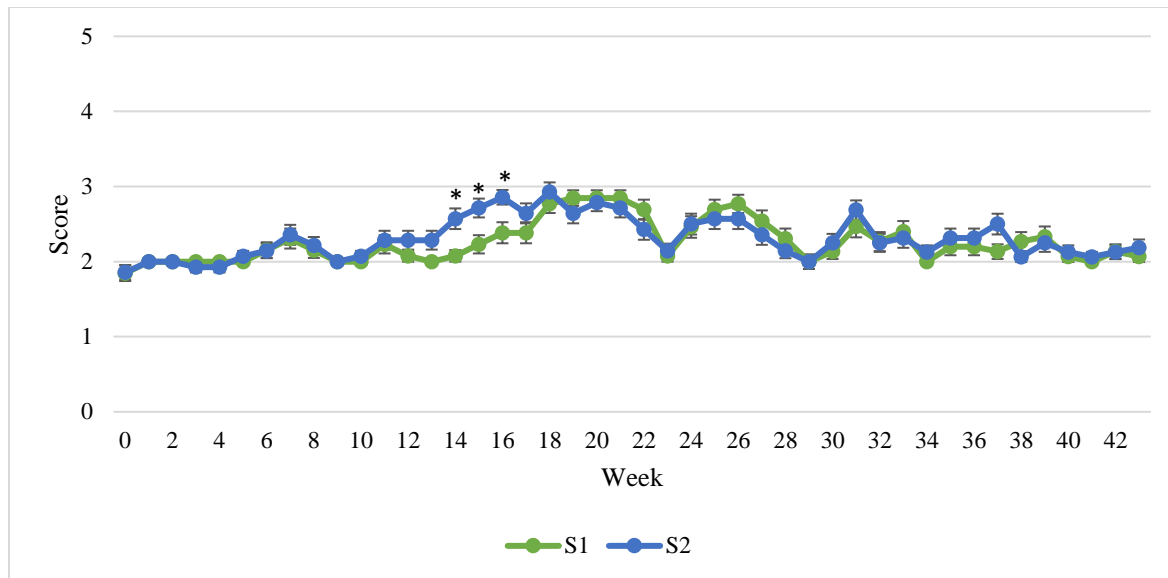


Figure 4. Year 1 mean ( $\pm$ SEM) FAMCHA© scores for strategy 1 (S1) ewes (n=15, supplemented with 0.91 kg/hd/d cracked corn) and strategy 2 (S2) ewes (n=16, supplemented with 0.91 kg/hd/d SL pellets). Arrows indicate start and end of supplemental feeding. \*Significant ( $p < 0.05$ ) difference. No dewormings were necessary.

At the beginning of yr 2 (January, 2012), ewes in both groups had similar ( $p > 0.05$ ) FEC through wk 16 (Figure 5). The FEC for S2 ewes was significantly ( $p < 0.05$ ) lower than the S1 ewes wk 17 and wk 42. On wk 22, supplement feeding for both groups was stopped. FEC remained similar for both groups through wk 39 and then increased for both groups through the end of the year; however, differences were still similar ( $p > 0.05$ ).

The PCV for both groups was similar ( $p > 0.05$ ) at the beginning of yr 2 until wk 4 (Figure 6). The PCV was significantly ( $p < 0.05$ ) lower for the S2 ewes than the S1 ewes on wk 5 and 7. However, the PCV was significantly ( $p < 0.05$ ) higher for the S2 ewes than the S1 ewes on wk 33.

The FAMCHA© scores for both groups were similar ( $p > 0.05$ ) at the beginning of the study through wk 4 (Figure 7). The FAMCHA© scores were significantly ( $p < 0.05$ ) higher for the S2 ewes than the S1 ewes on wk 5, 6, and 31. They remained similar for the rest of the year.

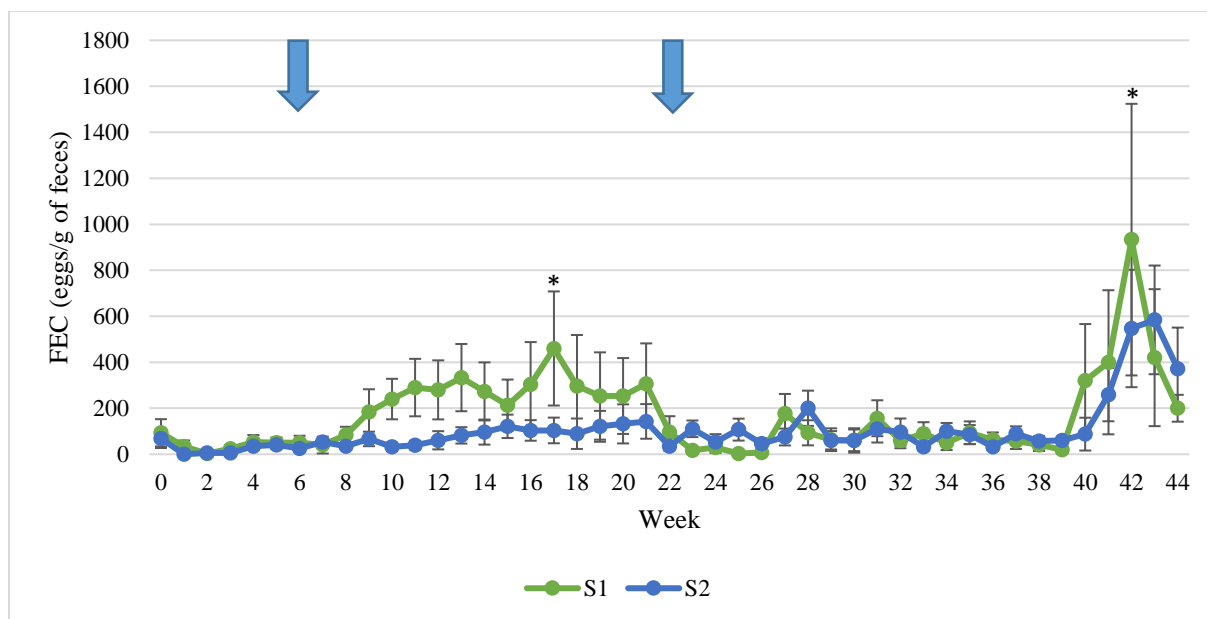


Figure 5. Year 2 mean ( $\pm$ SEM) fecal egg count (FEC) for strategy 1 (S1) ewes (n=15, supplemented with 0.91 kg/hd/d cracked corn) and strategy 2 (S2) ewes (n=16, supplemented with 0.91 kg/hd/d SL pellets). Arrows indicate start and end of supplemental feeding. \*Significant ( $p < 0.05$ ) difference. No dewormings were necessary.

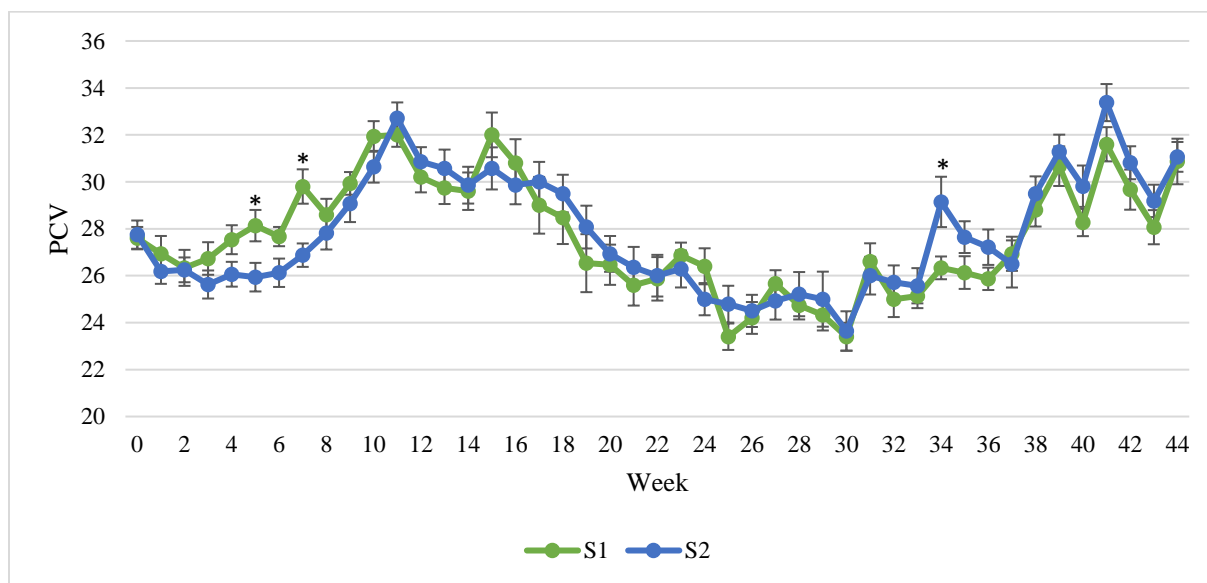


Figure 6. Year 2 mean ( $\pm$ SEM) blood packed cell volume (PCV) for strategy 1 (S1) ewes (n=15, supplemented with 0.91 kg/hd/d cracked corn) and strategy 2 (S2) ewes (n=16, supplemented with 0.91 kg/hd/d SL pellets). \*Significant ( $p < 0.05$ ) difference. No dewormings were necessary.

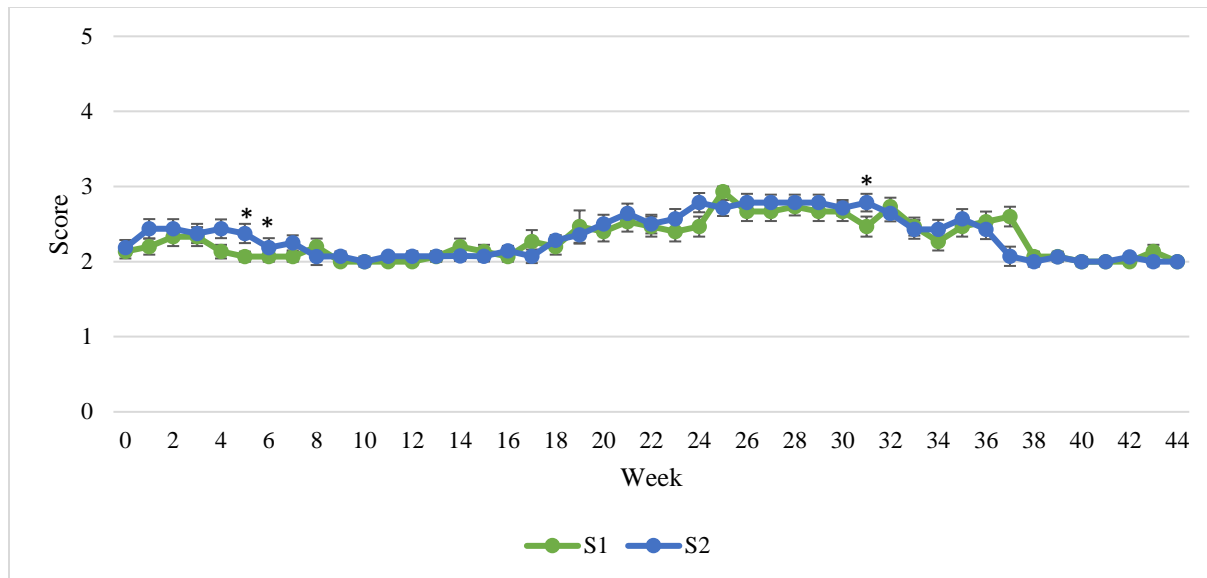


Figure 7. Year 2 Mean ( $\pm$ SEM) FAMCHA© scores for strategy 1 (S1) ewes (n=15, supplemented with 0.91 kg/hd/d cracked corn) and strategy 2 (S2) ewes (n=16, supplemented with 0.91 kg/hd/d SL pellets). Arrows indicate start and end of supplemental feeding. \*Significant ( $p < 0.05$ ) difference. No dewormings were necessary.

At the beginning of year 3 (January, 2013), ewes in both groups had similar ( $p > 0.05$ ) FEC (Figure 8). From wk 4 through wk 13, FEC increased in both groups and the S1 FEC was significantly ( $p < 0.05$ ) higher than S2 ewe FEC for wk 6, 8, and 10-12. From wk 10 through wk 18, the FEC of both groups decreased, becoming similar ( $p > 0.05$ ) by wk 14. On wk 18, supplement feeding was stopped and there was no difference ( $p > 0.05$ ) in FEC between groups for the remainder of the year.

The PCV for the S2 ewes was significantly ( $p < 0.05$ ) higher than the S1 ewes at the beginning of year 3 (Figure 9). The PCV for the S2 ewes was significantly ( $p < 0.05$ ) higher than S1 ewes on wk 9 and 22. However, the PCV was significantly ( $p < 0.05$ ) lower for the S2 ewes than the S1 ewes on wk 15.

The FAMCHA© scores for both groups were similar ( $p > 0.05$ ) at the beginning of the study and remained similar for the duration of year 3 (Figure 10).

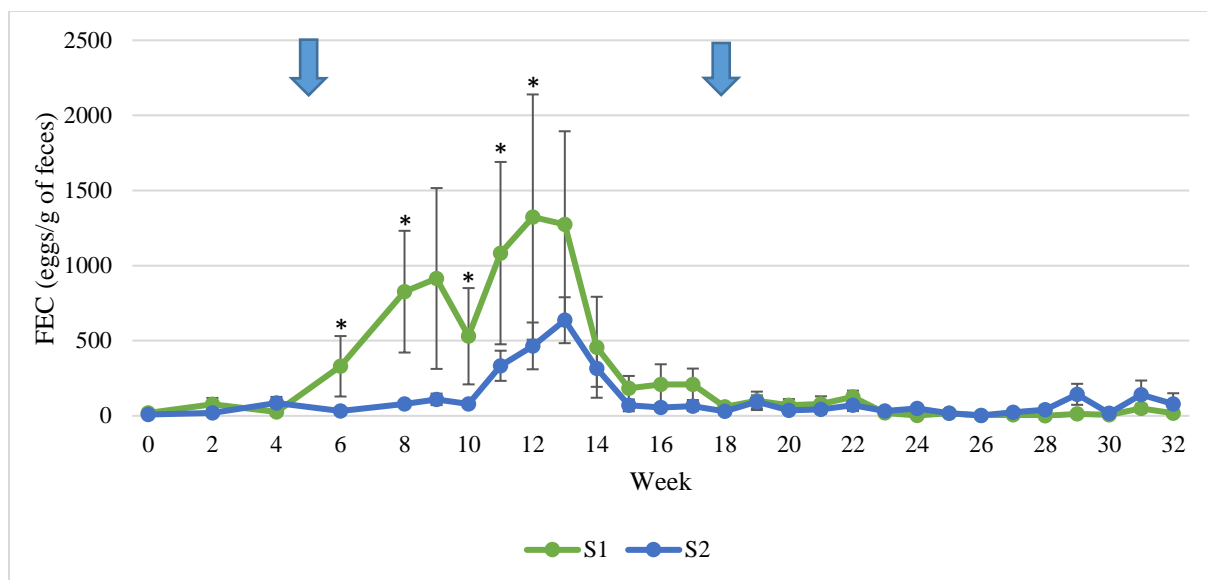


Figure 8. Year 3 mean ( $\pm$ SEM) fecal egg count (FEC) for strategy 1 (S1) ewes (n=15, supplemented with 0.91 kg/hd/d cracked corn) and strategy 2 (S2) ewes (n=16, supplemented with 0.91 kg/hd/d SL pellets). Arrows indicate start and end of supplemental feeding. \*Significant ( $p < 0.05$ ) difference. No dewormings were necessary.

