

EVALUATION OF FEEDING CHLAMYDOSPORES OF *DUDDINGTONIA*
FLAGRANS TO EWE/LAMB PAIRS AND WEANED LAMBS TO
BIOLOGICALLY CONTROL LEVELS OF *HAEMONCHUS CONTORTUS* ON
PASTURE

A Thesis

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ABSTRACT

Gastroenteritis caused by the nematode parasite *Haemonchus contortus* is a serious concern for small ruminants in the tropics and subtropics. Control is traditionally by anthelmintics and pasture management, however this specific nematode parasite has become resistant to many anthelmintics and research is now focused on novel control methods including biological control using the nematode-trapping fungus, *Duddingtonia flagrans*. The objective of this study was to investigate the effectiveness of feeding chlamydospores of the fungus at a dosage of 5×10^5 spores/kg of BW to ewe/lamb pairs (Trial 1) and weaned lambs (Trial 2) for a total of 17 and 8 weeks, respectively.

For Trial 1, 34 pregnant ewes were randomly allocated into Treated and Control groups of 17 each and each group grazed on separate pastures for 21 weeks. For Trial 2, 30 lambs from Trial 1 were randomly allocated into Treated and Control groups of 15 each and each group grazed the same pastures as in Trial 1. In both Trials, results from fecal cultures, pasture larval recovery and tracer animals showed that the Treated group pasture had lower infectivity. This reduced pasture infectivity did not translate into reduced infection levels in the ewe/lamb pairs in Trial 1, but infection levels were reduced in Trial 2. There was no effect on weight gain in either Trial. It can be concluded from this study that *Duddingtonia flagrans* can be used as a biological control agent to reduce pasture infectivity, however, infection levels and productivity may not be affected in sheep during one grazing season.

CHAPTER 1 INTRODUCTION

Gastroenteritis caused by parasitism is an important cause of production losses in small ruminants (Eysker and Ploeger, 2000). The main parasite that affects small ruminants in the tropic and subtropical regions is the bloodworm *Haemonchus contortus* that causes the disease haemonchosis (Preston and Allonby, 1979; Waruiru et al., 1993). Research is now focused on the use of non-chemical methods to control this parasite because of the problems with the use of traditional chemotherapeutic methods (Peña et al., 2002). The overuse and/or inappropriate use of anthelmintics have resulted in the development of resistance (Howell, 1999). In addition, there has been an increased public concern about chemical residues in animal products and in the environment (Grønvold et al., 1993a). One of the non-chemical methods under investigation is biological control (BC) by nematode trapping fungi (Waller, 1992).

The nematode trapping fungi showing the most promise as a biological agent over the last ten years is the fungus *Duddingtonia flagrans* (Larsen, 2000). This fungus belongs to a heterogeneous group of microfungi that are unique because they can trap nematodes and use them as a main or supplementary source of food (Waller et al., 1996). *Duddingtonia flagrans* is a predacious nematode fungus that produces adhesive three-dimensional hyphal networks during development, which trap the larval stages of nematodes (Cooke, 1969). This fungus has shown its ability to survive passage through the digestive system of ruminants as a resistant spore (chlamydospore) form (Manueli et al., 1999) and has also demonstrated its potential to reduce the transmission of infective larvae from feces to herbage (Fernandez et al., 1999a).

There is an indication that within the near future, BC by means of predacious fungi will be incorporated into parasite control strategies (Larsen, 1999). The objective of a control strategy utilizing nematode-trapping fungi as a BC agent is to reduce the larval population on herbage and thus reduce the worm burden in the animals (Larsen et al., 1997). The objective of this research is to investigate the effectiveness of feeding *D. flagrans* chlamydospores to grazing ewe/lamb pairs and weaned lambs to reduce pasture infective larvae levels.

CHAPTER 2 LITERATURE REVIEW

2.1. Epizootiologic Cycle

In many parts of the world infections with the blood-feeding nematode *H. contortus* are a major constraint on sheep and goat health and production (Schallig, 2000). It is commonly referred to as the barber-pole worm because the white ovaries wind spirally around the red intestines, giving the females a barber-pole appearance (Levine, 1980). The parasite can be found in both mature and growing animals. However, growing lambs suffer more severely than mature sheep (Stear et al., 1999). Adult worms feed on blood and can cause severe anemia, associated edema and diarrhea resulting in poor growth rate and weight loss and heavy infections can result in death (Schallig, 2000; Eysker and Ploeger, 2000). *Haemonchus* species live in the abomasum of ruminants and their life cycle is direct.

The life cycle of *Haemonchus* involves four phases – contamination, free-living, infective and parasitic. The contamination phase begins with eggs that are passed out in the feces of the animal. The eggs hatch and develop through three larval stages and these stages constitute the free-living phase. These free-living stages are affected by general climate and weather conditions, as well as the microclimate of their habitats (Rees, 1950). Under favorable conditions, the L₃ may show remarkable longevity (Gordon, 1948).

The first-stage (L₁) and second-stage (L₂) larvae feed on bacteria and other organic material within the feces. These two stages are not protected and are vulnerable to adverse microclimatic conditions within the fecal pat (Levine, 1963). The third-stage (L₃) larvae retain the L₂ cuticle and this ensheathed larval form is relatively resistant to

adverse microclimate conditions in the fecal pat and climatic conditions after leaving the pat onto the pasture herbage. Climate conditions that influence the level of L₃ on pasture are relative humidity, illumination, and temperature (Krecek et al., 1992). Under ideal conditions development from the egg to L₃ can happen in as little as a week. The L₃ cannot feed and migrate out of the fecal pat which requires a moisture medium such as rain or heavy dew, thus becoming the infective phase of the cycle ready to be ingested by a new host (Hsu and Levine, 1997).

The parasitic phase begins with the ingestion of the L₃. After ingestion, exsheathment of the L₃ occurs in the rumen and the larvae migrate to the abomasum where they penetrate into the mucosa. In the mucosa, another molt occurs to the fourth-stage (L₄) larvae. The L₄ emerges back into the lumen where they molt to the adult stage. Adults mate and eggs are passed out in the feces, thus completing the life cycle. The time period for parasitic development from ingestion of the L₃ to egg-laying adults is about two to four weeks and is called the pre-patent period.

2.2. Pasture Contamination

Grazing on infective pastures provide the connection between the free-living and parasitic stages of nematode parasites (Waller, 1997a). Therefore, if daily grazing on infective pastures is constant, intake of L₃ by the animal will be comparative to the concentration on the herbage (Barger, 1999). Composition of pasture herbage species will also contribute to the development and survival of the free-living phase and will be an indicator of the intensification of parasitism, which in turn, will affect the establishment of the parasitic phase (Waller, 1997a). Based on visual assessment, Beiser and Dunsmore

(1993) reported that the best indicators of the suitability of conditions for the development of *H. contortus* were the proportion of green pasture herbage present and the availability of moisture in the pasture.

Survival and availability of free-living and infective stages on pasture is also dependent on existing climatic conditions and geographical locations. Banks et al. (1990) observed that in a tropical environment, *H. contortus* developed on pastures throughout the year in wet zones but development was more sporadic in the dry zone. In temperate zones, infection level has been reported to be seasonal with increased infection level in the spring and autumn months (Thomas and Boag, 1972; Boag and Thomas, 1979). Sources of infection level in the spring are attributed to the ability *H. contortus* L₃ to survive the harsh conditions of winter (Rose, 1963), and in the fall from ewe spring rise and lamb egg output (Thomas and Boag, 1972).

The nematode burden in grazing animals is determined by the infection rate, which is related to the number of L₃ ingested with herbage each day (Barger, 1999). Therefore, there is a direct relationship between pasture infectivity and infection level. Strategic control methods must incorporate the role of pasture infectivity to effectively control nematode burden in grazing animals.

