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Efficacy of *Duddingtonia flagrans* spores fed in trace mineral mix to lambs in reducing the development of gastrointestinal nematode larvae in feces

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ABSTRACT

Providing spores of the nematode-trapping fungus, *Duddingtonia flagrans*, in the diet of livestock leads to fewer gastrointestinal nematode (**GIN**) larvae on pasture. The objective was to determine the efficacy of *D. flagrans* (**Df**; BioWorma®) fed in a mineral mix compared with a feed supplement to naturally infected lambs (predominantly *Haemonchus contortus* and *Trichostrongylus/Teladorsagia* spp.) to reduce the number of GIN larvae cultured. Initially, the efficacy of Df spores was examined in a preliminary experiment in which lambs were fed a control supplement without Df (**CON**) or with Df (n = 8/treatment) for 7 days. Feces were collected every other day for three coprocultures. Feeding Df led to a 73 % reduction in stongylid third-stage larvae ($P = 0.005$). In the second experiment, weaned lambs were fed a 12 % crude protein grain supplement with trace mineral added without Df (CON), Df mixed in feed (**DfC**) or in trace mineral (**DfM**; n = 8/treatment) for 16 days. Strongylid larval recovery was reduced in both Df groups ($P < 0.001$), and the third-stage larval recovery was lower in DfM than DfC ($P = 0.001$). In a field study, similar treatments were used (n = 16/treatment including 2 reps/treatment) using weaned lambs for a 30-day period. The sheep were fed a 16 % crude protein commercial grain mix and a commercial mineral mix; for the DfM group, Df spores were mixed with the mineral mix 7 days prior to feeding. Similarly, strongylid larval recovery was reduced in both Df groups ($P < 0.001$) but was similar between Df treatments ($P = 0.62$). No treatment effect on *Strongyloides* larvae recovered was noted ($P = 0.181$). Including Df in a trace mineral mix offers promise in control of GIN on pasture or range for flocks that do not offer grain supplements.

1. Introduction

Duddingtonia flagrans, a nematode trapping fungus, can be used in small ruminants for parasite control on pasture, targeting third-stage (**L3**) gastrointestinal nematode (**GIN**) larvae (Githigia et al. 1997; Waller et al., 2001; Peña et al., 2002; Terrill et al., 2004). In recent years, *D. flagrans* for control of GIN on pasture was commercialized in the U.S. (BioWorma®, Int. Anim. Health Prod. Pty. Ltd., Australia) which includes 5×10^5 chlamydospores/g of *D. flagrans* as a free-flowing fine meal (Healey et al., 2018a,b). The spores pass through the digestive tract of ruminant livestock and are deposited in the feces (Larsen et al., 1992, 1998; Larsen, 2000; Grønvold et al., 1993; Faedo et al., 1997). In feces deposited on pasture, the fungus develops along with the GIN larvae, trapping and killing the larvae, including *Haemonchus contortus* (Fontenot et al., 2003; Waghorn et al., 2003; Chandrawathani et al., 2004), *Trichostrongylus* spp. and *Teladorsagia* spp. (Paraud et al., 2004; 2006). Thus, pasture infectivity of *H. contortus* was greatly reduced in sheep pastures with *D. flagrans* feeding (Fontenot et al., 2003; Chandrawathani et al., 2004). There are fewer studies examining the effect of *D. flagrans* on *Strongyloides* spp. Campos et al. (2017) and Braga et al. (2020) reported an efficient capture of *S. papillosus* in the absence of other GIN by *D. flagrans*.