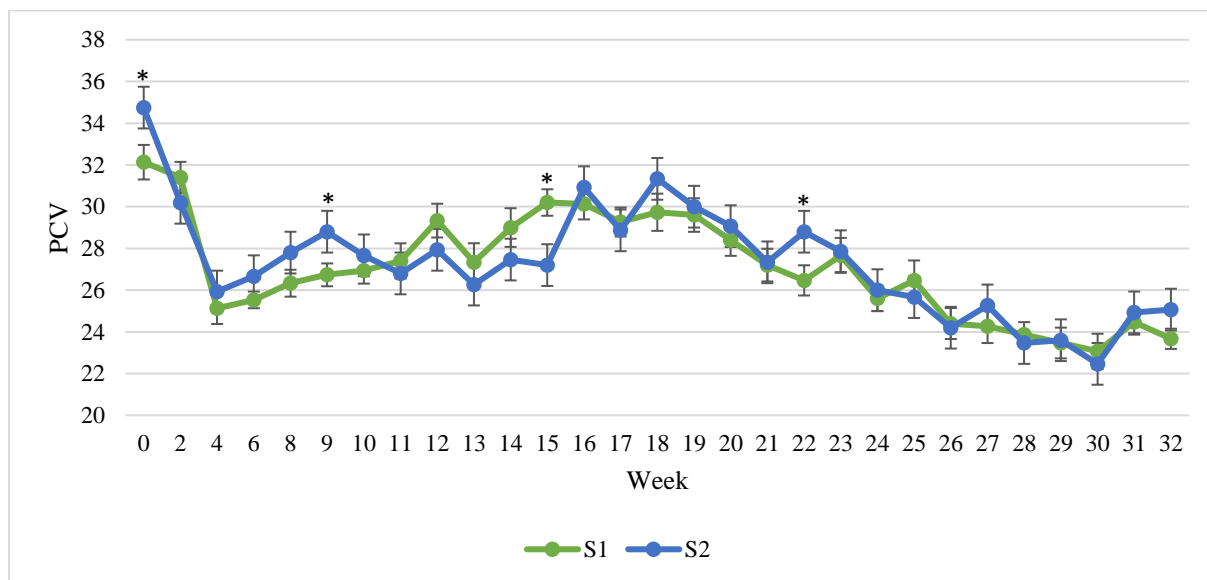


Figure 9. Year 3 mean ( $\pm$ SEM) blood packed cell volume (PCV) for strategy 1 (S1) ewes (n=15, supplemented with 0.91 kg/hd/d cracked corn) and strategy 2 (S2) ewes (n=16, supplemented with 0.91 kg/hd/d SL pellets). \*Significant ( $p < 0.05$ ) difference. No dewormings were necessary.

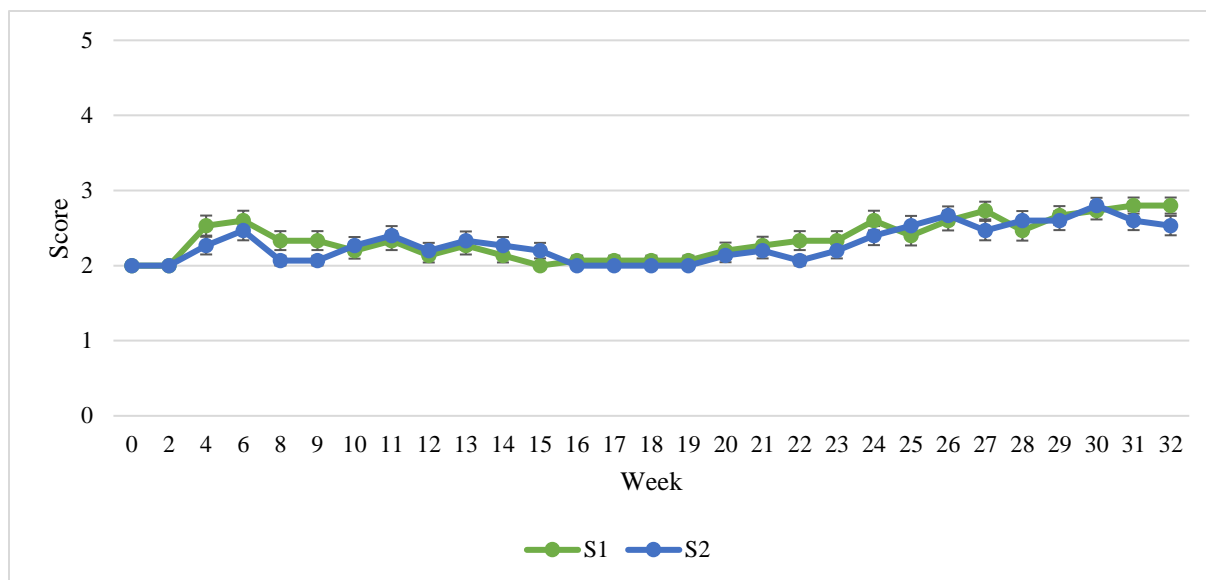


Figure 10. Year 3 Mean ( $\pm$ SEM) FAMCHA© scores for strategy 1 (S1) ewes (n=15, supplemented with 0.91 kg/hd/d cracked corn) and strategy 2 (S2) ewes (n=16, supplemented with 0.91 kg/hd/d SL pellets). Arrows indicate start and end of supplemental feeding. Differences were not significant ( $p > 0.05$ ). No dewormings were necessary.

Fecal cultures indicated that the three predominant genera of L3 were *Haemonchus*, *Trichostrongylus*, and *Cooperia*. In yr 1, there were 64% *Haemonchus*, 35% *Trichostrongylus*, and 1% *Cooperia*. In yr 2, no samples for culture were collected due to low FEC. In yr 3, there were 84% *Haemonchus*, 15% *Trichostrongylus*, and 1% *Cooperia*.

In yr 1, *Haemonchus* and *Trichostrongylus* L3 were recovered from S1 and S2 pasture forage (Figure 11). In April, the S1 group had more *Haemonchus* L3 than the S2 group. There were *Trichostrongylus* L3 on the S1 pasture and none on the S2 pasture. In May of yr 1, there were *Haemonchus* and *Trichostrongylus* L3 on S1 pasture but no L3 on the S2 pasture. In June, there were *Haemonchus* L3 on the S1 pasture and none on the S2 pasture. In July, there were higher numbers of *Haemonchus* L3 on the S2 pasture than the S1 pasture. Also, there were *Trichostrongylus* L3 on the S2 pasture and none on the S1 pasture. There were no L3 found

from August through November. In December, there were *Haemonchus* L3 found on S1 pasture but none on S2 pasture.

In yr 2, in February there were *Haemonchus* L3 found on the S1 pasture but not S2 pasture (Figure 12). There were no L3 found from March through August on the S1 pasture and no L3 found from March through October on S2 the pasture. In September, there were *Haemonchus* L3 on the S1 pasture and in November, there were *Haemonchus* and *Trichostrongylus* L3 on the S1 pasture while the S2 pasture had *Trichostrongylus* L3 in November. The *Trichostrongylus* L3 were higher on the S2 pasture than the S1 pasture. There were no L3 found in December on either pasture.

In yr 3, L3 on both pastures were found in April (Figure 13). In April, there were more *Haemonchus* and *Trichostrongylus* L3 on the S1 pasture when compared to the S2 pasture. Furthermore, there were *Haemonchus* and *Trichostrongylus* L3 on the S1 pasture and none on the S2 pasture for May.

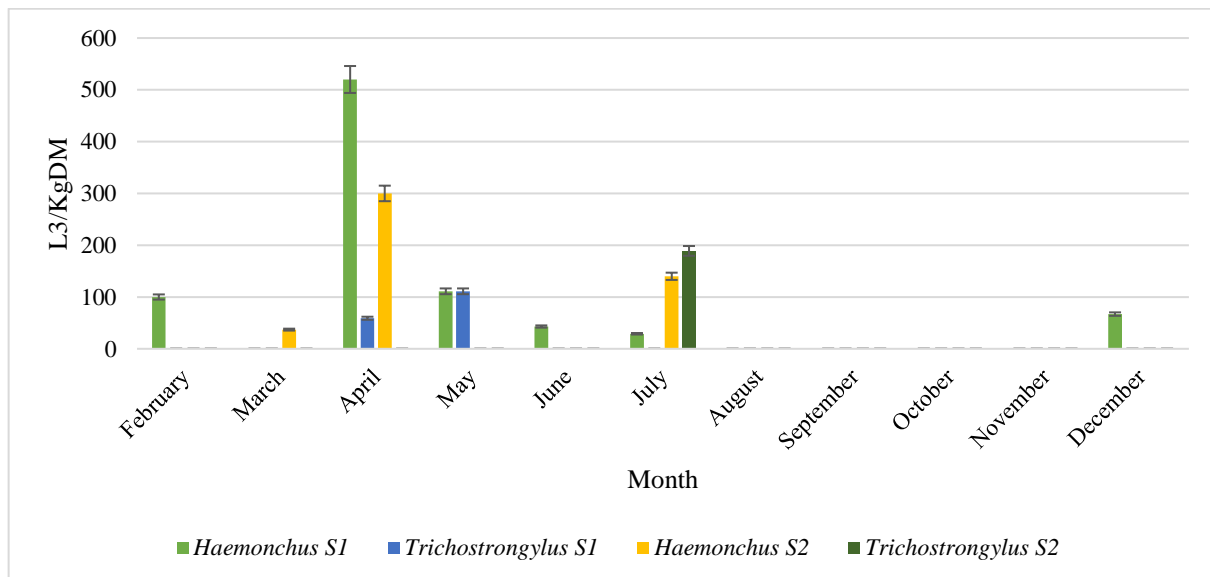


Figure 11. Larvae (L3) per kg dry matter on S1 and S2 ewe pastures from February through December in year 1.

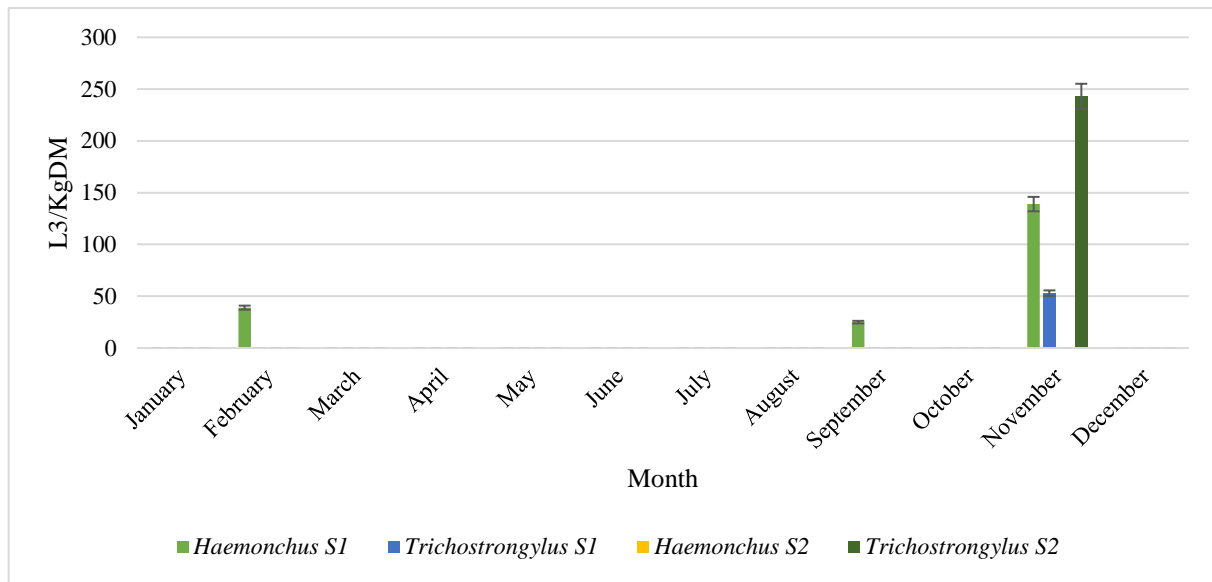


Figure 12. Larvae (L3) per kg dry matter on S1 and S2 ewe pastures from January through December in year 2.

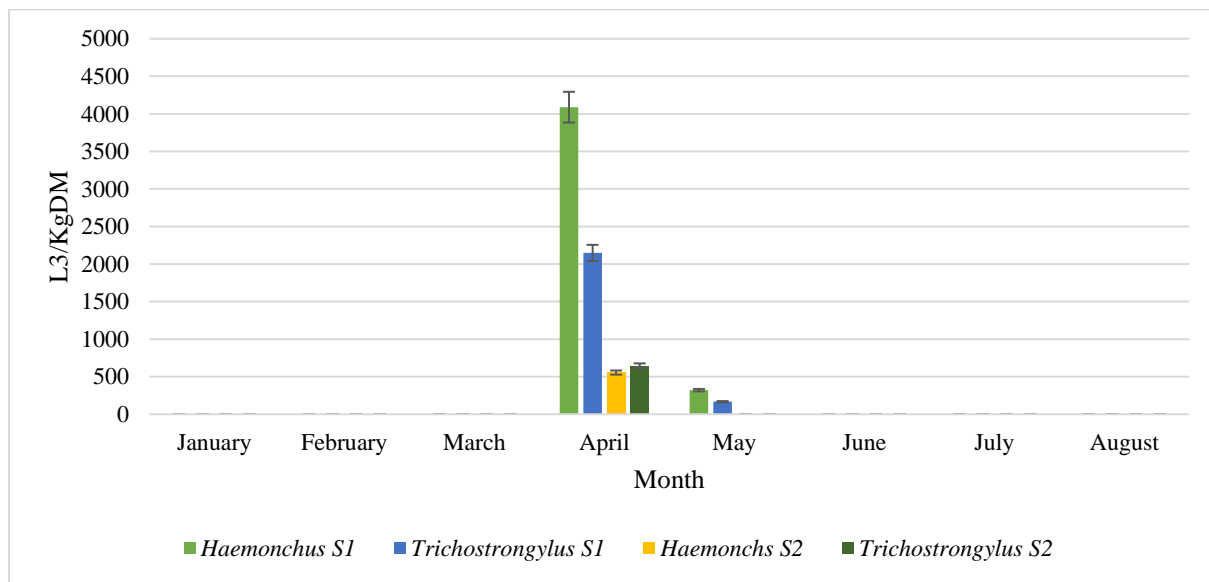


Figure 13. Larvae (L3) per kg dry matter on S1 and S2 ewe pastures from January through December in year 3.

### 3.6 Discussion

For all three yr, both groups exhibited a peri-parturient rise (PPR) in FEC and that rise was lower for S2 ewes than S1 ewes. The PPR is primarily due to the relaxation of immunity

during times of stress, in this case parturition and lactation. Adult female worms sense this relaxation which results in an increase in egg excretion and, thus, FEC. The results of this study indicated that supplementing with SL (for a 12 week period) suppressed the PPR possibly due to an adverse effect on female worm fecundity counteracting the relaxation of immunity. The effect on fecundity is supported by the substantial increase in FEC of S2 ewe FEC when SL supplementation was stopped in yr 1. However, for yr 2 and 3, after the supplement feeding stopped, the FEC did not increase for the S2 ewes but remained lower than the FEC in the S1 ewes. This suggests that SL suppressed FEC during the PPR. To support these results, Lange et al. (2006) found that feeding SL hay to lambs reduced FEC during the time of feeding, and once the feeding of SL stopped, FEC increased. This suggested that SL had an effect on fecundity. Terrill et al. (2007) and Shaik et al. (2006) found that SL hay and pelleted SL hay was effective in reducing FEC in goats. In addition, Terrill et al. (2006) reported the effect was also on adult worms in that worm burden was reduced by 67.2%.

It is important to know the GIN population distribution can be instrumental in interpretation of pattern of infection. The yr 1 population was predominantly *Haemonchus* (63%) followed by *Trichostrongylus* (36%). During yr 1, there were unusual high temperatures from July to September and ample rainfall for July (WeatherSpark, 2013). The yr 2 population could not be determined in that FEC was too low for culture to be meaningful. The yr 3 population was again predominately *Haemonchus* (84%, greater than yr 1) followed by *Trichostrongylus* (15%, less than yr 1). When found, *Cooperia* spp. was present in very low numbers (1%). This represented what has been the common historical pattern of infection at the study site with *Haemonchus* consistently being the predominant GIN followed by *Trichostrongylus*.