2.3. Control

Traditionally, control of nematode infection has been the use of anthelmintics and pasture management (Newland et al., 2001). Anthelmintics have been used intensively in the form of drenches, injections and feed additives. This reliance on anthelmintics has led to the development of nematode parasites that are resistant to the mechanism of action of

anthelminitics (Howell et al., 1999) and as such is a growing concern in animal husbandry worldwide (Sutherland et al., 2000). Prichard et al. (1980) defined the presence of resistance when there is a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal population of the same species and that resistance is heritable. The escalation of the development of resistant nematodes in small ruminants has thus provided the need for research into novel (non-chemical) control methods such as host genetic resistance, improved grazing management schemes, vaccines and BC (Waller, 1997a).

The novel control method showing the most promise to date is BC. Biological control has been used in the management of arthropods pests and there are indications that this will also be useful for animal nematode control (Waller et al., 1996). Biological control of nematodes is no longer a novelty and is fast becoming an area of applied research with the possibility of becoming an important integrated element in developing new sustainable control strategies (Larsen, 1999). Biological control is directed at the free-living stages and will not have an effect on the parasitic phase within the host (Waller, 1997b), therefore, the objective of BC is to reduce the availability of L3 on pasture and thus reduce parasite establishment (Fernandez et al., 1997).

Complete comprehension of the epidemiology of the parasite is required to enforce appropriate control strategies. This would provide a better insight into the natural process controlling parasite populations and lead to more effective control methods (Donald, 1968). Biological control agents under consideration are diatomaceous earth, earthworms, dung beetles and nematode trapping fungi, mainly, *D. flagrans*.

Diatomaceous earth (DE) is a powdery natural material formed almost entirely from the skeletons of diatoms. It is mined and quarried for industrial use and for insect pest control because the broken cell walls of silica tear the insect gut. The principle behind the mode of action of DE as a pest control is logical however; there is little or no data available from a refereed source on using DE as a control measure for parasitic nematodes. A previous study by Villamil (2000) analyzed the efficacy of feeding DE at 5% of the daily ration and concluded that DE was not effective in controlling nematode parasites. However Nuti et al. (2000) concluded that feeding DE at 2.5% of the concentrate ration was equal to a marginally effective anthelmintic in controlling parasitic disease. These reports are conflicting and therefore the efficacy of DE is inconclusive.

Since earthworms are soil inhabitants they consume large volumes of soil and organic matter including animal feces and thus play an important role in the biology and epidemiology of several parasitic nematodes as intermediate hosts, reservoir hosts or as mechanical vectors (Ryšavý, 1969). During feeding, they inevitably consume nematode larvae present in the soil and feces (Grønvold et al., 1996a). Holter (1979) showed that earthworms play an important role in the structural decomposition and disappearance of cowpats in Denmark. However, for earthworms to be an effective BC method, they must be able to tolerate climatic conditions conducive to larval development and must be present in sufficient quantities at the site of fecal deposit.

Evidence suggested that dung beetle activity did reduce the number of L₃ found in feces and herbage (Waller et al., 1996). When dung beetles bury large amount of fecal

pat, their activity cause the fecal pats to become aerated and with unfavorable climatic conditions desiccation of the L₃ occurs (Bryan, 1973). Dung beetles lowered the chance of pasture infection because affected pats no longer served as an effective reservoir for larvae (Bryan and Kerr, 1989).

Climatic conditions optimum for the development of nematode larvae may not be ideal for dung beetle activity. In a study on the effects of dung beetles on the free-living stages of horse strongylids, English (1979) observed that beetle activity was reduced during periods of heavy rainfall. Tembley et al. (1997) have shown that rainfall and other conditions have the most important effect on the development and survival of eggs and free-living stages of nematodes. Therefore, if optimal climatic conditions for larval development reduces beetle activity, then fecal pats may not be aerated enough to become desiccated (Bryan, 1973) and hence will not be an effective method of control.

For a fungus to be used as an effective BC agent it must be able to produce ensnaring nets in a timely manner in the feces and it must be present in the feces where eggs hatch and develop into L₃ (Waller et al., 1994; Faedo et al., 1998). Therefore, one major requirement of the fungus is the ability to survive gut passage in ruminants, so that oral administration can be pursued as a practical means of deploying these organisms (Waller et al., 1994). This requirement was met by the cultivation of nematophagous fungi spores on barley grain, which were then able to withstand passage through the intestinal conditions and be isolated in the feces (Larsen et al., 1992).

Hay et al., (1997) showed that a variety of nematophagous fungi are present in a significant proportion of dung samples at 3 days after deposition which indicated that

fungi in pasture soil could play an important role in reducing populations of gastrointestinal nematodes on pasture. However, Grønvold et al. (1993b) challenged this theory because it is unlikely that under natural conditions, fungi that enter the dung from the soil will be in sufficient numbers to effectively reduce the parasite larval population.

Duddingtonia flagrans has proven to be the most resilient and superior nematophagous fungi and this was attributed to the ability to produce large numbers of thick-walled spherical chlamydospores (Larsen et al., 1991). Experiments conducted by several researchers have demonstrated that *D. flagrans* can survive passage through the gastrointestinal tract and effectively trap larvae present in the feces (Faedo et al., 1998; Llerandi-Juarez and Mendoza de Gives, 1998; Mendoza de Gives et al., 1998). The rate of trap formation in *D. flagrans* has an optimum at 30°C, producing 700-800 traps/cm² /2 days, when induced by 20 nematodes/ cm² on agar (Grønvold, 1996b). The ability of viable spores to germinate, spread in feces and capture larvae before they migrate out is vital for BC (Faedo et al., 1997). Therefore, fungal spores that pass through the gastrointestinal tract germinate and trap free-living larval stages developing in the fecal pat and, thus, theoretically reduce pasture larval populations (Wolstrup et al., 1994).

The dosage of spores required to achieve adequate control depends on the livestock species. Sarkunas et al. (2000) concluded that dosing calves with 10⁶ *D. flagrans* chlamydospores/kg BW daily resulted in significantly reduced herbage infectivity and subsequently infection levels. Baudena et al., (2000) reported that a dosage of 2 x10⁶ spores/kg⁻¹ BW effectively reduced transmission of equine cyathostome L₃ from fecal pats to surrounding herbage year round. Peña et al. (2002) conducted dose-

titration and target dose trials and reported that dosages from 2.5×10^4 to 5×10^5 spores/kg⁻¹ BW fed daily effectively controlled larval stages of *H. contortus* in sheep feces.

CHAPTER 3 MATERIALS AND METHODS

3.1. Project Site and Experimental Animals

The location for this study was the Ben Hur Research Farm Sheep Unit, Louisiana State University Agriculture Experimental Station, Baton Rouge, Louisiana.

Experimental animals used were 34 pregnant two to four year old Gulf Coast Native (GCN) x Suffolk F₁ ewes, with an average weight of 50.76 kg, 34 Suffolk lambs (both during the neonatal period and as weaners) and 4 GCN lambs. There were two trials conducted where Trial 1 commenced on January 30, 2001 and ended on June 21, 2001 (20 weeks) and Trial 2 commenced on July 3, 2001 and continued through to November 30, 2001 (21 weeks).

3.2. Experimental Design: Trial 1

In the fall, 2000, a pasture of approximately 1.62 hectares that had not been grazed for at least a year was seeded with rye grass and divided into two equal parts. The ewes were randomly allocated into Treatment and Control groups of 17 each based on fecal egg count (FEC). Because the FECs were very low, approximately 5,000 *H. contortus* L₃ were administered orally to all animals in the second week of grazing. Each treatment group was assigned to a pasture where they remained for the duration of Trial I.

Animals were fed a pelleted sheep ration (500 g/animal) on a daily basis and *D. flagrans* spores were mixed with treated group's ration at the dosage of 500,000 spores/kg of body weight from weeks 5 to 20. Animals were weighed every two weeks to adjust fungal spore dosage. Analysis of blood to determine the pack cell volume (PCV) was conducted on a weekly basis and salvage anthelmintic treatment with albendazole

(Valbazen[®], 10mg/kg) and moxidectin (Cydectin[®], 0.2mg/kg) was administered to individual animals if the PCV was below 20 percent. Fecal samples were collected on a weekly basis to determine FEC. Every two weeks, feces from any ewe with a FEC above zero eggs per gram (EPG) were cultured to evaluate the effect of the fungus on the larval population. Because lamb fecal samples were too small to do individual cultures, on weeks 19 and 20, lamb feces were pooled and cultured in bulk. Alternating with fecal cultures, herbage samples were collected from each pasture at two-week intervals.

