



Research paper

Coadministration of nematophagous fungi for biological control over gastrointestinal helminths in sheep in the semiarid region of northeastern Brazil



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ABSTRACT

This study aimed to evaluate coadministration of *Duddingtonia flagrans* and *Monacrosporium thaumasium* in a sodium alginate matrix for controlling gastrointestinal helminths in young and adult sheep in the semiarid region of northeastern Brazil. An area of 1 ha was divided into two paddocks, in which two experimental groups (fungus and control) were formed, each consisting of six adult females and ten young males. In each group, two subgroups were formed in accordance with the animal category (adult or young). In the fungus group, each animal received 3 g of pellets containing 0.6 g of fungal mycelium, with 0.3 g of *D. flagrans* and 0.3 g of *M. thaumasium* for each 10 kg of body weight, in their feed twice a week, for six months. In the control group, each animal received 3 g of pellets without fungus for each 10 kg of body weight, in their feed twice a week, for six months, serving as a witness group. Reductions in numbers of eggs per gram of feces of 76% among the adult sheep in the fungus group and 83% among the young sheep in the fungus group were observed, in comparison with their respective control subgroups. The groups that received these fungi needed less salvage deworming and presented better packed cell volume percentages, better weight gain and lower levels of L3/kg dry matter in their paddock than the control groups. Thus, it was concluded that coadministration of *D. flagrans* and *M. thaumasium* was effective in controlling gastrointestinal helminths of adults and young sheep in the semiarid region of northeastern Brazil.

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1. Introduction

Research on new alternatives capable of controlling gastrointestinal helminths in small ruminants has been widely encouraged. In this regard, use of nematophagous fungi has shown promise in several experiments worldwide (Paraud et al., 2007; Silva et al., 2009; Sagués et al., 2011; Vilela et al., 2013).

After passage through the gastrointestinal tract, the fungi are eliminated together with the feces into the environment, where they colonize the dung and come into contact with the hatched

larvae. The fungi produce traps that lead to death among the larvae, thus decreasing the numbers of infective larvae on the pasture and preventing animal reinfection (Silva et al., 2009).

The species *Duddingtonia flagrans* and *Monacrosporium thaumasium* are the ones that have been studied most and that have the greatest potential for commercialization. Both of these species produce chlamydozoospores that are highly resistant and capable of passing through the gastrointestinal tract of animals in sodium alginate matrices. However, there may be differences in the action of different isolates of these species, as has been mentioned in several investigations in different regions (Araújo et al., 2004; Tavela et al., 2013; Braga and Araújo, 2014).

The effectiveness of fungal isolates of *D. flagrans* and *M. thaumasium* has already been proven in several experiments in Brazil (Braga et al., 2009; Tavela et al., 2011; Assis et al., 2013), and also,

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more specifically, in the semiarid region of northeastern Brazil (Araújo et al., 2007; Vilela et al., 2012, 2013). However, until now, these fungi have been evaluated separately and have not been tested on sheep in this region.

Braga et al. (2009) reported that it was unknown whether using these species in combination might add some kind of advantage from a biological point of view. Furthermore, according to Mota et al. (2003), it would be important to assess the action of these fungi under *in vivo* conditions, irrespective of whether administered separately or in combination, so that their action could be observed in the fecal environment. In this regard, Tavela et al. (2013) observed the viability of coadministration of *D. flagrans* and *M. thaumasium* on L3 cyathostomins after passage through the gastrointestinal tract of horses. However, subject to certain particularities, there have not been any reports showing that coadministration of these fungi on nematodes was capable of controlling these parasites in sheep in fields.

The objective of this study was to evaluate coadministration of *D. flagrans* and *M. thaumasium* in sodium alginate matrix on gastrointestinal helminths in young and adults sheep in the semiarid region of northeastern Brazil.

2. Material and methods

2.1. Fungi and production of mycelial mass

Isolates from two species of fungus that are predators of nematodes were used: *D. flagrans* (AC001) and *M. thaumasium* (NF34). These isolates were obtained from soils in the Zona da Mata region of the state of Minas Gerais, Brazil.

Mycelium was obtained by transferring cultured disks (approximately 4 mm in diameter) of the fungal isolates (*D. flagrans* and *M. thaumasium*) in 2% water agar (2% WA) to Erlenmeyer flasks of capacity 250 mL containing 150 mL of liquid glucose, sodium and yeast extract (GPY) medium. These were then incubated under agitation at 120 rpm in the dark at 26 °C for ten days. After this period, the mycelium was removed, filtered and weighed on an analytical balance. All the procedures followed the methodology of Araújo et al. (2010).