As would be expected, both groups of ewes experienced a decrease in FEC after the lambs were weaned. This usually occurs when the stress is removed and immune status returns to normal, thus again restricting egg output by female worms. Another explanation for the decrease is the self-cure phenomenon. A self-cure occurs when adult *H. contortus* are abruptly expelled from the abomasum. This usually occurs with a large influx of L3 over a short period of time which results in an inflammatory response that affects the ability of worms to remain in situ, thus they are expelled. With the heavy contamination of pasture with eggs and then L3 during lactation, the conditions could be right for heavy reinfection that would trigger the self-cure. However, regaining immune function is a more plausible explanation for the decrease in FEC. In addition, the infection conditions were such that no selective treatments had to be administered to either group which indicated low level of reinfection.

In conclusion, supplementation with SL alone appeared to be enough to control the PPR and thus might be a viable means to reduce pasture contamination over time.

## **CHAPTER 4**

### **LAMB FARM STUDY**

#### **4.1 Methods and Materials**

##### **4.1.1 Location and Animals**

The lamb studies were conducted at the Ben Hur Research Farm Sheep Unit of the Louisiana Agricultural Experiment Station in Baton Rouge, Louisiana from June-September, 2011, 2012, and 2013.

##### **4.1.2 Experimental Design**

##### **4.1.3 Farm: Year 1-3**

In this 3 yr study, two integrated control strategies were compared, one strategy (S1) used a conventional anthelmintic/drug approach and the other strategy (S2) used a non-anthelmintic/drug approach in lambs. Lambs, after weaning, were placed in different pastures from their dams, either in the S1 group, or S2 group for yr 1, yr 2, and yr 3.

The 15 heaviest lambs from each S1 and S2 ewe groups were moved to separate pastures to acquire natural GIN infections for the summer. Each yr, lambs grazed the same 2 separate pastures. Lambs were housed separately at night, and had water and hay available at all times for the duration of the study. There were 13 male and 2 female S1 lambs (2 triplet born males, 8 twin born males, 3 single born males, and 2 twin born females) and 8 male and 7 female S2 lambs (7 twin born males, 1 single born male, 4 twin born females, and 3 single born females) in yr 1. In yr 2, there were 7 male and 8 female S1 lambs (6 twin born males, 1 single born male, 5 twin born females, and 3 single born females) and 10 male and 5 female S2 lambs (8 twin born males, 2 single born males, 4 twin born females, and 3 single born females). Additionally, there were 9 male and 6 female S1 lambs (9 twin born males, 4 twin born females, and 2 single born females) and 11 male and 4 female S2 lambs (9 twin born males, 2 single born males, 2 twin born females, and 2 single born females) in yr 3.

#### **4.1.4 S1 Lambs**

The S1 lambs grazed a Bermuda grass pasture and were supplemented with Purina® Honor® Show Lamb™ Showlamb Grower (15% CP and 8.5% CF, 0.91 kg/hd/d) to meet nutrient needs based on National Research Council requirements (2007).

Individual lambs were selectively dewormed with levamisole (LEV, Levasole®, 8 mg/kg drench) and albendazole (ABZ, Valbazen®, 10 mg/kg drench) when FAMACHA© score was 4/5. In August of yr 1, moxidectin (MOX, Cydectin®, 0.2 mg/kg drench) was added for subsequent dewormings. This combination approach was needed, because of drug resistance issues at the farm. All lambs other than replacement ewe lambs were sold after the summer grazing period.

#### **4.1.4 S2 Lambs**

S2 lambs were managed similarly to the S1 lambs. Lambs grazed a pasture that was predominantly SL, but also had some other mixed grasses. Lambs were supplemented with SL pellets (Sims Brother Inc., Union Springs, AL; 12% CP and 20% CF; 0.91 kg/hd/d). Individual lambs were selectively dewormed with copper oxide wire particles (COWP, Copasure®, 1g orally in gel caps) when FAMACHA© score was 4/5. All lambs other than replacement ewe lambs were sold after the summer grazing period.

For yr 1, a coccidiostat was added to the water to control coccidiosis in lambs as part of a management practice at the farm. Collaborators in Arkansas had noted SL reduced coccidia fecal oocyst count (FOC) in lambs and kids; therefore, for yr 2 and 3, no coccidiostat was used and coccidia FOC was monitored and a separate efficacy study was conducted (See Chapter 5).

## **4.2 Techniques**

### **4.2.1 General**

Collection and analytical techniques used in this experiment were the same techniques as described in Chapter 3. One addition was that the level of coccidia (*Eimeria* spp.) infection was determined by FOC. Oocysts were counted along with GIN eggs with the McMaster technique. Animals were weighed monthly to monitor weight gain for production purposes.

### **4.3 Statistical Analysis**

Data were analyzed using SAS® (version 9.4, SAS Institute, Cary, NC) as a repeated measures analysis of variance using Proc Mixed. Fixed effects included Group, Week and the Group\*Week interaction. The random effect was Animal (Group). The response variables were EPG, logepg, PCV, FAMACHA, COCCIDIA, logcoccidia, and WEIGHT. Differences were considered significant when  $p \leq 0.05$ .

### **4.4 Results**

At the beginning of yr 1, the FEC for the S2 lambs was significantly ( $p < 0.05$ ) higher than S1 lambs, but then were significantly ( $p < 0.05$ ) lower for wk 5, 6, and wk 9 through 12 (Figure 14). As the FEC increased after wk 5 for the S1 lambs, deworming was required for 17 lambs in July (3 on wk 5, 2 on wk 6, 9 on wk 7, and 3 on wk 8). In August, 8 lambs required deworming and when that deworming lacked efficacy, 16 lambs required deworming with MOX. Thereafter, MOX was added to the combination. Deworming was required for 2, 6, 6, 3 and 7 lambs on wk 9, 10, 11, 12 and 13, respectively. At the end of yr 1 (wk 16), 8 lambs required deworming. After wk 4, the FEC of S2 lambs was significantly ( $p < 0.05$ ) lower than S1 lambs. Deworming was required for 1, 2, 3, 2, 2 and 5 lambs on wk 7, 8, 9, 11, 12 and 13, respectively. At the end of yr 1 (wk 16), 6 lambs required deworming. During yr 1, 49 and 21 treatments were given to S1 and S2 lambs, respectively.

At the beginning of yr 1, the PCV for both groups was similar ( $p > 0.05$ , Figure 15). From wk 5 through wk 14, the PCV for S1 lambs was significantly ( $p < 0.05$ ) lower than the PCV for S2 lambs for the duration of yr 1. The PCV for the S1 lambs decreased from 25 to 16 and remained lower than S2 lambs. The PCV for S2 lambs decreased from 26 to 22 after wk 8 and remained stable until the end of the year.

There was no significant difference ( $p > 0.05$ ) in weight between S1 and S2 lambs (Figure 16).

At the beginning of the study, the FAMACHA© score was similar ( $p > 0.05$ ) for both groups and remained similar through wk 5 (Figure 17). The FAMACHA© scores for the S2 lambs were significantly ( $p < 0.05$ ) lower than the FAMACHA© scores for the S1 lambs on wk 6, 7, 9, 10, 12, and 16.

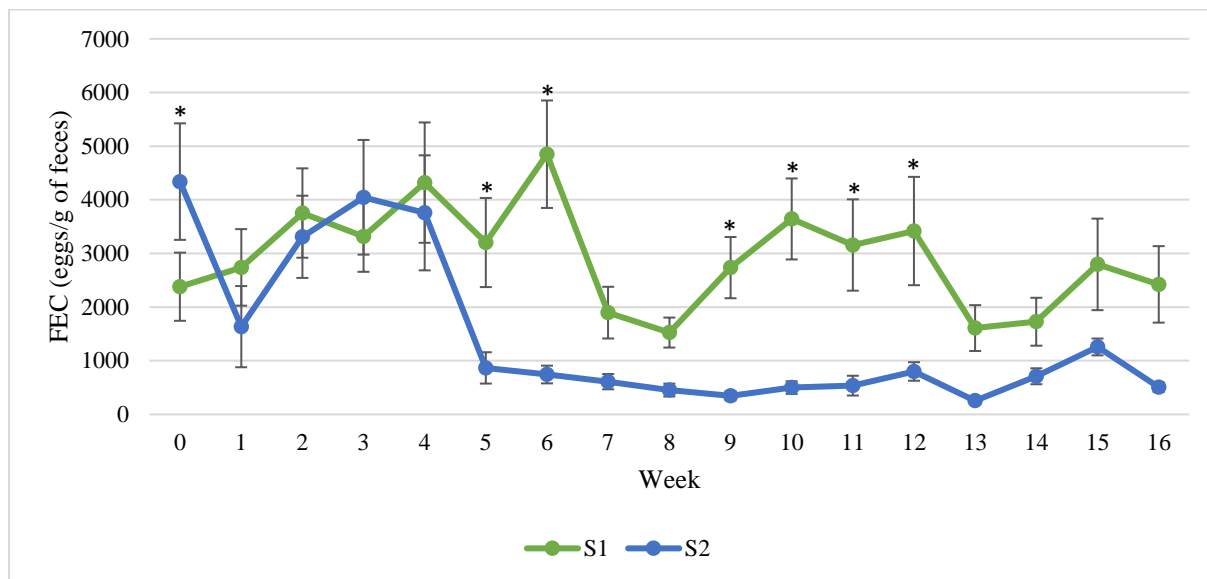


Figure 14. Year 1 mean ( $\pm$ SEM) fecal egg count (FEC) for strategy 1 (S1) lambs ( $n=15$ , supplemented with 0.91 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower) and strategy 2 (S2) lambs ( $n=15$ , supplemented with 0.91 kg/hd/d SL pellets). \*Significant ( $p < 0.05$ ) difference.

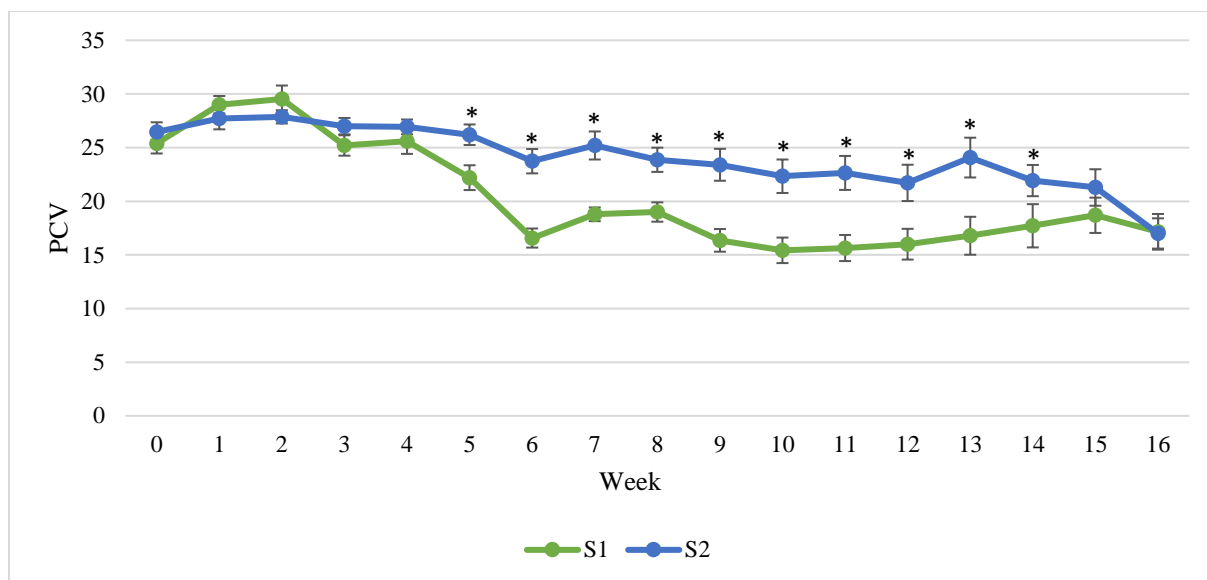


Figure 15. Year 1 mean ( $\pm$ SEM) blood packed cell volume (PCV) for strategy 1 (S1) lambs (n=15, supplemented with 0.91 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower) and strategy 2 (S2) lambs (n=15, supplemented with 0.91 kg/hd/d SL pellets). \*Significant ( $p < 0.05$ ) difference.

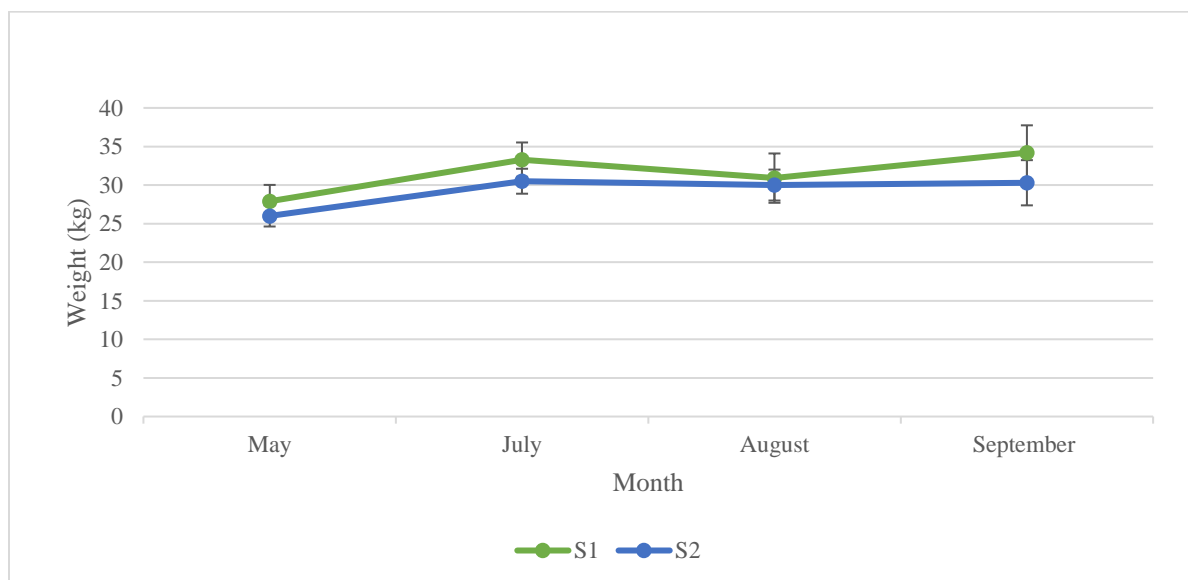


Figure 16. Year 1 mean ( $\pm$ SEM) lamb weights for strategy 1 (S1) lambs (n=15, supplemented with 0.91 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower) and strategy 2 (S2) lambs (n=15, supplemented with 0.91 kg/hd/d SL pellets). There was no significant difference ( $p > 0.05$ ).

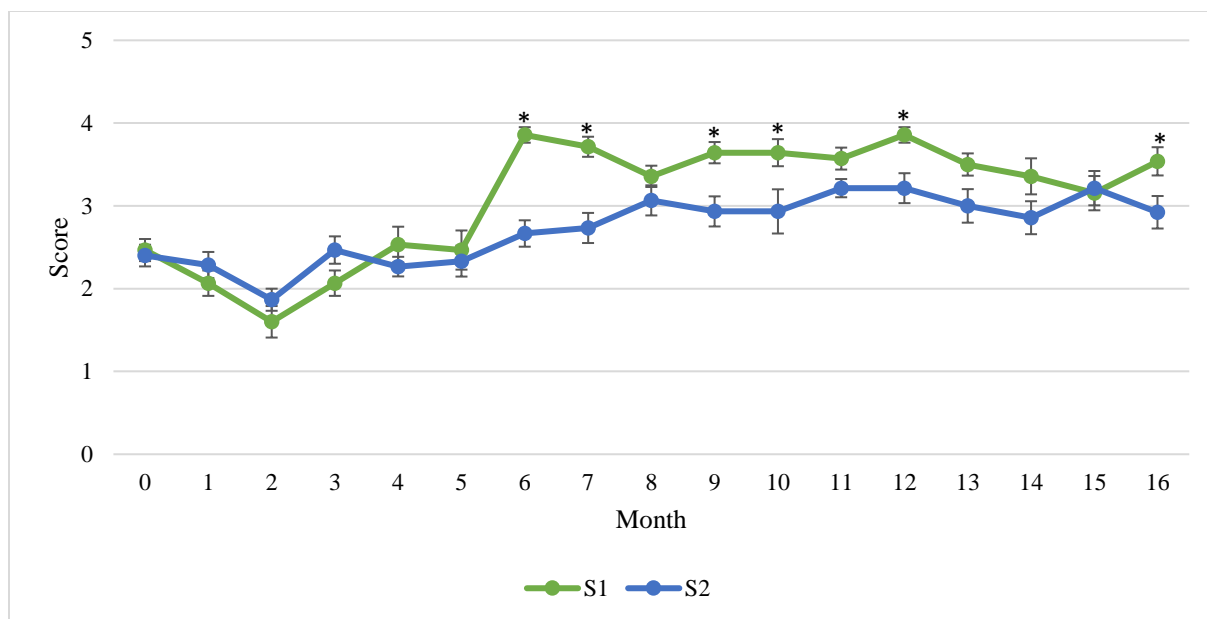


Figure 17. Year 1 mean ( $\pm$ SEM) FAMCHA© scores for strategy 1 (S1) lambs (n=15, supplemented with 0.91 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower) and strategy 2 (S2) lambs (n=15, supplemented with 0.91 kg/hd/d SL pellets). \*Significant ( $p < 0.05$ ) difference.