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The commercial product used in the U.S. (BioWorma®, Int. Anim. Health Prod. Pty Ltd, Australia) was designed to be incorporated in the feed or as a top dressing. Forage-based sheep operations are limited in the use of *D. flagrans* in a feed supplement when concentrated feeds are not allowed or available but could clearly benefit from this technology for control of GIN. The advantages of including *D. flagrans* in a feed supplement rather than in a mineral mix (as we have examined in this manuscript) is having greater control over the number of spores given to reduce GIN larvae in feces and protection of chlamydospores from environmental extremes by feeding fresh product daily. The advantage of including *D. flagrans* in a mineral mix, assuming chlamydospores remain viable in a trace mineralized salt mix, would be that no feed supplement is necessary and that the product would not have to be provided daily since mineral mix is normally provided in bulk for consumption ad libitum. Disadvantages of this system would be that all animals may not receive the targeted minimum number of spores to reduce GIN larvae in feces and any potential effects of exposure to the elements such as animal saliva. The objective of this study was to determine whether the commercial product containing *D. flagrans* could be included in a trace mineral mix to maintain efficacy in GIN larval reduction compared with incorporating it in a feed supplement. The null hypothesis was that including *D. flagrans* in a mineral mix would be similar to the control diet without *D. flagrans*, or there would be no reduction in larvae in feces; the alternative hypothesis was that *D. flagrans* would be as effective as its inclusion in a feed supplement, or there would be a significant reduction in larvae in feces compared with the control diet without *D. flagrans*. Efficacy of *D. flagrans* to trap GIN larvae was examined in coprocultures originating from naturally GIN-infected lambs.

2. Materials and methods

Experiments 1 and 2 took place at the USDA, Agricultural Research Service, Dale Bumpers Small Farms Research Center in Booneville, AR (35°N, 94°W) using the Katahdin sheep breed. Experiment 3 was conducted at the Louisiana State University Agricultural Center Doyle Chambers Central Research Station in Baton Rouge, LA (30°N, 91°W) using Katahdin and Hampshire cross sheep. All lambs in these studies were naturally infected with GIN from previously grazed grass pastures. The experimental procedures for Exp. 1 and 2 were reviewed and accepted by the Institutional Animal Care and Use Committee at the USDA Agricultural Research Service while those for experiment 3 were reviewed and approved by the Louisiana State University Agricultural Center Institutional Animal Care and Use Committee.

2.1. Experimental procedures

2.1.1. Experiment 1: validation of efficacy of D. flagrans

The first objective was to determine the efficacy of *D. flagrans* (**Df**) spores in BioWorma® (lot no. 3167101, manufactured in Oct 2022; Int. Anim. Health Prod. Pty Ltd, Australia) in reducing L3 in cultured feces. This objective was necessary to validate the efficacy of BioWorma® because an experiment using product with a different lot number previously was conducted, with the aim of addressing the main objective of the present studies, but no larval reduction in feces from Df-fed sheep was observed (data not shown or published). In Jan 2023, an equal number of ram and ewe lambs per treatment (97 ± 1.2 days of age; 19.5 ± 1.3 kg body weight; mean ± standard error) were randomly assigned to a control (**CON**; no Df) or Df-fed treatment (8/treatment; 2 lambs/pen for feeding). All lambs were fed 900 g/lamb daily of a 50:50 mix of soy hull pellets and dried distillers' grain (Farmers Coop, Van Buren, AR, USA) and 7.1 g trace mineral mix [salt (87.7 %), Sheep Trace Mineral Premix (8.8 %), and Vitamin ADE mix (3.5 %), Premier 1, Washington, IA, USA]. The Df lambs were fed the manufacturer-recommended 60 mg BioWorma®/kg body weight daily (3×10^4 viable chlamydospores per kg body weight; Healey et al., 2018a,b). Lambs were housed together and brought into pens in pairs for feeding daily then returned to ~1-ha pasture after feeding, and had free choice bermudagrass hay, water, and access to shelter/shade. Lambs were acclimated to pen feeding for 7 days before beginning treatment diets for 7 days. Rectal fecal samples were collected on first day of diet acclimation (day 0) and days 7, 9, and 11 to determine fecal egg count (**FEC**). Feces were cultured to examine L3 recovery rate (L3 larvae/FEC) relative to control group and determine the predominant GIN in feces. Blood was collected one time on day 0 to determine packed cell volume (**PCV**).