2.2. Experimental assay and animals

The experiment was conducted on the Farinha farm, located in the municipality of Patos, Paraíba, northeastern Brazil, at latitude 7° 1' 28" S, longitude 37° 16' 48" W, from April to September 2013. This region has a semiarid climate with two seasons: a rainy season from January to May, when an average of 98.6% of the annual precipitation occurs, and a dry season encompassing the rest of the year (Vilela et al., 2008).

A grazing area of 1.0 ha planted with Tifton grass (*Cynodon dactylon*) that was maintained under daily irrigation was divided into two paddocks. Each enclosure had previously been allowed to become infested with nematodes through 30 days of grazing by ten five-month-old male Dorper sheep. The mean number of eggs per gram of feces (EPG) was 4870 ± 990, and the nematodes comprised 70% *Haemonchus* sp., 22% *Trichostrongylus* spp., 6% *Strongyloides* sp. and 2% *Oesophagostomum* sp.

In order to choose an anthelmintic agent for subsequent use in the experiment, 24 sheep were divided into four groups and were subjected to the Fecal Egg Count Reduction Test (FECRT), as described by Coles et al. (1992). The anthelmintics used were moxidectin 0.2%, albendazole 5%, ivermectin 0.08% and levamisole hydrochloride 5%, which showed higher FECR (95%) with the chosen drug.

Thirty-two sheep of the Dorper breed were used in the experiment: 12 adult females (mean age 48 months) and 20 young males (mean age four months). Fifteen days before the start of the experiment, the animals received the anthelmintic agent levamisole hydrochloride (5 mg/kg body weight) orally for three consecutive days. Seven days after the first deworming, EPG counts were made using the technique of Gordon and Whitlock (1939). Three examinations were made on each sample and all the animals presented negative results.

Two experimental groups (fungus and control) were formed, each consisting of six adult females and ten young males. In each group, two subgroups were formed in accordance with the animal type (adult or young). In the fungus group, each animal received 3 g of pellets containing 0.6 g of fungal mycelium, with 0.3 g of *D. flagrans* and 0.3 g of *M. thaumasium* for each 10 kg of body weight, in their feed twice a week, for six months. In the control group, each animal received 3 g of pellets without fungus for each 10 kg of body weight, in their feed twice a week, for six months, serving as a witness group. Each group was kept in a paddock at a stocking rate of 1.5 animals per hectare. Every day, all the animals received supplementation consisting of protein-energy concentrate at the rate of 0.75% of body weight, along with balanced mineral salt and water *ad libitum*. To avoid mortality, salvage dewormings were conducted on animals that individually presented packed cell volume (PCV) less than 16%, using levamisole hydrochloride (5 mg/kg body weight).

Blood and fecal samples were collected from all the animals every 15 days for EPG analysis, fecal cultures (Roberts and O'Sullivan, 1950) and PCV analysis (Ferreira Neto et al., 1981). Every 15 days, the young animals of both subgroups were weighed to monitor their weight gain.

To determine the levels of environmental infestation by infective larvae (L3), each month, five samples of around 200 g of leaf mass each were collected from different areas in each paddock, thus totaling approximately 1000 g of material (Raynaud and Gruner, 1982). The samples were then placed individually in plastic buckets of capacity 10 L that had been filled with water at 37 °C. The buckets were left standing for four hours for the larvae to be released. The leaf mass was then removed carefully so that the larvae would not remain suspended in the water and the supernatant water was discarded. The precipitate was strained in a sieve of mesh size 200 µm for subsequent decantation into a Hoffman glass. The sediment was examined under an optical microscope and the larvae were quantified and identified. Subsequently, an arithmetic average of the total number of larvae recovered was taken from the five samples analyzed from each paddock. Immediately after the leaf mass had been removed from the plastic buckets, it was packed into metal containers and placed in a forced-air oven at 45 °C for 48 h. Subsequently the dry matter was weighed to determine the ratio of L3/kg of dry matter.

Meteorological data such as temperature, relative humidity and rainfall were collected monthly at a specialized station of the Federal University of Campina Grande, Patos, state of Paraíba. During the experiment, the temperature ranged from 22 to 36 °C and the relative humidity from 51 to 93%. The monthly rainfall was 150 mm³ in April, 80 mm³ in May, 70 mm³ in June and 45 mm³ in July, August and September 2013.

