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The Use of Duddingtonia Flagrans Aged in Mineral for Gastrointestinal Parasitic Nematode Control in Small Ruminants

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**THE USE OF *DUDDINGTONIA FLAGRANS* AGED IN MINERAL
FOR GASTROINTESTINAL PARASITIC NEMATODE
CONTROL IN SMALL RUMINANTS**

A Thesis

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

in

The School of Animal Sciences

By
Elisa Preston
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ABSTRACT

Gastrointestinal nematodes (GIN) are a major constraint to small ruminant health and production throughout the world. *Haemonchus contortus* commonly known as the “barber pole worm” is a major concern for producers and veterinarians because of the severe anemia, and other clinical symptoms these worms cause. For decades, anthelmintics have been used to control the GIN burden small ruminants face. Katahdin (n=36) and Hampshire sheep (n=12) were used in a randomized block design experiment over a 30-day period. The objective of this study was to evaluate the effectiveness of *Duddingtonia flagrans* spores, the active ingredient in a commercial product, Bioworma® (International Animal Health Products Pty Ltd, Australia), when thoroughly combined with a mineral mix and aged for seven days prior to feeding. Three treatment groups were utilized: control, Bioworma fed in concentrate (BWC) and Bioworma fed in mineral (BWM). Coprocultures were set up twice a week in order to allow the nematode eggs in the feces to develop to third-stage larvae (L3s) which were then counted. The percentage of larvae recovered was then calculated per culture and least squares means by treatment were reported. The percentage of larvae recovered from Bioworma-treated animals was consistently lower than the percentage of larvae recovered from animals in the control group. There was no difference in the percentage of larvae recovered when BWC and BWM treatments were compared. These data support the effectiveness of Bioworma when it has previously been mixed with a mineral supplement at least seven days prior to feeding.

CHAPTER I. INTRODUCTION

The United States Department of Agriculture has published that as of January 1st, 2024, the sheep and lamb inventory in the United States totaled 5.03 million head and the goat and kid inventory totaled 2.47 million head, a two percent decrease from 2023 (NASS, 2024). There is still much demand for products such as dairy, meat, and fiber derived from small ruminants. One of the most prevalent challenges a small ruminant producer faces is internal parasites, specifically gastrointestinal nematodes (GIN) (Zajac, 2013). A heavy parasitic infection will reduce the intake and utilization of feed, resulting in decreased growth, inferior quality product, and less product overall (Sutherland et al., 2010). To combat parasitic burdens, small ruminant producers rely heavily on the use of anthelmintics. The frequent use of anthelmintics was beneficial for many years, but resistance has developed to multiple classes of anthelmintics (Kaplan, 2013). Many studies have been conducted in the southeastern United States and in other warm, tropical climates that favor parasite growth. In these areas which include Alabama, Florida, Georgia, Louisiana, Puerto Rico, St Croix and the US Virgin Islands, anthelmintic resistant *Haemonchus contortus* was reported. Out of 46 farms tested, *H. contortus* nematodes were resistant to at least one class of anthelmintics (Howell et al., 2008). As anthelmintic resistance continues to intensify, new anthelmintics are not expected on the market any time soon. Efforts have switched to finding alternatives to anthelmintics. The use of nematode-trapping fungi shows promise in controlling larval development on pasture. One of these fungi, *Duddingtonia flagrans*, produces spores which can withstand the ruminant gastrointestinal tract when ingested, are passed out in the feces, and effectively use sticky traps and loops to restrain the larval stages of nematodes in feces before killing them by feeding on the nematodes (Miller

et al., 2021). Bioworma®, a product manufactured by International Animal Health Products Pty Ltd (Australia), contains spores of *D. flagrans*. The product is recommended to be fed thoroughly mixed with feed supplements, premixes, concentrates, or loose mixes (www.bioworma.com/bioworma). Studies have proven the efficacy of Bioworma when mixed with grain and fed daily. However, many small ruminant producers rely on forages to finish their animals on pastures and only supplement their animals with minerals. The objective of this study was to determine the efficacy of Bioworma when mixed with a mineral supplement and stored for at least 7 days prior to feeding.

CHAPTER II. LITERATURE REVIEW

2.1. Parasite of Most Concern, *Haemonchus contortus*

The superfamily Trichostrongyloidea contains the most important gastrointestinal nematodes (GIN) that affect grazing small ruminants. Nematodes in this family include *Haemonchus contortus*, *Teladorsagia* spp., *Trichostrongylus* spp., *Cooperia* spp., and *Oesophagostomum* spp. (Pugh and Baird, 2012). These species of nematodes can cause clinical disease such as diarrhea, weight loss, anemia, poor production, and poor reproduction.

Within the Trichostrongyloidea superfamily, *Haemonchus contortus* is the parasite of most concern for producers. *Haemonchus contortus* has a direct lifecycle, similar to the other nematodes in this superfamily (Arsenopoulos et al., 2021). Within the direct lifecycle there are two phases, the free living, which is in the environment, and the parasitic, which is in the animal. In the free-living stage, eggs produced by the worms are excreted by the animal in the feces. In this regard, *H. contortus* is the most abundant egg layer in this nematode superfamily. Once eggs are laid on pasture, they will hatch and the first larval stage (L1) emerges. This stage molts to the second stage larva (L2) and, in turn, this L2 molts to the third stage larva (L3). The third stage larvae are ingested by the animal, making the L3 the infective stage of *H. contortus*. When the larvae are inside the animal, the parasitic stage of the life cycle begins, where L3s will migrate to the abomasum of the small ruminant. The L3s will molt to give rise to the fourth-stage larvae (L4s) (Arsenopoulos et al., 2021). Inside the abomasum, the L4 molts one last time into the immature adult stage, which then develop into mature, reproductive adults. *Haemonchus contortus* has a buccal hook which acts as a tooth or lancet to puncture and suck blood directly from the host (Zajac, 2013; Flay et al., 2022). Blood-sucking can cause significant anemia in hosts infected with *H. contortus*, from the loss of ingested red blood cells and blood proteins. A

mature female worm is able to produce approximately 10,000 eggs per day, which serves to contaminate the pasture. One complete lifecycle takes approximately three weeks from ingestion of infective larvae to production of eggs passed in the feces, the so-called prepatent period (Delano et al., 2002). *Haemonchus contortus* has the ability to undergo hypobiosis when conditions do not favor development of the larval stages on pasture. This involves the larvae arresting their development within the host and remaining in this state within the mucosal tissues of the abomasum until conditions on pasture are suitable for the lifecycle to continue. Hypobiosis can occur for months at a time, ensuring larvae are available for development to the egg-laying adult stages months later, which will serve to ensure the pastures are again contaminated when conditions are favorable for the continuation of the free-living phase of the lifecycle (Delano et al., 2002; Arsenopoulos et al., 2021; López et al., 2023).

Haemonchus contortus is commonly known as the “barber pole worm” because of the female’s appearance after ingesting blood from the abomasum. The female’s white uterus wraps around the red blood-filled digestive tract giving the female this characteristic barber-pole appearance (Arsenopoulos et al., 2021). Animals with acute haemonchosis present with severe anemia and edema. Hyperacute infections can result in death within a week of infection without significant symptoms presenting themselves (VanHoy, 2023). Edema in small ruminants infected with *H. contortus* is usually dependent edema which is noted in the intermandibular space and is commonly referred to as “bottle jaw” (Flay et al., 2022).

Infections of *H. contortus* are extremely common in the southern United States because of the climate in this region. The warm temperatures and moisture in the air favor the development of *H. contortus*. Other regions with similar climates such as the tropical regions of Africa, tropical islands of the Caribbean, and southern Asia also battle ongoing *H. contortus* infections.

Many of these regions also have high levels of summer rainfall. Warm temperatures and the high humidity that results from frequent rainfall promote continuous development of the larval stages on pasture (Arsenopoulos et al., 2021). As temperatures fall in these regions during the winter, hypobiosis begins and allows for a dormant period in nematode activity outside the host. When the environment begins to warm, hypobiosis ends, and an ideal environment supports infections again. While a warm, wet environment is ideal for *H. contortus*, these nematodes are able to survive in several other climates for seasons at a time. In areas such as Western Australia, south-west Africa and south-east Australia infections are bi-phasic. Summers in these regions are hot and dry, and winters are cold. Infections of *H. contortus* are highest in the autumn and spring when conditions are favorable between the conditions of extreme heat and cold. *Haemonchus contortus* has found ways to proliferate in many regions of the world causing problems for producers worldwide (Arsenopoulos et al., 2021).

2.2. Classes of Anthelmintics Available in the United States

Efficient use of anthelmintics is important for the control of nematodes such as *H. contortus*. Anthelmintics is the name given to drugs that treat infections of animals with parasitic worms including trematodes, cestodes, and nematodes (Holden-Dye and Walker, 2014). There are three common classes of anthelmintics which are used frequently: benzimidazoles, nicotinic agonists, and macrocyclic lactones which producers and veterinarians rely on to treat infections.

2.2.1. Benzimidazole Class

Benzimidazoles were introduced in the U.S. in the 1960's with thiabendazole being the first in its class (Abongwa et al., 2017). Drugs in this class have a broad spectrum of use and a large safety margin. When the flow-rate of ingesta is slowed, such as by withholding feed, the absorption of the drug may be enhanced. The rate of passage of the drugs is inherently slowed

through the rumen or cecum, making the benzimidazoles very suitable for use in ruminants and horses. In the U.S., these drugs are formulated as a suspension and should be administered by mouth, but in other countries, they are available in other formulations such as slow-releasing boluses or topical formulations (Vercruyse and Claerebout, 2022a).

The basic structure of the benzimidazoles is a benzene fused with an imidazole ring (Veerasingh et al., 2021). Common drugs in this class are fenbendazole (Safeguard and Panacur, Merck Animal Health, Rahway, New Jersey), albendazole (Valbazen, Zoetis, Parsippany, New Jersey) and oxfendazole (Synanthic, Boehringer Ingelheim Animal Health USA Inc., Duluth, Georgia) (Schoenian, 2012). When thiabendazole was first introduced, the mechanism of action was believed to target metabolic pathways of parasites (Prichard, 1970). It has since then been established that the actual mechanism of action involves beta tubulin. Beta and alpha tubulin molecules form a dimer that is used by cells to create microtubules which are necessary for cell structure, cell growth and division, and transportation (Binarová and Tuszynski, 2019). Benzimidazoles bind the beta tubulin in parasites which prevents the polymerization of microtubules, resulting in cell destruction and death of the parasite (Abongwa et al., 2017).