2.1.2. Experiment 2: D. flagrans included in trace mineral mix to determine reduction of GIN larvae in feces – controlled pen study

The objective was to determine efficacy of Df included in a mineral mix on GIN larval reduction compared with Df mixed in a feed supplement in lambs. This experiment addressed the main objective of the present studies in a controlled manner by ensuring the targeted number of Df spores per animal (3×10^4 viable chlamydospores per kg body weight) for the two Df treatments were consumed. In Feb 2023, weaned ram lambs (94.6 ± 2.1 days of age; 24.1 ± 1.6 kg body weight) were acclimated to pen feeding and diets (control) for 7 days before randomization to treatment diets for 16 days. During the experimental period (days 0–16), lambs were supplemented in pairs to minimize social distress with a 12 % crude protein sweet feed (450 g/lamb daily or 900 g/pair; Producer's Pride 12 % All Stock Sweet Feed, Tractor Supply Co., Brentwood, TN, USA) that was thoroughly mixed daily with trace mineral mix [7.1 g of salt (87.7 %), Sheep Trace Mineral Premix (8.8 %), and Vitamin ADE mix (3.5 %), Premier 1]. The three treatments (n = 8/treatment; 2/pen while feeding) were 1) control (**CON**; no Df), 2) Df (BioWorma®, same lot and dose as in Exp. 1) and trace mineral mixed into the supplement as recommended (**DfC**; weighed daily at 2.1 g/lamb fed to heaviest lamb which was 34.5 kg), or 3) Df added in mineral (**DfM**; 56.8 g trace mineral mix was mixed with 16.6 g Df for 8 lambs then stored for 7 days in a Ziploc® bag prior to feeding) which was then added to the supplement on the day of feeding. Pair feeding lasted 1 hour (no feed refusals remained), then lambs had access to free choice bermudagrass hay and water as a group with access to shelter and shade. Lambs were weighed on days 0 and 16. Fecal samples were collected for FEC and coprocultures (4 g/lamb) in pairs twice weekly (every 3–4 days), and blood collected in Vacutainer® tubes (Becton Dickinson, Franklin Lakes, NY, USA) via jugular venipuncture every 7 days to determine PCV between days 0 and 16. Larval recovery (see description in methods below) was calculated per pair: [(L3/g feces)/average FEC) × 100].

2.1.3. Experiment 3. Efficacy of ad libitum feeding of trace mineral mix containing D. flagrans for reduction of GIN larvae in feces of lambs on pasture

This third experiment addressed the main objective of the present studies, but allowed the sheep free choice access to the trace mineral mix that contained the Df similar to what might occur on farms or ranches. A preliminary or pilot study was conducted to ensure that the Df used in Exp. 2 had remained efficacious.

A 6-day pilot study was initially conducted at Louisiana State University on Katahdin lambs to test the efficacy of *D. flagrans* (BioWorma®; same lot as in experiment 1 but had been shipped and potentially exposed to warm temperatures) to be used in the experiment. Three lambs were fed BioWorma® in a grain/mineral mix (Df), according to the label directions, and three lambs were assigned as controls (CON). Fecal samples were collected daily between days 3 and 6. Equal amount of feces per lamb were pooled by treatment, FEC were performed in duplicate for each lamb, and 10-g coprocultures were made for the Df and control groups. Larvae were recovered and counted for each coproculture. All methods were performed as described below for Exp. 3. The percentage larval recoveries from the controls were 42.1 %, 42.3 %, 8.1 %, and 33.6 % on days 3, 4, 5, and 6, respectively, while the respective recoveries from the Df treatment were 2.2.%, 4.2 %, 5.7 %,

and 6.2 %. These results provided sufficient evidence for the efficacy of the Df to be used in Exp. 3.

The primary objective of Exp. 3 was to determine the efficacy of Df fed to weaned lambs on GIN larval reduction when included in a mineral mix fed *ad libitum* compared with Df included in a feed supplement. In Jul 2023, 36 Katahdin lambs (~172 days of age; 35.4 ± 0.7 kg body weight) and 12 Hampshire cross lambs (~132 days of age; 29.1 ± 1.2 kg body weight) were randomly blocked by FEC, body weight, sex, and breed for a randomized block design into three treatment groups (n = 16 lambs per group or n = 8 per replicate). Two barns and adjacent pastures were utilized, split into three pens with adjoining pastures each. Each treatment was replicated twice in each barn, with each replicate including 6 Katahdin sheep and 2 Hampshire crosses. Each pasture was approximately 0.34 ha. 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 group fed a 16 % crude protein commercial grain mix (Kentwood Co-Op, Kentwood, LA). Grain was initially fed at 675 g/head daily, split evenly into morning and evening feedings. On Day 22, feed was increased to 900 g/head fed daily split evenly, based on average body weight. Clean, fresh water was provided *ad libitum* to animals through the entire study. Purina® Wind and Rain® Sheep Mineral (Purina Anim. Nutr. LLC, Arden Hills, MN) was fed as the mineral mix as described in the following paragraph. Animals were allowed to graze in their respective pastures during the day and housed in the pens through the night. Pastures were predominantly grass in abundant quantity, requiring mowing to maintain a vegetative state.

