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Copper oxide wire particles in feed pellets for controlling gastrointestinal nematode infection in ewes and lambs

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**COPPER OXIDE WIRE PARTICLES IN FEED PELLETS FOR
CONTROLLING GASTROINTESTINAL NEMATODE INFECTION IN
EWES AND LAMBS**

A Thesis

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

in

The Interdepartmental Program in the School of Animal Science

**By
Sarah T. Orlik
B.S., Southeastern Louisiana University, 2007
August 2010**

DEDICATION

Mr. Henryk and Mrs. Angela Orlik

Vanessa, Henryk, and Mandy

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ABSTRACT

Gastrointestinal nematode parasites cause extensive damage to small ruminants, and *Haemonchus contortus* is a major concern to production. In the past, small ruminants were dewormed at regular intervals and control methods were based primarily on the use of anthelmintics. At present, anthelmintic resistance has been reported worldwide and has developed into a serious problem for small ruminant management programs. In view of this, alternate control methods are needed. One alternative method is the use of copper oxide wire particles (COWP). Three independent trials were conducted during Spring, Summer, and Fall of 2008. Two trials evaluated the effect of COWP in food pellets in reducing *H. contortus* infection in crossbred ewes and Suffolk lambs. The third trial compared the effect of Copasure[®] COWP with that of an industrial-grade COWP. Trials consisted of similar protocols where ewes and lambs were allocated into groups based on fecal egg count (FEC). Copasure[®] COWP in feed pellets were fed to ewes at mid-lactation when FEC increased to over 1000 eggs per gram, and to individual lambs when FAMACHA[®] score was 4/5. Ewe/lamb infection was monitored weekly by FEC and blood packed cell volume. Results of Trial 1 indicated that Copasure[®] COWP in feed pellets was effective in reducing the peri-parturient rise in ewes, based on FEC. Trial 2 indicated that Copasure[®] COWP in feed pellets was just as good as the levamisole/albendazole treatment when the FAMACHA System[®] was used to determine when to treat lambs. Trial 3 indicated that Copasure[®] COWP effectively reduced parasite infection while the industrial-grade COWP did not. The results from these trials demonstrated that the use of Copasure[®] COWP in feed pellets reduced *H. contortus* infection and may be useful alone or when used with other control methods.

CHAPTER 1 INTRODUCTION

Sheep play an important role in agricultural production worldwide. Larger flocks are usually found in Australia, China, India, and the Middle East. These countries use sheep for the local market but also export wool and meat (Cuming, 2008). New Zealand has smaller flocks but the economic impact is quite large due to exportation of sheep goods. Sheep also make an impact on local economies where the market focus is on “organic” and/or “sustainable” production (Severson, 2008).

There are production concerns when raising sheep and gastrointestinal nematode (GIN) parasitism is a major constraint to production globally (Fleming et al., 2006). Across the southeastern United States GIN parasitism is comparatively high because environmental conditions are ideal for development and survival. GIN have caused production losses due to cost of prophylaxis, cost of treatment, and loss in production. Heavily infected animals are less efficient which results in less wool or meat for market (Hartwig, 2009).

Losses due to GIN parasitism estimated by governmental organizations extend into millions of dollars per year, but it is difficult to assess these losses because there are many factors involved. Such factors and their interaction include nutrition, environmental stress, genetics, management and concurrent disease. There is no published data available in the United States on the estimated dollar value of annual losses; however, there is information for other countries. For example, in New Zealand, it was estimated that NZ\$200 million of production annually was still not being realized after taking into account the cost of nematode parasite control (Vlassov et al., 2001); in Australia, with increasing anthelmintic resistance, estimated losses were up to AD\$700 million annually (Besier and Love, 2003); and Ethiopian meat

markets and the export of livestock have an estimated annual loss of US\$400 million (Kumsa 2006).

The GINs of concern for sheep producers include *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus colubriformis*, *Trichostrongylus axei*, *Nematodirus* spp., and *Cooperia* spp. *Haemonchus contortus* is considered the most pathogenic and is known as the barberpole worm. It is found in the abomasum, feeds on blood and can readily cause death. *Teladorsagia circumcincta*, the brown stomach worm, is also found in the abomasum and can cause major mucosal pathology which results in impaired digestive processes and the subsequent loss of protein and fluids leading to diarrhea (Schoenian, 2003). *Trichostrongylus 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 essential to maintain a productive and profitable operation. The most common method of control has been the use of anthelmintics. When anthelmintics are effective, improved production (e.g. weight gain, wool quality, etc.) and body condition can be expected especially in young growing animals. Anthelmintics available in the United States 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 are levamisole (Levisole[®]) and Tramisol[®]) and morantel tartrate (Rumatel[®]). The macrocyclic lactones, commonly called “mectins”, are ivermectin (Ivomec[®]), doramectin (Dectomax[®]) and moxidectin (Cydectin[®]). 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, 2003).

The constant dependence on anthelmintics over the past several decades has led to GIN populations that have developed resistance, which has been recognized worldwide (Prichard, 1990; Waller, 1994; Sangster, 1999; Fleming et al., 2006; Papadopoulos, 2008). *Haemonchus contortus* is the most prominent GIN showing resistance. 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 is replaced (Papadopoulos, 2008). Resistance to all 3 classes of anthelmintics has been reported in the southeastern United States (Howell et al., 2008). Due to the high level of resistance that *H. contortus* has developed, alternative methods of control are necessary to maintain production of small ruminants.

Alternative methods and integrated strategies can help to decrease reliance on anthelmintics which can lead to more sustainable parasite management. Such alternative methods might include selecting nematode-resistant individuals for breeding, nematode trapping fungi, condensed tannin containing plants, smart drenching, the FAMACHA System[®], vaccines and copper oxide wire particles (COWP).

Copasure[®] COWP are marketed for copper deficiency in cattle in the United States, but have also been shown to have the potential for controlling *H. contortus* infection (Burke et al., 2004; Burke and Miller, 2006). It should be noted that sheep are sensitive to copper and can suffer from toxicity due to accumulation in the liver. Once the liver can't accumulate any more copper, the excess copper remains in the blood and causes a hemolytic crisis destroying red blood cells which leads to severe anemia and death. Fortunately, the copper in Copasure[®] COWP is not well absorbed and therefore reduces the potential for toxicity (Burke et al., 2004; Hale et. al, 2007).

The objectives of this study were: 1) to determine if Copasure® COWP in feed pellets is an effective and efficient way to control *H. contortus* infection in lactating grazing ewes, 2) to determine when to treat grazing lambs, based on FAMACHA[©], with Copasure® COWP in feed pellets to control *H. contortus* infection and 3) to determine if industrial-grade COWP are as effective as Copasure® COWP to control *H. contortus* infection.

CHAPTER 2 LITERATURE REVIEW

2.1. *Haemonchus contortus*

Haemonchus contortus is the predominant GIN found in small ruminants in tropical and subtropical regions of the world, but it can also be found in more temperate regions where sheep are housed during the cold season (Cameron, 1956). *H. contortus* is potentially very harmful because it can cause severe anemia and death. It disrupts the abomasal mucosa and small capillaries and feeds on the blood. Symptoms depend upon infection level and include anemia, emaciation, edema, poor growth, and death (Burke, 2005). In female *H. contortus*, the white uteri and ovaries are twisted around the red intestine which gives it a barberpole appearance; hence, its common name is the barberpole worm (Urquhart, 2007). It causes extensive damage to its host and most commonly infects small ruminants such as sheep and goats, but can also infect cattle and wild ruminants (Soulsby, 1968).