3.2.1. Fungus Administration

Duddingtonia flagrans spores were obtained from Christian Hansen Biosystems (Horsholm, Denmark). Spores were cultivated on millet seeds and came packaged at a concentration of 1.0×10^7 spores per gram of material. Animals in the Treated group received their daily dosage with feed that was given in feed bins that had adequate space to allow each animal free access to feed. In addition, animals were monitored to ensure complete consumption of the ration. Feeding of the fungal spores began when the FEC started to increase (week 5) and continued for the duration of the project.

3.3. Experimental Design: Trial 2

After weaning, the 34 lambs from Trial 1 were removed from pasture, dewormed with albendazole (Valbazen[®], 10mg/kg) and moxidectin (Cydectin[®], 0.2mg/kg) and kept in confinement under parasite free conditions for a 3 week stabilization period. Thirty lambs were then randomly allocated to Treatment and Control groups consisting of 15 animals each.

The Treatment and Control groups were then assigned to the same Treatment and Control pastures previously grazed by the ewes. Animals were housed at night to avoid attack by predators. Grazing was for a period of 18 weeks and the fungal spores were fed from week 2 to week 10. PCV, FEC, fecal culturing, pasture sampling and weighing were identical to Study I. Salvage anthelmintics treatment was also used for this study.

Twelve tracer lambs were used during three specific strategic periods to assess pasture infectivity. For each period, tracers were randomly assigned in sets of two, to each pasture and after a three week grazing period they were held in confinement under parasite-free conditions for an additional three weeks to allow infections to mature. The first group of tracers was the extra lambs from the resident flock of weaners, and they began grazing at the beginning of Trial 2 and were dewormed with albendazole (Valbazen[®], 10mg/kg) and levamisole (Tramisol[®], 8mg/kg). The second group of tracers was GCN wethers, which were dewormed with albendazole (Valbazen[®], 10mg/kg) and levamisole (Tramisol[®], 8mg/kg) and kept in confinement under parasite-free conditions for three weeks before being turned out to pasture at the end of the 8-week fungal feeding period. The final group of tracers was also selected from the resident flock dewormed with albendazole (Valbazen[®], 10mg/kg) and levamisole (Tramisol[®], 8mg/kg) and turned out to pasture three weeks prior to end of the 18-week grazing period. Tracers were necropsied to evaluate nematode burdens.

3.3.1. Fungus Administration

Dosage and administration were identical to Trial 1, however, the fungus was fed for only eight weeks.

3.4. Techniques

3.4.1. Pasture Sampling

Grass samples were taken to determine the level of pasture infectivity. The first sample was taken one week prior to the beginning of grazing for both studies. Samples were collected from each pasture and kept separate for analysis. Equipment used for sampling included a square hoop (400 cm²), grass shears and two large plastic collecting bags. Pasture sampling was conducted by walking a W-shaped route through each pasture. At approximately equal intervals along the route, 25 samples were collected. At each point, the hoop was thrown in the air and all the herbage within the hoop was cut to ground level and put in the bag.

In the lab, a small sample was then removed from each bag, weighed and placed in a drying oven for 7 days. The dried grass was then re-weighed to determine dry matter percent. The remaining grass was weighed and used for larval recovery.

The process of larval recovery involved placing the grass from each pasture into separate wash tubs. Tubs were labeled for proper identification. Grass was covered with water and a small amount of liquid detergent was added which aids in reducing the surface tension and allows larval release from the grass. Grass was soaked overnight and then rinsed in a smaller bucket and discarded. Contents of the smaller bucket were added to the larger bucket and grass debris was removed with a tea strainer. The contents were allowed to settle overnight.

After settling, the supernatant is poured off, without disturbing the sediment, and the contents transferred into a smaller bucket. The process of sedimentation and

removing the supernatant was repeated into smaller and smaller containers until reaching a volume 500 ml. From this 2-25 ml aliquots were removed, while being thoroughly mixed with a magnetic stir bar, and then transferred to 50-ml screw top centrifuge tubes. Five ml of formalin was added to preserve the contents. For larval counting, the sample was thoroughly mixed and 100 μ l was taken, placed on a microscope and the number of larvae was counted using a compound microscope.

3.4.2. Fecal Cultures

Individual animal fecal cultures for larval recovery were made if their FEC was greater than zero EPG. Cups were labeled carefully with individual animal data, sample date and culture date. Culture preparation included weighing 10g of feces into a 100 ml plastic cup and adding enough vermiculite and water to make the mixture crumbly but not wet or sticky. Cheesecloth was used to cover the cup, which was then inverted into a 200 ml plastic cup with about 10 ml of water. The inverted cup did not touch the water and was kept in a sealed plastic bag for 10 days. After 10 days, cultures were removed from the bag and the 200 ml cup was filled with warm water completely immersing the culture material. After 24 hours of immersion, the 100 ml cup was removed and the contents discarded. The supernatant in the 200 ml cup was removed by vacuum until approximately 14 ml of liquid remained. The sediment and liquid was then transferred to a 15-ml centrifuge tube and 1 ml of formalin was added to preserve the contents.

Supernatant from preserved samples was further vacuumed off to a volume of 1 ml and a 100 μ l subsample was removed for larval counting under a compound microscope. The results were reported as larvae per gram (LPG) of feces. LPG was

calculated by extrapolating the number of larvae counted in the subsample to the total volume. Percent larval recovery was computed by dividing (LPG /EPG) x 100. Percent reduction to determine the difference between control and fungus animals was then calculated by the following formula:

$$\text{Percent Reduction} = \frac{\text{Mean recovery of control} - \text{Mean recovery of treated}}{\text{Mean recovery of control}} \times 100$$

3.4.3. Necropsy of Animals

Nematode parasite burden is used in tracer animals to evaluate relative pasture infectivity level between groups. Animals were euthanized and then the gastrointestinal (GI) was removed. The protocol for processing the GI tract began with the opening of the entire length of the abomasum and emptying its contents into a wash tub of approximately 10 liters. The abomasums was thoroughly rinsed three times with one to two liters of water for each rinse. After each rinse, the water and contents were transferred to another tub. The volume was then brought to five liters. After thorough mixing, four 125 ml aliquots were taken and transferred to a 500 ml labeled plastic bottle. After the contents settled an hour, 100 ml was poured off and 100 ml of formalin was added for preservation of the contents. The abomasum was placed in a plastic dish with approximately one liter of warm water. After soaking overnight, the abomasum is then rinsed two times in one to two liters of water for each rinse. After each rinse, the rinse water was transferred to a 10 liter tub and the soaked contents were brought to a volume of five liters. The soaked contents were then processed the same as the abomasal wash.

The small intestine (SI) was stripped and opened along its entire length and the contents emptied into a washtub of approximately 10 liters. Water was then added to

about five liters and the SI was thoroughly washed before being discarded. The contents were then processed the same as the abomasal wash. Processing of the large intestine (LI) included obtaining a fecal sample from the rectum and then stripping the LI as close to the serosal surface as possible. The LI is then processed by the same procedure as the SI.

3.4.4. Classification and Enumeration of Nematodes

Sample bottles containing preserved contents of the GI tract were processed to ascertain species and sex of nematodes found in each group of tracer animals. A 100 ml sample was poured through a 200-mesh sieve and washed. The sieved contents were transferred to beaker and diluted with water. Small amounts were transferred to a petri dish for scanning with a dissecting microscope. Worms were counted and the first 100 were picked out and transferred to a glass slide in a drop of lactophenol. Slides were then labeled and scanned under a compound microscope to determine species and sex. The number of worms counted was extrapolated back to obtain the total number present in each organ.

3.4.5. Fecal Egg Count

Rectal fecal samples were collected on a weekly basis and began the week animals commenced grazing. Fecal egg count (FEC) was determined using the modified McMaster technique (Whitlock, 1948), where two grams of feces were mixed with 30 ml of saturated salt solution. A sample was pipetted out immediately and read in the McMaster chamber and results were reported as eggs per gram (EPG). Slides were read within 15 minutes to prevent errors that might be caused by egg disruption due to the hypertonic salt solution.