Treatment 1 (**CON**) consisted of feeding mineral *ad libitum* and standard grain. Treatment 2 (**DfC**) involved mixing Df (BioWorma®; same lot and dose/kg body weight as Exp. 1) thoroughly with grain once per day in the afternoon feeding. The Df was administered according to the manufacturer's recommendation at 60 mg BioWorma®/kg body weight. Treatment 3 (**DfM**) was fed grain and Df at the same rate as treatment 2 (though because mineral mix was fed ad lib, lambs may have consumed Df in excess of the recommended amount), but the Df was "aged" in the commercial mineral mix. Minerals were weighed out seven days prior to the start of administration. On this day Df was thoroughly mixed into the mineral to allow it to age. Batches were produced every seven days such that this group was fed Df that had been aged for at least 7 days and up to 14 days, to simulate field conditions. To prepare the batches, estimates for the amount of mineral-Df mixture to offer were based on the recommended administration rate for the mineral of 625 mg mineral/kg body weight.

Lambs were weighed, body condition scored (1 = emaciated; 5 = obese), and rectal fecal samples for FEC and blood for PCV were collected on days 0, 7, 14, 21, and 30. Rectal fecal samples for culture were collected on days 0, 3, 7, 10, 14, 17, 21, 24, 28, and 30.

2.2. Sample collection and analyses

2.2.1. FEC and PCV

Fecal egg counts of strongylid eggs were conducted using the modified McMaster technique with a multiplication factor of 50, that is, one egg counted represented 50 eggs/g (Whitlock, 1948). Packed cell volumes were determined using the microhematocrit method.

2.2.2. Exp. 1 and 2: larval recovery

For larval recovery, the FEC of individual lambs was determined first. Feces collected from individual lambs were pooled into a group of two per culture replicate using 8–10 g feces. Initial FEC was averaged within a group. Equal fecal mass per individual animal within the rep was used. Otherwise, coprocultures were prepared according to Peña et al. (2002) and incubated at 25°C for 7 days. For counting and speciation, two 100 μl aliquots were placed on a microscope slide and covered with a cover slip. The L3 were counted at 50 X power. The number of L3 per 100 μl was the mean of the two aliquot counts and the total number of L3 recovered estimated by extrapolation. The total number of L3 was divided grams of feces per culture to calculate L3/g feces. Recovery was calculated as [1 – (FEC – larval count)/FEC)]. GIN were identified to the genus level (Peña et al., 2002) and presence of *Strongyloides* spp. noted.

2.2.3. Exp. 3: larval recovery

For preparing larval cultures, the lambs in each pasture were divided into two subsets consisting of four animals each. The lambs in 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 FEC, and then a representative sample was taken to make a culture. The amount of feces included in each culture was 4 g for days 0, 3, and 7; 6 g for days 10 and 14; and 8 g for days 17, 21, 24, 28, and 30. The mass of feces cultured was increased out of concern that inadequate larvae were being recovered at the lower weights and that meaningful comparison between treatments might not be possible if larval recovery numbers were low overall.

Preparation of the cultures was performed as previously described (Peña et al., 2002) with the following modifications: the feces were placed in a 100 ml "culture" cup and 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 7-day incubation period. The cultures were incubated at 25°C for 7 days. On day 7 the 250 ml water cup was flooded to the top with warm water and allowed to sit at room temperature 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 strongylid larvae were counted and identified to genus using published keys (Dunn, 1969; Van Wyk and Mayhew, 2013) and the remaining strongylid larvae were enumerated. *Strongyloides papillosus* larvae were counted separately.