2.2. Life Cycle

Haemonchus contortus goes through a typical trichostrongyle life cycle. It is a direct life cycle where no intermediate host is required. Females are considered very fecund because of their abundant egg production, depositing between 5,000 to 10,000 eggs per day which pass out of the host via feces (Leite-Browning, 2006; Urquhart, 2007). The first stage larvae hatch from the eggs in the feces and while feeding on organic matter develop to the second and then the third stage larvae (L3) which do not feed and are infective to the vertebrate host. Migration of the L3 from feces to forage requires moisture (rain, irrigation, dew, etc.) and the ruminant becomes infected by ingesting the L3 while grazing. The L3 exsheath in the rumen and move to the abomasum with the ingesta. Once there, they penetrate the mucosa and develop to the fourth

stage larvae (L4). They then migrate into the lumen of the abomasum. Before they become adults, they develop a sharp lancet at the anterior oral opening which is used to disrupt the mucosa and small capillaries for feeding purposes (Urquhart, 2007). Both the L4 and adult worms feed on blood. The life cycle continues by adults mating and the female excreting her eggs. Considerable numbers of L3 may develop on heavily grazed pastures (Leite-Browning, 2006). In sheep, the prepatent period, 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).

2.3. Haemonchosis

When infected, ruminants display clinical signs, it is generally called haemonchosis. There are three forms that can take place, the hyperacute, acute, and chronic form of the disease. In the hyperacute form, the animal is exposed to thousands of L3 over a short period of time. The infection results in the loss of 200–600 ml of blood per day and death can occur in a week. When acute disease occurs, animals of all ages show anemia, bottle jaw (pendulant edema under the lower jaw), and dark feces. The first phase of anemia takes place during the prepatent period, where blood PCV drops severely. This is caused by immature L4. Within one or two months, the second stage begins where the PCV does not decrease any further but iron levels are depleted. This is the beginning the third stage of the anemia which is marked by another drop in PCV. The third form, chronic haemonchosis, can last for two to six months. During this phase, between 100 and 1000 adult worms can account for blood loss of about 50 ml/day. The animal appears malnourished, with weight loss and poor wool quality in adult animals and poor growth in lambs. Generally, when an infected animal has a FEC of 10,000 eggs per gram or more, the animal is deemed to suffer from haemonchosis (Mehlhorn, 2008).

2.4. Anthelmintic Control of Gastrointestinal Nematodes

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, 2009).

Small ruminants are predisposed to worms due to their grazing behavior and slow development of immunity. When considering deworming, the key is to target animals which are most susceptible to parasitism, which are lactating ewes and lambs. Therefore, it is important to consider deworming ewes before parturition or during lactation because during this time the immune system is compromised. In addition, it reduces the risk of passing on the infection to the offspring. Furthermore, to reduce pasture contamination ewes may be dewormed prior to summer grazing (Schoenian, 2003).

The three classes of anthelmintic drugs recognized by the sheep industry are the benzimidazoles, nicotinic antagonists, and macrocyclic lactones.

2.4.1. Benzimidazoles

Benzimidazoles are anthelmintics whose names end in "-azole" and are commonly referred to as the white dewormers. Benzimidazoles are heterocyclic aromatic organic compounds consisting of the synthesis of benzene and imidazole. They were the first modern class of anthelmintic developed. In addition, they are broad spectrum dewormers effective against all the GINs including larvae and adults, and some show effectiveness against liver flukes. Benzimidazoles bind to the protein tubulin. Tubulin, found in worm cells, combines to form long tubes that are important to the worm's survival (Sangster and Hennessy, 2007). Benzimidazoles prevent polymerization of tubulin and the formation of microtubules which reduces the uptake of glucose and deprives the parasite of energy (Arundel, 1985). In other

words, benzimidazoles impede the formation of microtubules causing the worms to die (Sangster and Hennessy, 2007).

In 1962, the first benzimidazole introduced was thiabendazole (Arundel, 1985). Other benzimidazoles include mebendazole, flubendazole, fenbendazole, oxfendazole, oxibendazole, albendazole, albendazole sulfoxide, thiophanate, febantel, netobimin, and triclabendazole (Kahn, 2005). Thiabendazole (TBZ[®]) and albendazole (Valbazen[®]) are approved anthelmintics for sheep by the Food and Drug Administration (FDA). However, thiabendazole is no longer available due to widespread resistance and decrease in popularity. Fenbendazole and oxfendazole (Synanthic[®]) are commonly used “extra- label” because of current resistance issues (Rook, 2009).

2.4.2. Nicotinic Antagonist

The nicotinic antagonists are anticholinergic and impede the action of nicotinic acetylcholine receptors (Kahn, 2005). They are levamisole, morantel tartrate, and pyrantel pamoate and are commonly known as the clear dewormers. Levamisole and morantel act on ganglions. Levamisole acts by affecting the nerve ganglion of the worm which causes the worm to have rapid muscle contraction leading to paralysis. Morantel tartrate works differently by “depolarizing” the neuromuscular system (Arundel, 1985). Levamisole is also known as an imidazothiazole and morantel tartrate is often classified as a tetrahydropyrimidine which also includes pyrantel pamoate (Schoenian, 2008).

Only levamisole (Levasol[®] and Tramisol[®]) in this group is FDA approved for sheep. Morantel tartrate (Rumatel[®]) and pyrantel pamoate (Pyrantel Pamoate Horse Dewormer[®]) are often used “extra- label” because of current resistance issues (Rook, 2009).

2.4.3. Macrocyclic Lactones

The macrocyclic lactones are anthelmintics that end in “ectin” and are called avermectins. They are the most recent anthelmintic family. They include ivermectin, doramectin, and moxidectin. They work by affecting the reproduction of the parasite and paralyzing the worm. This is accomplished by blocking gamma aminobutyric acid (GABA) and mediated transmission of nerve ganglions (Arundel, 1985). 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 current resistance issues (Rook, 2009).

2.5. Factors Affecting Efficiency

Anthelmintics can be given to sheep orally or by subcutaneous injection. When given orally, a drench gun or syringe (with drench adapter) is used to administer the correct dose. The sheep needs to be held properly and the dewormer needs to be delivered over the back of the tongue so that it is swallowed rapidly. If the drench is delivered incorrectly, it may not be swallowed and instead inhaled which makes it potentially dangerous and increase risk of respiratory issues (Arundel, 1985). Also, if the drench is not delivered over the back of the tongue, it may stimulate closure of the esophageal groove and bypass the rumen, thus full effect of the anthelmintic will be lost.

2.6. Anthelmintic Resistance

Control of GIN in sheep has relied on the use of anthelmintics. Over the past few decades, anthelmintics were used in an attempt to eliminate all infection which in return leads to frequent use. However, the constant reliance on anthelmintics has caused GIN to become

resistant. Resistance occurs when there is a reduction in efficiency of an anthelmintic against parasites that are usually susceptible (Sangster, 1999).

Resistance is very common in tropical or sub-tropical regions where *H. contortus* prevails. In the United States this occurs primarily in the southeast. Resistance was first documented in the United States and is now a common occurrence all over the world (Coles, 1986; Flemming et al., 2006). The first reports of resistant strains of *H. contortus* was to thiabendazole which was documented in 1964 in the United States (Conway, 1964) and in 1968 in Australia (Smeal et al., 1968). In addition, resistance to benzimidazoles has been recognized in parts of Australia, New Zealand, South Africa, and England. Other species of nematodes (*Teladorsagia*, *Trichostrongylus*, *Cooperia*, *Nematodirus*) have developed resistance to anthelmintics as well (Waghorn et al., 2006). In a study conducted in Louisiana, Suffolk lambs infected with *H. contortus* showed resistance to ivermectin when given in both oral and injectable forms (Miller and Barras, 1994). Recently, total failure to all three classes of anthelmintics was reported in an Arkansas goat herd (Kaplan et al., 2005). In addition, the extent of resistance on sheep and goat farms in the southeast has been reported (Howell et al., 2008).

Resistance has been documented to all of the broad spectrum anthelmintics. Multiple resistance and cross resistance are a common occurrence. Multiple resistance occurs when nematodes are exposed to two or more anthelmintics and resistance develops to both. Cross resistance takes place when resistance occurs as a result of being exposed to another anthelmintic with a similar mode of action (Arundel, 1985).