2.2.2. Nicotinic Agonist Class

The second class of anthelmintics commonly used is nicotinic agonists, which can be split into two different groups, imidazothiazole and tetrahydropyrimidines. The only drug included in the grouping of the imidazothiazoles is levamisole (Prohibit, Huvepharma, Inc., Peachtree City, Georgia). Within the group of the tetrahydropyrimidines there are drugs such as morantel (Rumatel, Phibro Animal Health Corporation, Teaneck, New Jersey) and pyrantel (Strongid, Zoetis, Parsippany, New Jersey) (Schoenian, 2012). The nicotinic agonist class inhibits nematodes and causes paralysis by acting on the nicotinic acetylcholine receptors

(Martin and Robertson, 2007).

Tetramisole was the first drug introduced in the imidazothiazole group and is a racemic mixture, meaning that the drug contains 50% L- or S- and 50% D- or R- isomers (Abongwa et al., 2017). While tetramisole was being studied, it became evident that the most potent portion of the drug was the L-isomer. This resulted in the removal of the D-isomer from the racemic mixture and gave rise to levamisole (Abongwa et al., 2017). Levamisole has a broad spectrum, a wide safety margin, and has not shown connections to developmental abnormalities; it is effective against adult and larval stages of certain parasites. The drug can be administered as a pour on or as a slow-release bolus in small ruminants (Vercruyse and Claerebout, 2022b), but in the U.S. it is available only for oral administration by itself or as an injectable formulation in combination with doramectin (Valcor, Zoetis, Parsipany, New Jersey).

The mechanism of action of nicotinic agonists includes selectively targeting the membrane ion channels of parasites, and not the host. These targeted ion channels are activated by nicotinic acetylcholine receptors in the somatic musculature of nematodes. Acetylcholine is a neurotransmitter that is responsible for signal transmission between neurons and it traditionally binds to nicotinic acetylcholine receptors (Purves et al., 2001). Drugs in the nicotinic agonist class act in a similar manner to acetylcholine and cause ion channels to stay open for a prolonged period, leading to extended muscle contraction. This continued contraction causes spastic paralysis of the parasite (Williamson et al., 2009).

2.2.3. Macrocyclic Lactone Class

The most recently introduced class of anthelmintics is the macrocyclic lactones, introduced in the 1980s. This class is also split into two groups, the avermectins and the milbemycins. The avermectins include ivermectin (Ivomec, Boehringer Ingelheim Animal Health USA Inc.,

Duluth, Georgia) and doramectin (Dectomax, Zoetis, Parsippany, New Jersey), and the milbemycins include moxidectin (Cydectin, Elanco Animal Health, Greenfield, Indiana). One characteristic that sets macrocyclic lactones apart from the other classes is that these drugs can also treat some external parasites (Schoenian, 2012). Macrocyclic lactones can treat immature and hypobiotic nematodes at low doses and are broad-spectrum drugs. The drugs are formulated to be administered orally and as pour-ons (Vercruyse and Claerebout, 2022c).

Macrocyclic lactones work by targeting the glutamate-gated chloride channels (GluCl_s) that are present on the neurons of nematodes, but not the host (Abongwa et al., 2017). These channels are responsible for controlling the locomotion, feeding, and sensory inputs that influence behavior. Ivermectin binds between two cell membrane-spanning domains of adjacent subunits making up the glutamate-gated chloride channel. When binding occurs to these units in the somatic musculature and the pharynx of the nematode, paralysis and inhibition of pharyngeal pumping ensues, respectively (Wolstenholme, 2012; Abongwa et al., 2017). Macrocyclic lactones will also act as an antagonist of gamma-aminobutyric acid (GABA) and nicotinic receptors. The role of GABA within parasites is not extremely clear, but it is proposed that GABA acts as an intracellular signal or as a cell-to-cell signal, mediating environmental and host stress (Nugraha et al., 2022).

2.3. Anthelmintic Resistance

For decades, anthelmintics have been effective in treating parasitic infections, but in current days, the efficacy has greatly decreased, owing to the development of anthelmintic resistance, with no promise of new anthelmintics on the horizon. Anthelmintic resistance is defined by many parameters but can best be described as “the heritable loss of sensitivity of an anthelmintic in a parasite population that was in the past susceptible to the same anthelmintic” (Fissiha and

Kinde, 2021). Through genetic mutations which are passed on via offspring and selected for by anthelmintic treatment, nematode populations have been able to evolve such that they are able to escape the killing actions of anthelmintics, and this resistance is now present worldwide.

Several mutations have occurred across the three classes of anthelmintics which have led to the occurrence of resistance. Nematodes exhibiting resistance to the benzimidazoles were noted as early as the 1990's. In benzimidazole resistance, mutations have occurred that prevent the connections between beta tubulin and the benzimidazoles from happening. Recently, the specific mutations that occurred in *H. contortus* so that the worm has become resistant to the benzimidazoles have been found. A single amino acid mutation will cause inhibition of the binding by benzimidazoles, and three prominent single nucleotide mutations have been identified as likely causes of resistance. Mutations occurring at positions 200 and 167 (TTC to TAC) cause a substitution of phenylalanine to tyrosine, and position 198 (GCA to GAA) results in the change of glutamate to alanine. These point mutations result in different isotype formations of beta tubulin (Arsenopoulos et al., 2021; Fissiha and Kinde, 2021), which confer resistance. Resistance to imidazothiazoles has also been seen since the 1990's, stemming from the mutation of genes that encode for the nicotinic acetylcholine receptor subunit (Sangster and Bjorn, 1995). Genetic mutations of the Hco-unc-63 and Hco-acr-8 genes lead to the expression of a protein that will bind to the acetylcholine receptor, blocking the binding of the imidazothiazoles (Arsenopoulos et al., 2021). *Haemonchus contortus* has also exhibited resistance to the macrocyclic lactones. Different mechanisms of resistance have been proposed, one being the mutation of a glycine residue. The glycine residue on the gene encoding for the glutamate-gated chloride ion channels is mutated and this is thought to play a role in *H. contortus* resistance to this anthelmintic class (Arsenopoulos et al., 2021).

Besides the genetic mutations in *H. contortus* which have resulted in the development of resistance, other factors have led to the levels of anthelmintic resistance facing producers today. There was once a theory that the frequent use of anthelmintics was the best way to battle GIN infection. It is now clear that this approach was not feasible for the long-term sustainable use of anthelmintics (Kaplan, 2013). Frequent use, along with preemptive mass treatment with anthelmintics, has selected strongly for resistance because these practices have significant effects on reducing the susceptible worms in refugia, which is the group of nematodes not exposed to treatment (Greer et al., 2020). Treatment of all animals in a group will lead to the death of the majority of the anthelmintic-susceptible worms. When the concept of maintaining anthelmintic-susceptible individuals in refugia is followed, for example, by treatment of only half the herd, some susceptible alleles will be left in the herd. This allows for a dilution of the resistant nematodes by susceptible nematodes. Making effective use of the concept of refugia was not followed until recent years but is now central to strategies used to slow further development of resistance (Hodgkinson et al., 2019).

Another reason for the development of resistance is the repeated use of the same classes. When anthelmintics in the same classes are used frequently, resistance develops rapidly. Said differently, the more often an anthelmintic of a particular class is used, the greater the likelihood that anthelmintic resistance will emerge. Use of one singular drug selects for nematodes with the mutations needed to combat the effects of the anthelmintic in question, leading to resistance (Shalaby, 2013). Underdosing of anthelmintics has also allowed for the development of resistance. Administering doses less than the therapeutic dose allows for the survival of nematodes with resistant alleles, which may be passed on to their offspring. The bioavailability of anthelmintics in different hosts also accounts for anthelmintic resistance. Producers have

dosed goats and sheep at the same rate for years although it is now known goats need to be dosed at a rate of 1.5 to 2 times higher than sheep. Dosing goats at the sheep dosage is effectively equivalent to underdosing the animals.

2.4. Alternative Methods

While anthelmintic resistance is an ongoing battle, the discovery and development of new anthelmintics does not appear to be promising. Finding alternative methods to the use of anthelmintics is one of the keys to controlling parasites in small ruminants.

2.4.1. Pasture Management

Managing pastures that small ruminants graze may be a beneficial strategy for controlling nematodes. Methods such as burning pastures and pasture rotation are two ways producers can manage parasitic infection (Kumar et al., 2013). Ideally animals would be able to graze completely “clean” pastures. In the southeastern U.S., pastures could be considered clean by routine burning, rotation of grazing lands with field crops, or removing animals for several years. (A reduction in infectivity may be achieved by removing animals from pastures for periods of a year, though the pastures would not be “clean.”) Another strategy, likely more practicable in most situations, involves rotational grazing. Here animals are removed from one pasture and placed on another, leaving a period in between before switching back to the original pasture. Pasture rest should last at least three to six months to reduce levels of infection (Kumar et al., 2013). All pasture management techniques are likely beneficial to some degree or another, but they may not be feasible to implement by all producers.

2.4.2. *Sericea lespedeza*

Sericea lespedeza (*Lespedeza cuneata*), also known as “poor man’s alfalfa,” is a warm-season legume that is able to grow in many adverse conditions (Mosjidis and Terrill, 2013).

Another benefit of this legume is that it is high in tannins which have been shown to have an antiparasitic effect. Several mechanisms have been proposed for this anthelmintic effect such as reducing host invasion by infective L3s, reducing production of eggs by mature nematodes, and reducing the development of eggs to L3s (Huang et al., 2018). Several researchers have shown a reduction in nematodes after feeding sericea lespedeza. In a study by Min et al. (2005) where feeding sericea lespedeza was practiced, mean fecal egg count was lower for animals fed sericea lespedeza than animals which were fed *Festuca arundinacea* (tall fescue, which has low tannin levels) or animals which followed a rotational grazing strategy. Burke et al. (2012) showed that animals who grazed sericea lespedeza tended to have a lower fecal egg count and a higher packed cell volume. Recommendations include that sericea lespedeza should be fed as at least 50% of the diet, but not for a period longer than eight weeks for young animals (Mosjidis and Terrill, 2013). Feeding sericea lespedeza may not be practical for all producers because of the large amounts needed to be fed and its low palatability during certain seasons.