After the larvae were counted, the percentage larval recovery was calculated for the strongylid larvae. The average of the two FEC 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 was divided by the estimate of the total number of eggs incubated and the product multiplied by 100 to give the percentage larval recovery. Because the *Strongyloides* eggs were not counted, the percentage recoveries could not be calculated in this manner. The number of *Strongyloides* larvae counted was divided by the number of grams of feces in the culture, to give a value of *Strongyloides* larvae/g of feces.

2.3. Statistical analyses

Data were analyzed using the proc mixed models procedure with an autoregressive covariance structure (SAS Inst. Inc., Cary, NC (SAS/STAT®). For Exp. 1 and 2, the model included PCV, FEC, or L3 recovery, and for Exp. 3, the model included PCV, FEC, strongylid L3 recovery, *Strongyloides* larval counts, body weight and body condition scores, as dependent variables; and treatment (Exp. 1, 2, and 3), sex (Exp. 1 and 3) and breed (Exp. 3), day, and interactions as independent variables with a repeated statement for day of measurement (sex and breed were not included in models for L3 recovery or larval counts). If interactions were not significant, they were removed from the model.

Orthogonal contrasts included CON vs. DfC and DfC vs. DfM (Exp. 2 and 3). FEC were log transformed as ln(FEC + 25) and *Strongyloides* larval counts were log transformed as ln(recovery). Statistical inferences were made on transformed data and back-transformed least squares means and standard errors (Notter et al., 2017) presented. For other variables, least squares means are presented and variation around the means is denoted by the standard errors of the means.

Although the study in Exp 3 was conducted over a 30-day period, certain data (FEC, PCV, body weight, and body condition score) collected after day 21 were removed. At this point, 7 of the 12 Hampshire crosses were removed from pasture and study because of severe anemia. With several missing samples, ~~various~~ non-estimable parameters for FEC, PCV, body weight, and body condition score were found at day 30. Because pooled samples were used for coprocultures (described above), larval counts and recovery were not affected.

3. Results

3.1. Exp. 1

A short-term study was conducted to determine efficacy of a new lot of BioWorma®. The GIN were 89 % *H. contortus*, 5 % *Trichostrongylus* spp., and 6 % *Oesophagostomum* spp. Mean PCV of lambs was 26.5 ± 1.4 % and similar between treatments ($P > 0.10$). Mean FEC were similar between treatments ($P > 0.10$) and ranged from 800 to 20,400 eggs/g; least squares FEC means were 8.54 and 8.71 ± 0.22 (log transformed) or 5215 and 6163 eggs/g (back-transformed) in CON and Df fed lambs, respectively ($P > 0.10$). Feeding BioWorma® at the recommended dose rate led to a 73.3 % reduction in L3 recovered, or least squares means for recovery of 8.5 ± 1.1 % compared with 2.3 ± 1.1 % recovery in CON and Df groups, respectively, over the 3-day sample period ($P = 0.005$). There was no effect of day or an interaction ($P > 0.10$).

3.2. Exp. 2

The GIN were 88 % *H. contortus*, 6 % *Trichostrongylus* spp., and 5 % *Oesophagostomum* spp. Nearly all samples contained *S. papillosus* which were not included in total counts. The FEC ranged from 1350 to 12,300 eggs/g on day 0 and averaged 5792 eggs/g. The log-transformed FEC was similar among treatment groups ($P > 0.10$; 8.3 ± 0.32 eggs/g; 4048 eggs/g, back-transformed). The PCV (least squares mean for both groups, 24.8 ± 1.1 %) were similar among treatment groups ($P > 0.10$). Larval recovery was reduced in both Df groups as expected compared with the control group ($P < 0.001$; Fig. 1), and the recovery was lower in DfM than DfC ($P = 0.001$).