3.4.6. Packed Cell Volume

Blood samples were collected on a weekly basis commencing on the week before animals were placed on pasture. Blood was collected by jugular venipuncture into EDTA vacutainer tubes. Packed cell volume (PCV) was determined by microhematocrit centrifugation and PCV for each animal was obtained by reading the scale.

3.4.7. Weight Analysis

Weighing of animals in both studies was done at two-week intervals. Weight data was used to adjust the fungal spore dosage and to monitor weight gain differences between the groups.

3.5. Statistical Analysis

Differences between groups for FEC, PCV, weight, pasture larval recovery and culture percent reduction, were determined by using the SAS Systems Software[®] (1999-2001) (SAS Institute, Cary, NC) GLM Repeated Measure and student *t*-test procedures. Means were reported as least square (LS) means. FEC was transformed by $\log_{10}(n + 1)$ to normalize data. All differences were considered significant at $P < 0.05$.

CHAPTER 4 RESULTS

4.1. Trial 1

4.1.1. Pasture Larval Recovery

Larval recovery from both pastures was relatively even for the first five pasture samplings however, the Control increased after week 8 and remained higher throughout the grazing period (Fig. 1). Overall, there was a significant difference in larval recovery from Treated 18.36 ± 5.14 and Control 56.64 ± 16.78 pastures.

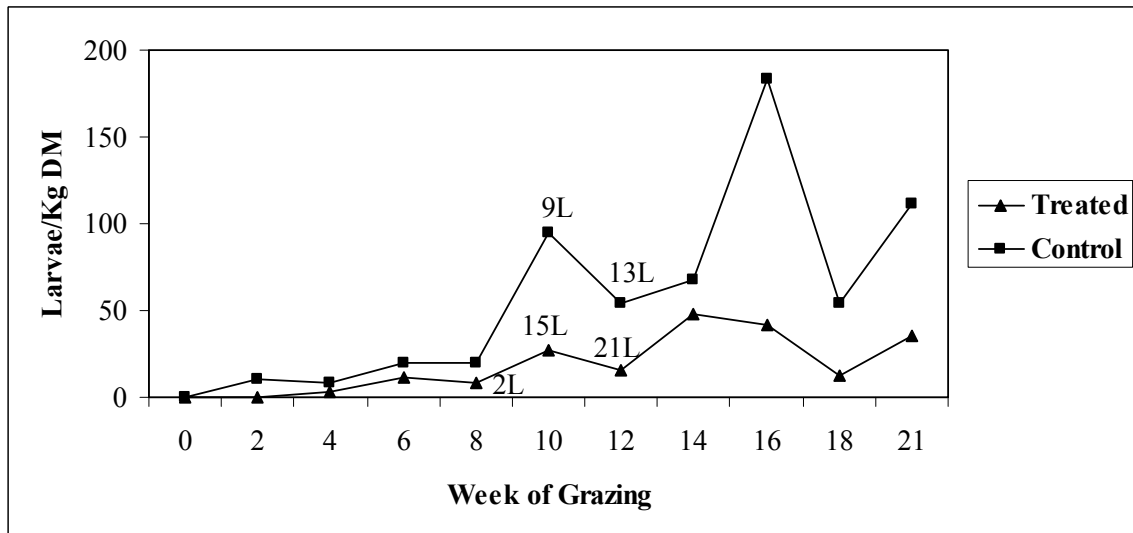


Fig. 1. Trial 1. Pasture larval recovery for ewe/lamb pairs comparing larvae/gm DM between Treated and Control pastures. L denotes the total number of lambs on each pasture.

4.1.2. Fecal Cultures

Fecal culture for ewes began on week 4 and for lambs on week 19 of grazing and continued for the duration of the project (Fig. 2). Percent recovery for the Treated group remained low throughout the study with the lowest recovery at week 13 with a mean of 0.5 and highest recovery at week 5 with a mean of 27.0. The Control culture remained

higher than the Treated during the study and had the highest recovery at week 1 with a mean of 37.3 and lowest at week 19 with a mean of 18.6. Overall, there was a significant difference between groups in mean percent recovery of larvae, Treated 6.47 ± 2.7 and Control 27.87 ± 2.05 , from fecal cultures. Percent recovery for bulk cultures from lambs was higher in the Control for both weeks (Table 1).

The highest percent reduction for ewe fecal cultures was seen at week 13 of 98% and lowest was in week 5 where no reduction occurred (Table 1). Reduction ranged from 0 to 98% with maximum reduction occurring during weeks 9-13 where percent reduction ranged from 90.5%-98.0%. The highest percent reduction for the lamb culture was at week 21 when the reduction was 100%.

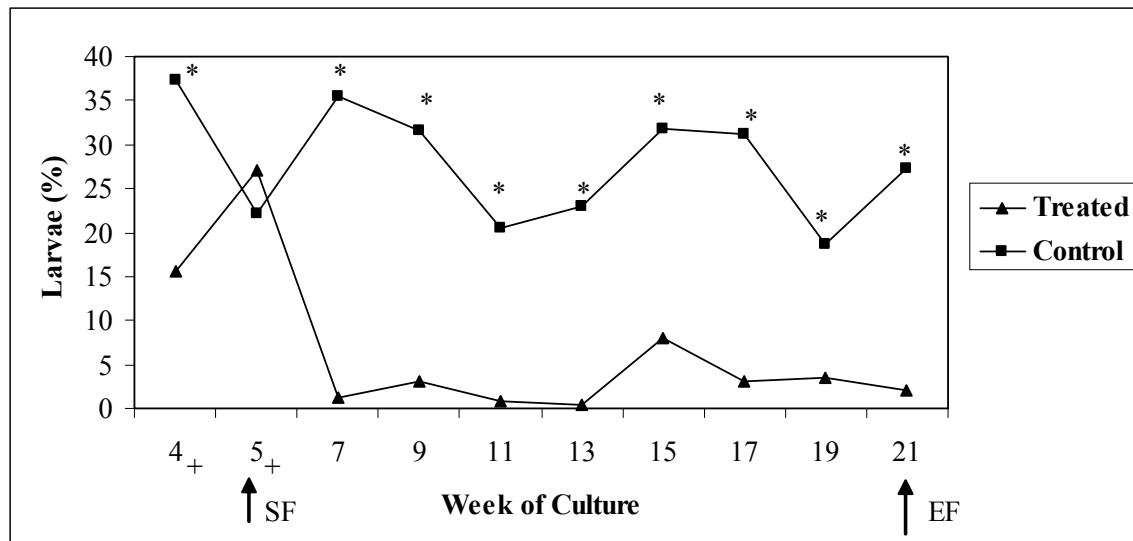


Fig. 2. Trial 1. Mean percent of larvae recovered from ewe fecal cultures. The + represents pre-fungal feeding periods. SF and EF denote start and ending of fungus feeding period, respectively. * $P < 0.05$.

4.1.3. FEC Analysis

For ewes, FEC in both groups started relatively low at the beginning and increased steadily for 13 weeks at which point there was a decline and then an increase

from week 16 and another decline after week 18 (Fig. 3). There was no significant difference in FEC between groups for any week over the 21-week grazing period and no overall significance between groups.

Table 1. Trial 1. Mean percent recovery and fecal percent reduction for ewe/lamb pairs. * represents lamb bulk culture.