2.4.3. Copper Oxide Wire Particles

Copper oxide wire particles (COWP) have also been shown to have antiparasitic effects in small ruminants. COWP were originally produced to combat a copper deficiency in small ruminants and cattle. The particles can be administered in a gelatin capsule containing the appropriate dose the animal needs. When administered in a bolus or feed, the capsule travels through the rumen and to the folds of the abomasum. Copper may provide antiparasitic effects by increasing the levels of copper in the abomasum, directly affecting the cuticle of the nematode, specifically *H. contortus*. Lesions have been seen on the cuticle of *H. contortus* in animals treated with COWP (Burke et al., 2013). In studies evaluating the difference in anthelmintic effects based on treatment dose, boluses containing zero, two, four, or six grams of COWP were

fed to hair breed lambs. Copper levels found in the liver in that study were not expected to have toxic effects on the animals. Lambs who received four or six grams of COWP had elevated copper concentrations in the liver which may have led to copper toxicity eventually. All these doses of COWP had an effect on *H. contortus* worm burdens. Animals which received two grams of COWP needed an extra seven days to reach an adequate decrease in fecal egg count compared to animals which received four or six grams of COWP (Burke et al., 2004). COWP shows promise in effectively battling nematodes, but producers face the possibility of copper toxicity and that for all practical purposes, only *H. contortus* will be affected by COWP.

2.5. *Duddingtonia flagrans*

The use of nematode-trapping fungi has shown great promise in controlling GIN in small ruminants. The fungus is found as a natural part of the environment where it feeds on naturally occurring nematodes (Miller et al., 2021). These fungi can also be used to trap the larval stages of parasitic nematodes that infect livestock. The trapping and killing of nematode larvae on pasture causes a break in the parasitic lifecycle, so that reinfection will not occur (Blair and Biddle, 2020). Since the nematode lifecycle takes place in two phases, control of the parasites during the pasture phase has proved to be beneficial in diminishing worm burdens. Anthelmintics may be used to control nematodes inside the host, while fungi may be used to control pasture contamination. One particular species of fungus, *Duddingtonia flagrans*, has demonstrated promising results in the control of nematodes. One of the beneficial factors of *D. flagrans* is that its spores are able to withstand the conditions of the small ruminant gastrointestinal tract (Miller et al., 2021).

Duddingtonia flagrans produces chlamydospores. These spores have a thick wall which allows for protection from high temperatures and, when ingested, from varying pH levels

throughout the GI tract (Céspedes-Gutiérrez et al., 2021). The spores are found at the hyphae or hyphal tips of the fungi; the hyphae act as a means for growth (Lin and Heitman, 2005; Steinberg, 2007). Multiple hyphae form the mycelium, which is considered the body of the fungus. Spores of *D. flagrans* that are ingested are later defecated where they can germinate in the feces and begin to create mycelia (Burke and Miller, 2021). The mycelia grow into “sticky traps” and loops that become intricate enough to trap any developing larval stages of nematodes present in the feces. Trapping structures can be seen within a few hours of defecation. A sticky portion of the trap which helps to grasp the larvae and then penetrate the larval cuticle wall will be seen within 48 hours (Burke and Miller, 2021). When developing larvae are caught in a trap, the hyphae will infiltrate the larval cuticle and fill the majority of the larval body by digesting the contents. Since larvae are stuck in these traps, they are unable to move out of the fecal pad and onto the pasture and are not ingested by animals (Burke and Miller, 2021).

2.5.1. Bioworma®

Bioworma® is a commercially available product produced in Australia by International Animal Health Products Pty Ltd (IHAP). The product consists of *D. flagrans* chlamydospores and has the consistency of a fine powder, meal-like substance that can be added to the feed or feed supplements. Each gram of Bioworma contains a minimum of 50,000 chlamydospores (International Animal Health Products, 2022). IHAP claims Bioworma will control *Haemonchus* spp., *Trichostrongylus* spp., *Teladorsagia* spp., and *Oesophagostomum* spp., along with some other nematodes found in small ruminants (International Animal Health Products, 2022). The recommended dose of Bioworma is 0.1 ounces per 100 pounds of body weight per animal. Bioworma retails for as much as \$355.00 for 10 pounds according to Premier1, an authorized Bioworma retailer.

The primary method of administration for Bioworma is to mix the product with grain daily and feed it to the animals. This works for many production farms, but other producers do not feed grain, only mineral mix, and finish their sheep on pastures. Where grain is not fed, Bioworma could be mixed with the mineral supplement. Feeding Bioworma with the mineral mix might mean that it does not need to be fed daily which would cut down on labor costs. In this case, the mineral mix should be kept covered and dry (Burke and Miller, 2021). There is a lack of studies to date evaluating the efficacy of Bioworma when administered in a mineral mix, rather than mixed with grain, and the effect of allowing the Bioworma to “age” in the presence of minerals has not been evaluated.

CHAPTER III. MATERIALS AND METHODS

3.1. Animals and Treatments

Forty Katahdin (25 female, 15 male; mean age = 172 days) and twelve Hampshire cross (2 females, 10 males; mean age = 132 days) sheep were utilized in a study to assess the efficacy of a commercial product consisting of *Duddingtonia flagrans* spores (Bioworma®, International Health Products Pty Ltd, Australia; lot number 3167101) supplemented in minerals fed *ad libitum* over a 30-day period spanning July and August 2023. Katahdin sheep were obtained from the USDA Dale Bumpers Small Farms Research Center (Booneville, AR) and were quarantined for a minimum of 30 days before use. Hampshire cross sheep were obtained from the LSU AgCenter Research Station, Baton Rouge, Louisiana, which is where the study was conducted. This study was approved by the LSU AgCenter Institutional Animal Care and Use Committee.

Animals were randomly blocked by fecal egg count (FEC), body weight, sex and breed for a randomized block design. Two barns and adjacent pastures were utilized, split into three pens with adjoining pastures each. Each treatment was replicated in each barn. All pens and pastures were approximately the same size. Animals were given a 14-day acclimation period. During this time, they were exposed to feed troughs, mineral feeders, and other animals that would be used during the study.

All animals were fed a 16% crude protein commercial grain mix (Table 3.1; Kentwood Co-Op, Kentwood, LA). Grain was initially fed at one pound per head per day, split evenly into morning and evening feedings. Feed was increased to two pounds per head per day split evenly, based on average daily gain calculated weekly. Clean, fresh water was provided *ad libitum* to animals through the entire study. Purina® Wind and Rain® mineral mix (Kentwood Co-Op,

Kentwood, LA) was fed *ad libitum* to all animals. Animals were allowed to graze in their respective pastures during the day and housed in the pens through the night.

Table 3.1. Composition of Grain Mix

<i>Ingredient</i>	<i>Percent of Mixture</i>
Rolled Corn	64.10%
Cottonseed Meal 41	12.00%
Soybean Meal 48	8.00%
Soy Hull Pellets	10.00%
NB Ammonium Chloride	0.80%
MB Mold X 41	0.10%
Molasses	5.00%

A pilot study was conducted, and this confirmed the effectiveness of the batch of Bioworma used in the study (see Appendix). Treatment 1 (Control) consisted of feeding standard grain and mineral. Treatment 2 involved mixing Bioworma thoroughly with grain once per day in the afternoon feeding. Bioworma was administered according to the manufacturer’s recommendation at 2.835 grams per 45.359 kilograms of live body weight. Treatment 3 consisted of feeding Bioworma aged in the commercial mineral mix. Minerals were weighed out seven days prior to the start of administration. On this day 2.835 grams of Bioworma was thoroughly mixed into 0.4535 kilograms of mineral to allow it to “age”. The aged mineral mix was then fed at the rate of 28.3495 grams of mineral mix per 45.359 kilograms of body weight. Batches were produced every seven days such that this group was fed Bioworma that had been aged for at least 7 days and up to 14 days, to simulate field conditions.

3.2. Body Weight and Body Condition Scoring

Body weights were measured, and body condition scores were assigned on days 0, 7, 14, 21, and 30. Animals were weighed, and body condition scores were assigned based on a scale of 1 (emaciated) – 5 (obese). Areas including the spinous and transverse processes of the lumbar

vertebrae were palpated for muscle and fat covering to assign an appropriate score (Thompson et al. 1994). Given that Katahdins are a hair breed, attention was also paid to fat deposits around the tail and sternal area.

3.3. Blood and Packed Cell Volume (PCV)

Blood samples were collected on days 0, 7, 14, 21, and 30 via the jugular vein with a 21-gauge one inch needle into Vacutainer® tubes containing EDTA. Samples were kept on ice until they were used to determine packed cell volume. Two blood samples from each animal were aliquoted into capillary tubes and the tubes were sealed with putty (Critoseal® Tube Closures, McCormick Scientific). The tubes were then centrifuged in a microhematocrit centrifuge (Autocrit Ultra 3 Microhematocrit Centrifuge, Becton, Dickson, and Company) for five minutes. Once completed, packed cell volume was read using a hematocrit chart placed directly on the centrifuge.

3.4 FAMACHA©

The FAMACHA technique was used to assess the level of anemia in animals. A FAMACHA card consists of five color categories ranging from 1 (non-anemic) to 5 (severely anemic). Colors on the card were compared to the conjunctival mucous membrane of the animal (Vatta, 2013). Animals were placed in direct sunlight where the conjunctival mucous membrane was exposed and compared to the color of the card. The opposite eye was done subsequently, in cases where FAMACHA score differed between eyes, the higher score was reported.

3.5. Fecal Samples and Fecal Egg Count

Fecal samples were collected on days 0, 7, 14, 21 and 30. Samples were collected directly from the rectum, placed in individual cups, and kept in a cooler on ice or in the refrigerator until laboratory analysis. Samples were analyzed using the modified McMaster technique for fecal

egg count. Two grams of feces were weighed off and combined with 30 mL of salt solution (sodium chloride mixed with 1 L water to give a specific gravity of 1.18). The mixture was then strained through a tea strainer. A transfer pipette was used to place a portion of the sample into a two-chambered McMaster slide (Chalex, LLC, Park City, Utah). The slides were observed under 10x magnification. Trichostrongyle-type eggs were counted. The number of eggs per gram (epg) for each sample was calculated using the formula:

$$\# \text{ of eggs} \times 50 = \text{epg}$$

3.6. Coprocultures

Coprocultures were used to allow for the development of larvae from the egg to the third-stage larva (L3). Fecal samples for culture were collected on days 0, 3, 7, 10, 14, 17, 21, 23, 28 and 30. The sheep in each pasture were divided into two subsets consisting of four animals each. These subsets remained the same throughout the entire study. The same amount of feces from each animal was weighed out and mixed thoroughly. From there 2 g of feces were aliquoted for a FEC, this was repeated to give two FECs, and then a representative sample was taken to make a culture. The amount of feces included in each culture was increased from 4 g to 6 g to 8 g to ensure adequate larval recovery (Table 3.2), after the initial recoveries appeared to be low in numbers. Once feces were placed in a 100 mL “culture” cup, they were mixed with vermiculite at a 1:1 ratio with a small amount of water to moisten the sample. A piece of double layer cheese cloth and a rubber band were used to secure the top of the cup. A 250 mL cup was filled with approximately 50 mL of warm water. The culture was inverted and placed into the larger cup. This allowed for a moist environment for a seven-day incubation period. On day 7 the 250 mL water cup was flooded to the top with warm water and allowed to sit for at least 24 hours. After that time, the culture cup was removed, and the contents of the 250 mL cup were given another

24 hours to settle. The contents of the 250 mL cup were then reduced to 50 mL, transferred to a 50 mL tube, and left at room temperature for an additional 24 hours, to allow the contents to settle. Following this, the contents were then reduced to 15 mL and transferred to a 15 mL tube. Once all viable larvae had settled to the bottom of the tube following an additional 24-hour period, water was pipetted off leaving approximately 1-2 mL of fluid in the tube (leaving the plugs of larvae intact). The first 200 trichostrongyle-type larvae were counted and identified to genus and the remaining trichostrongyle-type larvae were enumerated. All the *Strongyloides* larvae were counted separately.