3.3. Exp. 3

From day 0 to day 21, least squares FEC means increased (day 0: 2430 eggs/g, and back-transformed, 1312 ± 339 eggs/g; day 21: 3412 eggs/g, and back-transformed 1400 ± 365 eggs/g; $P < 0.001$ for day for log-transformed values), and least squares PCV means decreased (day 0: 25.6 ± 0.58 %; day 21: 21.7 ± 0.58 %; $P < 0.001$). Lambs gained weight between day 0 (32.3 ± 0.69 kg) and day 21 (35.0 ± 0.69 kg; $P < 0.001$); mean body condition score varied between 2.6 ± 0.1 and 2.7 ± 0.1 ($P = 0.012$). As expected, there was no effect of treatment on these parameters measured ($P \geq 0.47$) though the treatment × day interaction was significant for FEC and PCV ($P \leq 0.02$). An effect of breed was noted for PCV and body weight in which the Katahdin lambs had higher PCV than Hampshire crosses (Katahdins: 25.7 ± 0.49 %; Hampshire crosses: 20.7 ± 0.89 %; $P < 0.001$) and body weights (Katahdins: 37.4 ± 0.66 kg; Hampshire crosses: 30.7 ± 1.20 kg; $P < 0.001$).

The strongylid larvae recovered were 69.9 % *H. contortus*, 27.9 % *Teladorsagia circumcincta* or *Trichostrongylus* spp. (which were not differentiated from each other), 2.1 % *Oesophagostomum* spp., and 0.2 % *Cooperia* spp. The percentage larvae recovered was consistently lower in the feces of the Df groups throughout the study compared to the control group (CON: 46.7 ± 2.3 %; DfC: 16.9 ± 2.3 %; DfM: 15.2 ± 2.3 %; $P < 0.001$; Fig. 2), but there was no difference in percentage larvae recovered between the two methods of Df delivery ($P = 0.62$). The effect of day was significant ($P < 0.001$) but not the interaction of treatment × day ($P = 0.22$).

There were no differences detected in the least squares mean log-transformed *Strongyloides* larvae/g of feces between treatments ($P = 0.31$; Fig. 3). A day effect was present ($P < 0.001$) and the treatment × day interaction was significant ($P = 0.008$). *Strongyloides* appeared to develop better in the DfC cultures than in the cultures for

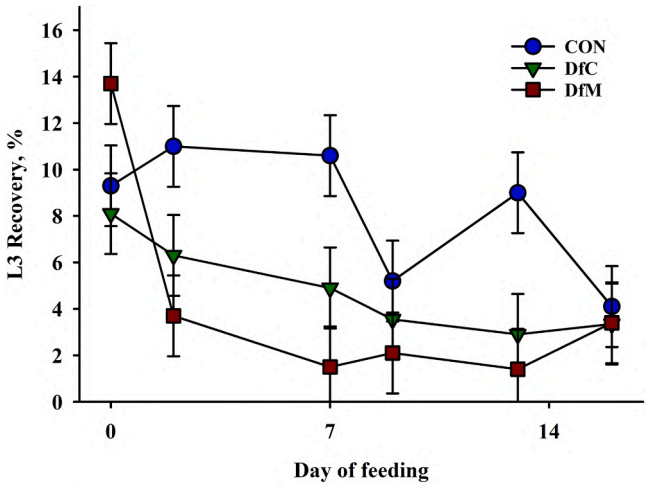

Fig. 1. Experiment 2. Effect of no *Duddingtonia flagrans* (Df; CON) or Df in BioWorma® top dressed on feed supplement (DfC) or included in trace mineralized salt (DfM; n = 8 lambs/treatment) on least squares means of percentage of larval recovery of gastrointestinal nematodes in cultured feces between Day 0 (first day of feeding) and Day 16. There was a treatment × date effect ($P < 0.02$) and orthogonal contrasts revealed a difference between CON and Df groups ($P = 0.01$) and between the Df groups ($P = 0.001$).