2.3. Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) and Tukey's test at 5% probability. The EPG values were analyzed using logarithmic transformation, *i.e.* log ($x + 1$), but they are presented in the figures as unprocessed arithmetic mean val-

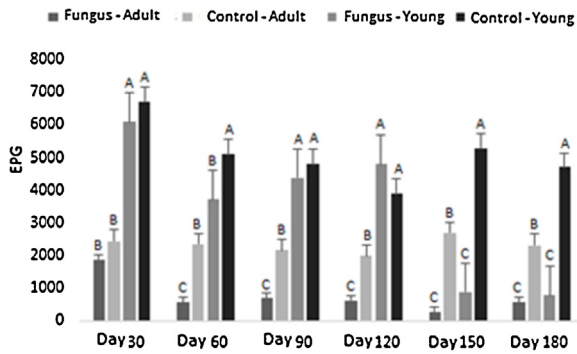


Fig. 1. Means and standard deviations of Eggs Per Gram of feces (EPG) of sheep in the fungus and control groups (adult and young subgroups) during 180 days in the semiarid region of northeastern Brazil. Values with the same letters are statistically similar by Tukey's test ($p < 0.05$).

ues. Analyses were performed using the BioEstat software 5.0 (Ayres et al., 2003).

3. Results

It was observed that the EPG of the group of adults that received the combination of fungi (adult subgroup of fungus group) differed statistically from the adult subgroup of the control group, on day 60 ($p < 0.05$). The average EPG count was 1890 on day 30, and reduced to 560 on day 180 (Fig. 1). The reduction in EPG count in the young subgroup of the fungus group was even more pronounced, reducing from 6090 on day 30–780 on day 180. This differed significantly from the young subgroup of the control group on day 60. The EPG levels in both control subgroups (adult and young) remained high throughout the experiment.

In the adult subgroup of the fungus group, no animal needed salvage deworming at any time during the experiment. However, adult subgroup of the control group, some animals required deworming on or after day 90: two sheep were dewormed once (one on day 90 and another on day 150), one sheep was dewormed twice (days 90 and 150) and another was dewormed three times (days 90, 120 and 180), thus totaling seven dewormings.

In the young subgroup of the fungus group, five lambs (5/10) required salvage deworming (one lamb dewormed one time; three lambs, three times; and one lamb, four times, totaling 14 dewormings, of which 11 were on day 90 of the experiment. In the young subgroup of the control group, all the animals (10/10) were dewormed at least once: two lambs were dewormed once; five lambs, two times; and three lambs, three times, totaling 21

Table 1

Percentage of infective larvae of *Haemonchus* sp. (H), *Trichostrongylus* spp. (T), *Strongyloides* sp. (S) and *Oesophagostomum* sp. (O) in fecal cultures of sheep treated with the combination of *D. flagrans* and *M. thaumasium* (fungus group- adult and Young subgroups) and not treated (control group-adult and Young subgroups) during 180 days in the semiarid region of northeastern Brazil.

Groups		Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
Fungi-Adults	H	85	78	93	90	89	89
	T	12	15	6	9	7	11
	S	–	6	–	1	2	–
	O	3	1	1	–	2	–
Control-Adults	H	87	93	90	78	75	96
	T	13	5	10	20	20	4
	S	–	1	–	2	2	–
	O	–	1	–	–	3	–
Fungi-Young	H	75	72	72	79	86	81
	T	20	15	18	11	10	13
	S	4	12	10	10	3	6
	O	–	1	–	–	1	–
Control-Young	H	78	82	75	73	79	82
	T	10	4	16	14	21	15
	S	12	13	9	3	–	2
	O	–	1	–	–	–	1

dewormings that were well distributed across the experimental period (up to day 90: 10 dewormings; days 91–180: 11 dewormings).

In fecal cultures, it was observed that *Haemonchus* sp. was the most prevalent helminth genus, with percentages ranging from 72% to 96% (Table 1). *Trichostrongylus* spp. was the second most prevalent, followed by *Strongyloides* sp. and *Oesophagostomum* spp.

The young subgroup of the fungus group showed weight gains that were statistically greater ($p < 0.05$) than those of the young subgroup of the control group of the 90-day experiment, such that the animals reached the end of the experiment with a mean weight of 29.8 kg (mean weight gain of 18.7 kg), in comparison with 19.4 kg (mean weight gain of 8.1 kg) in the young subgroup of the control group (Fig. 2).