Group	Week											
	4	5	7	9	11	13	15	17	19	19*	21	21*
Treat	15.5	27.0	1.3	3.0	0.9	0.5	8.0	3.0	3.5	6.1	2.0	0.5
Control	37.3	22.2	35.4	31.5	20.6	23.0	31.7	31.2	18.6	31.7	27.2	100
Reduction	49.6	0.0	96.3	90.5	95.7	98.0	74.4	91.0	81.4	81.0	92.7	100

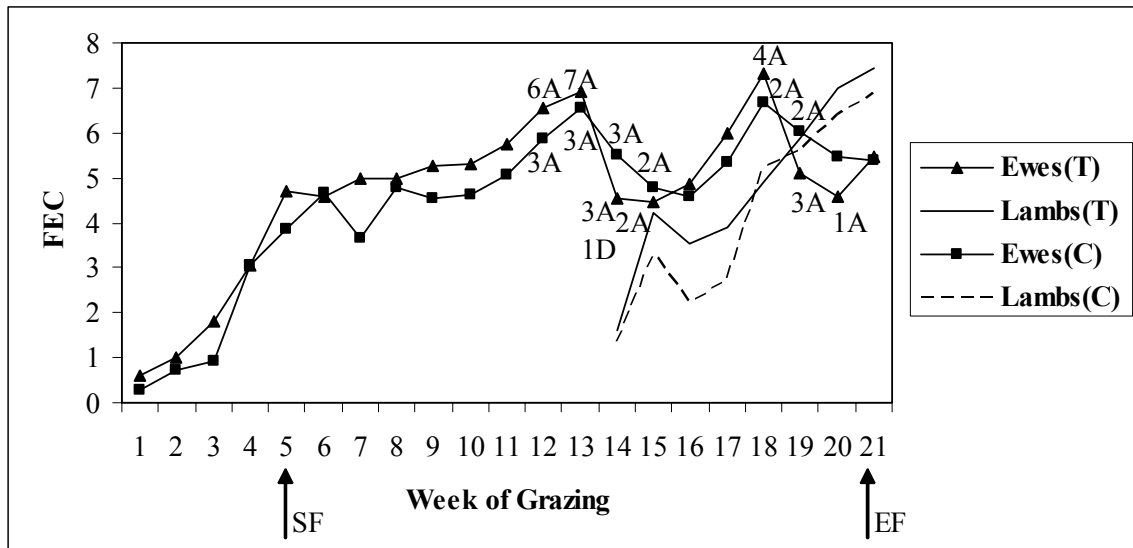


Fig. 3. Trial 1. Mean log fecal egg count of Treated and Control ewes/lambs pairs (n=17 for control ewes; n=17 for treated ewes up to week 14 then n=16 weeks 15-21) while grazing separate pastures. Animals with PCV below 20% were treated with albendazole (Valbazen[®], 10mg/kg) and moxidectin (Cydectin[®], 0.2mg/kg) and are denoted by A. Animals that died are denoted by D. SF and EF denote start and ending of fungus feeding period respectively.

4.1.4. PCV Analysis

PCV levels were relatively even at the beginning of the study with the Control group having a slightly higher PCV and this trend continued to week 10 at which point

PCV levels for both groups began to decline (Fig. 4). The decline in the Treated group was greater than the Control group. At week 15, PCV levels started to increase and this trend continued for the duration of the study with the Control group maintaining the higher PCV. There was an overall significant difference between the groups, Treated 25.4 ± 0.26 and Control 25.5 ± 0.30 . Signs of haemonchosis (bottle jaw) were observed in both groups and the one animal that died had a low PCV and a high FEC.

Lambs in the Control group began with a higher PCV level than the Treated group and PCV in both groups subsequently declined over the grazing period. There were no significant differences between groups at any time.

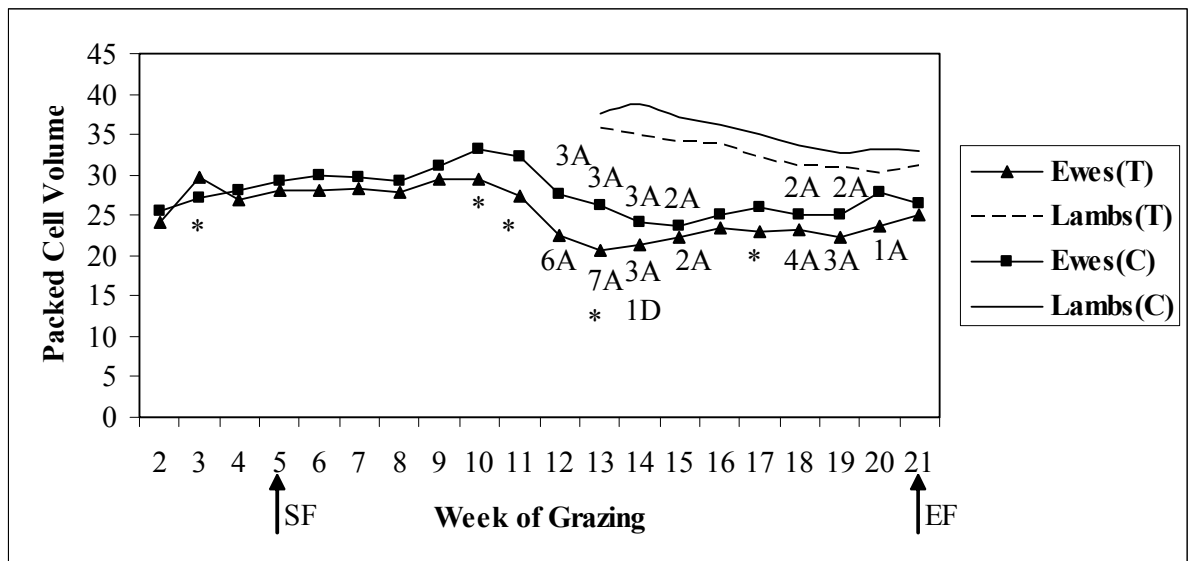


Fig. 4. Trial 1. Mean blood packed cell volume (PCV) of Treated and Control ewes/lamb pairs (n=17 for control ewes; n=17 for treated ewes up to week 14 then n=16 weeks 15-21) while grazing separate pastures. Animals with PCV below 20% were treated with albendazole (Valbazen[®], 10mg/kg) and moxidectin (Cydectin[®], 0.2mg/kg) and are denoted with A. Animals that died are denoted by D. SF and EF denote start and ending of fungus feeding period, respectively. *P<0.05.

4.1.5. Weight Analysis

Control ewes started out heavier than Treated ewes and remained higher throughout the study (Fig. 5). Weight for both groups followed the same trend increasing from weeks 1 to 9, a decrease from weeks 9 to 11, and then remained relatively constant from week 11 to 21. There was no significant difference between weight for Treated and Control groups throughout the study. Mean birth weight for lambs was 4.8 kg for both Treated and Control groups. Mean weaning weight was 21.2 kg and 21.9 kg in the Treated and Control groups, respectively.

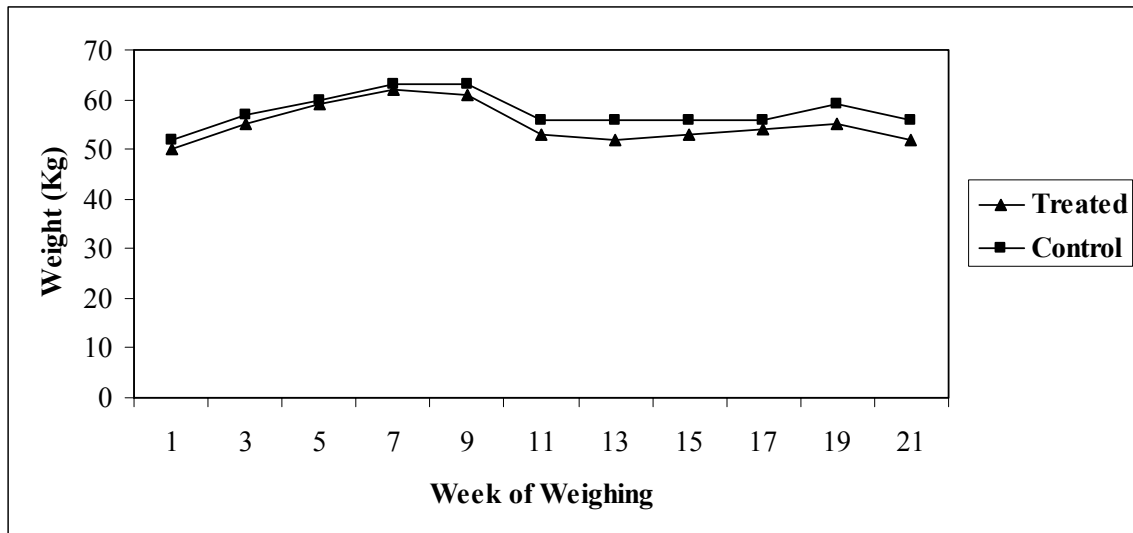


Fig. 5. Trial 1. Mean weight of Treated and Control ewes (n=17 for control; n=17 for treated up to week 14 then n=16 weeks 15-21).