At the beginning of yr 2, the S2 lambs FEC was significantly ( $p < 0.05$ ) lower than the FEC for the S1 lambs and remained significantly ( $p < 0.05$ ) lower on wk 1, 2 and 4 -8 (Figure 18). The FEC for S1 and S2 lambs was 2842 EPG and 949 EPG, respectively. After wk 8 there was a steady decrease, after which there was no difference ( $p > 0.05$ ) between groups. One S1 lamb required deworming (LEV/ABZ/MOX) after the first wk in June and 2 lambs required deworming after wk 5 in July. In July, August, and September, one S2 lamb required deworming (COWP).

The PCV for the S2 lambs was significantly ( $p < 0.05$ ) higher than the S1 lambs for wk 1 and wk 4 (Figures 19). The PCV for both groups remained similar for both groups for the rest of the study.

Lamb weights were similar ( $p > 0.05$ ) for both groups at the beginning of the study through August (Figure 20). Weight for the S2 lambs was significantly ( $p < 0.05$ ) lower than the weight of the S1 lambs in September.

The FAMACHA© score was similar ( $p > 0.05$ ) for both groups at the beginning of the study (Figure 21). The FAMACHA© scores for the S2 lambs were significantly ( $p < 0.05$ ) lower than the FAMACHA© scores for the S1 lambs on wk 1, 3, and 11.

In addition, it was noted that the S2 lambs had significantly ( $p < 0.05$ ) lower coccidia fecal oocyst counts (FOC) than the S1 lambs for the first 5 wk after weaning (Figures 22). Overall FOC for S1 and S2 lambs was 1557 oocysts/g (OPG) and 275 OPG, respectively. Subsequent to wk 4, FOC for both groups were similar ( $p > 0.05$ ).

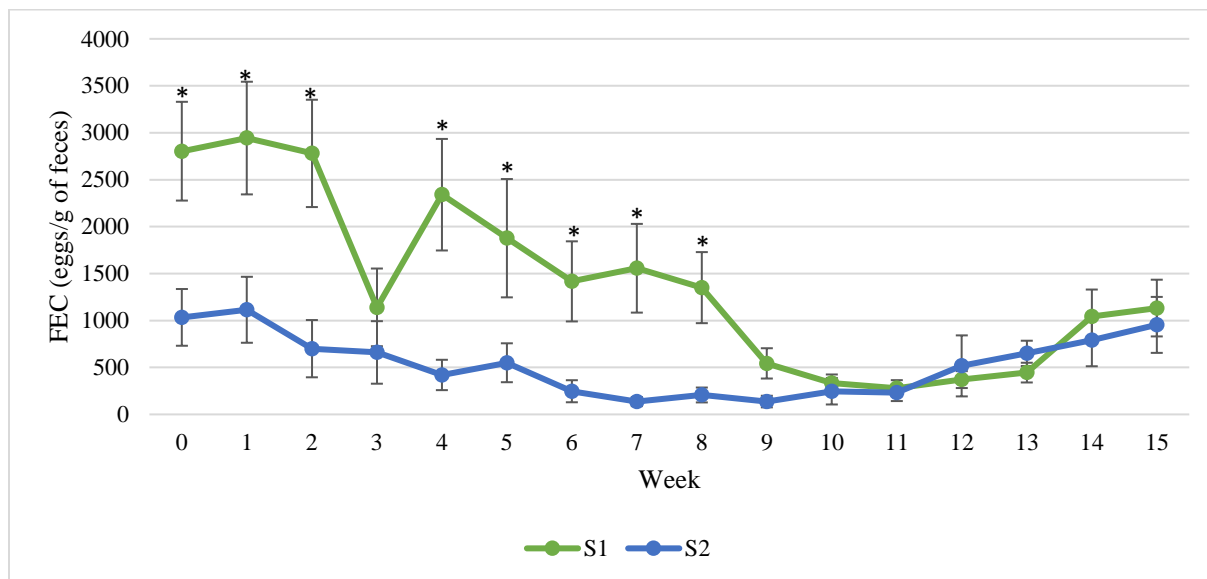


Figure 18. Year 2 mean ( $\pm$ SEM) fecal egg count (FEC) for strategy 1 (S1) lambs ( $n=15$ , supplemented with 0.91 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower) and strategy 2 (S2) lambs ( $n=15$ , supplemented with 0.91 kg/hd/d SL pellets). \*Significant ( $p < 0.05$ ) difference.

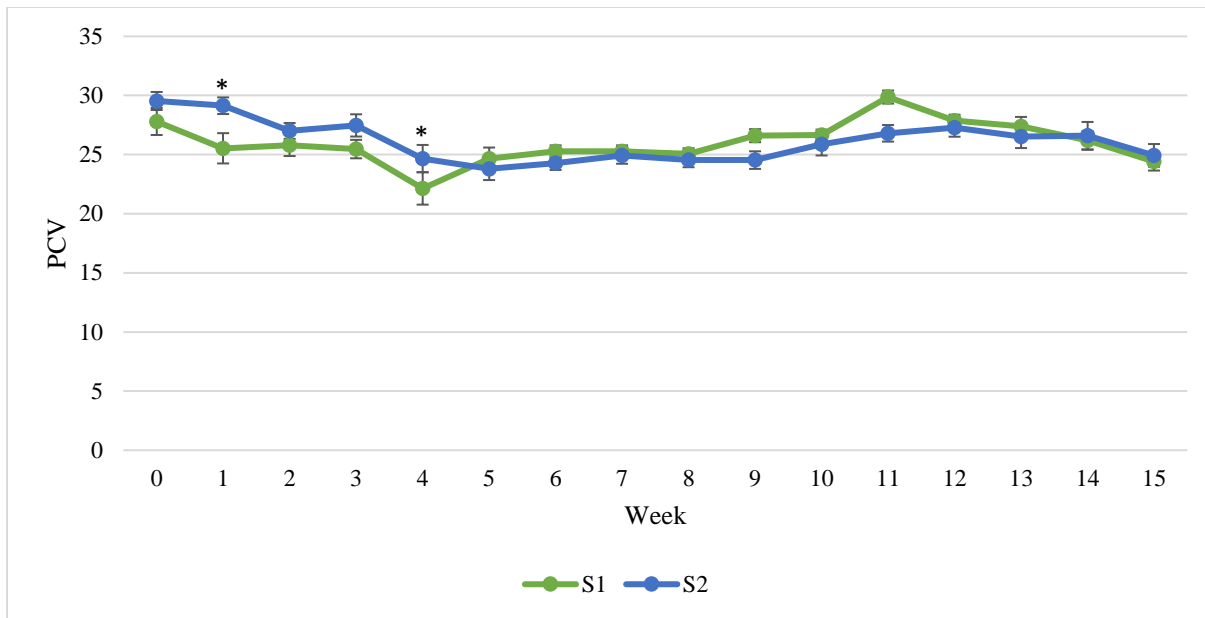


Figure 19. Year 2 mean ( $\pm$ SEM) blood packed cell volume (PCV) for strategy 1 (S1) lambs (n=15, supplemented with 0.91 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower) and strategy 2 (S2) lambs (n=15, supplemented with 0.91 kg/hd/d SL pellets). \*Significant ( $p < 0.05$ ) difference.

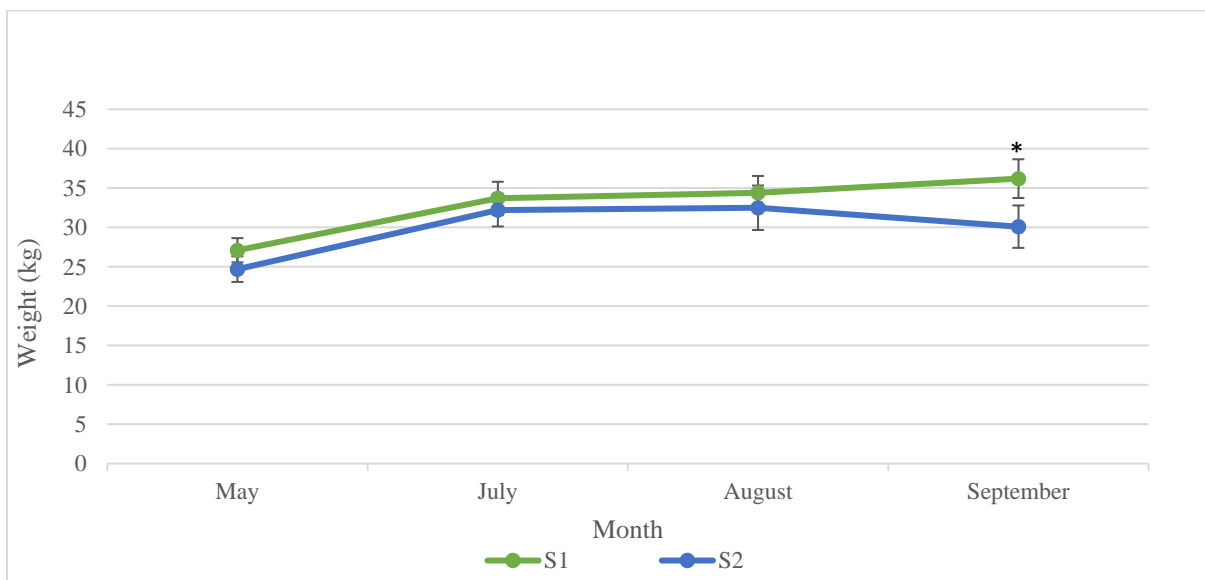


Figure 20. Year 2 mean ( $\pm$ SEM) weight for strategy 1 (S1) lambs (n=15, supplemented with 0.91 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower) and strategy 2 (S2) lambs (n=15), supplemented with 0.91 kg/hd/d SL pellets. \*Significant ( $p < 0.05$ ) difference.

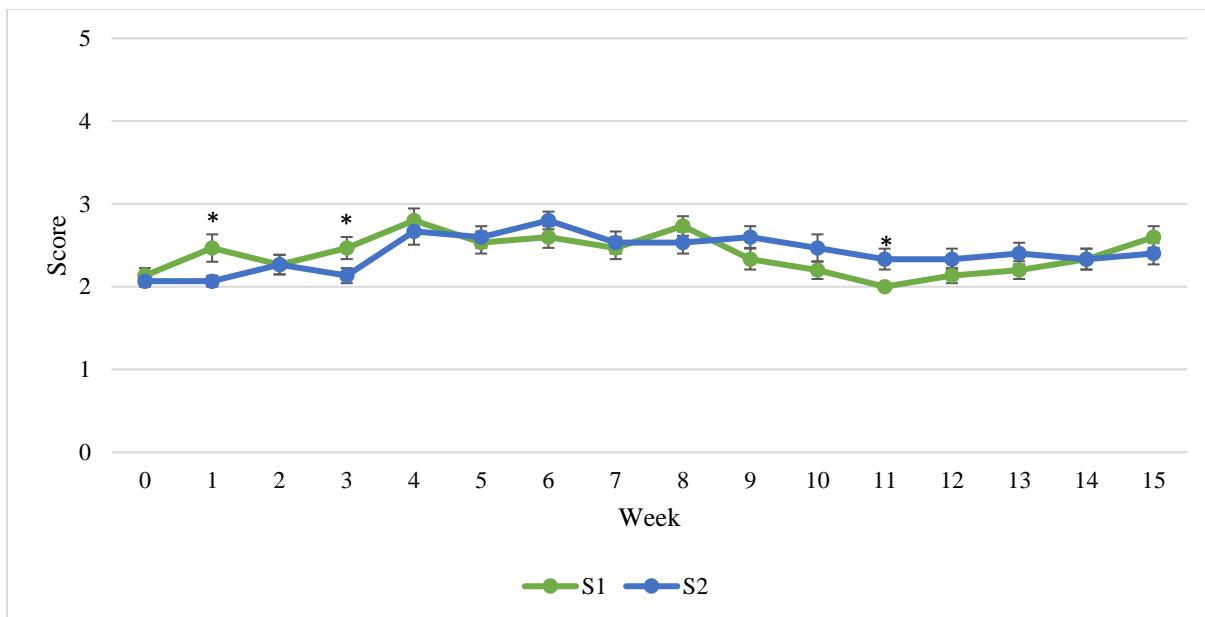


Figure 21. Year 2 mean ( $\pm$ SEM) FAMCHA© scores for strategy 1 (S1) lambs (n=15, supplemented with 0.91 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower) and strategy 2 (S2) lambs (n=15, supplemented with 0.91 kg/hd/d SL pellets). \*Significant (p < 0.05) difference.

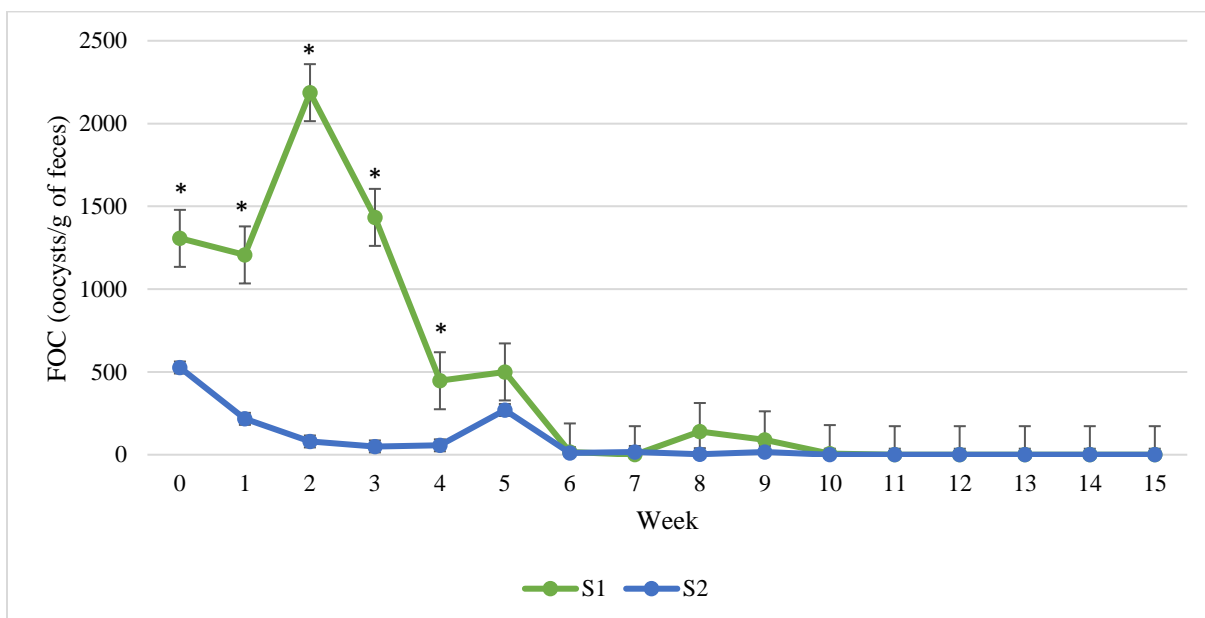


Figure 22. Year 2 mean ( $\pm$ SEM) fecal oocyst count (FOC) for strategy 1 (S1) lambs (n=15, supplemented with 0.91 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower) and strategy 2 (S2) lambs (n=15, supplemented with 0.91 kg/hd/d SL pellets). \*Significant (p < 0.05) difference.