The groups that received the combination of nematophagous fungi (adult and young subgroups of the fungus group) showed PCV percentages that were statistically greater ($p < 0.05$) than those of the control groups on days 150 and 180 (Fig. 3).

There was a high rate of environmental infestation in the paddock at the beginning of the experiment. However, the mean L3/kg of dry matter found from the grass that had been sampled from the pasture presented statistically significant differences ($p < 0.05$) on days 60, 150 and 180 (Fig. 4). The results from day 180 can be highlighted, in which the paddock used by the adult and young sub-

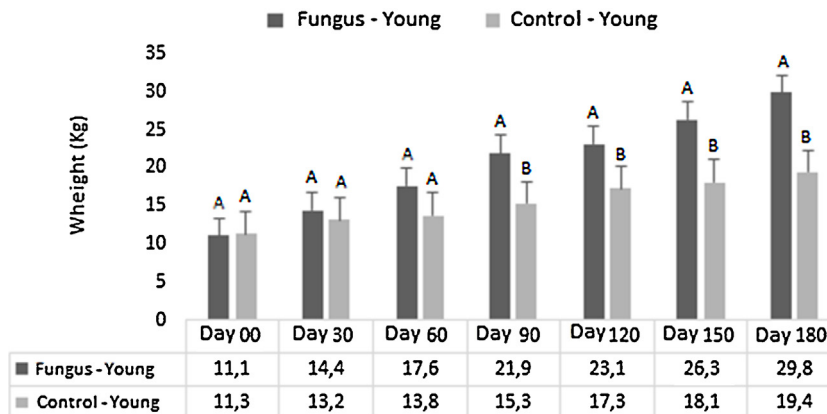


Fig. 2. Means and standard deviations of body weight (kg) of sheep in the young subgroups of the fungus and control groups during 180 days in the semiarid region of northeastern Brazil. Values with the same letters are statistically similar by Tukey's test ($p < 0.05$).

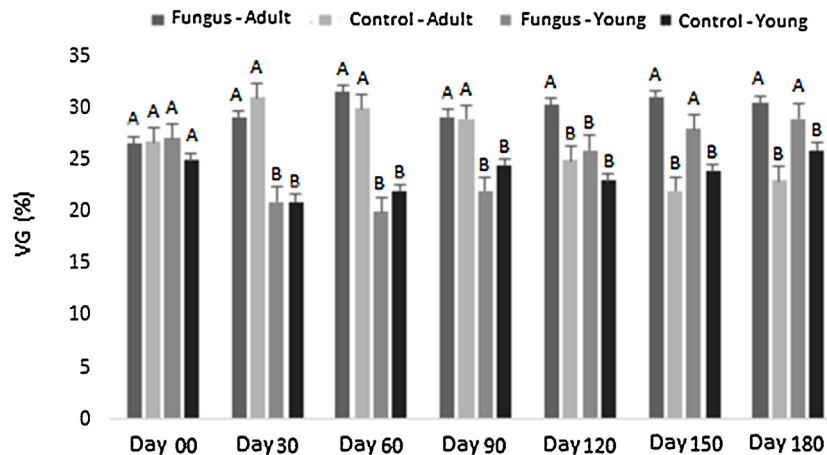


Fig. 3. Percentage of sheep Packed Cell Volume (PCV) of sheep in the fungus and control groups (adult and young subgroups) during 180 days in the semiarid region of northeastern Brazil. Values with the same letters are statistically similar by Tukey's test ($p < 0.05$).

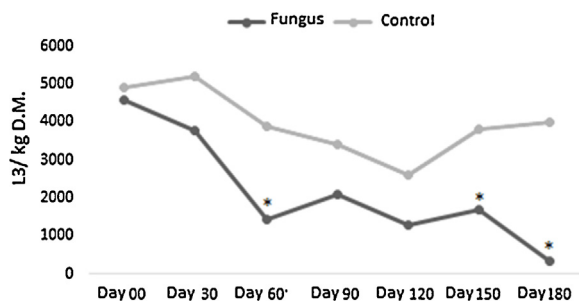


Fig. 4. Arithmetic means of the number of infective larvae/kg of dry matter (L3/kg D.M.) in paddocks grazed by sheep treated with the combination of *D. flagrans* and *M. thausasium* (fungus group) and not treated (control group). Asterisks indicate days with statistically significant differences between the groups—Tukey's test ($p < 0.05$).

groups of the fungus group showed 91.6% less L3/kg of dry matter than the paddock used by the control group.