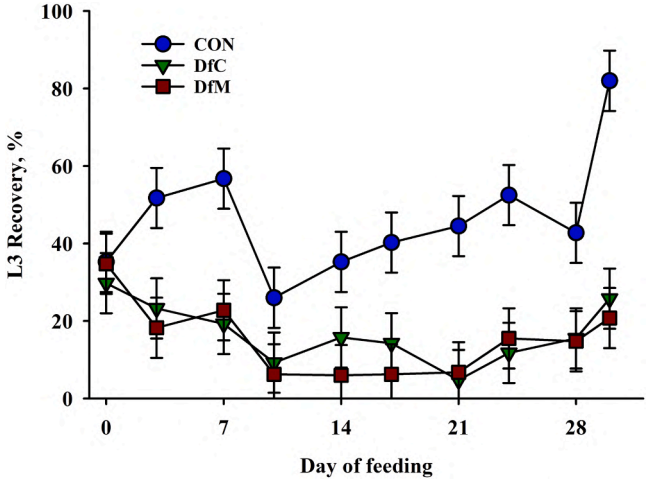

Fig. 2. Experiment 3. Effect of no *Duddingtonia flagrans* (Df; CON) or Df in BioWorma® top dressed on feed supplement (DfC) or included in trace mineralized salt (DfM; n = 4 cultures/treatment) on least squares means of percentage of recovery of strongylid larvae in cultured feces between Day 0 (first day of feeding) and Day 30. There was a treatment ($P < 0.001$) and day effect ($P < 0.001$), but no interaction ($P = 0.22$). Orthogonal contrasts showed a difference between CON and Df groups ($P < 0.001$) but no difference between the Df groups ($P = 0.62$).

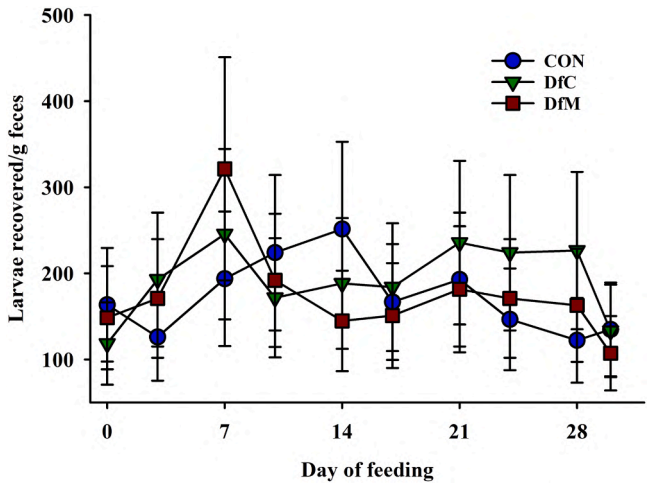

Fig. 3. Experiment 3. Effect of no *Duddingtonia flagrans* (Df; CON) or Df in BioWorma® top dressed on feed supplement (DfC) or included in trace mineralized salt (DfM; n = 4 cultures/treatment) on back-transformed least squares means of *Strongyloides* larvae/g feces cultured between Day 0 (first day of feeding) and Day 30. There was a treatment × day interaction ($P = 0.008$). Within day, treatments that were different ($P < 0.05$) from each other occurred on Day 0 (DfC < CON), 3 (CON < DfC), 14 (DfM < CON), 28 (CON < DfC), and 30 (DfM < both CON and DfC).

the other two treatments, but differences were only significant between CON and DfC on days 3 and 28 ($P < 0.05$).

4. Discussion

Experiment 1 was conducted to determine the efficacy of a newly produced lot of BioWorma®, Exp. 2 was performed to determine, under pen conditions, the efficacy of Df when "aged" in a mineral mix, and Exp. 3 was run to determine the efficacy of Df aged in a mineral mix and fed *ad libitum* under field conditions. Lambs used for these experiments were naturally infected with a mixed population of GIN, predominantly *H. contortus* and *T. circumcincta* or *Trichostrongylus* spp. *Strongyloides papillosus* was often detected in fecal samples but either not included in the total counts of GIN (Exp. 1 and 2) or counted separately from the strongylid larvae (Exp. 3). *Duddingtonia flagrans* previously has been shown to reduce the percentage of GIN in culture (Larsen et al., 1998; Braga et al., 2020), including *S. papillosus* (Braga et al., 2020), and chlamydospores of the fungus are now produced on an industrial scale and marketed as the commercial product, BioWorma®, or as part of a nutritional supplement, Livamol® with BioWorma®, in Australia, New Zealand, and the United States.