Table 3.2. Week, Date of Culture, and Weight of Coprocultures

<i>Week</i>	<i>Culture Date (2024)</i>	<i>Culture Weight</i>
1	July 11	4g
1	July 14	4g
2	July 18	4g
2	July 21	6g
3	July 25	6g
3	July 28	8g
4	August 1	8g
4	August 4	8g
5	August 8	8g
5	August 10	8g

After the larvae had been counted, the percentage larval recovery was calculated. To do this, the average of the two FECs performed for each pasture subset was calculated and multiplied by the amount of feces cultured to estimate the total number of eggs that were incubated for that culture. Then, the total number of larvae recovered from that culture were divided by the estimate of the total number of eggs incubated and the product multiplied by 100 to give the percentage larval recovery.

3.7. Statistics

Data were analyzed using the SAS PROC MIXED procedure with an autoregressive covariance structure. The model included PCV, FEC, L3 recovery, FAMACHA, body weight, and BCS as dependent variables and treatment, sex, breed, barn, day, and interactions as independent variables and a repeated statement for day of measurement. Orthogonal contrasts included control vs. BWC and BWC vs. BWM. FEC were log transformed $\ln(\text{FEC}+25)$ for the analyses, but untransformed means are reported in the results. Statistical inferences were made on transformed data and untransformed least squares means were presented. PDIFF was used to determine differences among treatments if $P < 0.05$. Although the study was conducted over a 30-day period, data collected after day 21 were removed. At this point, 7 of the 12 Hampshire sheep were removed from pasture and study because of severe anemia. With several missing samples, various non-estimable parameters were found at day 30.

CHAPTER IV. RESULTS

4.1. Larval Recovery

As noted previously, coprocultures were made approximately every three days to allow for recovery of L3s. Least squares means for percentage larval recovery are presented in Figure 4.1. The percentage larvae recovered from cultures was consistently lower in the feces of the Bioworma-fed animals throughout the study compared to control animals. Day ($P=0.004$) and treatment ($P<0.0001$) effects were significant, but there was no treatment by week interaction ($P=0.2161$). The least squares means of larvae recovered were 46.7% for the control group, 16.9% for the Bioworma-combined-with-concentrate (BWC) group, and 15.2% for the Bioworma-mixed-with-mineral (BWM) group, overall. Orthogonal contrasts showed an overall difference in control versus Bioworma-fed in concentrate animals ($P<0.01$), but there was no difference between the two methods of delivery ($P=0.6211$).

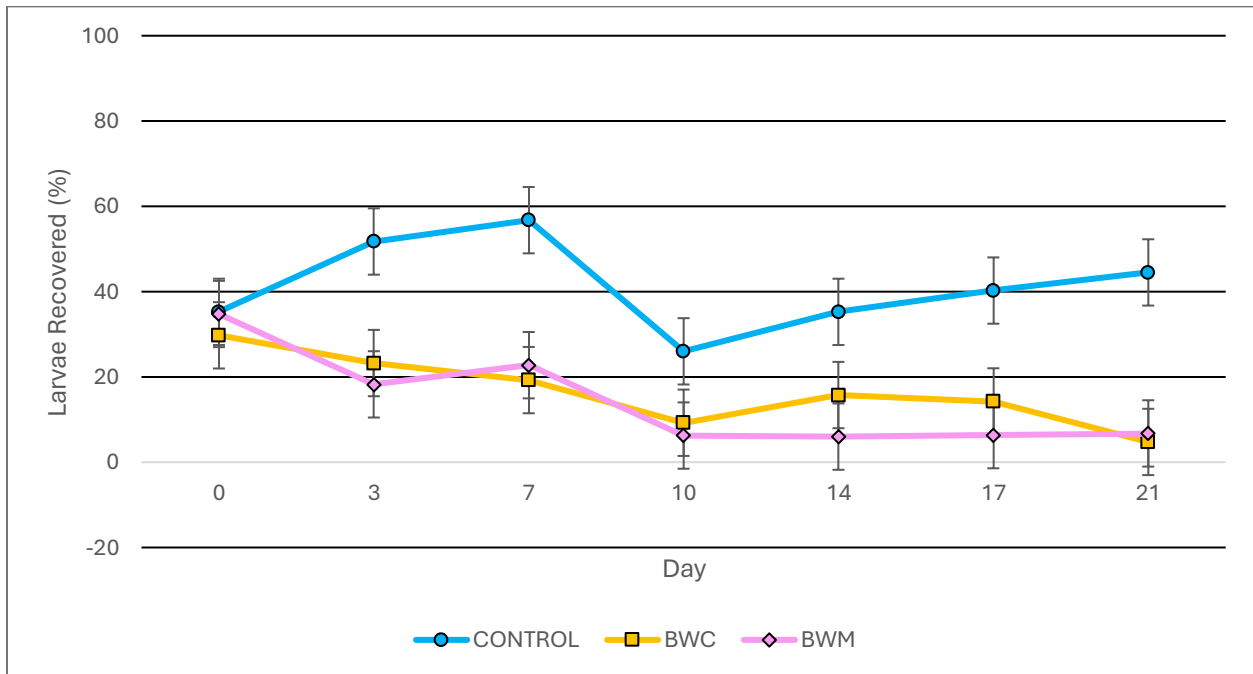


Figure 4.1. Least squares means for percent of third-stage larvae recovered from control, BWC, and BWM treatments ($n=4$ per treatment). Main effect of treatment ($P<0.0001$).

4.2. Fecal Egg Count

Fecal egg counts were assessed every seven days to monitor the parasite burden in animals. There was no effect of treatment on FEC with means of 3,852 epg for control animals, 2,744 epg for BWC animals, and 3,435 epg for BWM animals ($P=0.7501$ based on log-transformed values). A treatment by day interaction was observed over the 21-day period for mean fecal egg counts ($P<0.01$; Figure 4.2) along with an overall effect of day ($P<0.0001$) as fecal egg counts increased during the experimental period (Figure 4.3).

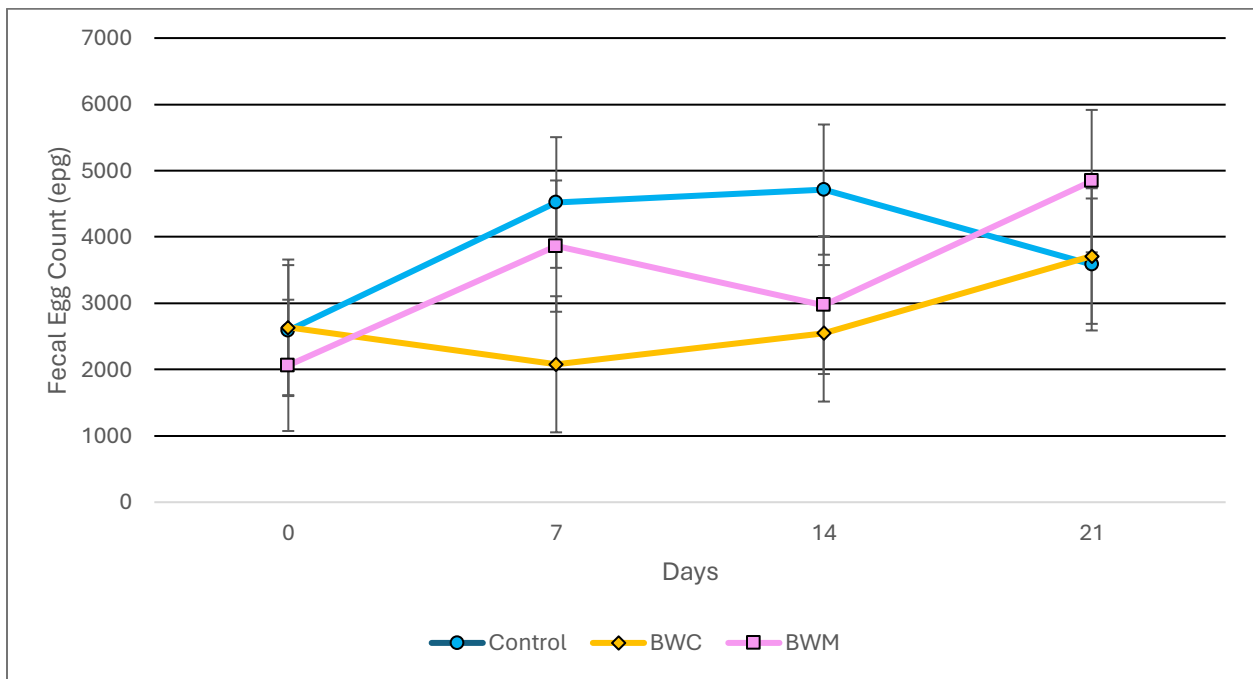


Figure 4.2. Least squares means for fecal egg counts for animals in control, BWC, and BWM treatments. Treatment by day interaction ($P<0.01$).