In order to maintain profitable small ruminant production it is important to have healthy animals. With anthelmintic resistance on the rise, it is imperative 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. New alternative methods are being developed to combat GIN infection in small ruminants.

2.7. Alternate Parasite Control

Efforts must be made to impede the progression of resistance and move away from the frequent use of anthelmintics. Newer alternate approaches are being investigated, which have the potential to reduce morbidity and mortality of small ruminants infected with GIN. Such methods include nematode trapping fungi, condensed tannins, selecting resistant individuals for breeding, vaccines, pasture management, smart drenching, FAMACHA system[®], and Copasure[®] COWP.

2.7.1 Nematode Trapping Fungus

Nematode trapping fungi have been studied and found to be effective in reducing the development of the free-living parasitic larvae to the L3 form (Larsen, 2006). The most promising fungus is *Duddingtonia flagrans* whose chlamydospores are fed and are able to withstand the environmental conditions of the gastrointestinal tract and thus are able to successfully grow in feces producing hyphal loops that trap developing larvae (Faedo et al., 1977). *D. flagrans* was reported to be effective in reducing L3 in the feces of sheep with high FEC when given continuously (Pena et al., 2002) and when fed with supplement feed to grazing mature dry ewes resulting in a reduction of L3 in feces and on the pasture (Fontenot et al., 2003). Commercial farms in Malaysia have relied mainly on anthelmintic use and with the rising concern of anthelmintic resistance, it was reported that pasture rotation and feeding *D. flagrans* spores at night when sheep were housed resulted in a decrease of L3 and a manageable situation for large scale sheep operations (Chandrawathani et al., 2004). An additional study examined

the interaction with Copasure[®] COWP, which may act as a fungicide against *D. flagrans* (Burke et al., 2005). That study suggested that Copasure[®] COWP did not have a negative effect on *D. flagrans* and did show that when used concurrently, FEC and L3 were reduced.

2.7.2. Condensed Tannins

Another alternative approach to control GIN infection is with condensed tannin containing plants, of which sericea lespedeza (SL, *Lespedeza cuniata*) is one. SL is a perennial legume that grows best in warm/hot areas and acclimates well to acidic poor soils. Grazing SL was reported to yield lower FEC in goats (Min et al., 2004). Sericea lespedeza is easily grown and can be a cost effective way of controlling parasitic infections (Shaik et al., 2006). Furthermore, the effects of feeding SL as hay has been reported to be effective in controlling the egg, larval, and adult phases of GIN in the abomasum and to some extent the small intestine of sheep and goats (Lange et al., 2006; Shaik et al., 2006). The feeding of SL as hay to goats and sheep could be a problem as the leaf is very fragile and much of the active plant material can go to waste. Therefore, SL has been pelleted to see if there was any lose of efficiency during the pelleting process. The pellets were effective in controlling GIN infections (Terrill et al., 2006).

Another condensed tannin plant with promise is *Acacia molissima*. Santa Ines sheep infected with *H. contortus* and *T. colubriformis* were drenched with an *A. molissima* condensed tannin extract which effectively decreased infection (Minho et al., 2008). Therefore, *A. molissima* extract may be another viable alternative to traditional anthelmintics.

Further studies need to be conducted before these condensed tannin containing plant products are made commercially available.

2.7.3. Breeding for Resistance

Another alternative method which can help reduce losses to GIN in small ruminants is using breeds that have shown resistance or incorporating resistant breeds into a crossbreeding program. One can also select individuals within a breed for resistance, and this approach has been successful (Woolaston and Baker, 1996). In the United States, Gulf Coast Native (GCN) sheep were compared to Suffolk sheep to confirm that GCN sheep were more resistant than Suffolk sheep. Both breeds were exposed to the same environmental conditions and infection levels were significantly higher in Suffolk sheep than GCN sheep (Miller et al., 1998). Furthermore, a previous study showed nursing GCN lambs were better able to cope with GIN infection at an earlier age than Suffolk lambs (Bahirathan et al., 1996).

Currently, FEC is the best measure to evaluate and select for resistance. In the future, parasite antigen assays, host antibody, and DNA markers might provide additional information on satisfactory selection methods. The sheep industry would profit from selecting resistant individuals for breeding programs because it would be another way to reduce the reliance on anthelmintics (Dominik, 2005).

2.7.4. Vaccines

The effectiveness of vaccines and the development of vaccines to protect small ruminants against *H. contortus* and other GIN have been ongoing for a long time (Knox et al., 2003). The two approaches that have been used are natural and hidden antigens. Natural antigens are antigens recognized by the immune system while the host is infected. This immune response includes antibody and cellular responses and are triggered by surface and/or excretory/secretory products of the various stages of the worm (including irradiated larvae) and studies have shown that protection has been limited (Smith and Zarlinga, 2006).

Hidden gut antigens are not recognized by the immune system when the animal is infected. The first hidden gut antigen, H11 is an integral membrane glycoprotein attained from intestinal microvilli of *H. contortus*. It has been reported to be effective against *H. contortus* infection by reducing FEC by more than 90% and worm burden by more than 75% (Smith and Smith, 1993). Furthermore, H11 was effective in young lambs of a range of breeds and anthelmintic resistant worms (Newton, 1995). Animals were protected for at least twenty three weeks. Subsequent to H11, another hidden gut antigen (H-gal-GP) was shown to be similarly protective (Smith et al., 1994). In addition, the combination vaccine consisting of H11 and H-gal-GP was reported to be effective in reducing FEC by more than 82% in grazing sheep (Kabagambe et al., 2000; Smith et al., 2001). A similar study was conducted when exposure to infection was very high and FECs were reduced by only 65% (Knox et al., 2003). Since infection is not monospecific (e.g. all *Haemonchus*) in grazing lambs, variability in infection and response to vaccination is to be expected.

Problems are also associated with the making of hidden gut vaccines. Using natural antigens that must be derived from worm gut cells make large scale extractions very time consuming and impractical. Therefore, research efforts have been directed at development of recombinant antigens for commercial use. Unfortunately, such recombinant antigens have not induced adequate protection to be considered for commercialization as a vaccine against *H. contortus* (Smith and Zarlenga, 2006). However, with more research and technology the outlook for vaccines is optimistic.

2.7.5. Copper Oxide Wire Particles

Another method to control GIN are COWP. There have been a number of reports indicating a direct association between COWP and decreasing FEC and numbers of the abomasal

worms *H. contortus* and *Teladorsagia circumcincta* in small ruminants (Bang et al., 1990; Knox, 2002; Burke et al., 2004; Burke and Miller, 2006; Soli et al., 2010). Initially, COWP were developed to treat copper deficiency in sheep and subsequently the effect on the major abomasal worms was noted. After administration, COWP adhere to the gastrointestinal tract mucosa and can remain for about 4-5 weeks (Dewey, 1977). In the acidic abomasal environment, copper is released over time allowing slow absorption to occur.

Early studies used 5 g of COWP once (Bang et al., 1990; Knox, 2002), but repeated treatments would be expected for longer term control. Because excessive copper accumulates in the liver of sheep, toxicity is a concern with longer term use. Therefore a dose titration study (0, 2, 4 and 6 g Copasure[®] COWP), administered orally in gelatin capsules, was conducted to determine what might be 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, and copper accumulation in the liver was the least for 2g and the greatest for 6 g. However, liver copper level for all 3 doses was within normal limits. Field use of Copasure[®] COWP in grazing animals requires repeated use. Another study that used even lower doses (0.5 and 1 g), administered orally in gelatin capsules, were also effective in reducing infection, and it was observed 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. Serum aspartate aminotransferase (liver enzyme) level was similar for both Copasure[®] COWP and untreated animals and liver copper levels were higher in Copasure[®] COWP treated animals, but within normal limits. The conclusion was that multiple low doses of Copasure[®] COWP could be used repeatedly without inducing copper toxicity. The mechanism

of action of Copasure[®] COWP has not been definitively established, but one study showed that worms from Copasure[®] COWP treated lambs had cuticular damage which might lead to disruption of the ability of worms to maintain metabolic function (Moscona et al., 2008). Thus, they become weak and die or unable to maintain position and are eliminated.