At the beginning of yr 3, the S2 lambs FEC was significantly ( $p < 0.05$ ) lower than the S1 lambs (Figure 23). The FEC for the S1 and S2 lambs was 2819 EPG and 637 EPG, respectively. The FEC remained high for the S1 lambs through wk 9 but steadily decreased to the level of the S2 lambs and were similar for the rest of the yr. The FEC remained low for the S2 group through wk 11 and then increased slightly on wk 12. The S2 group FEC was significantly ( $p < 0.05$ ) lower than the S1 group FEC on wk 0, 1, 3, 4, and 6-8. Dewormings for S1 lambs included 2 lambs on wk 1, 1 lamb on wk 9 and 2 lambs on wk 13. Dewormings for S2 lambs included 1 lamb on wk 10 (repeated on wk 11) and 1 lamb on wk 13.

The PCV for both groups was similar at the beginning and steadily decreased over the 14 wk period (Figures 24). The PCV for the S1 lambs was significantly ( $p < 0.05$ ) lower than the PCV from the S2 lambs on wk 3.

Weight for the S1 and S2 lambs was significantly different in July, August, and September ( $p < 0.05$ , Figure 25).

The FAMACHA© score was similar ( $p > 0.05$ ) for both groups at the beginning of the study and for the duration of the study (Figure 26).

As in yr 2, it was noted that S2 lambs had a significantly ( $p < 0.05$ ) lower coccidia FOC than S1 lambs following weaning (Figure 27), but were similar after wk 1. Overall FOC for S1 and S2 lambs was 1726.7 OPG and 546.7 OPG, respectively.

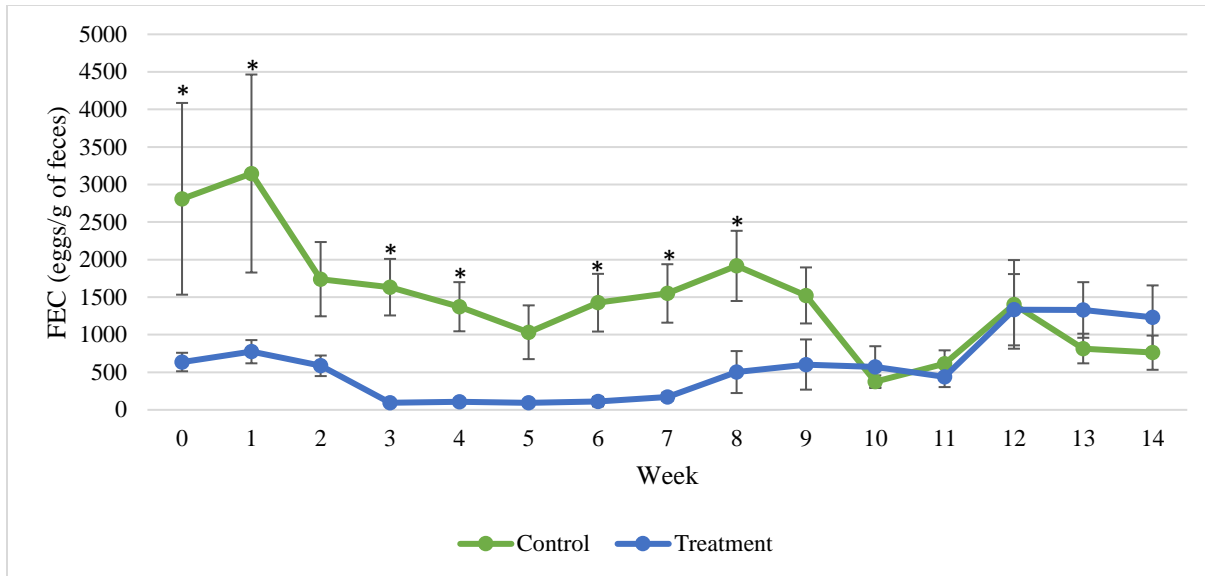


Figure 23. Year 3 mean ( $\pm$ SEM) fecal egg count (FEC) for strategy 1 (S1) lambs (n=15, supplemented with 0.91 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower) and strategy 2 (S2) lambs (n=15, supplemented with 0.91 kg/hd/d SL pellets). \*Significant ( $p < 0.05$ ) difference.

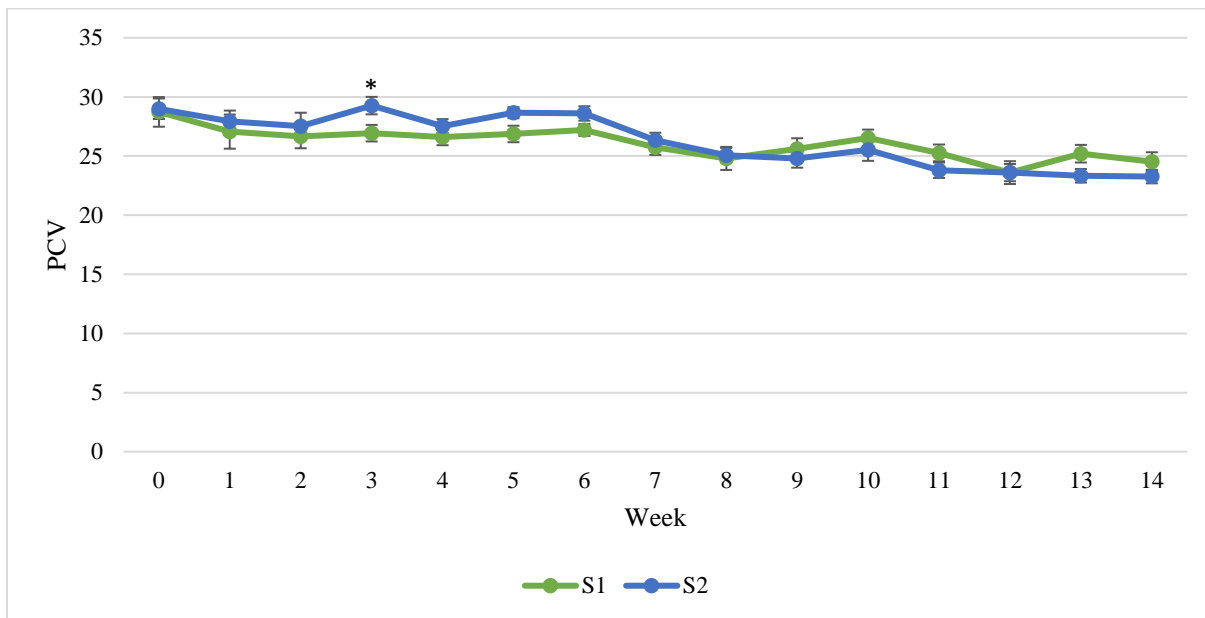


Figure 24. Year 3 mean ( $\pm$ SEM) blood packed cell volume (PCV) for strategy 1 (S1) lambs (n=15, supplemented with 0.91 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower) and strategy 2 (S2) lambs (n=15, supplemented with 0.91 kg/hd/d SL pellets). \*Significant ( $p < 0.05$ ) difference.

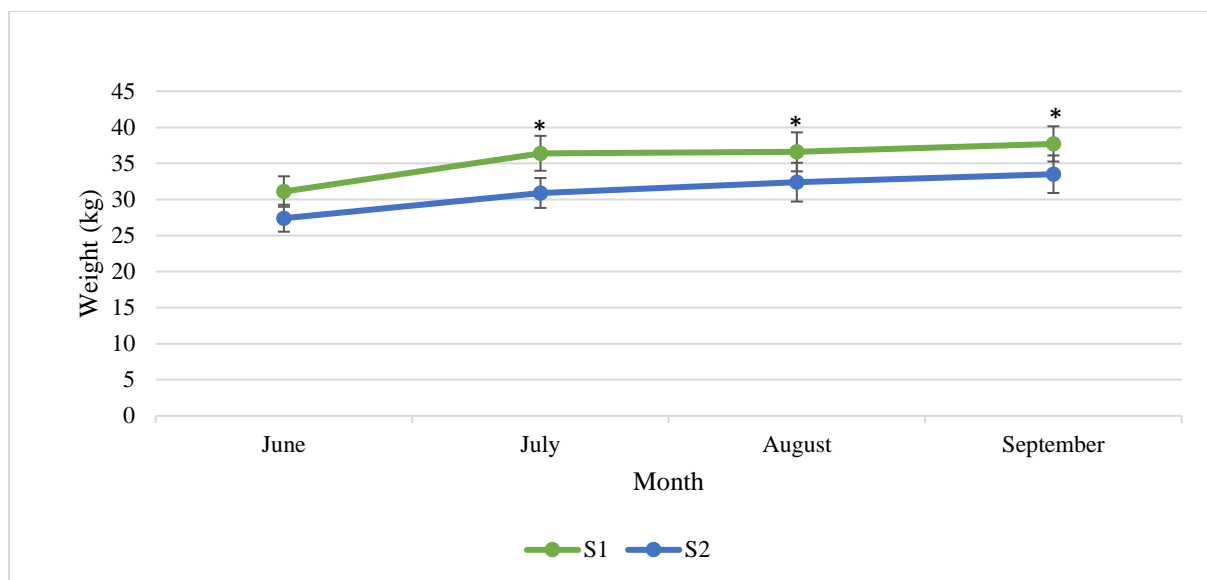


Figure 25. Year 3 mean ( $\pm$ SEM) lamb weight for strategy 1 (S1) lambs (n=15, supplemented with 0.91 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower) and strategy 2 (S2) lambs (n=15, supplemented with 0.91 kg/hd/d SL pellets). \*Significant ( $p < 0.05$ ) difference.

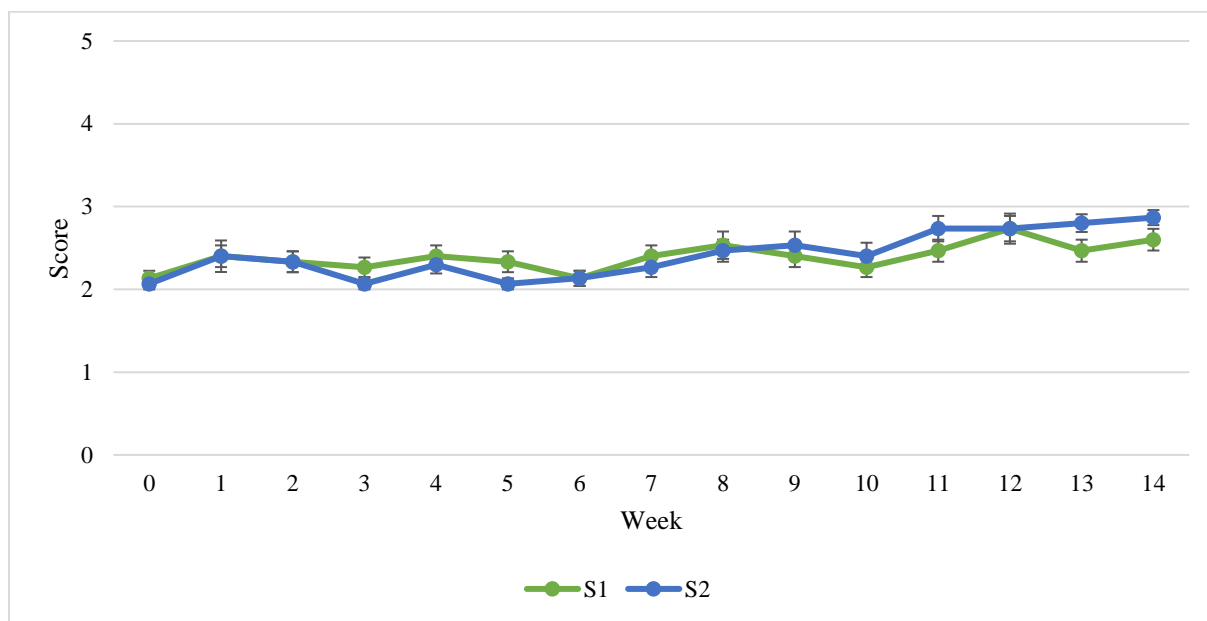


Figure 26. Year 3 mean ( $\pm$ SEM) FAMCHA© scores for strategy 1 (S1) lambs (n=15, supplemented with 0.91 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower) and strategy 2 (S2) lambs (n=15, supplemented with 0.91 kg/hd/d SL pellets). There was no significant difference ( $p > 0.05$ ) between groups.

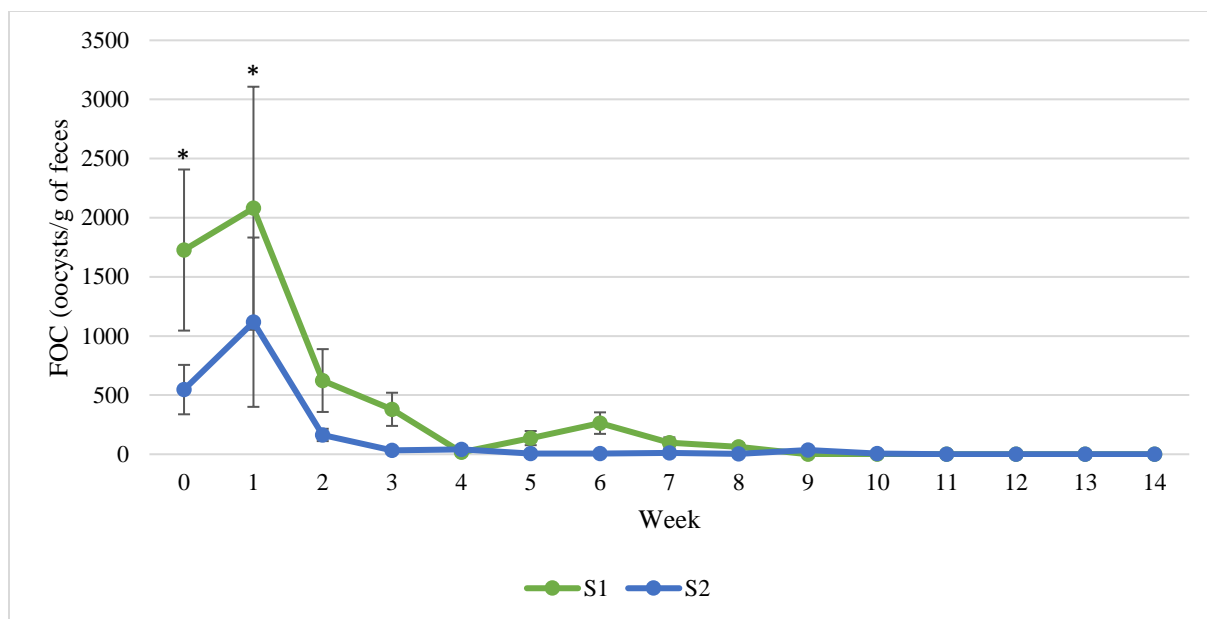


Figure 27. Year 3 mean ( $\pm$ SEM) fecal oocyst count (FOC) for strategy 1 (S1) lambs (n=15, supplemented with 0.91 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower) and strategy 2 (S2) lambs (n=15, supplemented with 0.91 kg/hd/d SL pellets). \*Significant ( $p < 0.05$ ) difference.

As observed for the ewe study (Chapter 3), fecal cultures showed that the three predominant genera of GIN L3 were *Haemonchus*, *Trichostrongylus* and *Cooperia*. In yr 1, there were 84% *Haemonchus*, 15% *Trichostrongylus* and 1% *Cooperia*. In yr 2, there were 28% *Haemonchus*, 69% *Trichostrongylus* and 3% *Cooperia*. In yr 3, there were 55% *Haemonchus*, 42% *Trichostrongylus* and 3% *Cooperia*.