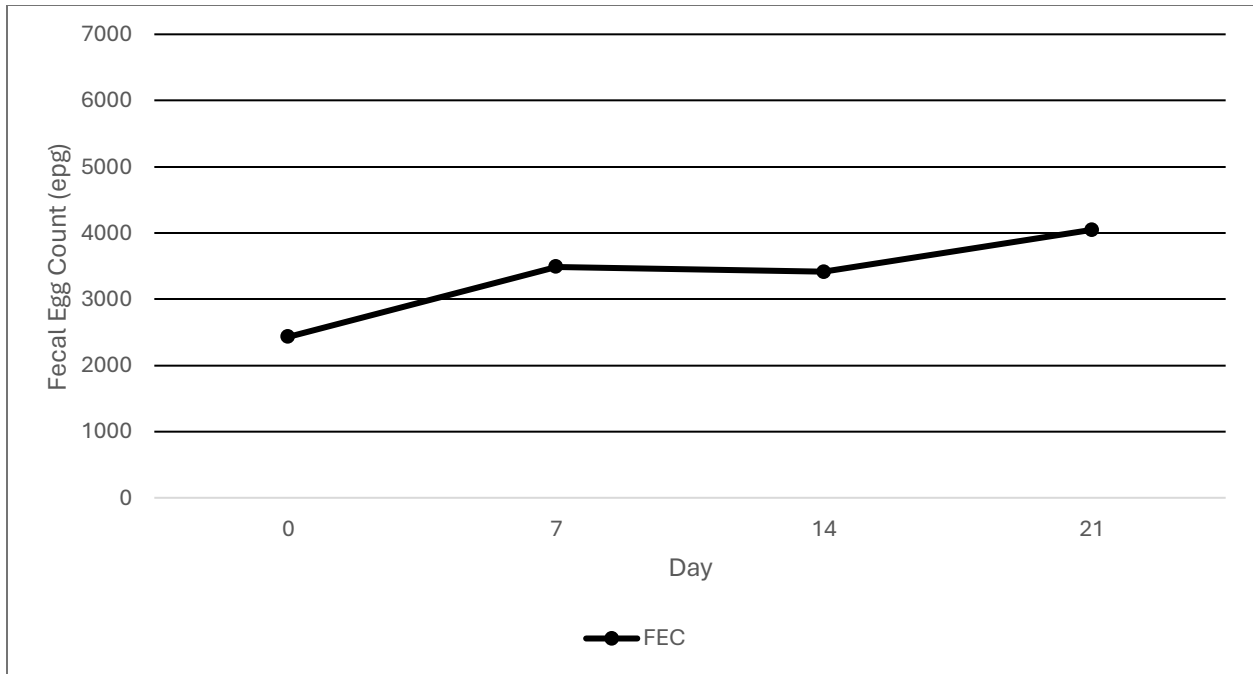


Figure 4.3. Fecal egg count over the 21-day period. Main effect of day ($P < 0.0001$).

4.3. Packed Cell Volume

Packed cell volume was measured every seven days to monitor for the development of anemia in the animals. There was no effect of treatment with overall least squares means of 23.3% for control, 23.9% for BWC, and 22.4% for BWM ($P = 0.4658$). A treatment by day interaction was observed over the 21-day period ($P = 0.0217$). There was also an overall main effect of day ($P < 0.0001$; Figure 4.3) with values declining as the study progressed.

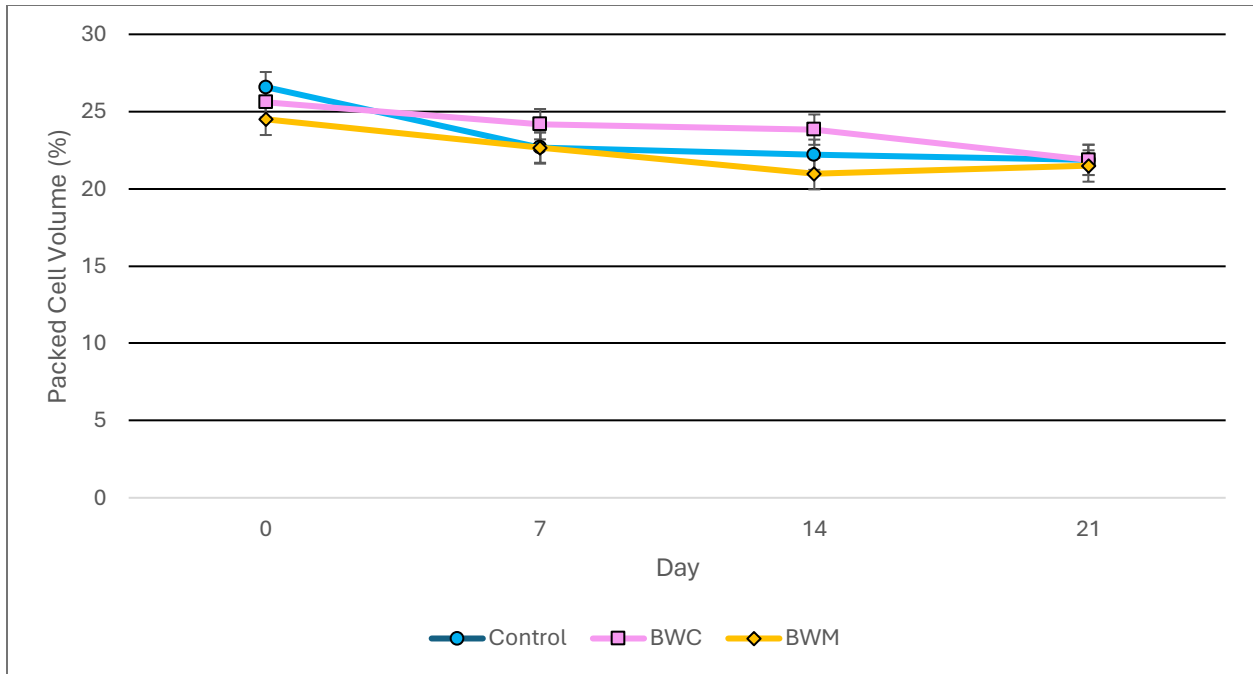


Figure 4.4. Least squares means for packed cell volume in control, BWC and BWM treatments. Treatment by day interaction (P=0.0217).

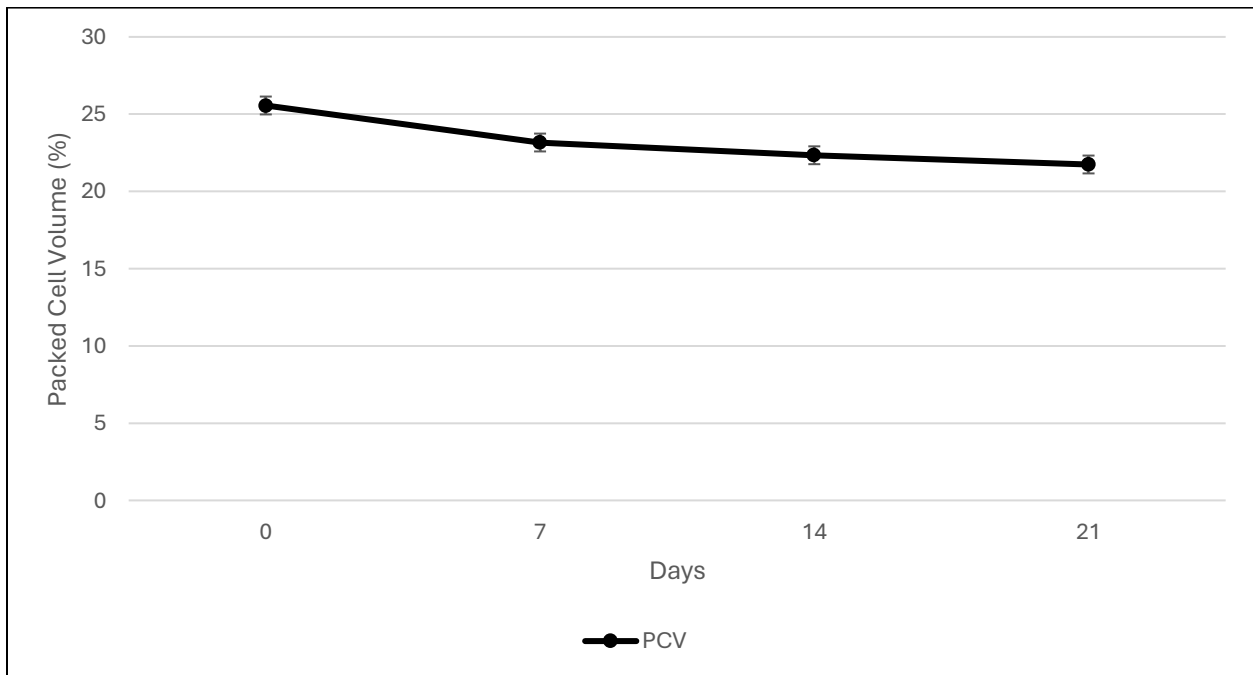


Figure 4.5. Overall least squares means for packed cell volume over the 21-day period of the study. Main effect of day (P<0.0001).

4.4. FAMACHA

FAMACHA was observed every seven days as another indicator of the development of anemia. The overall least squares means for control (3.1), BWC (3.4), and BWM (3.3) showed no significant difference between each treatment ($P=0.4531$). The treatment by week interaction was not significant (Figure 4.6; $P=0.4505$), but there was an effect of day for FAMACHA measured across each treatment ($P=0.0113$).

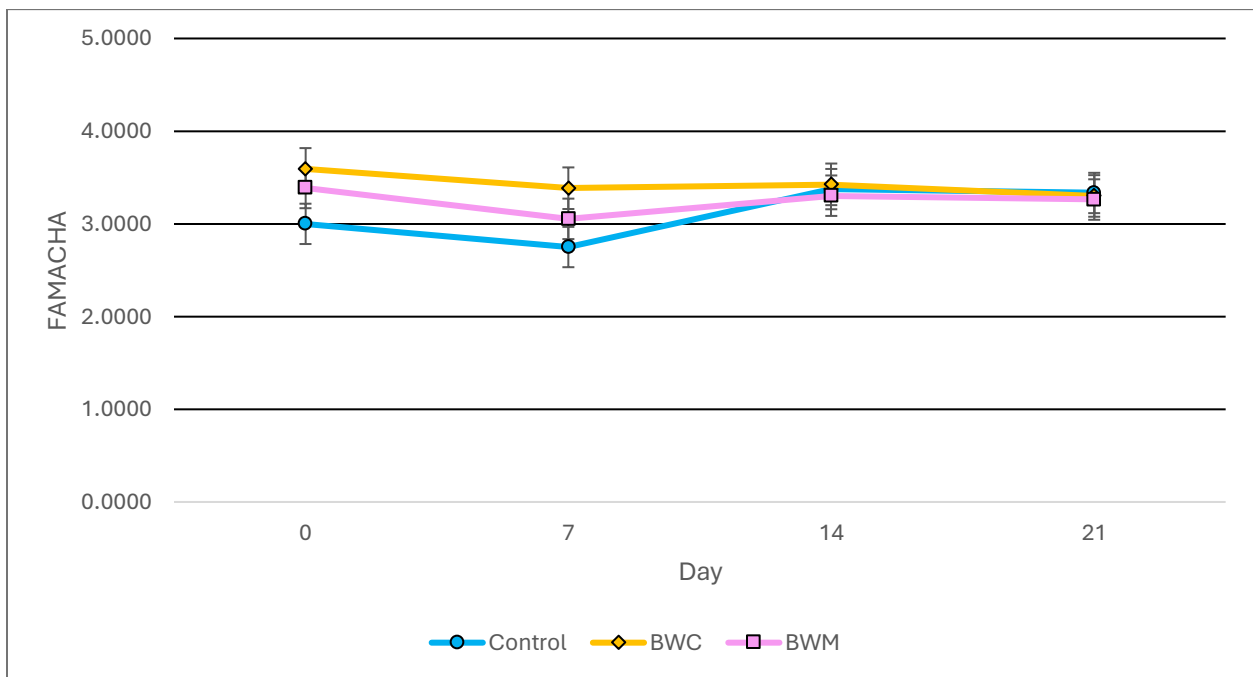


Figure 4.6. Least squares means for FAMACHA for control, BWC, and BWM. Treatment by day interaction ($P=0.4505$).