4.2. Trial 2

4.2.1. Pasture Larval Recovery

Larval recovery from pasture was higher in Control pastures than Treated for the first seven pasture samplings (Fig. 6). Recovery was relatively even for the remainder of the samplings. Overall, there was a significant difference in larval recovery from Treated, 4.1 ± 1.62 and Control, 12.1 ± 3.25 pastures.

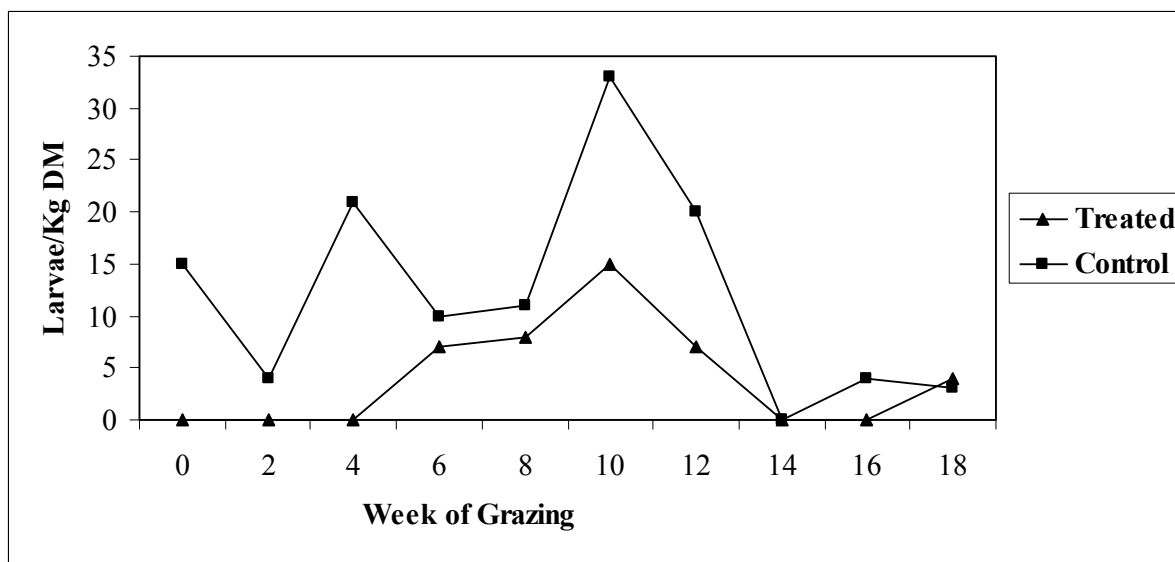


Fig. 6. Trial 2. Pasture larval recovery comparing larvae/gm DM between Treated and Control pastures.

4.2.2. Fecal Cultures

Lamb fecal culture began on week 2 of grazing and continued until week 12 (Fig. 7). Week 2 was pre-fungus feeding and weeks 11 and 12 were post-fungus feeding. The percent recovery for the Treated group remained low throughout the study with the lowest recovery at week 9 with a mean of 2.9% and highest recovery at week 2 with a mean of 56.8%. The Control group remained high during the study and had the highest recovery at week 2 with a mean of 50.6% and lowest at week 5 with a mean of 17.4%. Overall, the difference in mean percent recovery of larvae from Treated, 20.26 ± 7.75 and Control 37.56 ± 4.05 fecal cultures was significant.

The highest percent reduction for lamb fecal cultures was seen at week 9 (92%) and lowest was at week 2 where no reduction occurred (Table 2). Reduction ranged from 0 to 92% and maximum reduction occurred at week 7 and 9 weeks where percent reduction was 88.7% and 92.4%, respectively.

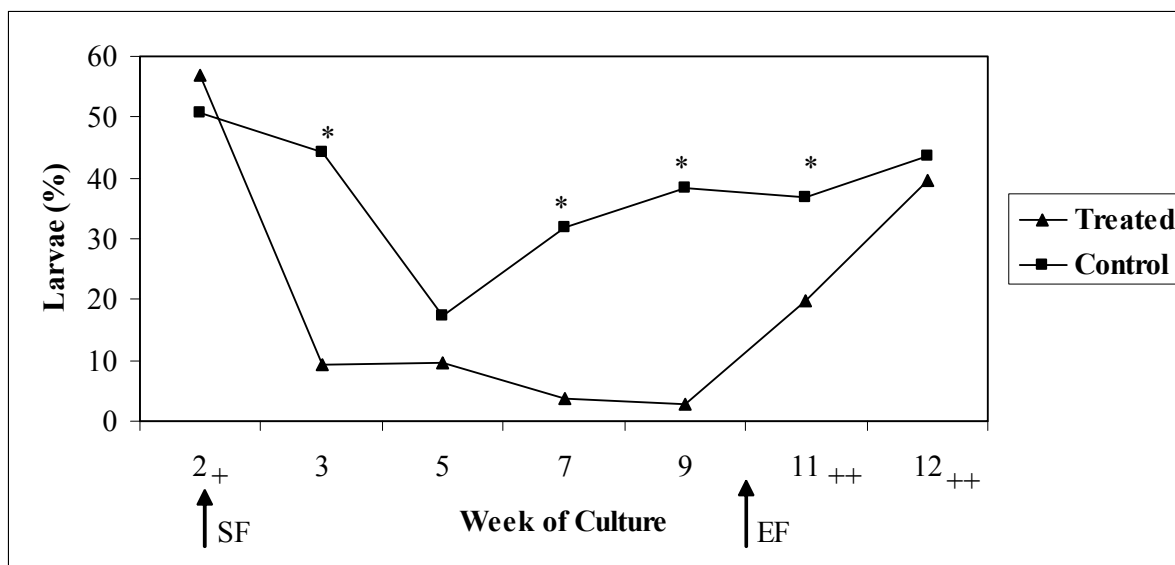


Fig. 7. Trial 2. Mean percent of larvae recovered from lamb fecal cultures. The + and ++ represent pre-fungus and post-fungus periods respectively. SF and EF denote start and ending of fungus feeding period, respectively. * $P < 0.05$.

Table 2. Trial 2. Mean percent recovery and fecal percent reduction for lambs.

Group	Week						
	2	3	5	7	9	11	12
Treat	56.8	9.4	9.6	3.6	2.9	19.9	39.6
Control	50.6	44.2	17.4	31.8	38.5	36.9	43.5
Reduction	0.0	78.7	44.5	88.7	92.4	46.1	9.0

4.2.3. Tracer Animals

All tracers had a FEC of 0 EPG on the first day of grazing (Table 3). PCV level was similar for all three groups of tracers. At necropsy, the first group of Treated and Control group tracers had a mean FEC of 7,625 EPG and 13,025 EPG, respectively. At necropsy, the second group of Treated and Control group tracers had a mean FEC of 75 EPG and 2,375 EPG, respectively. At necropsy, the third group of Treated and Control group tracers had a mean FEC of 1,675 EPG and 4,225 EPG, respectively. The

predominate nematode recovered from the abomasum of the tracer animals was *H. contortus*, where substantially fewer were recovered from the first and second set of Treated group tracers than Control group tracers (Table 3). *Haemonchus contortus* recovered from the third set of tracers were essentially the same for both Control and Treated groups. A few adult *Trichostrongylus colubriformis* were recovered from the small intestine. No nematodes were recovered from the large intestine.

4.2.4. FEC Analysis

Mean FEC for lambs in both groups was zero at the beginning of the trial (Fig. 8). The Control group maintained a higher FEC for the first eight weeks and then FEC reversed for the following six weeks where the Control group was lower than the Treated group. There was no overall significant difference in FEC between groups for the 18-week grazing period but significant differences were at weeks 2 and 3.