The effectiveness of Copasure[®] COWP in pregnant ewes and how it might affect their offspring has also been reported (Burke et al., 2005). In that study, ewes were given 0, 2 or 4 g Copasure[®] COWP, administered orally in gelatin capsules, about 30 days before parturition. Both doses effectively reduced infection, based on FEC. At birth, lambs from Copasure[®] COWP treated ewes had higher serum AST levels and weighed less than lambs from untreated ewes. However, by 30 days of age, AST 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. The conclusion was that a 2 g dose of Copasure[®] COWP appeared to be safe for production.

Goats are also susceptible to gastrointestinal nematode (specifically *H. contortus*) infection and anthelmintic resistance is common. Similar studies to those described above for sheep have been reported. In the US, infection in mature grazing does was controlled with 4-10 g doses of Copasure[®] COWP (administered orally in gelatin capsules) and doses as low as 0.5-1 g were effective in grazing kids (Burke et al., 2007). In South Africa, kids were experimentally infected with *H. contortus* and then given 2 and 4 g doses of Copasure[®] COWP (administered orally in gelatin capsules) and both doses effectively reduced (93-95%) infection (Vatta et al., 2009). This study also looked at the levels of copper in tissues and effects on meat quality. Liver, kidney and muscle copper levels were similar in both control and Copasure[®] COWP treated kids, which indicated that copper may not accumulate as much in goats as it does in

sheep. Therefore, copper toxicity may not be much of an issue and meat quality was not affected (Vatta et al., 2009).

In all of the studies mentioned, Copasure[®] COWP was effective in reducing infection, based on FEC (and worm burdens in some cases), in both adult and young sheep and goats when administered in the form of a bolus (gel capsules).

With the interest in Copasure[®] COWP as an alternate control method, the use of copper sulfate generated interest as well. A study was conducted to determine if copper sulfate mixed with a daily feed supplement could be used to control *H. contortus* infection in goat kids (Burke et al., 2008). After 9 weeks of feeding copper sulfate, there was no difference in infection level, based on FEC, between treated and control grazing goat kids. The study concluded that copper sulfate was not effective.

2.7.1 Integrated Control Strategies

In view of the anthelmintic resistance problem, effective control of GIN now calls for using a combination of different control strategies to reduce the reliance on traditional anthelmintic-based control.

Smart Drenching used in conjunction with the FAMACHA System[®] is very useful as part of a management scheme (Kaplan et al., 2004). It has been recognized that deworming all animals in a population is no longer advantageous because not all animals harbor the same number of worms. In fact, the minority of animals harbor the majority of worms (Galvani, 2003; Sreter et al., 1994). It is common to find 70-80% of the worms in 20-30% of the animals. Deworming all animals eliminates susceptible worms and leaves resistant ones, therefore, little or no refugia (susceptible worms) is left to compete with resistant worms for survival. This leads to increased populations of resistant worms. The FAMACHA System[®] is a five point color

scale (red to white) depicted on a card which is matched to the lower eyelid membrane color. The color reflects the level of the anemia and thus is only good for *H. contortus*. The animals that require deworming should then be given an effective anthelmintic at the correct dose. It also needs to be delivered over the back of the tongue to ensure it is deposited in the rumen. The use of all anthelmintics should be monitored; effectiveness can be tested by a FEC reduction test. Sometimes fasting animals for 12-24 hours prior to deworming is beneficial as fasting slows down rumen motility and allows increased contact of the anthelmintic with the worms. In cases where resistance has developed to all available anthelmintics, combinations of two or more may help to increase efficacy, and repeated treatments may be necessary for 2-3 days. In addition to proper use of anthelmintics, proper pasture management should be incorporated. This might include reducing stocking rates, keeping forages longer than 3-4 inches (larvae seldom migrate higher than that), co-grazing small ruminants with cattle, or rotating grazing pastures between cattle and small ruminants (Hale, 2006).

Ultimately, all available methods for control (smart drenching, FAMACHA[®], nematode trapping fungi, condensed tannin containing forages, Copasure[®] COWP, selection of resistant animals, pasture management, etc.) should be considered in some combination that fits a producer's production situation. Thus, reliance on anthelmintics will be reduced, which in turn will extend the useful life of those anthelmintics that are effective.

CHAPTER 3 MATERIALS AND METHODS

3.1. Location and Animals

The trials were conducted at the Ben Hur Research Farm Sheep Unit of the Louisiana Agricultural Experiment Station in Baton Rouge, Louisiana.

3.2. Experimental Design

3.2.1. Trial 1

This trial consisted of 20 pregnant/lactating grazing (ryegrass) crossbred ewes (Suffolk x Gulf Coast Native). Animals were housed at night and water was available at all times. The duration of the study was 13 weeks (2/20/08-5/13/08). At the beginning of the trial the ewes were close to parturition and lactated for the extent of the trial. Ewes were randomly allocated, based on FEC, into 2 groups of 10 each. Copasure[®] COWP incorporated into feed pellets were used to determine if they were an effective way of controlling the peri-parturient rise in FEC during the lactation period. Infection level was monitored by determining FEC and PCV at weekly intervals. At 6 weeks when FEC exceeded 1000 epg for both groups, Copasure[®] COWP (4 g/hd) feed pellets were administered to the treatment group. Ewes were housed until all the feed was consumed which took about 30 minutes. The control group was given an equivalent amount of supplement feed without Copasure[®] COWP. Both groups of sheep grazed the same pasture.

3.2.2. Trial 2

This trial consisted of 22 grazing (bermudagrass) 3 month old Suffolk lambs and the duration was 16 weeks (6/3/08-9/23/08). Lambs were dewormed with levamisole (Levasol[®], Schering-Plough, Union, NJ; 8 mg/kg) at weaning which was one week before the trial started. Animals were housed at night and water was available at all times. Lambs were randomly

allocated, based on FEC, into 2 groups of 11 each. Infection level was monitored by determining FEC, PCV and FAMACHA[®] at weekly intervals. This study compared the use of anthelmintics to Copasure[®] COWP incorporated into feed pellets to control GIN infection during the summer grazing season. Individual lambs in the anthelmintic group received treatment with levamasole (Levasol[®], Schering-Plough, Union, NJ; 8 mg/kg) and albendazole (Valbazen[®], Pfizer, New York, NY; 7.5 mg/kg) (LEV/ABZ) combination when lambs had a FAMACHA[®] score of 4/5. Individual lambs in the Copasure[®] COWP treatment group were treated with 2 g/hd, incorporated into feed pellets, when the FAMACHA[®] score was 4/5. Both groups grazed the same pasture.

3.2.3. Trial 3

This trial compared the use of Copasure[®] COWP (2 g/hd) to an industrial-grade COWP (elementar Analysensysteme GmbH, Hanau, Germany) of (2 g/hd) to control GIN infection. The trial started with 20 grazing (bermudagrass) Suffolk lambs and the duration was 3 weeks (10/1/08-10/14/08). Animals were housed at night and water was available at all times. Lambs were randomly allocated, based on FEC, into 3 groups: Control (n=6), Copasure[®] COWP (n=7) and industrial-grade COWP (n=7). Infection level was monitored by determining FEC and PCV at weekly intervals. One Control and two industrial-grade COWP lambs died the first week and one Control lamb had to be dewormed at week 1. Therefore, these 4 lambs were dropped from the trial. The COWP treatments were delivered by oral administration in gelatin capsules. The control group was not treated.