4.5. Body Weight

Body weight was measured to ensure adequate growth of animals throughout the study. Figure 4.7 shows the least squares means of body weight, and no treatment effect was observed ($P=0.8288$). There was a main effect of day ($P<0.0001$), as animals were gaining weight as the experiment progressed.

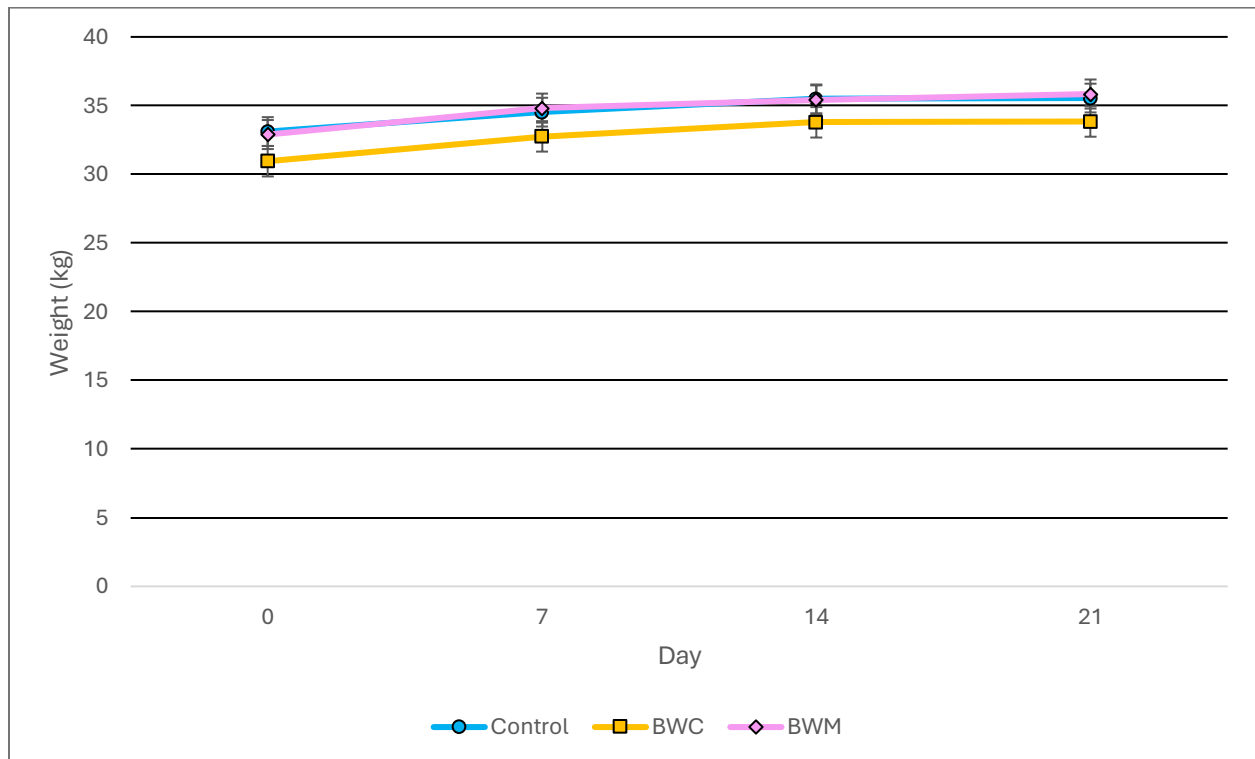


Figure 4.7. Least squares means for body weight (kg) for control, BWC, and BWM treatments. Main effect of day ($P<0.0001$).

4.6 Body Condition Score

Body condition score (BCS) was assigned for all animals, least squares means are presented in Figure 4.8. No treatment ($P=0.7298$) or treatment by day interaction was observed ($P=0.5715$). A main effect of day was observed ($P=0.0123$), with mean values of 2.6, 2.7, 2.6, and 2.6 for days 0, 7, 14, 21 respectively.

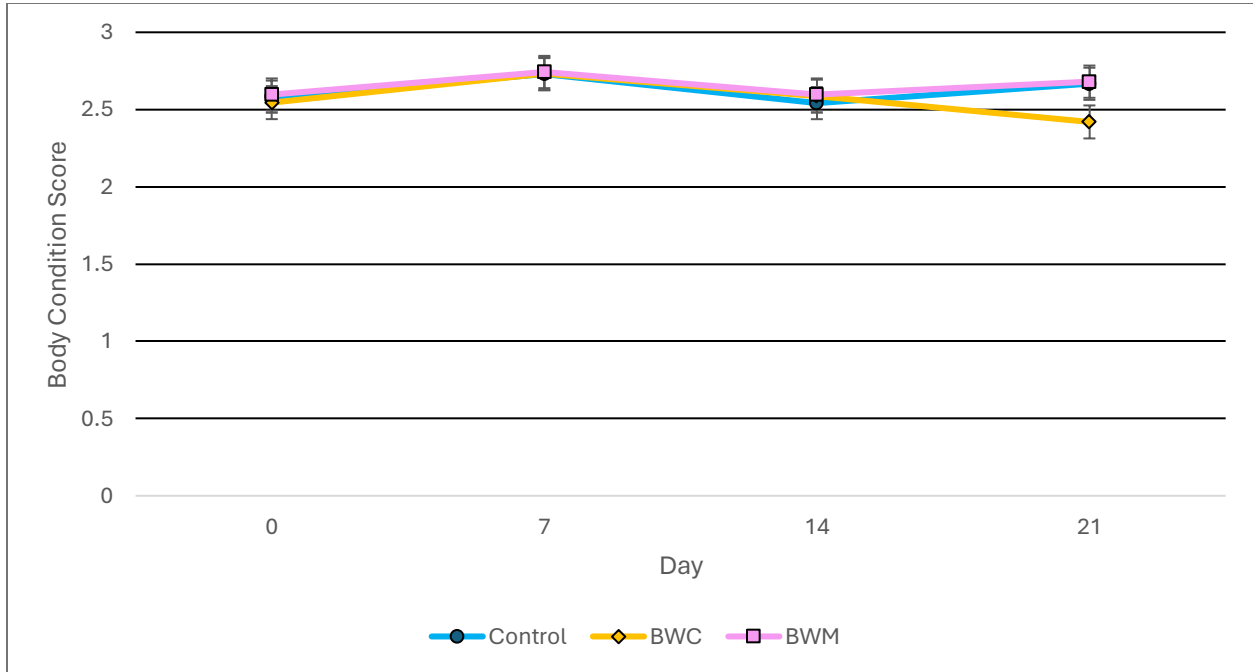


Figure 4.8. Least squares means for body condition scores for control, BWC, and BWM groups.

4.7. Breed Comparison

The Katahdin and Hampshire sheep breeds were compared using least squares means for each parameter. There was no difference amongst breeds for FEC and body condition scores (BCS) ($P>0.05$). Differences were observed between Katahdin and Hampshire breeds when PCV, FAMACHA and body weight were compared ($P<0.0001$). Least squares means for FEC, PCV, FAMACHA, body weight, and BCS are shown in Table 4.1.

Table 4.1. Least Squares Means for Katahdin and Hampshire Sheep

	<i>Katahdin</i>	<i>Hampshire</i>	<i>SEM</i>	<i>P Value</i>
FEC (epg)	2,969	3,719	903	0.8156
PCV (%)	25.7	20.7	0.89	<0.0001
FAMACHA	2.3	4.2	0.2	<0.0001
Body Weight (kg)	37.4	30.7	1.20	<0.0001
BCS	2.7	2.5	0.1	0.1091

SEM: standard error of the mean

A significant difference ($P < 0.05$) was observed for individual breed weight over time according to Figure 4.8. Katahdin sheep ($n=36$) had an overall larger weight when compared to Hampshire sheep ($n=12$) throughout the entirety of the study. The overall least squares means for Katahdin sheep was 37.2972 kg, while Hampshire sheep had an overall least squares means of 30.6980 kg ($P < 0.01$).

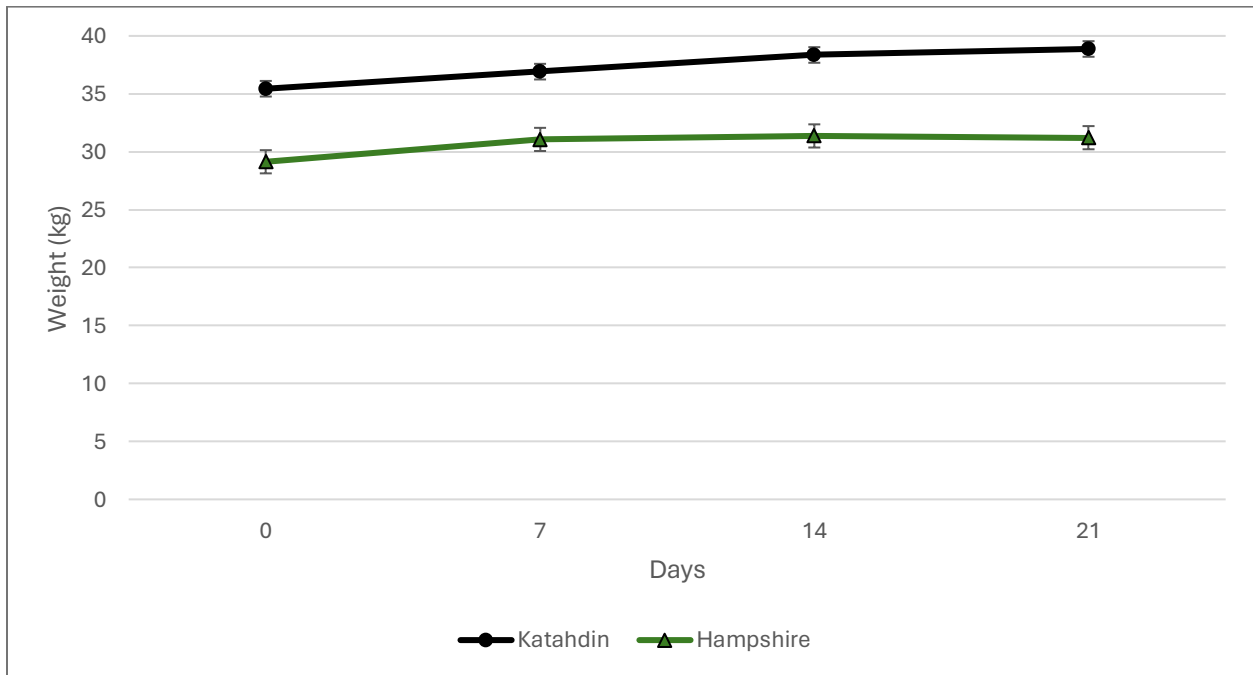


Figure 4.9. Least squares means for weight of Katahdin sheep and Hampshire sheep. Main effect of breed ($P < 0.0001$).