4.2.5. PCV Analysis

Mean PCV for lambs was relatively similar for the first three weeks of grazing with a general decrease for both groups after week 2 (Fig. 9). The decrease continued until week 8, and then stabilized through week 12. Subsequently, PCV increased through week 16 and remained relatively steady for weeks 17 and 18. Through week 8 PCV for the Control group was consistently lower than the Treated group and subsequent to week 10, vice versa. At weeks 4, 6, 8, 11, and 18 there was a significant difference in PCV between groups, but there was no overall significance difference between groups. One animal in the Treated group was lost due to an attack by a wild dog.

Table 3. FEC and nematode burden of the tracer animals that grazed pastures of control and fungus-fed animals.

Grp ²	Pasture	Animal Turnout	FEC ¹		Nematodes		
			Necropsy	Abomasum ³	SI ⁴	Total	
1	Treated	1	0	5000	1200	2	1202
		2	0	10250	2000	2	2002
		Mean	0	7625	1600	2	1602
	Control	1	0	10550	950	50	1000
		2	0	15500	4350	0	4350
		Mean	0	13025	2650	25	2675
2	Treated	1	0	100	250	50	300
		2	0	50	500	75	575
		Mean	0	75	375	62.5	437.5
	Control	1	0	1600	1950	0	1950
		2	0	3150	2300	50	2350
		Mean	0	2375	2125	25	2150
3	Treated	1	0	2000	3350	75	3425
		2	0	1350	1200	75	1275
		Mean	0	1675	2275	75	2350
	Control	1	0	1850	2300	50	2350
		2	0	6600	3050	100	3150
		Mean	0	4225	2675	75	2750

¹ Eggs per gram of feces.

² 1 = After ewe/lamb 16 week fungus feeding period; 2 = After weaner 8 week fungus feeding period; 3 = After 8 weeks of no fungus feeding at the end of the study.

³ All were *Haemonchus contortus* (mostly late L₄ and immature adults).

⁴ All were *Trichostrongylus* spp.

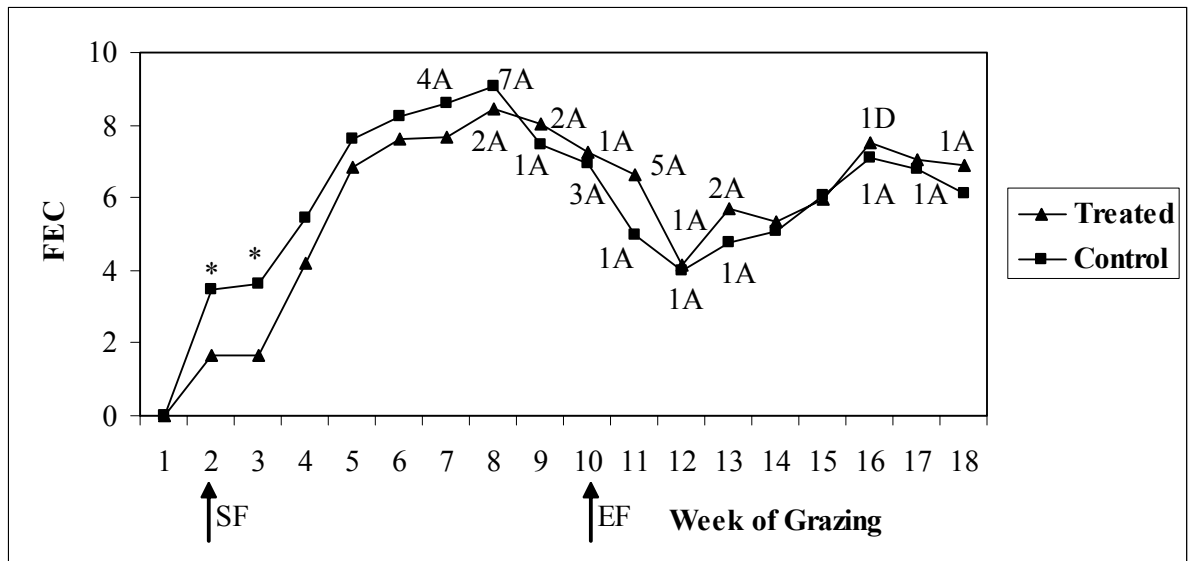


Fig. 8. Trial 2. Mean log fecal egg count (EPG) of Treated and Control lambs (n=15/group up to week15; for Treated n=14 for week 16 then n=12 for weeks 17-18; for Control n=15 for week 16 and n=13 for weeks 17-18). Animals with a PCV below 20% were treated with albendazole (Valbazen[®], 10mg/kg) and moxidectin (Cydectin[®], 0.2mg/kg) and are denoted by A. Animals that died are denoted by D. SF and EF denote start and ending of fungus feeding period respectively. * P<0.05.

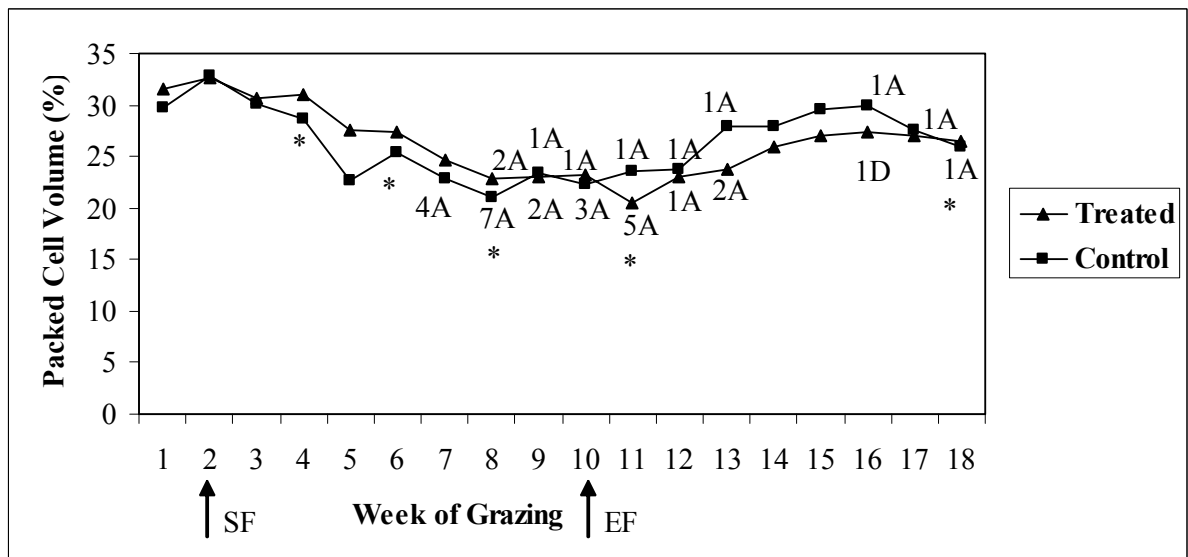


Fig. 9. Trial 2. Mean blood packed cell volume (PCV) of Treated and Control lambs (n=15/group up to week15; for Treated n=14 for week 16 then n=12 for weeks 17-18; for Control n=15 for week 16 and n=13 for weeks 17-18). Animals with a PCV below 20% were treated with albendazole (Valbazen[®], 10mg/kg) and moxidectin (Cydectin[®], 0.2mg/kg) and are denoted by A. Animals that died are denoted by D. SF and EF denote start and ending of the fungus-feeding period, respectively. * P<0.05.

4.2.6. Anthelmintic Administration

Individual deworming began to be required in the Control group at week 7 when four animals were dewormed (Fig.8 and 9). The following week, seven more Control group and two Treated group animals required deworming. Subsequently, 9 and 12 dewormings were required for Control and Treated group animals, respectively. A total of 20 and 14 dewormings were required, respectively.

4.2.7. Weight Analysis

Mean weight for the Control group started out heavier than the Treated group and remained higher until the end of the study (Fig.10). Weights for both groups followed the same trend throughout the study and there was no overall significant difference between groups.