For yr 1, there were no L3 found on either the S1 or S2 pastures in June, September, and October (Figure 28). In July, there were *Haemonchus* and *Trichostrongylus* found on the S2 pasture but no L3 on the S1 pasture. However, in August, there were no L3 found on the S2 pasture, but *Haemonchus* and *Trichostrongylus* L3 were found on the S1 pasture.

For yr 2, there were no L3 found on either pasture in June, August, or September. In July, there were *Haemonchus* L3 found on the S1 pasture (Figure 29). There were no L3 found on the S2 pasture.

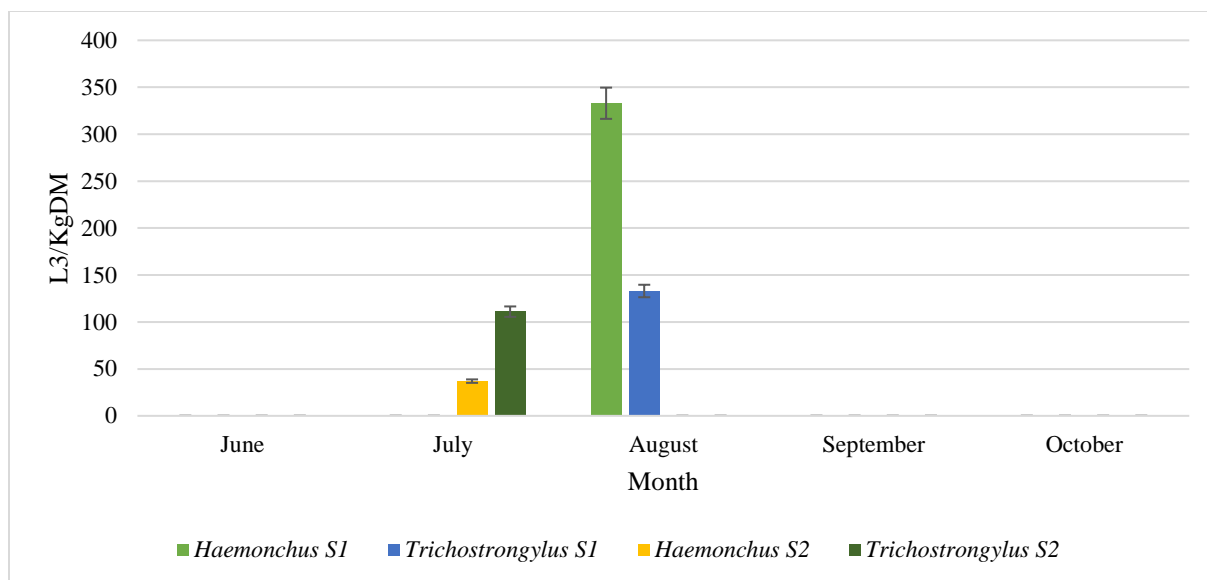


Figure 28. Larvae (L3) per kg dry matter on S1 and S2 lamb pasture from June through October in year 1.

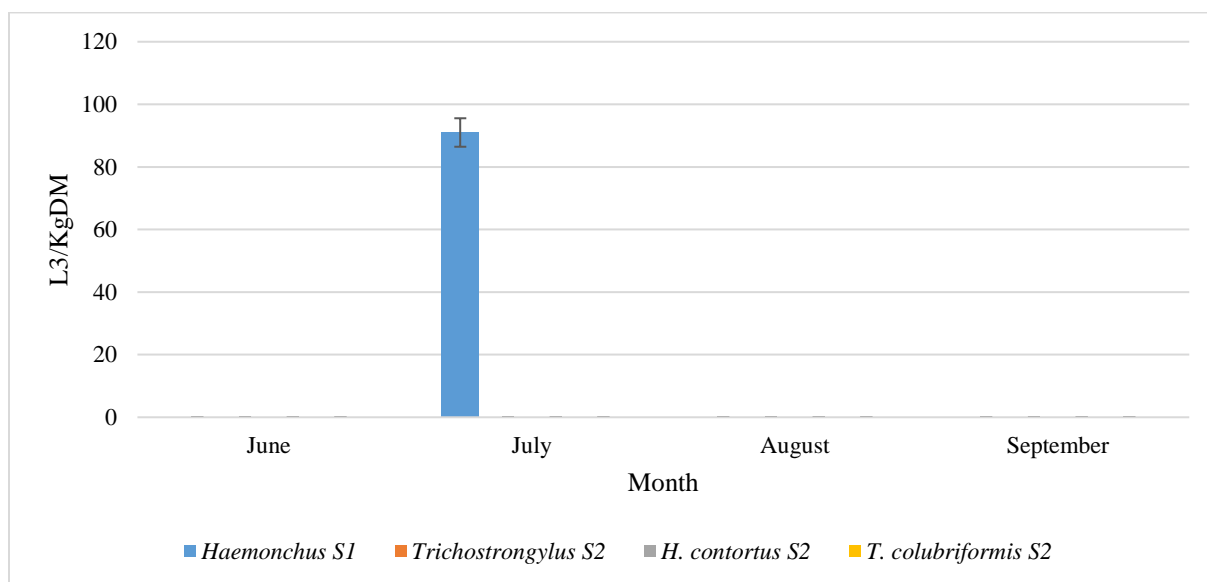


Figure 29. Larvae (L3) per kg dry matter on S1 and S2 lamb pasture from June through October in year 2.

For yr 3, there were L3 found on both pastures in July, August, and September (Figure 30). In July, there were more *Haemonchus* L3 found on the S1 pasture than on the S2 pasture. In August, there were similar numbers of *Haemonchus*. However, there were more

*Trichostrongylus* L3 on the S1 pasture than the S2 pasture. In September, there were more *Haemonchus* L3 found on the S2 pasture than the S1 pasture. There were *Trichostrongylus* L3 on the S1 pasture and none on the S2 pasture.

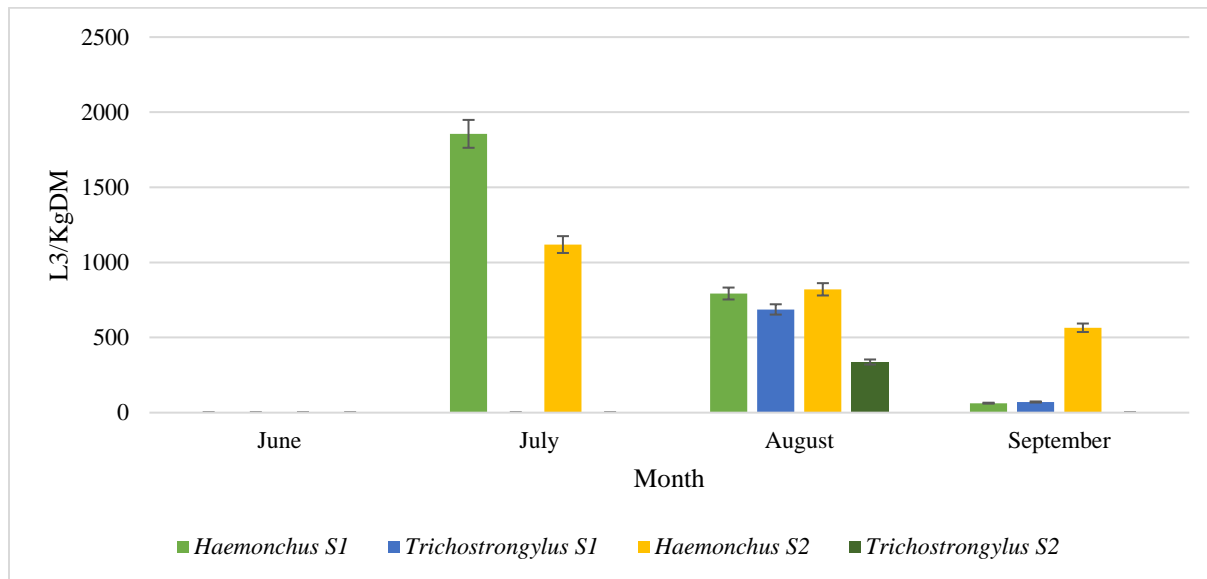


Figure 30. Larvae (L3) per kg dry matter on S1 and S2 lamb pasture from June through October in year 3.

#### 4.5 Discussion

In this study, each yr weaned lambs from S1 and S2 ewes were placed on separate pastures and were managed similar to their dams. The one addition was that S2 lambs had SL to graze and S1 lambs didn't. For yr 1, both groups had similar FEC for the first 4 wks, after which the S2 lamb GIN infection was controlled better than S1 lamb GIN infection. For each of yr 2 and 3, FEC started at the same level for S1 lambs and, in contrast, FEC for S2 lambs started lower in yr 2 and lower again in yr 3. This suggested that 1) lambs from SL supplemented ewes had lower infection level than lambs from normal supplemented ewes, and/or 2) the SL pasture plus SL supplement reduced pasture contamination and infectivity from yr to yr. Because targeted selective treatment is now considered the method of choice to conserve the useful life of

available anthelmintics, lambs in both groups were dewormed if their FAMACHA® score was 4/5. Historically, resistance (unpublished observations, Ben Hur Research Farm Sheep Unit, Louisiana Agricultural Experiment Station, Baton Rouge, LA) to LEV and ABZ used individually had reduced efficacy and the combination had been used successfully to reduce infection about 95%. As such, the combination became the deworming protocol of choice. However, due to observed resistance using this combination in yr 1, MOX was added for subsequent dewormings. In yr 1, 49 and 21 dewormings were administered to the S1 and S2 groups, respectively. Eleven out of 15 S1 lambs and 6 out of 15 S2 lambs were dewormed multiple times. In yr 2, 3 and 3 dewormings were administered to the S1 and S2 groups, respectively, followed by 5 and 3 dewormings, respectively, in yr 3. One lamb in the S2 group required a repeat dosing of COWP in year 3. It was noticed that the lamb chewed and broke the capsule, thus resulting in the COWP being spit out.

In this study, S2 lambs had lower coccidia FOC than S1 lambs for yr 2 and 3. This supports the findings of Burke et al. (2013) where SL was shown to be successful in preventing and controlling coccidiosis and GIN infections in lambs.

Results of this study indicate that selective deworming with LEV/ABZ/MOX combination and COWP were both effective in reducing FEC in lambs. Of note is that yr 1 S2 lambs required fewer dewormings than S1 lambs. Burke et al. (2012) also found that lambs grazing SL pastures needed fewer dewormings than lambs grazing mixed pastures.

For all 3 yr, S2 lambs required deworming later in the year than S1 lambs where S1 deworming began in July while S2 deworming began in mid-August. This suggests that the S2 pasture was not as heavily infective as the S1 pasture, therefore, delaying reinfection and the

need to deworm. Also, the lower initial FEC of S2 lambs in yr 2 and 3 may have contributed to further reduction of pasture infectivity.

## **CHAPTER 5**

### **COCCIDIA SERICEA LESPEDEZA STUDY**

#### **5.1 Introduction**

It was noted that there was a reduction in clinical signs of coccidiosis in SL supplemented lambs compared to non-SL supplemented lambs in yr 1 (June-September 2011) (Chapter 4). The same observation was noted in studies conducted by our collaborators in Arkansas (Burke et al., 2013). The study in Arkansas consisted of two experiments. In experiment 1, weaned lambs received either 2% alfalfa pellets (control) or SL with or without amprolium and in experiment 2, twin born lambs were either fed a control creep supplement (16% CP; n = 40) or SL pellets (14% CP; n = 32) a month before weaning. Feces were collected for FEC, FOC, and fecal score for experiment 1. For experiment 2, feces were collected for FEC, FOC, fecal score, dag score and blood was collected for PCV. Results for experiment 1, showed similar FEC and FOC in both control and SL fed lambs, however, control lambs had higher fecal score than the SL lambs. In experiment 2, FEC, FOC, fecal score, and dag score were similar for both groups at the beginning of the trial, however the SL fed lambs had a lower FEC, FOC, fecal score, and dag score after weaning. Furthermore, no SL fed lamb required treatment for coccidia infections while a third of the control lambs required treatment.

To follow up on this observation, coccidia infection was monitored by FOC on lambs during yr 2 and 3 (Chapter 4). In addition, this independent study was conducted to evaluate the effect of feeding SL to lambs that were given an experimental *Eimeria* spp. infection.

#### **5.2 Methods and Materials**

##### **5.2.1 Location and Animals**

This study was conducted for 35 d at the Louisiana State University, School of Veterinary Medicine in Baton Rouge, Louisiana from June 18, 2012-July 23, 2012 (yr 2).

### 5.2.2 Experimental Design

Twenty-four three month old weaned lambs (10 crossbred F2, Suffolk x Gulf Coast Native and 14 Katahdin) were randomly divided by FEC into two groups: 1) control (C) and 2) treatment (T). There were 6 replicates, 2 lambs for each treatment. Lambs were housed 2 per pen with water available at all times. Pens were cleaned daily. The C lambs were fed a commercial sheep ration (Purina® Honor® Show Lamb™) and T lambs were fed SL pellets. Both groups were fed 0.91-1.1 kg/hd/d. Feeding began on d 3 and ended on d 14. Animals were dewormed at the beginning of the study with levamisole (Levisole, 8 mg/kg drench), albendazole (Valbazen, 10 mg/kg drench), and moxidectin (Cydectin, 0.2 mg/kg drench). All lambs were inoculated with 30,000, 5,000, and 15,000 *Eimeria* spp. oocysts on d 11, 14, and 17, respectively.

### 5.3 Techniques

Collection and analytical techniques used in this study were the same techniques as described in Chapter 3. Fecal samples (5-10 g) were collected directly from the rectum every 2-3 d for FOC and FEC using the modified McMaster technique.

### 5.4 Statistical Analysis

Data were analyzed using SAS® (version 9.4, SAS Institute, Cary, NC) as a repeated measures analysis of variance using Proc Mixed. Fixed effects included Group, Day and the Group\*Day interaction. The random effect was Animal (Group). The response variables were EPG, logepg, FOC, and logfoc. Differences were considered significant when  $p \leq 0.05$ .

### 5.5 Results

At the beginning of the study (d 0), lambs in both groups had similar ( $p > 0.05$ ) mean FEC of approximately 50 epg which indicated that the deworming was effective (Figure 31). The FEC for the T group was significantly ( $p < 0.05$ ) lower than the FEC for the C group on d 17 and 18. After d 18 the FEC for both groups remained relatively low and similar ( $p > 0.05$ ).

Initially, the T group lambs did not readily eat the SL feed, but after d 5, they readily ate the feed.

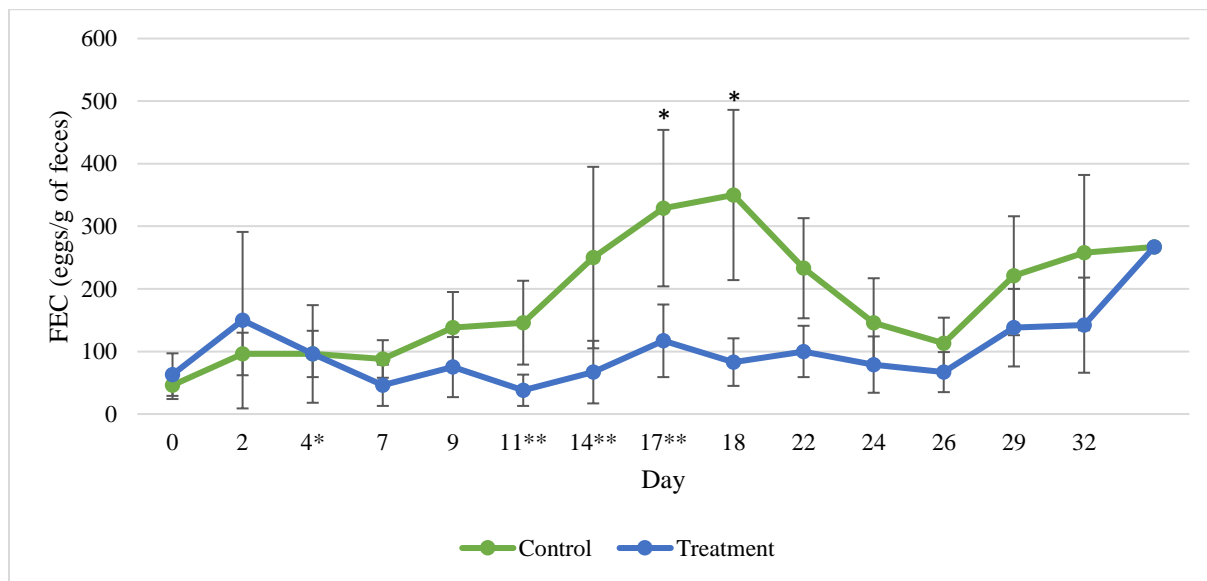


Figure 31. Mean ( $\pm$ SEM) fecal egg count (FEC) for Control (n=12, fed 0.91 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower) and Treatment (n=12, fed 1.1 kg/hd/d sericea lespedeza, SL, pellets) lambs. \*Started SL feeding. \*\*Inoculated with 30,000, 5,000 and 15,000 oocysts on d 11, 14 and 17, respectively. \*Significant ( $p < 0.05$ ) difference.