CHAPTER V. DISCUSSION AND CONCLUSION

Duddingtonia flagrans has shown great efficacy in trapping GIN of small ruminants (Healey et al., 2018). The commercial product Bioworma allows for a convenient method of administration of *D. flagrans* spores for producers. Research has shown that when Bioworma is fed mixed in a grain concentrate, GIN larvae are effectively reduced in the fecal pellets (Miller et al., 2021). No studies have been conducted assessing the efficacy of Bioworma when thoroughly combined with a commercial mineral mix. Combining Bioworma in a mineral mix would be beneficial for producers who do not feed their animals concentrates, but rather finish their animals solely on pasture. Allowing Bioworma to age in mineral for seven days before feeding mimics the producer experience when mineral is fed *ad libitum*.

The objective of the study was to assess the efficacy of Bioworma which had been thoroughly mixed in a mineral supplement and aged as such for at least seven days. Larval recovery was used to assess the ability of *D. flagrans* to trap GIN. The percent of L3s recovered from feces of sheep fed Bioworma mixed with concentrate (BWC) and Bioworma mixed with minerals (BWM) was significantly less than the percent recovery from feces of control animals. Treatments of BWC and BWM did not differ from each other and provides objective evidence for what Miller et al. (2021) stated when they wrote that Bioworma can be fed using different administration methods such as mixed with feedstuffs and minerals. The data support feeding Bioworma when mixed in a mineral supplement and that the fungal spores will retain their effectiveness after being in contact with the minerals for at least 7 days.

Parameters such as fecal egg count, packed cell volume, FAMACHA, body weight and body condition score were recorded every seven days to confirm the status of each animal's health. The least squares means for FEC, packed cell volume, FAMACHA, BCS, and body

weight did not differ between treatments. The lack of significant differences in fecal egg counts between treatments serves to confirm further that Bioworma has no known effectiveness against parasites in the animal (Zegbi et al., 2021). Use of the product is aimed at management of larvae on pasture. An increase in FEC overall was not unexpected in this study given the season and climate in which the study was conducted (main effect of day: $P < 0.0001$). As the parasite burden increased, a decrease in PCV and an increase in FAMACHA were noted and this is to be expected because of the large population of hematophagous *H. contortus*. The increase in body weight seen overall was also expected, since the lambs were growing and were expected to have been steadily gaining body weight. Body condition scoring is a technique used to assess the health and nutritional status of the animal. All scores were within the ideal range (2.5-4) for growing sheep.

Since two breeds of sheep were used within this study, conclusions can be drawn about the ability of Katahdin and Hampshire sheep to withstand GIN infection. Studies have shown that certain sheep breeds are better at handling parasitic burden than others. Katahdin are hair sheep and are known to exhibit the development of immunity at an earlier age than wool breeds, such as Hampshire sheep (Bielek, 2014). Multiple different breeds of hair sheep have been evaluated and Katahdin sheep have been found to have a higher PCV, along with a lower FEC, than other breeds such as Dorset or Dorper sheep (Vanimisetti et al., 2004). When Katahdin sheep were compared to St Croix and Dorper lambs, Katahdin sheep handled parasitic infection better than Dorper lambs, but not as well as St Croix, which have been shown to have great resistance to GIN (Burke and Miller, 2004). St Croix sheep, like Katahdins, are also hair sheep adapted to living in tropical and subtropical regions. Sheep breeds have become adapted to different climates, where wool sheep thrive in more temperate climates, and hair breeds do better

in hotter climates (McManus et al., 2020). The wool on sheep can trap heat, while the short thick hairs that are found densely compacted on hair sheep can allow for better dissipation of heat (McManus et al., 2020). Since this study was conducted in a hot, humid climate it is to be expected that the wool sheep, the Hampshires, would not tolerate the heat as well as the Kathadins apparently did and were more susceptible to the parasites on pasture. After 21 days, seven Hampshire sheep had to be treated with anthelmintics because they were anemic and had high egg counts. They were removed from pasture and placed on a dry lot to prevent further infection with parasites on pasture.

This study provides support for the efficacy of Bioworma when mixed with a mineral supplement and allowed to age for at least 7 days. This conclusion should be of practical use to producers who finish their sheep on pasture and do not provide supplements other than a mineral mix. The determination that aging the *D. flagrans* chlamydospores with the mineral for at least 7 and up to 14 days has no apparent deleterious effects on the fungal spores will provide producers with the convenient option to offer Bioworma to sheep on a weekly basis, or once every two weeks, rather than daily. Use of Bioworma along with other parasite management strategies such as pasture and grazing management and the selective use of COWP should result in lowered infectivity of pastures, which would be an especially useful outcome given the high prevalence of anthelmintic resistance.

APPENDIX. PILOT STUDY

A six-day pilot study was conducted to test the efficacy of the Bioworma® used in the study (lot number 3167101). Six Katahdin sheep were used. Three sheep were randomly assigned to a treatment group which consisted of feeding Bioworma in a grain/mineral mix according to the label instructions (2.835 grams per 45.359 kilograms of live body weight). Three sheep were assigned as controls. They were fed a grain/mineral mix with no additions. Fecal samples were collected on days 3, 4, 5, and 6. The feces were pooled by treatment, fecal egg counts performed in duplicate, and ten-gram coprocultures made for the Bioworma and control groups. Larvae were recovered and counted for each coproculture. All methods were performed as described in the materials and methods chapter of this thesis (Chapter III).

The results for the larval recovery are given in Table A.1.

Table A.1. Pilot study: Percent larval recovery from coprocultures of Bioworma-fed and control sheep by date

	6/22/2023	6/23/2023	6/24/2023	6/25/2023
Control	42.1%	42.3%	8.1%	33.6%
Bioworma	2.2%	4.2%	5.7%	6.2%

Except for 6/24/2023, the percent larval recovery from the controls was at least five times greater than the recovery from the Bioworma-fed animals. These results provided sufficient evidence for the efficacy of the *D. flagrans* in the batch of Bioworma to be used on study.

REFERENCES

- Abongwa, M., R. J. Martin, and A. P. Robertson. 2017. A brief review on the mode of action of antinematodal drugs. *Acta Vet. (Beogr.)* 67:137–152. doi:10.1515/ACVE-2017-0013.
- Arsenopoulos, K. V., G. C. Fthenakis, E. I. Katsarou, and E. Papadopoulos. 2021. Haemonchosis: A challenging parasitic infection of sheep and goats. *Animals* 11:1–29. doi:10.3390/ANI11020363.
- Bielek, K. 2014. Blueprint for selecting parasite resistant sheep: a shepherd’s perspective. American Consortium for Small Ruminant Parasite Control. <https://www.wormx.info/blueprint> (accessed 3.12.24).
- Binarová, P. and J. Tuszynski. 2019. Tubulin: Structure, functions and roles in disease. *Cells* 8:1294. doi:10.3390/cells8101294.
- Blair, J., and A. Biddle. 2020. Stimulating *Duddingtonia flagrans* chlamydospore production through dehydration. *Parasitol. Res.* 119:123–128. doi:10.1007/S00436-019-06499-0.
- Burke, J. M., J. E. Miller, D. D. Olcott, B. M. Olcott, and T. H. Terrill. 2004. Effect of copper oxide wire particles dosage and feed supplement level on *Haemonchus contortus* infection in lambs. *Vet. Parasitol.* 123:235–243. doi:10.1016/j.vetpar.2004.06.009.
- Burke, J. and J. Miller. 2004. Resistance to gastrointestinal parasites in Dorper, Katahdin and St. Croix lambs in the southeastern region of the United States. *Small Rumin. Res.* 54:43–51.
- Burke, J. and J. Miller. 2021. Worm-trapping fungus. American Consortium for Small Ruminant Parasite Control. https://www.wormx.info/files/ugd/6ef604_dd01da101d8b42f0bfd0d807912be378.pdf (accessed 3.12.24).
- Burke, J.M., Miller, J.E., Mosjidis, J.A., Terrill, T.H. 2012. Grazing *Sericea lespedeza* for control of gastrointestinal nematodes in lambs. *Veterinary Parasitology.* 186:507-512.
- Burke, J. M., J. E. Miller, and T. H. Terrill. 2013. Copper oxide wire particles to control *Haemonchus contortus* in sheep and goats. American Consortium for Small Ruminant Parasite Control. <https://60f7303d-ac52-4cac-b7fb->

6050f500b0b6.filesusr.com/ugd/aded98_b8d159de1b3b43b783bf0305c8c30213.pdf
(accessed 3.12.24).