3.4. Techniques

3.4.1. Fecal Egg Count

Feces were obtained directly from the rectum and placed in Styrofoam cups and sealed with a lid. These samples were taken directly to the lab to be analyzed for FEC using a modified McMaster technique (Whitlock, 1948). Briefly, two g of feces from each animal were 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 was mixed using an electric drink mixer (Drinkmaster[®] Drink Mixer, Hamilton Beach Brands, Inc., Glen Allen, NC). A sample of the mixture was rapidly pipetted and transferred into both sides of a McMaster chamber and eggs were counted. The number of eggs was multiplied by 50 to obtain an estimate epg. The FEC was used to monitor the relative change in GIN infection in each animal.

Furthermore, for Trials 1 and 3, the FEC reduction test was used to determine the efficacy of treatments. This test compares the FEC of the treatment group to the control group 7 days after the treatment was administered. The formula for this calculation was: $\text{ControlFEC} - \text{TreatedFEC} / \text{ControlFEC} \times 100$.

3.4.1. 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 once the sample was collected. These samples were taken directly to the lab and PCV was determined by using blood filled micro-hematocrit capillary tubes which were sealed, and centrifuged in an Autocrit centrifuge (Autocrit Ultra 3 Microhematocrit Centrifuge, Becton, Dickson and Company) for five minutes. The PCV of each animal was read

directly from the centrifuge scale. PCV was done to check the level of anemia as a result of *H. contortus* blood feeding.

3.5 Statistical Analysis

Data were analyzed using SAS® (version 9.1.3) as repeated measures analysis of variance in a split-plot arrangement of treatments. Effects on the main plot included treatment and animal within treatment. Subplot effects included time and treatment by time interaction. The response variables were FEC, LOGFEC, and PCV. Pairwise comparison were conducted with Tukey's HSD test for main effects and with t-tests of least-square means for interaction effects. Differences were considered significant when $p \leq 0.05$.

CHAPTER 4 RESULTS

4.1. Trial 1 Ewes

At the beginning of the study, all animals had comparatively similar mean FEC (Figure 1). From week 1 through week 7 there was a steady increase in FEC for both the control and treated group and there was no significant ($p > 0.05$) difference between groups. At week 7, the FEC for the control and treated groups was 1,310 EPG and 1,300 EPG, respectively, and treatment was administered. After treatment, the FEC reduction test showed a 76.4% reduction in Copasure[®] COWP ewes compared to control ewes (Table 1). Subsequent to treatment, the FEC of the Copasure[®] COWP treated ewes remained consistently and significantly ($p < 0.05$) lower than the control ewes until the trial was terminated.

Table 1. Fecal egg count for control and copper oxide wire particle treated (4 g Copasure[®] COWP/ewe in pelleted feed) ewes before (Week 7) and 7 days (Week 8) after treatment.

Animal # Control	EPG (Week 7)	EPG (Week 8)	Animal # Treated	EPG (Week 7)	EPG (Week 8)
3228	2500	2250	2037	500	50
4209	8250	6050	2038	5150	200
5240	150	100	3251	2350	300
5242	0	0	4211	1200	300
6212	950	1000	5238	200	0
6225	250	150	5243	500	0
6246	200	150	6214	300	0
6247	50	150	7201	50	0
7238	50	50	9260	1500	450
9291	700	700	9282	1250	950
Mean	1310	1060	Mean	1300	250

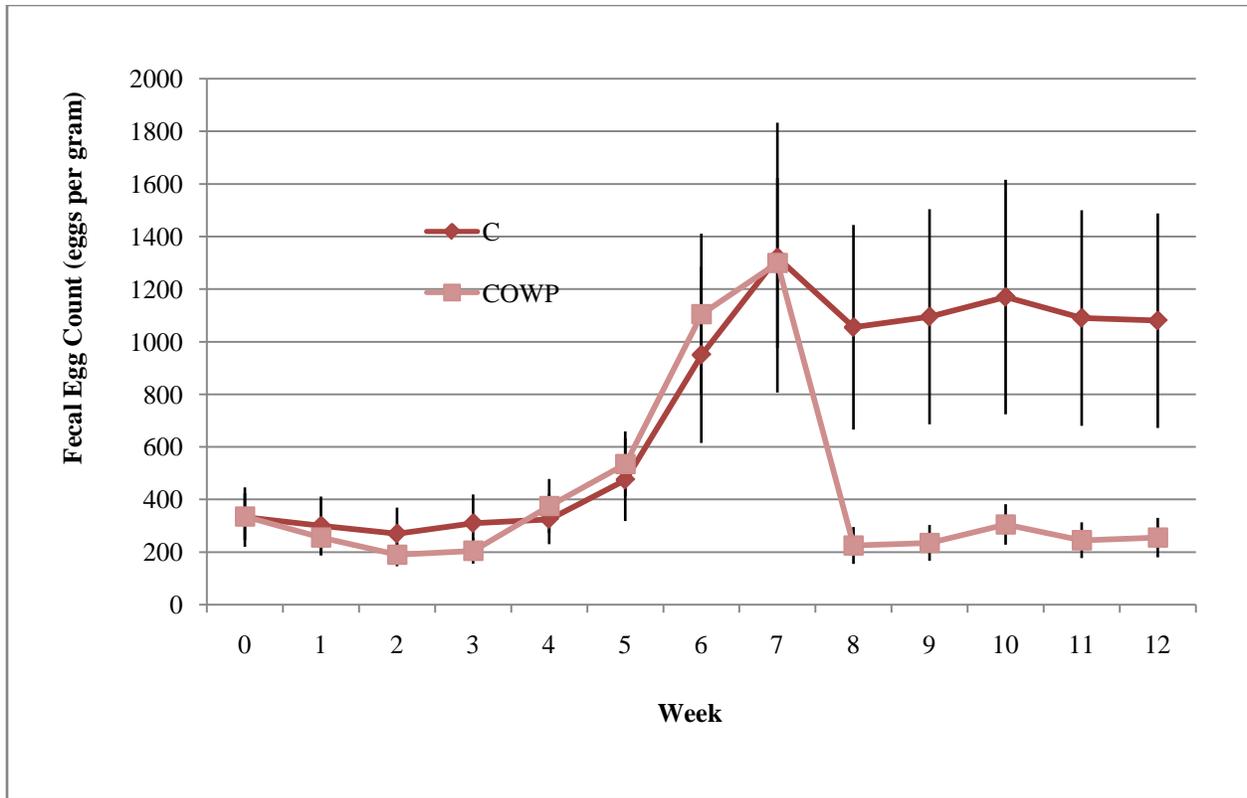


Figure 1. Fecal egg count for control (C) and treated (T, 4 g Copasur[®] COWP/ewe in pelleted feed) ewes (n = 10 per group). Differences were significant ($p < 0.05$) from week 8-12.

The PCV values for both the control and treated groups were similar ($p > 0.05$) throughout the trial; however, the treated group PCV was consistently higher than the control group after treatment (Figure 2).

4.2. Trial 2 Lambs

The FEC for the two groups of lambs was similar at the beginning of the trial (Figure 3). As the FEC increased after week 7, treatments were required for 2 and 1 LEV/ABZ lambs on week 8 and 9, respectively, and for 1 and 1 Copasur[®] COWP lambs on weeks 7 and 9, respectively. On week 10, the FEC in both groups had reached a level where clinical disease (haemonchosis) might be expected; therefore, all animals in each group were treated, excluding

those that had previously been treated. However, the FEC of the one Copasure[®] COWP lamb treated on week 9 actually increased on week 10 so it was treated again. Subsequently, FEC decreased in both groups and an additional 2, 3 and 3 LEV/ABZ lambs required treatment on weeks 12, 13 and 14, respectively, and 2 and 2 COWP lambs required treatment on weeks 13 and 14, respectively. A total of 18 and 15 treatments were given to LEV/ABZ and COWP lambs, respectively. There was no significant ($p > 0.05$) difference between groups at any time during the trial.