The C group and the T group had similar ( $p > 0.05$ ) coccidia FOC at the beginning of the trial (Figure 32). Lambs were inoculated with coccidia on day 11, 14, and 17. Coccidia counts for the control increased until d 17 and then decreased until d 24 and remained low until the end of the study. The T group remained low for the entire study. The FOC for the T group was significantly ( $p < 0.05$ ) lower than the FOC for the C group on d 14, 17, and 18.

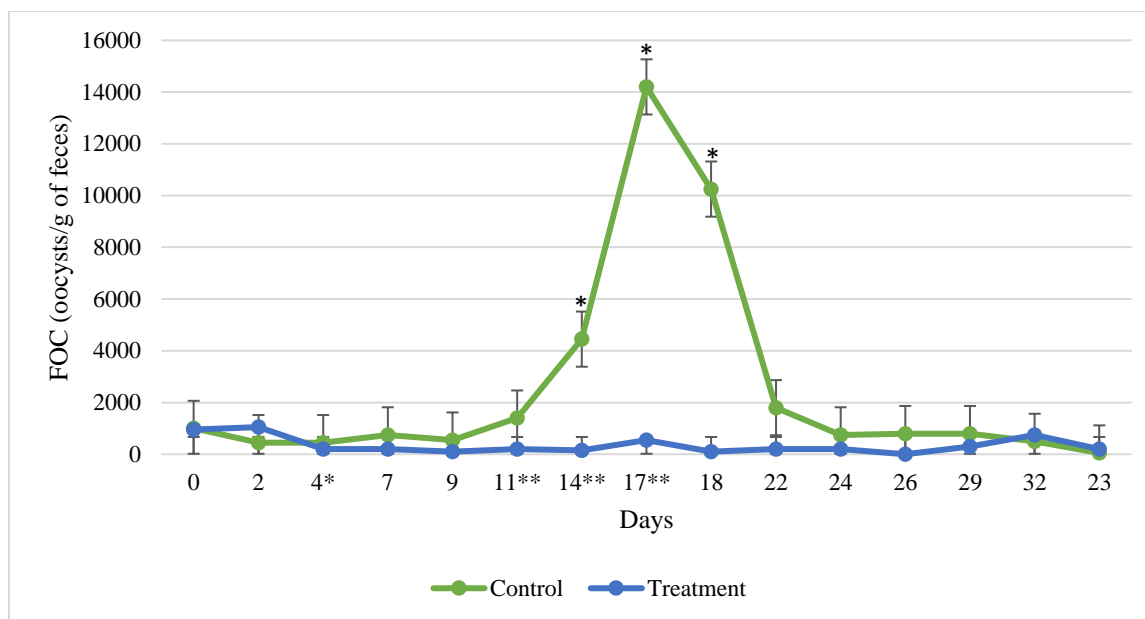


Figure 32. Mean ( $\pm$ SEM) fecal oocyst count (FOC) for control (C, n=12 fed 0.91 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower) and treatment (T, n=12 fed 1.1 kg/hd/d sericea lespedeza, SL, pellets) lambs. \*Started SL feeding. \*\*Inoculated with 30,000, 5,000 and 15,000 oocysts on d 11, 14 and 17, respectively. \*Significant ( $p < 0.05$ ) difference.

## 5.6 Discussion

Coccidiosis can be a devastating parasitic disease that affects lamb production around weaning. Lambs are stressed at that time, thus compromising immune function which leads to morbidity and often death. In this study, SL was fed to experimentally infected lambs to evaluate efficacy. The increase, peak and decline of FOC observed in the C lambs indicated a typical patent infection. The relatively unchanged FOC in the T lambs indicated that SL effectively controlled infection compared to the C lambs. In addition, FEC remained lower than C lambs which is in agreement with other reports of reduced FEC in SL fed animals (Lange et al., 2006; Shaik et al., 2006; Burke et al., 2012). This is mostly likely due to reduced fecundity of female worms. Under the conditions of this study, SL effectively controlled *Eimeria* spp. infection as well as reducing GIN FEC. The use of SL could be beneficial in a weaning management

program to control coccidiosis. This study was published as part of a multi-location report (Burke et al., 2013).

## **CHAPTER 6**

### **MOLYBDENUM STUDY**

#### **6.1 Introduction**

It was noted in 2011 (year 1) and 2012 (year 2) that S2 group lambs (Chapter 4) weighed less than the S1 lambs after about 8 weeks of grazing. In trying find an answer, serum concentration of trace minerals was determined which indicated very low molybdenum (Mo) levels in the S2 lambs compared to S1 lambs. In another study, lambs and kids that grazed SL or were supplemented with SL pellets also showed decreased weight gain towards the end of those studies (Acharya, 2014). They also had low Mo, and in addition low zinc (Zn) and selenium (Se) levels than control animals. This study was done to evaluate the effect of Mo supplementation on correcting Mo deficiency and the weight gain issue.

#### **6.2 Methods and Materials**

##### **6.2.1 Location and Animals**

This study was conducted over 38 days at the Louisiana State University, School of Veterinary Medicine in Baton Rouge, Louisiana from October 19, 2012-November 26, 2012 (end of year 2).

##### **6.2.2 Experimental Design**

Fifteen crossbred (Suffolk x Gulf Coast Native) lambs were housed individually in pens with water available at all times. Lambs were randomly divided into 3 groups according to FEC (n=5/group): 1) control (C), lamb growing ration; 2) SL pellets , and 3) SL pellets supplemented with Mo (SLM, sodium molybdate, 35mg/head/day). Lamb pens were cleaned daily. Lambs received the designated ration every morning (0.91-1.1 kg/hd/d). When SL feeding (and Mo supplementation) was stopped (d 24), lambs were fed the same ration as the C lambs for the rest of the study. On days 0, 12, 19, 24, 31 and 38, feces and blood were collected to monitor

infection and animals were weighed to determine weight gain. Serum trace mineral panels were done on days 0, 24 and 38.

### **6.3 Techniques**

Fecal collection, blood collection and analytical techniques used in this study were the same as described in Chapter 3. Animals were infected with GIN naturally while grazing before being placed in pens. Blood (5 mL) was also collected in royal blue EDTA tubes and sent to the Diagnostic Center for Population and Animal Health, Michigan State University for serum trace mineral analysis.

### **6.4 Statistical Analysis**

Data were analyzed using SAS® (version 9.4, SAS Institute, Cary, NC) as a repeated measures analysis of variance using Proc Mixed. Fixed effects included Group, Month and the Group\*Month interaction. The random effect was Animal (Group). The response variables were EPG, PCV, and WEIGHT. Differences were considered significant when  $p \leq 0.05$ .

### **6.5 Results**

At the beginning of this study, lamb weight for all 3 groups was similar ( $p < 0.05$ ), 37.4 kg, 35.8 kg, and 35.5 kg, respectively (Figure 33). The C lambs gained weight during the study and both SL and SLM lambs lost weight through d 24 when SL was being fed. On d 24, the weight of both SL and SLM lambs was significantly ( $p < 0.05$ ) lower than C lambs and trended towards significance on d 31 and 38. The final weight gain for the 3 groups was 2.0 kg, 1.0 kg, and 1.4 kg, respectively.

Lambs in all groups had similar ( $p > 0.05$ ) FEC at the beginning of the study (Figure 34). The FEC decreased for all 3 groups on d 12 with the greatest decrease in the SLM group. The FEC for the C lambs continued to decrease on d 19 and 24 while FEC increased for the SL and SLM lambs. On d 31 and 38, FEC continued to increase for the C and SL lambs while the SLM

lambs FEC remained constant. Overall, there was no significant ( $p > 0.05$ ) difference between the groups.

The PCV for all groups were similar ( $p > 0.05$ ) at the beginning of the trial and remained similar ( $p > 0.05$ ) for the duration of the study (Figure 35).

At the beginning of the study, all groups showed similar serum Mo concentration (Figure 36). By d 24, the C lambs had the highest Mo concentration while both SL and SLM lambs had significantly ( $p < 0.05$ ) reduced Mo concentration with SL being reduced more than SLM but not significant ( $p > 0.05$ ). After SL supplement feeding stopped, Mo concentration increased for both SL and SLM lambs and reached similar ( $p > 0.05$ ) levels with C lambs.

Similar to Mo, Se and Zn serum concentrations were similar ( $p > 0.05$ ) on d 0 and significantly ( $p < 0.05$ ) decreased in SL and SLM lambs on d 24, and then increased to C levels on d 38 ( $p > 0.05$ ) (Figures 37, 38). These decreases were not as pronounced as the Mo decrease.

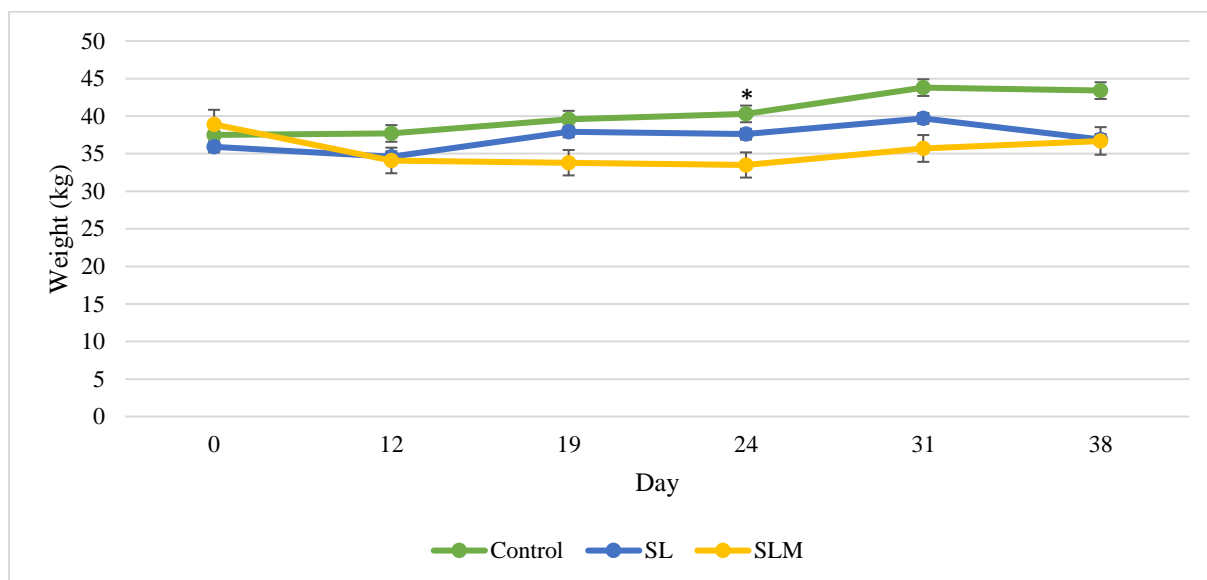


Figure 33. Mean ( $\pm$ SEM) weight for control (n=5, supplemented with 1.1 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower), sericea lespedeza (SL, n=5, 1.1 kg/hd/d SL pellets) and SL/molybdenum (SLM, n=5 1.1 kg/hd/d SL pellets and 35mg/hd/d sodium molybdate) fed lambs. \*Significant ( $p < 0.05$ ) difference.

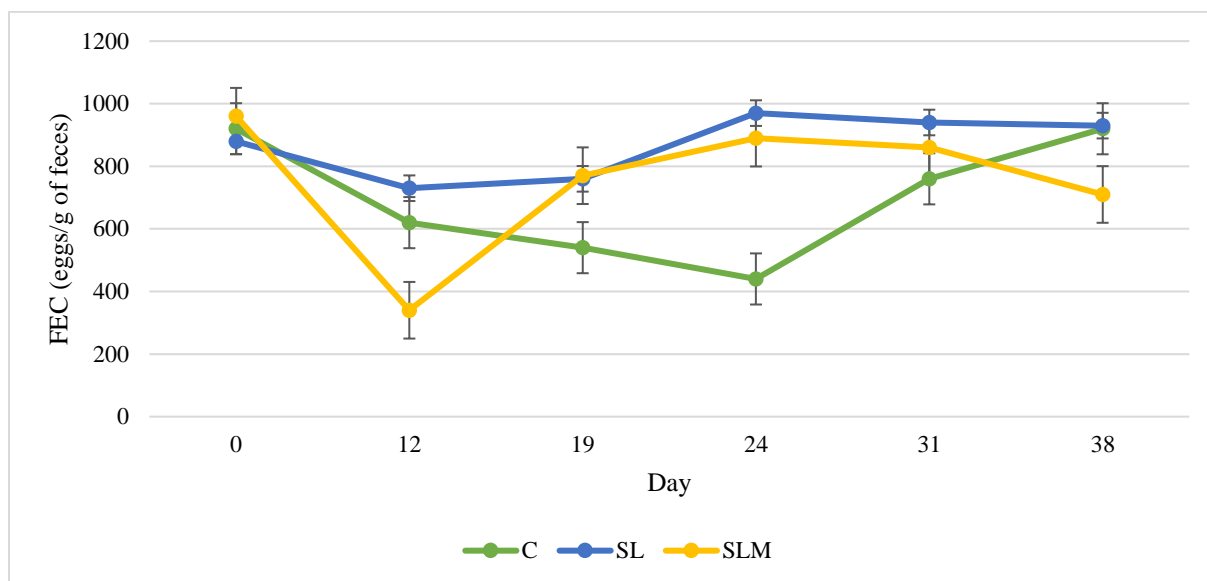


Figure 34. Mean ( $\pm$ SEM) fecal egg count (FEC) for control (n=5, supplemented with 1.1 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower), sericea lespedeza (SL, n=5, 1.1 kg/hd/d SL pellets) and SL/molybdenum (SLM, n=5, 1.1 kg/hd/d SL pellets and 35mg/hd/d sodium molybdate) fed lambs. There were no significant differences ( $p > 0.05$ ) between groups.