The validation trial (Exp. 1) demonstrated that the lot of BioWorma® used in the present studies contained viable chlamydospores, reducing L3 in coprocultures by 73 %. After consulting with the manufacturer, a previous lot was determined to have a low spore count and experiments conducted by the current authors using the previous lot led to results that showed no differences in strongylid larval recovery between no Df and Df treatments (data not shown). The pilot study conducted prior to Exp. 3 provided further evidence that the batch of product used in these experiments was performing as expected.

There is a large range in larval recovery using coprocultures which is influenced by temperature, humidity, and time (Coyne and Smith, 1992) and nematode species present (Dobson et al., 1992). Our L3 recovery in Exp. 2 was on the lower end of the range, and we speculate that this may possibly be due to higher pH of the tap water used in the cultures than we would normally expect for a laboratory water supply.

In addressing the main objective of these studies (Exp. 2 and 3), namely, the evaluation of the efficacy of Df chlamydospores after aging in mineral, it was uncertain whether the salts in the trace mineral would affect the quality of chlamydospores during the 7-day storage period. The results of Exp. 2 demonstrated that providing Df in a trace mineralized salt resulted in a 48 % reduction of strongylid L3 in cultured feces which was an even greater reduction than the 41 % reduction from Df when fed mixed directly with the feed according to manufacturer recommendations. The results of the field study (Exp. 3) confirmed the efficacy of the Df even after aging in mineral mix for 7–14 days, with the reduction in percentage larval recovery approximately 30 percentage points lower in the Df-fed treatments than the control, though no difference was noted between feeding the Df aged in mineral as a free-choice supplement compared with feeding it directly mixed with feed.

It is unclear why the BioWorma® had no apparent effect in reducing the *Strongyloides* larvae in the Df-fed treatments versus the controls in Exp 3. The BioWorma® label lists *Strongyloides* as one of the nematodes that it has efficacy in trapping and studies by others have demonstrated efficacy of Df against *S. papillosus* in goat (Campos et al., 2009, 2017; De Araújo et al. 2006) and sheep coprocultures (Braga et al., 2020) in the absence of other GIN. It may be that Df preferentially traps *H. contortus* over *S. papillosus*. Also, we speculate that the infection challenge by *Strongyloides* may have been substantial, particularly since the lambs were housed inside on dirt-floored barns at night, an environment which is extremely conducive to infection by the percutaneous route and likely the most important route of infection for the sheep in Exp 3.

To the author's knowledge, this is the first study to report on using Df in a trace mineralized salt. This offers producers who finish livestock on grass, and cannot offer grain or by-products, a technology to use Df to reduce infective larvae on pasture. Further research is needed to examine whether exposure of the trace mineral/Df mix offered in the field to moisture, by repeated licking by sheep or environmental sources of water would result in partial or complete sporulation of chlamydospores, rendering the product ineffective once deposited in GIN-infected fecal matter. The Df in Exp 3 was aged in the mineral mix for 7 days and that batch fed over the subsequent week, effectively testing the efficacy of the Df aged for up to 14 days in the mineral mix. While the effectiveness of BioWorma® in reducing pasture contamination relies on daily feeding, if producers follow a similar protocol to the one described, even if the chlamydospores were to become wet, fresh mineral mix would be offered within a week, potentially mitigating any major loss of the effectiveness of the control program.

5. Conclusions

In summary, a preliminary *in vivo* evaluation of the efficacy of Df in reducing GIN larvae is useful to confirm the efficacy, especially of a new lot, of the BioWorma® product. In both controlled intake and field studies, including Df either directly in the feed or in a trace mineral mix reduced larval recovery of strongylid GIN in lambs compared with a control. However, Df did not appear to reduce larval recovery of *S. papillosus*. A reduction in L3 recovery in Df offered in a trace mineral mix compared with dressing it on feed offers promise for the use of Df in farms that do not provide grain supplements.

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Declaration of Generative AI and AI-assisted technologies in the writing process

The authors declare that no AI-assisted technologies were used.

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Mention of trade names or commercial products in this manuscript is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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