The PCV values for both groups were similar ($p > 0.05$) throughout the trial and continuously decreased from about 33 to 20 through week 10 (Figure 4). After treatments on week 10, the PCV stabilized and remained about 20 for the remainder of the trial.

FAMACHA[®] scores for both groups were similar ($p > 0.05$) throughout the trial and continuously increased from about 1.7 to 2.5 through week 10 (Figure 5). After treatments on week 10, the scores continued to increase through week 15 to about 3.2 and then started to decrease at week 16 when the trial ended.

Weight gain for LEV/ABZ group and COWP treated groups was 5.9 and 6.4 kg, respectively, from weaning to the end of the trial which was not significant ($p > 0.05$, Figure 6).

4.3. Trial 3 Lambs

At 1 and 2 weeks after treatment, respectively, there was a significant ($p < 0.05$) decrease in FEC of 72.2% and 70.2% for Copasure[®] COWP treated lambs compared to control lambs and there was no difference ($p > 0.05$) for industrial-grade COWP treated lambs (Figure 7).

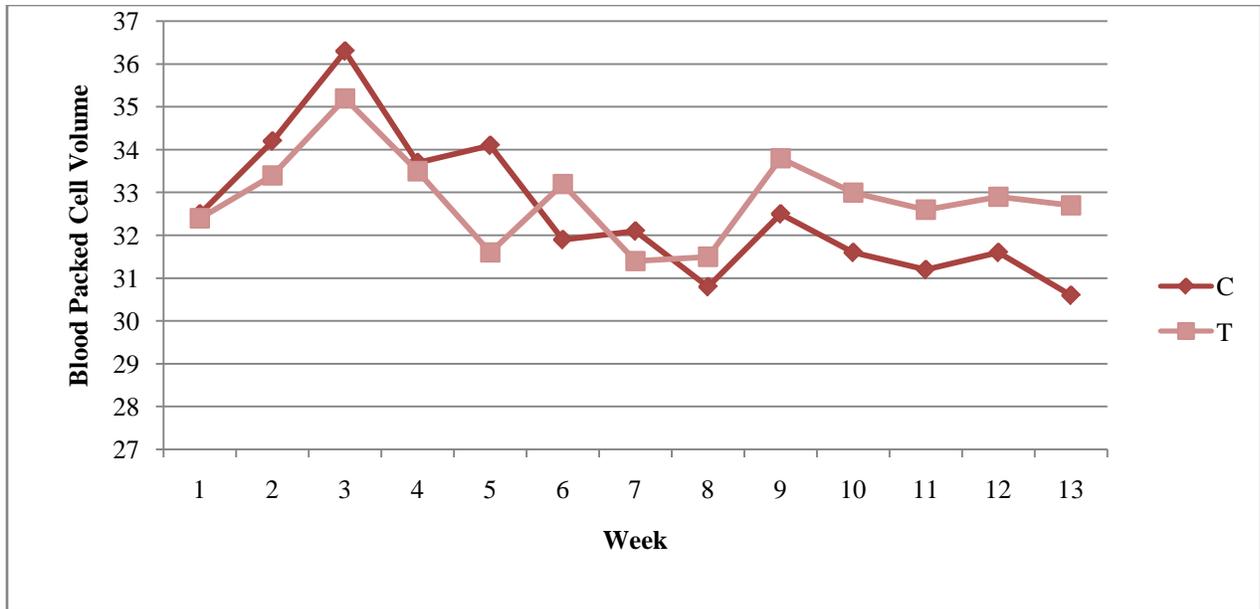


Figure 2. Blood packed cell volume for control (C) and treated (T, 4 g Copasure[®] COWP/ewe in pelleted feed) ewes (n = 10 per group). Differences were not significant ($p > 0.05$).

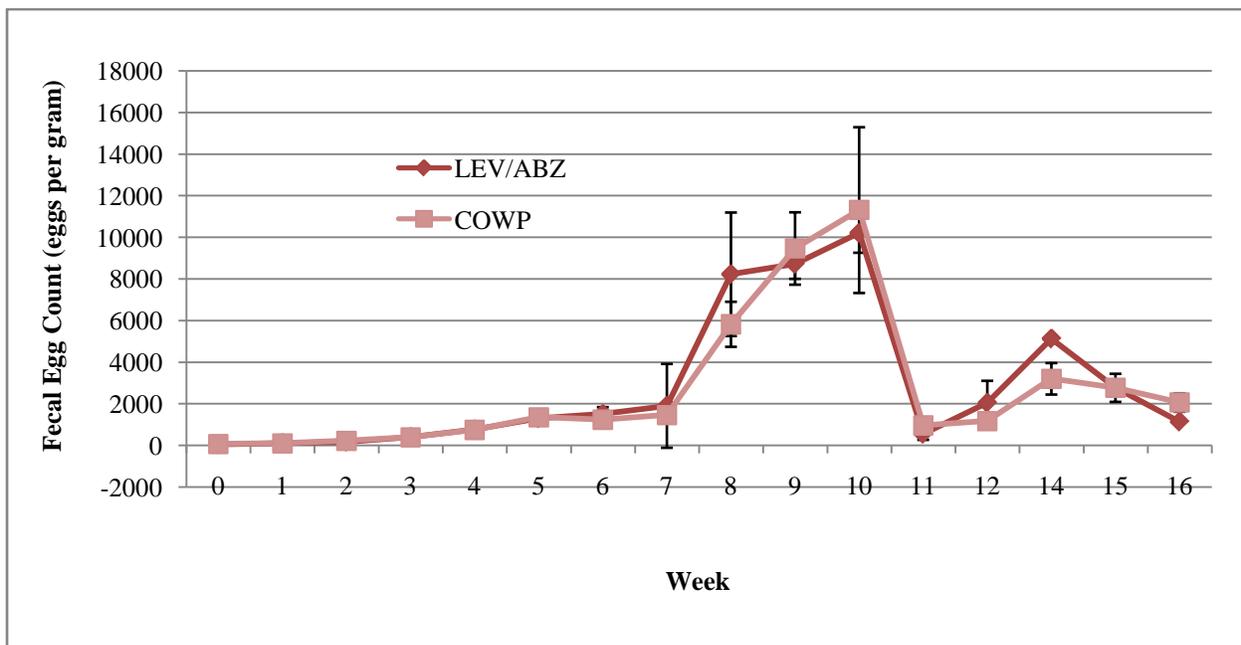


Figure 3. Fecal egg count for levamisole and albendazole (LEV/ABZ, n=10) and Copasure[®] copper oxide wire particle (COWP, n=10) treated lambs. Differences were not significant ($p > 0.05$).

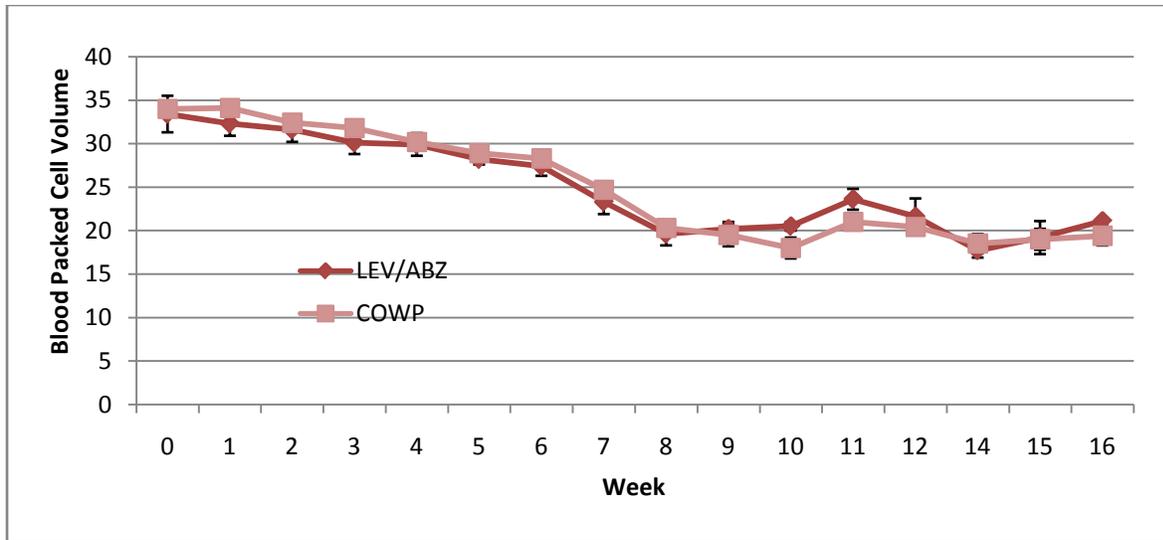


Figure 4. Blood packed cell volume for levamisole and albendazole (LEV/ABZ, n=10) and Copasure[®] copper oxide wire particles (COWP, n=10) treated lambs. Differences were not significant ($p > 0.05$).