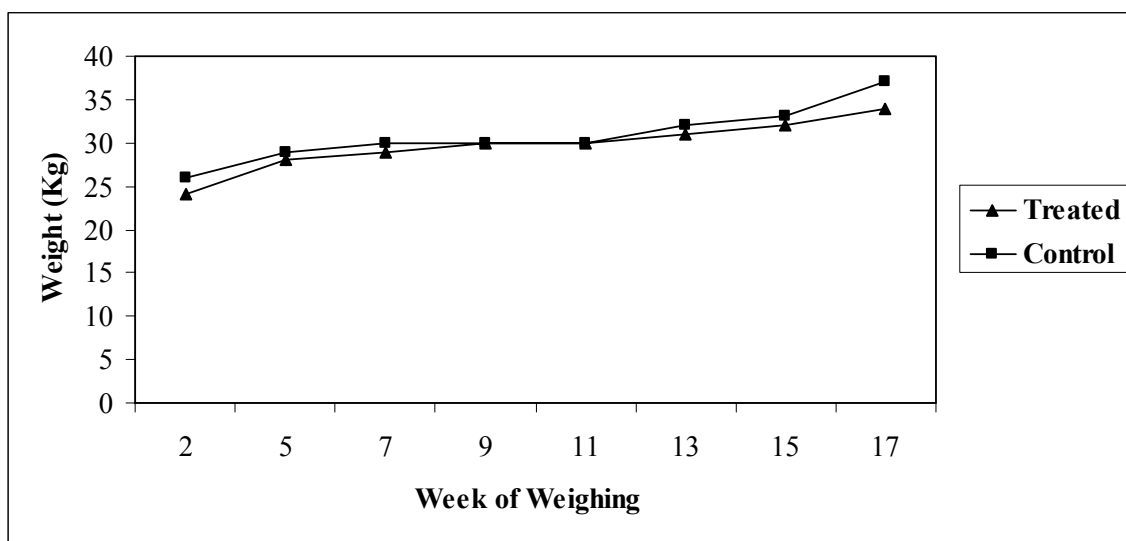


Fig.10. Trial 2. Mean weight of Treated and Control groups. (n=15/group up to week15; for Treated n=14 for week 16 then n=12 for weeks 17-18; for Control n= 15 for week 16 and n=13 for weeks 17-18).

CHAPTER 5 DISCUSSION AND CONCLUSION

5.1. Discussion

The results of this study indicated that *D. flagrans* has the ability to control pasture infectivity and kill larval stages of *H. contortus*. This concurs with Githigia et al. (1997) who conducted a study with three- to four-month old parasite naïve Dorset-crossbred lambs where it was concluded that by dosing with 10^6 chlamydospores/kg BW daily pasture infectivity may be limited and subsequently, intake of L₃ may be reduced.

Differentiation in the recovery of L₃ from grass samples indicated that density levels were lower in Treated pastures for both studies. Infection level in the first and second group of tracers concurs in that the Treated group tracers had substantially fewer nematodes from grazing after each of the fungus feeding periods. However, this advantage appears to have been lost during the eight weeks after fungus feeding was stopped as evidenced by the infection level in the final group of tracers and pasture L₃ recovery that indicated that pasture infectivity was relatively equivalent.

Fecal cultures were used to verify the effect of *D. flagrans* on the fecal pat larval population. Results supported the observed reduction in pasture infectivity, in that feces from the Treated groups in both studies had significantly reduced levels of L₃, during the entire period when fungal spores were fed. And, percent reduction, compared to the Control group, was as high as 98% in Study I and 92% in Study II. Fernandez et al. (1999b) also evaluated fecal cultures and showed that *D. flagrans* was able to significantly reduce the number of *Ostertagia ostertagi* L₃ in cattle and subsequently, the transmission of L₃ from feces to pasture herbage.

The dosage used in these studies was 5×10^5 spores/kg BW and was administered daily mixed with a small amount of supplement feed. This dosage and administration method has been reported to be highly effective in both a dose-titration study (Peña et al., 2002) and a field grazing study (Fontenot, 2001) using non-lactating ewes. Larval reductions in the dose-titration study, when animals were fed individually, were consistently higher than 95.8% during the fungal spore-feeding period. Larval reductions in the field grazing study, when animals were fed as a group, ranged from 78.9 to 89.3% during the first 10 weeks and higher than 95.4% during the last 8 weeks of the fungal spore-feeding period.

Results from Trial 1 were not that encouraging regarding the effect on infection level and performance. The ewes showed no significant difference between the two groups for FEC and this was expected initially as they were experimentally infected at the beginning of the trial and infection level was therefore not directly related to pasture infectivity. However, as infectivity increased on the Control pastures and was restricted on the Treated pasture, it was expected to see a greater increase in infection level in the Control group compared to the Treated group. Since this did not happen, one explanation is that the resident nematode population from the experimental infection was high enough to preclude establishment of any new additional larvae being consumed. In addition, the life span of the resident nematodes is longer than the trial period so there would not have been any substantial die off which would have allowed any infectivity differential to be observed. If the Treated group pasture infectivity was really reduced (as pasture grass L₃ recovery suggested), then the infection level in the Treated group lambs would have been

expected to be lower than the Control group lambs, but, again, there was no difference and the Control group lambs actually had a lower FEC overall. The only explanation is that the residual pasture infectivity in the Treated group pasture was still high enough to overcome any infection advantage.

The potential ability of *D. flagrans* to reduce pasture infectivity and thus infection level was seen in Trial 2 where FEC in the Treated group was significantly lower than that of the Control group during the 8-week fungal spore feeding period. This would seem logical as both groups grazed the same pastures that were previously grazed by the Control and Treated groups in Trial 1 and the Treated group should therefore have been exposed to fewer L₃. In support of this, five dewormings were required in the Treated group compared to 15 in the Control group during the spore-feeding period. And, the Control group dewormings were required sooner than the Treated group dewormings. However, during the 8-week period after spore feeding stopped, the advantage gained appeared to have been lost as pasture grass samples, fecal cultures, FEC, and tracer data were almost equivalent at the end of the trial. This suggested that the fungus does not have a long-term effect and may require continuous administration. Knox and Faedo (2001) have reported that FEC was reduced and weight gain improved when spore feeding was continuous over a six-month period. In this trial with only an 8-week spore-feeding period, there was no weight gain advantage.

A practical means of delivering fungal spores to where it can effectively reduce L₃ in feces is continually being investigated. Oral administration via daily feeding is the only method currently available and has been used successfully in other studies (Larsen

et al., 1992; Fernandez et al., 1997; Peña et al., 2002; Fontenot, 2001). Work investigating other potential spore delivery methods includes incorporation of spores into blocks or mineral salt mixes (Larsen, 1999) and via intra-ruminal controlled release devices (M. Larsen, personal communication). The latter will obviously be more expensive and require more work and skill in administration, but will probably provide more consistent results (Barger, 1996). Another method of delivery investigated by Waller et al. (1994) showed that unprotected conidia administered in very small volumes of water, produced results that indicated that a proportion of these minute and thin-walled structures were capable of survival.

Reduction in pasture infectivity will be an important advancement in small ruminant production. This reduction in L₃ on herbage will subsequently reduce the build-up of nematode burdens in hosts, which otherwise could cause subclinical or clinical disease especially in young animals (Larsen, 1999). *Duddingtonia flagrans* by itself should not be considered as an agent to completely control *H. contortus*, but must be a part of an integrated control management system utilizing minimum chemotherapy, grazing management, vaccines and other novel methods such as condensed tannins, copper wire boluses and using genetically resistant breeds or breeding for resistance. This later method may be enhanced when genetic markers become available which can identify those animals that have resistance genes (Gasbarre and Miller, 2000).

The advantage that *D. flagrans* will have is that it has the potential to reduce the dependence on anthelmintics thereby addressing the concerns of drug resistance development, residues in meat and increased environmental toxicity. However when

considering this novel method, pertinent questions that may be asked by producers include its effectiveness, cost, application and sustainability (Barger, 1996).

5.2. Conclusion

It can be concluded from this study that *D. flagrans* has the potential to be used as a BC agent to reduce pasture infectivity and thus nematode burdens in grazing sheep. However, *D. flagrans* cannot be the exclusive method of control as an integrated approach must be undertaken and *D. flagrans* can be an important element.

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VITA

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