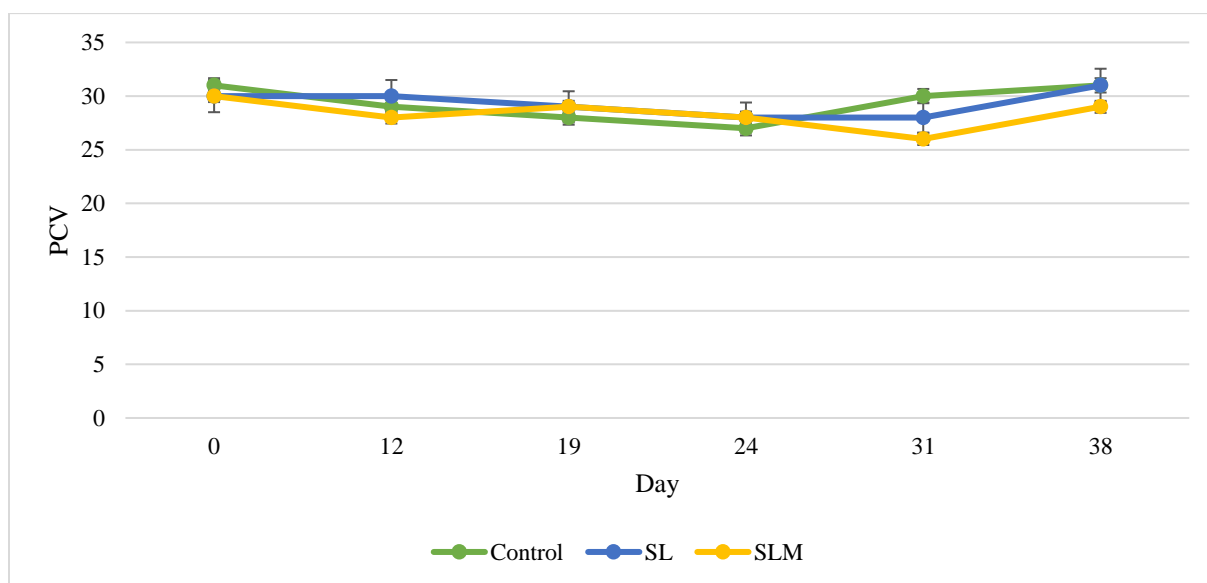


Figure 35. Mean ( $\pm$ SEM) blood packed cell volume (PCV) for control (n=5, supplemented with 1.1 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower), sericea lespedeza (SL, n=5, 1.1 kg/hd/d SL pellets) and SL/molybdenim (SLM, n=5, 1.1 kg/hd/d SL pellets and 35mg/hd/d sodium molybdate) fed lambs. There were no significant differences ( $p > 0.05$ ) between groups.

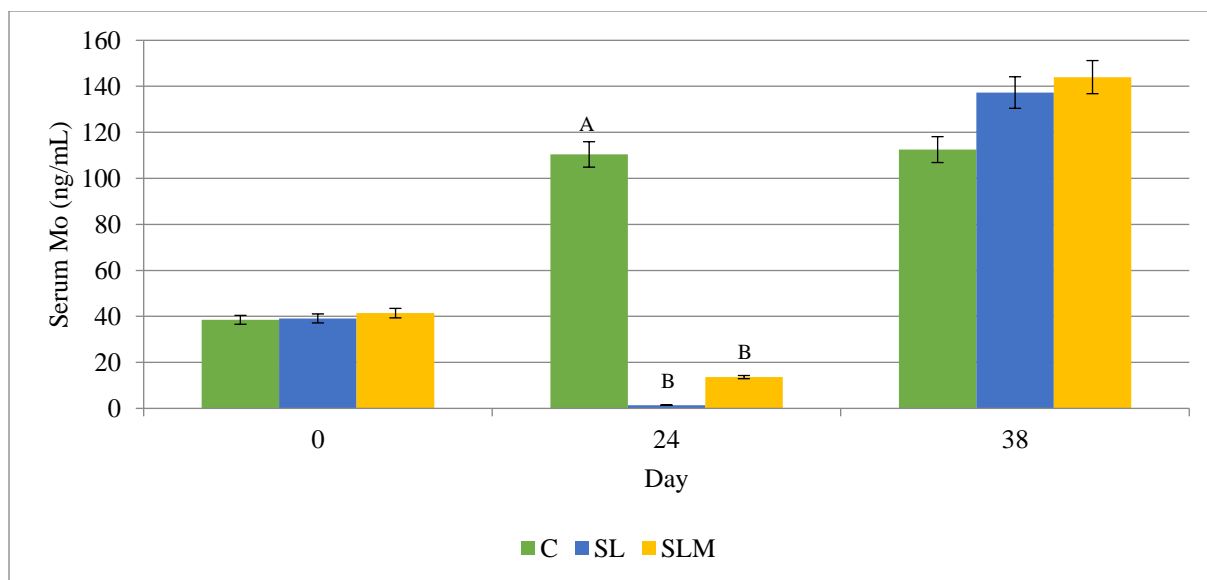


Figure 36. Mean ( $\pm$ SEM) molybdenum (Mo) serum concentration for control (n=5, supplemented with 1.1 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower), sericea lespedeza (SL, n=5, 1.1 kg/hd/d SL pellets) and SL/molybdenum (SLM, n=5, 1.1 kg/hd/d SL pellets and 35mg/hd/d sodium molybdate) fed lambs. A, B indicate significant ( $p < 0.05$ ) difference.

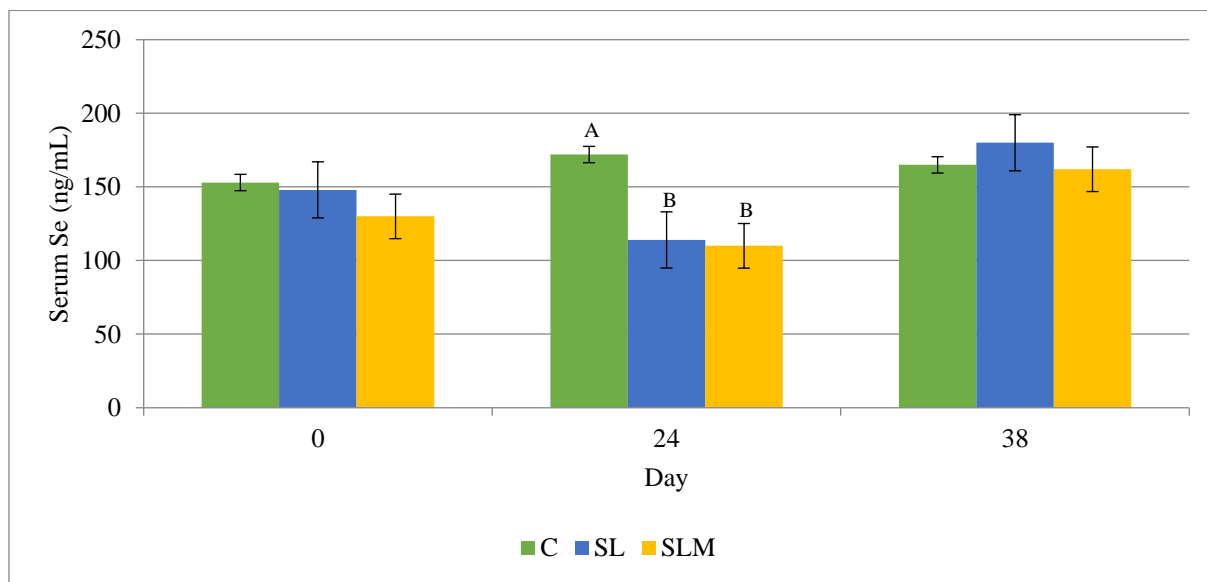


Figure 37. Mean ( $\pm$ SEM) selenium (Se) serum concentration for control (n=5, supplemented with 1.1 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower), sericea lespedeza (SL, n=5, 1.1 kg/hd/d SL pellets) and SL/molybdenum (SLM, n=5, 1.1 kg/hd/d SL pellets and 35mg/hd/d sodium molybdate) fed lambs. A, B indicate significant ( $p < 0.05$ ) difference.

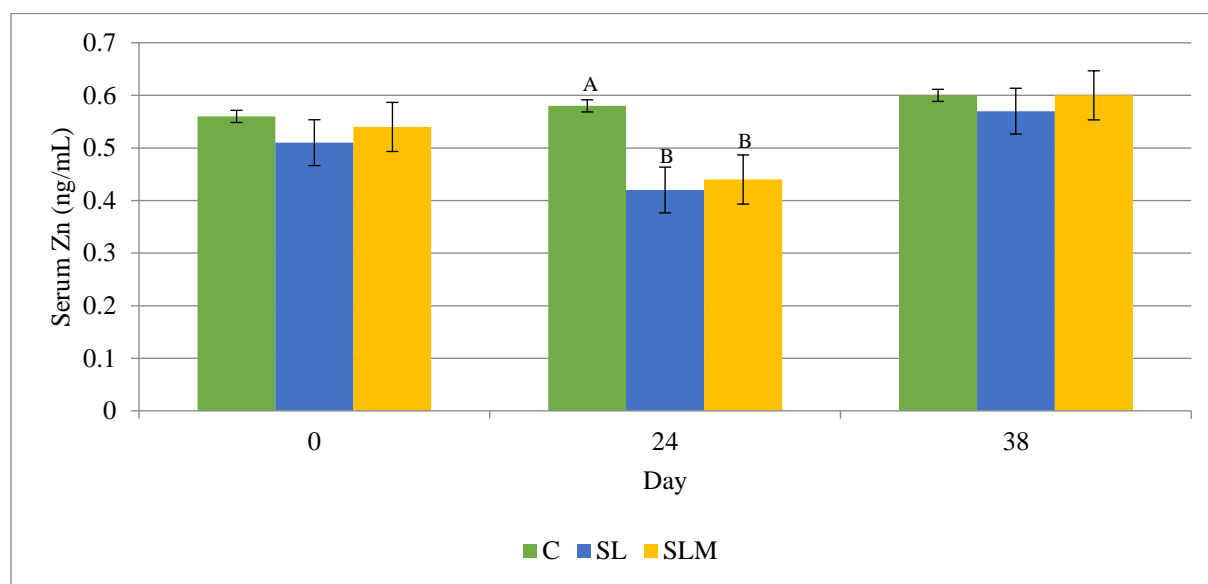


Figure 38. Mean ( $\pm$ SEM) zinc (Zn) serum level for control lambs (n=5, supplemented with 1.1 kg/hd/d Purina® Honor® Show Lamb™ Showlamb Grower), sericea lespedeza (SL, n=5, 1.1 kg/hd/d SL pellets), and SL/molybdenum (SLM, n=5, 1.1 kg/hd/d SL pellets and 35mg/hd/d sodium molybdate) fed lambs. A, B indicate significant ( $p < 0.05$ ) difference.

## 6.5 Discussion

As noted in Chapter 4, lambs did not gain weight after 8 weeks of SL grazing and supplementation compared to lambs not consuming SL. Serum trace mineral analysis revealed that Mo, Se and Zn concentrations were low, with Mo being severely low. This suggested that weight gain might be affected by this trace mineral deficiency. This study evaluated whether Mo supplementation could correct the Mo deficiency and improve weight gain. Results indicated that both SL and SLM lambs lost weight during SL feeding and then gained weight after SL feeding stopped; however, weight gain did not reach that of lambs that were not fed SL during the period of the study. Had the study gone longer, it is possible that weight gain may have improved more. The Mo serum concentration of SLM lambs was 10 fold higher than SL lambs which indicated that Mo supplementation did improve the deficiency, but it wasn't until SL feeding was stopped

that Mo (and Se and Zn) concentrations returned to normal. The level of Mo supplementation may have been less than what would be necessary to further correct the deficiency under SL feeding conditions. A study conducted in Arkansas noted that SL fed lambs and kids had decreased weight gain compared to lambs and kids not fed SL (Acharya, 2014). Serum concentrations of trace minerals in that study indicated a deficiency in Mo, Se and Zn, and also, manganese (Mn). Upon supplementation with Mo, Mn, Se and Zn serum concentrations of all increased but did not achieve normal values nor did it impact weight. The importance of trace minerals deficiencies certainly deserves more attention.

## **CHAPTER 7**

### **DISCUSSION AND CONCLUSION**

Anthelmintic resistance is very common in tropical or sub-tropical regions where *H. contortus* predominates. In the United States, this occurs primarily in the southeast, but with animal movement and climate changes, *H. contortus* is becoming more of a problem in northern temperate areas, including Canada (Barrere et al., 2013). It has been recognized that deworming all animals in a population is no longer constructive, because not all animals harbor the same number of worms. Deworming all animals removes susceptible worms and leaves resistant ones, as a result little refugia (population of susceptible worms) is left to compete with resistant worms for survival. This leads to an increased populations of resistant worms (van Wyk, 2001; Besier, 2008; Leathwick et al., 2009; Terrill et al., 2012). With anthelmintic resistance on the increase, it is necessary to find other methods to control GIN. Additionally, there is an increasing need for organic meats and other goods which means that the producer cannot use a chemical drug to prevent and treat GIN. Alternate methods and integrated strategies can reduce the dependence on anthelmintics which in turn will extend the usefulness of those anthelmintics that still might be effective. Thus, morbidity and mortality of small ruminants due to GIN infection can be reduced.

The objective of the studies in Chapters 3 and 4 was to compare chemical (anthelmintic) to non-chemical means of controlling GIN infection over a long term (3 yr). Results indicated that, for all 3 yr, ewes and lambs that grazed and/or were supplemented with SL and dewormed (only those that needed it) with COWP fared better than ewes and lambs that grazed conventional pasture and were dewormed (only those that needed it) with chemical anthelmintics

(ABZ, LEV and MOX) for controlling GIN. The peri-parturient rise in FEC was reduced in ewes, and lambs consistently had reduced infection level.

However, lambs that grazed and were fed SL supplement did not gain as well as those that grazed conventional pasture, especially in yr 1 and 2. Similar reduced weight gains were also observed in others studies (Acharya et al., 2015). A serum trace mineral panel was done to determine if there were any mineral deficiencies that might explain the impaired growth in SL fed lambs. Results showed that SL fed lambs were deficient in Mo, Se and Zn, and that Mo was by far the most deficient. An attempt was made to determine if Mo supplementation could correct the deficiency and improve growth (Chapter 6). Results of that study indicated that Mo supplementation improved serum Mo levels by about 10 fold compared to non-supplemented, but the level was still substantially below normal. The Mo supplemented group did gain more weight than the non-supplemented group by about 0.45 kg. This suggests that increasing Mo supplementation may further correct the deficiency and possibly increase weight gain. Similar results were observed by Acharya et al. (2015).

Another observation that was not expected was the reduction in clinical signs of coccidiosis in SL supplemented lambs compared to non-SL supplemented lambs in yr 1 (Chapter 4). The same observation was noted in Arkansas by Burke et al. (2013). To follow up on this observation, FOC were done on lambs during yr 2 and 3. Results showed that SL supplemented lamb FOC were reduced both yr, again with no signs of clinical disease. To further evaluate this, a controlled study was conducted to determine the effect of feeding SL pellets to experimentally infected lambs (Chapter 5). Results indicated that SL supplementation prevented an increase in FOC compared to non-supplemented lambs. This suggested that SL supplementation could be a valuable practice at weaning to control coccidia infection and associated losses.

The present studies indicated that integrated approaches were effective in sustaining the ewe and lamb flocks. The use of integrated methods that use a combinations of effective strategies were beneficial over the long term of this study. Incorporating SL pelleted feed suppressed the peri-parturient rise in ewes and infection was reduced in lambs that grazed SL and were also supplemented with SL pellets. Using SL in a GIN control program was shown to be effective. This control was also reflected in fewer dewormings needed for SL supplemented lambs, and COWP worked just as well as the LEV/ABZ/MOX combination, thus supporting the benefit of non-chemical control. Additionally, FAMACHA© served as a useful tool in identifying lambs that needed deworming for both integrated approaches. Results suggested that including SL pastures and/or feed supplement along with COWP could be a management practice to help reduce the use of and extend the effective/useful life of currently available conventional anthelmintics. The observation that SL supplemented lambs did not gain weight as well as conventional fed lambs suggested that grazing SL pastures and/or supplementing with SL pellets should be limited to short term (no more than 6-8 wks) early in the grazing season following weaning.

The overall results of these studies suggest that the use of SL and non-chemical integrated control may be better than or just as good as integrated control using conventional anthelmintics. However, SL may affect weight gain and additional supplementation with Mo (and possibly Zn, Se and Mn) may be necessary to overcome this issue. In addition, the use of SL, as an alternative for coccidia control in weaned lambs may be a valuable tool to include in the integrated approach to control.

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## **VITA**

Sarah Tammy Nicole Orlik-Keeton was born in Hof, Germany, in 1982 to Angela and Henryk Orlik. She has three younger siblings. While she was growing up, she attended various schools in different places in both Germany and the United States. After graduating with honors from Covington High School in Covington, Louisiana, in 2001, she attended Southeastern Louisiana University in Hammond, Louisiana. After graduating with a bachelor's degree in biological sciences in spring 2007, she attended Louisiana State University in Baton Rouge, Louisiana, and began her work on her master's degree in animal science. After graduating with a master's of science in summer 2010, she attended Louisiana State University in Baton Rouge, Louisiana, and started her work on her doctoral degree in animal science. She will graduate in August 2016.