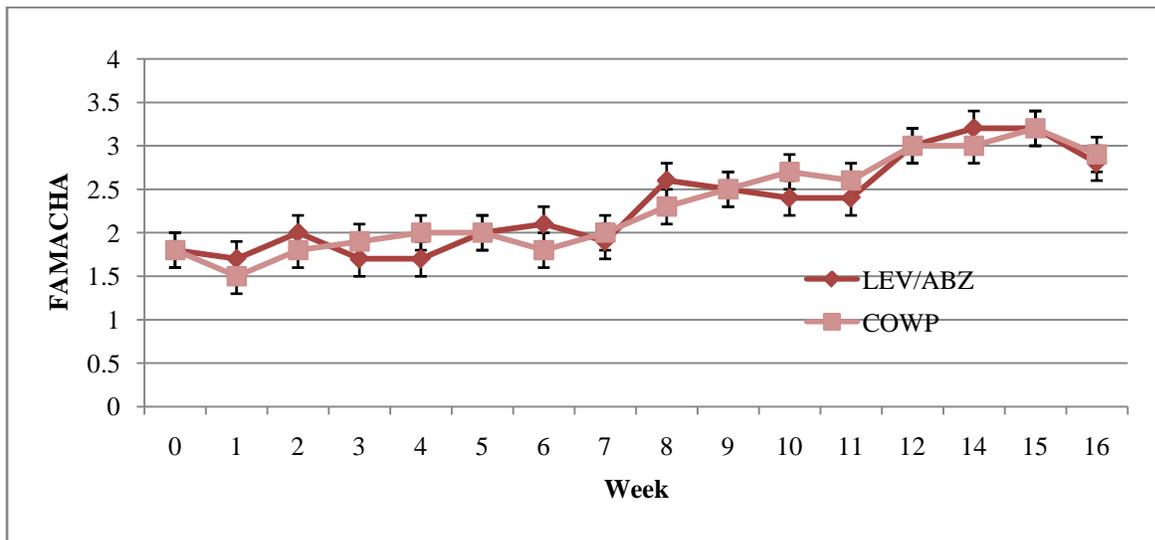


Figure 5. FAMACHA[®] score of levamisole and albendazole (LEV/ABZ, n=10) and Copasure[®] copper oxide wire particle (COWP, n=10) treated lambs. Differences were not significant ($p > 0.05$).

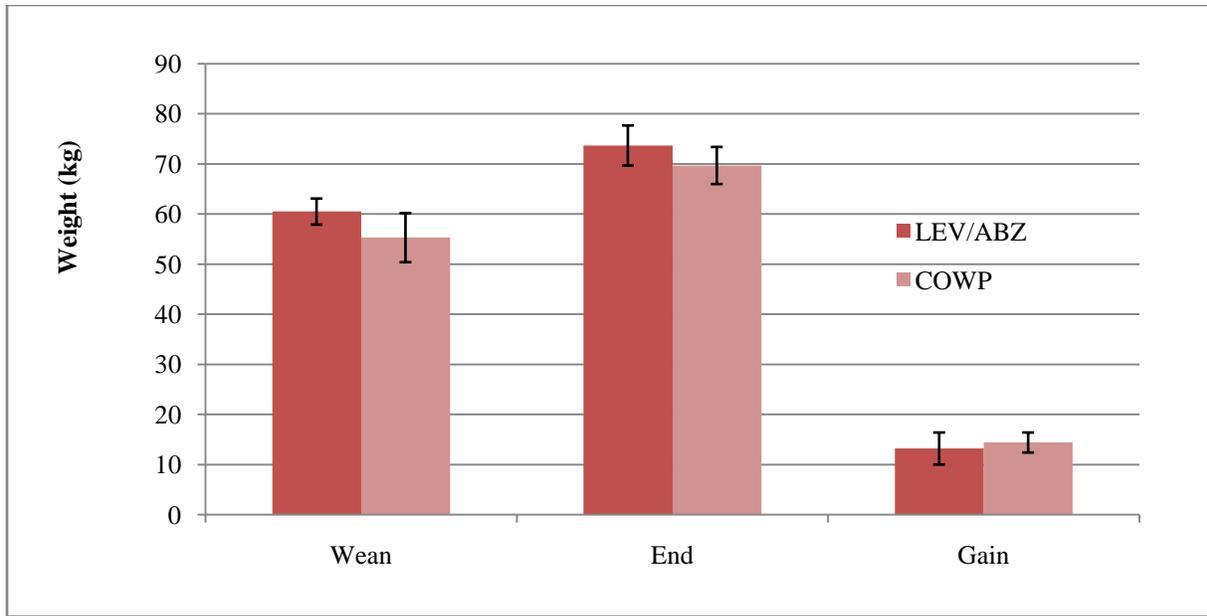


Figure 6. Weight at weaning and at end of trial for levamisole and albendazole (LEV/ABZ, n=10) and Copasure[®] copper oxide wire particle (COWP, n=10) treated lambs. Differences were not significant ($p > 0.05$).

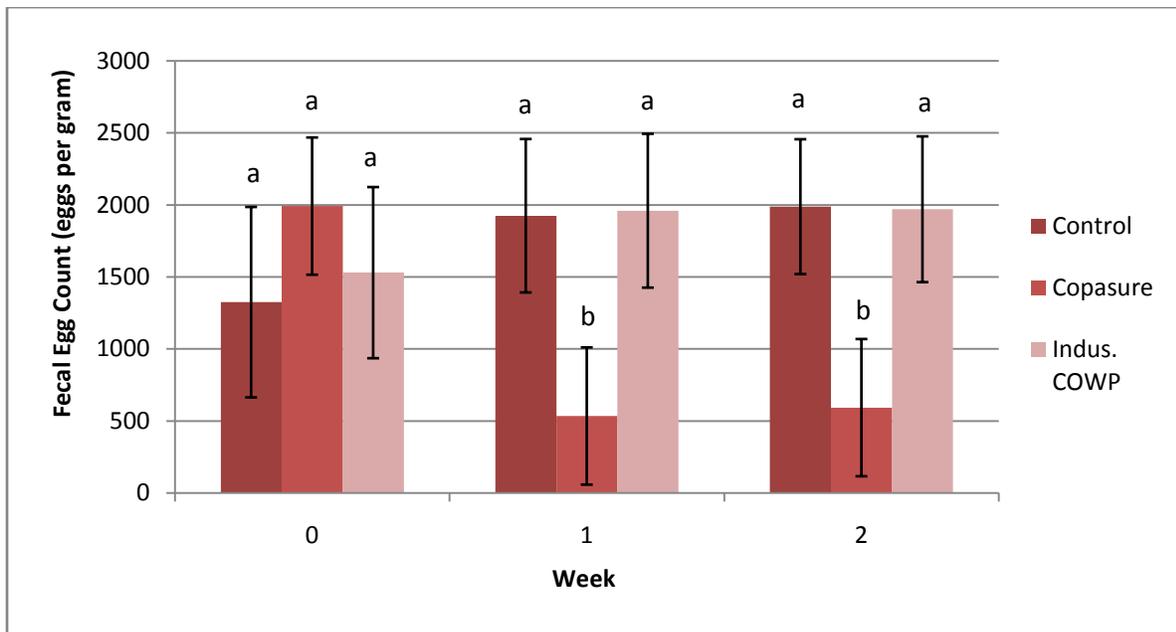


Figure 7. Fecal egg count for Control (C, n=4), Copasure[®] copper oxide wire particles (n=7), and industrial-grade copper oxide wire particles (n=5). Columns with different letters are significantly ($p < 0.05$) different.