- Céspedes-Gutiérrez, E., D. M. Aragón, M. I. Gómez-Álvarez, J. A. Cubides-Cárdenas, and D. F. Cortés-Rojas. 2021. Survival of the nematophagous fungus *Duddingtonia flagrans* to in vitro segments of sheep gastrointestinal tract. *Exp. Parasitol.* 231:108172. doi:10.1016/j.exppara.2021.108172.
- Delano, M. L., S. A. Mischler, and W. J. Underwood. 2002. Biology and Diseases of Ruminants: Sheep, Goats, and Cattle. *Laboratory Animal Medicine.* 519–614. doi:10.1016/B978-012263951-7/50017-X.
- Fissiha, W. and M. Z. Kinde. 2021. Anthelmintic resistance and its mechanism: A review. *Infect. Drug Resist.* 14:5403. doi:10.2147/IDR.S332378.
- Flay, K. J., F. I. Hill, and D. H. Muguero. 2022. A review: *Haemonchus contortus* infection in pasture-based sheep production systems, with a focus on the pathogenesis of anaemia and changes in haematological parameters. *Animals.* 12:1238. doi:10.3390/ANI12101238.
- Greer, A. W., J. A. Van Wyk, J. C. Hamie, C. Byaruhanga, and F. Kenyon. 2020. Refugia-based strategies for parasite control in livestock. *Vet. Clin. North Am. Food Anim. Pract.* 36:31–43. doi:10.1016/J.CVFA.2019.11.003. Available from: <https://pubmed.ncbi.nlm.nih.gov/32029187/>
- Healey, K., C. Lawlor, M. R. Knox, M. Chambers, J. Lamb, and P. Groves. 2018. Field evaluation of *Duddingtonia flagrans* IAH 1297 for the reduction of worm burden in grazing animals: Pasture larval studies in horses, cattle and goats. *Vet. Parasitol.* 258:124–132. doi:10.1016/j.vetpar.2018.06.017.
- Hodgkinson, J. E., R. M. Kaplan, F. Kenyon, E. R. Morgan, A. W. Park, S. Paterson, S. A. Babayan, N. J. Beesley, C. Britton, U. Chaudhry, S. R. Doyle, V. O. Ezenwa, A. Fenton, S. B. Howell, R. Laing, B. K. Mable, L. Matthews, J. McIntyre, C. E. Milne, T. A. Morrison, J. C. Prentice, N. D. Sargison, D. J. L. Williams, A. J. Wolstenholme, and E. Devaney. 2019. Refugia and anthelmintic resistance: Concepts and challenges. *Int. J. Parasitol. Drugs Drug Resist.* 10:51–57. doi:10.1016/J.IJPDDR.2019.05.001.
- Holden-Dye, L. and R. J. Walker. 2014. Anthelmintic drugs and nematicides: studies in *Caenorhabditis elegans*. *WormBook.* 1–29. doi:10.1895/wormbook.1.143.2.

- Howell, S. B., J. M. Burke, J. E. Miller, T. H. Terrill, E. Valencia, M. J. Williams, L. H. Williamson, A. M. Zajac, and R. M. Kaplan. 2008. Prevalence of anthelmintic resistance on sheep and goat farms in the southeastern United States. *J. Am. Vet. Med. Assoc.* 233:1913–1919. doi:10.2460/javma.233.12.1913.
- Huang, Q., X. Liu, G. Zhao, T. Hu, and Y. Wang. 2018. Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. *Animal Nutr.* 4:137–150. doi:10.1016/j.aninu.2017.09.004.
- International Animal Health Products. 2022. Bioworma. https://www.bioworma.com/___media_downloads/199381/BioWorma-USA-10lbs-20lbs-pails.pdf (accessed 3.12.24).
- Kaplan, R. 2013b. Biology of anthelmintic resistance: These ain't your father's parasites. American Consortium for Small Ruminant Parasite Control. https://www.wormx.info/files/ugd/6ef604_43a605eb522d4839859ce9b1cfedc599.pdf (accessed 3.12.24).
- Kumar, N., T. K. S. Rao, A. Varghese, and V. S. Rathor. 2013. Internal parasite management in grazing livestock. *J. Parasit. Dis.* 37:151–157. doi:10.1007/S12639-012-0215-Z.
- Lin, X. and J. Heitman. 2005. Chlamydospore formation during hyphal growth in *Cryptococcus neoformans*. *Eukaryot. Cell.* 4:1746. doi:10.1128/EC.4.10.1746-1754.2005.
- López, M., N. Carvalho, J. Henrique Das Neves, C. Santos Pennacchi, and A. F. Talamini Do Amarante. 2023. Hypobiosis and development of *Haemonchus contortus* and *Trichostrongylus colubriformis* infection in lambs under different levels of nutrition. *Ruminants.* 3:401–412. doi:10.3390/ruminants3040033.
- Martin, R. J. and A. P. Robertson. 2007. Mode of action of levamisole and pyrantel, anthelmintic resistance, E153 and Q57. *Parasitology.* 134:1093–1104. doi:10.1017/S0031182007000029.
- McManus, C. M., D. A. Faria, C. M. Lucci, H. Louvandini, S. A. Pereira, and S. R. Paiva. 2020. Heat stress effects on sheep: Are hair sheep more heat resistant? *Theriogenology.* 155:157–167. doi:10.1016/j.theriogenology.2020.05.047.

- Miller, J. E., J. M. Burke, and T. H. Terrill. 2021. BioWorma as an aid for controlling ruminant nematode parasites. *J. Anim. Sci.* 99:36–36. doi:10.1093/jas/skab096.065.
- Min, B. R., Hart, S. P., Miller, D., Tomita, G. M., Loetz, E., Sahl, T., 2005. The effect of grazing forage containing condensed tannins on gastro-intestinal parasite infection and milk composition in Angora does. *Vet Parasitol.* 130 (1-2):105-13. doi:10.1016/j.vetpar.2005.03.011.
- Mosjidis, J. A., and T. H. Terrill. 2013. *Sericea lespedeza*. American Consortium for Small Ruminant Parasite Control. https://60f7303d-ac52-4cac-b7fb-6050f500b0b6.filesusr.com/ugd/aded98_5c8066841cb64d86a990ed8d90d88e54.pdf (accessed 3.12.24).
- National Agricultural Statistics Board. 2024. Sheep and Goats. <https://downloads.usda.library.cornell.edu/usda-esmis/files/000000018/b8517891v/zw130s885/shep0124.pdf> (accessed 3.12.24).
- Nugraha, R. Y. B., G. Jeelani, and T. Nozaki. 2022. Physiological roles and metabolism of γ -aminobutyric acid (GABA) in parasitic protozoa. *Trends Parasitol.* 38:462–477. doi:10.1016/J.PT.2022.02.004.
- Prichard, R. K. 1970. Mode of action of the anthelmintic thiabendazole in *Haemonchus contortus*. *Nature.* 228:684–685. doi:10.1038/228684a0.
- Pugh, D. G., and A. N. Baird. (Eds.) 2012. *Sheep and Goat Medicine*. 2nd ed. Saunders, Maryland Heights, pp. 106-126.
- Purves, D., G. J. Augustine, D. Fitzpatrick, L. C. Katz, A.-S. LaMantia, J. O. McNamara, and S. M. Williams. 2001. Acetylcholine. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK11143/>
- Sangster, N. C., and H. Bjorn. 1995. Levamisole resistance in *Haemonchus contortus* selected at different stages of infection. *Int. J. Parasitol.* 25:343–348. doi:10.1016/0020-7519(94)E0068-X.
- Schoenian, S. 2012. Parasite Control in Dairy Sheep. American Consortium for Small Ruminant Parasite Control. [https://60f7303d-ac52-4cac-b7fb-](https://60f7303d-ac52-4cac-b7fb-6050f500b0b6.filesusr.com/ugd/aded98_5c8066841cb64d86a990ed8d90d88e54.pdf)

6050f500b0b6.filesusr.com/ugd/aded98_7335a3a25ec6427c8554413ac3785e8a.pdf
(accessed 3.12.24).

Shalaby, H. A. 2013. Anthelmintics resistance; How to overcome it? Iran J. Parasitol. 8:18–32.

Steinberg, G. 2007. Hyphal growth: a tale of motors, lipids, and the Spitzenkörper. Eukaryot. Cell. 6:351. doi:10.1128/EC.00381-06.

Sutherland, I. A., J. Shaw, and R. J. Shaw. 2010. The production costs of anthelmintic resistance in sheep managed within a monthly preventive drench program. Vet. Parasitol. 171:300–304. doi:10.1016/j.vetpar.2010.03.035.

VanHoy, G. 2023. Common Gastrointestinal Parasites of Small Ruminants . Merck Veterinary Manual. <https://www.merckvetmanual.com/digestive-system/gastrointestinal-parasites-of-ruminants/common-gastrointestinal-parasites-of-small-ruminants> (accessed 3.12.24).

Vanimiseti, H. B., S. P. Greiner, A. M. Zajac, and D. R. Notter. 2004. Performance of hair sheep composite breeds: resistance of lambs to *Haemonchus contortus*. J. Anim. Sci. 82:595–604. doi:10.2527/2004.822595X.

Vatta, A. 2013. The FAMACHA system-an aid in the Management of Haemonchosis in Small Ruminants. American Consortium for Small Ruminant Parasite Control. https://60f7303d-ac52-4cac-b7fb-6050f500b0b6.filesusr.com/ugd/aded98_be8f418e96684b588c2358b1bafb779c.pdf (accessed 4.1.24)

Veerasingh, R., A. Roy, R. Karunakaran, and H. Rajak. 2021. Structure–activity relationship analysis of benzimidazoles as emerging anti-inflammatory agents: An overview. Pharmaceuticals. 14:663 doi:10.3390/PH14070663.

Vercruyse, J., and E. Claerebout. 2022a. Benzimidazoles. Merck Veterinary Manual. <https://www.merckvetmanual.com/pharmacology/anthelmintics/benzimidazoles> (accessed 3.12.24).

Vercruyse, J., and E. Claerebout. 2022b. Imidazothiazoles. Merck Veterinary Manual. <https://www.merckvetmanual.com/pharmacology/anthelmintics/imidazothiazoles> (accessed 3.12.24).

- Vercruyse, J., and E. Claerebout. 2022c. Macrocyclic Lactones. Merck Veterinary Manual. <https://www.merckvetmanual.com/pharmacology/anthelmintics/macrocyclic-lactones> (accessed 3.12.24).
- Williamson, S. M., A. P. Robertson, L. Brown, T. Williams, D. J. Woods, R. J. Martin, D. B. Sattelle, and A. J. Wolstenholme. 2009. The nicotinic acetylcholine receptors of the parasitic nematode *Ascaris suum*: Formation of two distinct drug targets by varying the relative expression levels of two subunits. PLoS Pathog. 5:1000517. doi:10.1371/journal.ppat.1000517.
- Wolstenholme, A. J. 2012. Glutamate-gated chloride channels. J. Biol. Chem. 287:40232. doi:10.1074/JBC.R112.406280.
- Zajac, A. 2013. Biology of parasites. American Consortium for Small Ruminant Parasite Control. https://60f7303d-ac52-4cac-b7fb-6050f500b0b6.filesusr.com/ugd/aded98_32f9541f3a214e0aac905c3362e45f21.pdf (accessed 3.12.24).
- Zegbi, S., F. Sagües, C. Saumell, I. Guerrero, L. Iglesias, and S. Fernández. 2021. *In vitro* efficacy of different concentrations of *Duddingtonia flagrans* on varying egg densities of gastrointestinal nematodes of cattle. Exp. Parasitol. 230:108156. doi:10.1016/j.exppara.2021.108156.

VITA

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