CHAPTER 5 DISCUSSION AND CONCLUSION

Copper oxide wire particles were originally developed to treat copper deficiencies in cattle and sheep and later it was found that there was a direct association between COWP as an anthelmintic where FEC and numbers of abomasal nematodes (specifically *H. contortus*) were decreased in small ruminants. One initial report showed that 5 g of COWP were 96% effective against *H. contortus* (Bang et al., 1990), and studies since then with doses varying from 0.5-10 g have shown similar results (Knox, 2002, Burke et al., 2004, 2007; Burke and Miller, 2006). Because liver toxicity is an issue in sheep, the lowest effective dose should be the target dose. It has been shown that doses of 4 g or less for mature ewes and 2 g or less for lambs were effective and repeated treatments at 4 week intervals did not result in liver accumulation to toxic levels. However, liver copper level did increase with dose and long term use may be an issue. In addition, all those studies used COWP administered in bolus form to individual animals. The concept of using COWP in feed pellets has not been done. Industrial-grade COWP are also available and it is not known whether they might be just as effective as the Copasure[®] COWP.

Two of the trials that were conducted evaluated the effect of Copasure[®] COWP incorporated into feed pellets to control the peri-parturient rise in FEC of grazing ewes (Trial 1) and a comparison to anthelmintic treatment in grazing lambs (Trial 2).

In Trial 1, the typical peri-parturient rise in FEC was observed in both groups and after administration of the Copasure[®] COWP feed pellets there was a 76.4% reduction in FEC compared to control ewes. One concern that needs to be addressed when group feeding is that animals may consume different amounts of feed and those that get less feed may not get an effective dose. It was observed that the Copasure[®] COWP containing feed was consumed within 30 minutes and all animals ate some. The individual animal FEC before and 7 days after

treatment confirmed that the FEC of 9 of the 10 ewes was reduced by at least 70% and one ewe was reduced only 24%. So, that one ewe may not have consumed enough feed to provide the level of control observed for the other ewes. All ewe FECs in the Copasure[®] COWP group remained significantly reduced through weaning (4 more weeks). Infection monitoring was not continued beyond weaning so it is not known how long the reduction would have lasted. Results indicated that a one-time treatment of 4 g/hd Copasure[®] COWP in feed pellets at mid-lactation reduced infection in ewes for at least 4-5 weeks, thus pasture contamination and infection potential for their nursing lambs and subsequent post-weaning infection on the same pasture should be reduced.

Because targeted selective treatment is now considered the method of choice to conserve the useful life of available anthelmintics, the objective of Trial 2 was to use the FAMACHA System[®] to identify lambs that were anemic enough (from *H. contortus* infection) to warrant deworming and compare treatment with LEV/ABZ to Copasure[®] COWP in feed pellets. Due to observed resistance (unpublished data conducted at the Ben Hur Research Farm Sheep Unit of the Louisiana Agricultural Experiment Station in Baton Rouge, LA) to levamisole and albendazole used individually, the combination has been used successfully to reduce infection about 95% and, as such, has become the routine deworming protocol. After a few treatments in both groups, it became apparent that the FEC and PCV of many lambs was rapidly increasing and decreasing, respectively. FAMACHA[®] scores were also increasing, but had not reached the score of 4 that necessitated treatment. To avoid an impending clinical haemonchosis crisis, a salvage deworming was administered to all lambs that had not been previously dewormed. Subsequently, a similar number of treatments were given to individual lambs in each group. Different from Trial 1, the Copasure[®] COWP feed pellets were administered to individual

animals and all treated lambs consumed their feed, so any disparity in dosing was not a factor. Because infection increased quite rapidly, which was not picked up by attaining a FAMACHA[®] score of 4, it may be wise to use a score of 3 where heavy infection is expected. Results indicated that deworming with Copasure[®] COWP in feed pellets was just as effective as the LEV/ABZ treatment and production, as measured by weight gain, was similar for both groups.

In Trial 3, a comparison was made between Copasure[®] COWP and an industrial-grade COWP. It is important to consider the cost of treatment and the price of industrial-grade COWP is much lower than Copasure[®] COWP, but the efficacy of industrial-grade COWP has not been established. Both formulations of COWP were administered orally in geletin capsules and Copasure[®] COWP significantly reduced FEC while the industrial-grade COWP had no affect on FEC. In fact, the FEC increased in this group. What might account for this lack of efficacy is unknown, but it was observed that the industrial-grade COWP looked “dull” and were longer in length when compared to the Copasure[®] COWP. It may be that Copasure[®] COWP have been formulated differently than industrial-grade COWP which allows dissolution in the acidic environment of the abomasum.

Treatment with Copasure[®] COWP in feed pellets effectively reduced FEC in lactating ewes and could be used to suppress the peri-parturient rise in FEC. Also, Copasure[®] COWP in feed pellets worked as well as the combination of LEV/ABZ for deworming lambs on an individual basis. However, when using the FAMACHA system[®] to identify individuals anemic enough for deworming, consideration should be given to using a cutoff value of 3 rather than 4 especially under conditions of heavy infection. The results of these studies suggest that the use of Copasure[®] COWP in feed pellets may be a very useful tool for producers and help reduce reliance on the conventional use of anthelmintics alone for control.

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VITA

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Copper Oxide Wire Particles in Feed Pellets for Controlling Gastrointestinal Nematode Infection in Ewes and Lambs

Thesis directed by Professor Dr. James E. Miller

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ABSTRACT

Gastrointestinal nematode parasites cause extensive damage to small ruminants, and *Haemonchus contortus* is a major concern to production. In the past, small ruminants were dewormed at regular intervals and control methods were based primarily on the use of anthelmintics. At present, anthelmintic resistance has been reported worldwide and has developed into a serious problem for small ruminant management programs. In view of this, alternate control methods are needed. One alternative method is the use of copper oxide wire particles (COWP). Three independent trials were conducted during Spring, Summer, and Fall of 2008. Two trials evaluated the effect of COWP in food pellets in reducing *H. contortus* infection in crossbred ewes and Suffolk lambs. The third trial compared the effect of Copasure[®] COWP with that of an industrial-grade COWP. Trials consisted of similar protocols where ewes and lambs were allocated into groups based on fecal egg count (FEC). Copasure[®] COWP in feed pellets were fed to ewes at mid-lactation when FEC increased to over 1000 eggs per gram, and to individual lambs when FAMACHA[®] score was 4/5. Ewe/lamb infection was monitored weekly by FEC and blood packed cell volume. Results of Trial 1 indicated that Copasure[®] COWP in feed pellets was effective in reducing the peri-parturient rise in ewes, based on FEC. Trial 2 indicated that Copasure[®] COWP in feed pellets was just as good as the levamisole/albendazole treatment when the FAMACHA System[®] was used to determine when to treat lambs. Trial 3 indicated that Copasure[®] COWP effectively reduced parasite infection while the industrial-grade COWP did not. The results from these trials demonstrated that the use of Copasure[®] COWP in feed pellets reduced *H. contortus* infection and may be useful alone or when used with other control methods.