4. Discussion

Studies evaluating coadministration of nematophagous fungi are scarce, and this work was the first to evaluate combined use of the fungi *D. flagrans* and *M. thausasium* in sheep.

Use of these fungi at a dose of 3 g of pellets per 10 kg of body weight, twice a week, was shown to be effective in controlling gastrointestinal helminths in sheep, particularly in adult sheep. The EPG rates in the adult sheep of this study remained statistically lower ($p < 0.05$) throughout the experiment, without administration of salvage deworming, reaching a 76% reduction in EPG on day 180, in comparison with the control group, which still needed seven dewormings. Similar results were observed by Silva et al. (2009), who administered sodium alginate pellets containing *D. flagrans* at a dose of 1 g per 10 kg of body weight (0.2 g of mycelium), twice a week, for five months, in southeastern Brazil, and achieved a 71.6% reduction in EPG.

A marked reduction in EPG was also observed in the young subgroup of the fungus group, which reached day 180 with a 83% reduction in EPG, in comparison with the young subgroup of the control group. Half of the young sheep in the fungus group underwent salvage dewormings (10/21). The reductions in EPG obtained in this experiment were greater than those obtained using both fungi separately among goats at the same dosage, in the same semiarid environment under the challenge of grazing pressure that was five times lower, for six months. In that experiment, use of *D. fla-*

grans alone reduced EPG by 58.9% (Vilela et al., 2012) and use of *M. thausasium* alone reduced it by 34% (Vilela et al., 2013). However, the large reduction in parasite burden observed in the present experiment occurred gradually over a period of months. Deworming should be applied to young animals, especially during early use of nematophagous fungi and/or the first months of life of these animals, so as to avoid deaths.

It was observed that *Haemonchus* sp. was the most prevalent helminth in fecal cultures, thus confirming the results of Vieira et al. (2014), who studied the prevalence of gastrointestinal helminths of goats in the Sertão mesoregion of Paraíba. They observed that *Haemonchus* sp. was the most prevalent helminth in that region, accounting for 83.2% of the worm burden of these animals.

The young subgroup of the fungus group showed an average weight gain of 18.7 kg, which was statistically greater ($p < 0.05$) than that of the young subgroup of the control group, with a mean weight gain of 8.1 kg. In a similar study on young goats, in which pellets of the fungus *D. flagrans* alone were administered, Vilela et al. (2012) observed an average weight gain of 9.3 kg, while the control group showed a reduction in weight of 1.1 kg. Silva et al. (2009) found no statistically significant difference ($p > 0.05$) in weight gain among ewes, between those treated with *D. flagrans* and the control group, probably because their animals were adults (between 24 and 48 months of age) and were receiving supplementation for weight maintenance. The high weight gain observed in the present experiment was due not only to the good response from combined use of the fungi, but also to the fact that the animals used (Dorper breed) have high feed conversion and carcass yield when they are kept under good feeding and sanitary conditions (Snowders and Duckett, 2003).

The groups that received the combination of nematophagous fungi (adult and young subgroups of the fungus group) showed PCV percentages that were statistically greater ($p < 0.05$) than those of the control groups over the 60 days of the experiment. Similar results were observed by Vilela et al. (2013): in that study, in which pellets containing *M. thausasium* alone were administered to goats, PCV levels that were statistically greater than those of the control group were observed. These results are not in line with those of Silva et al. (2010), in which the PCV levels in sheep that received pellets containing *D. flagrans* alone were slightly lower than those of the control group.

There was a marked reduction in infestation levels in the paddock where the fungus group was kept, especially on day 180, when the level of L3/kg of dry matter was 91.6% lower than in the paddock of the control group. Rainfall data may not have influ-

enced the results obtained in this experiment, since the paddocks were irrigated daily. However, this explains the high environmental infestation in the control group paddock in the last 90 days of the experiment, when the rain levels observed did not exceed 45 mm³ per month. These results demonstrate that there was a beneficial interaction between the fungi *D. flagrans* and *M. thausasium*, as predators of larvae in the environment. In this manner, the numbers of larvae were significantly reduced, along with the degree of reinfection of animals. This made it possible for the animals' parasite loads to become lower, which was shown by the 76% reduction in EPG among these animals. Almeida et al. (2012) observed a reduction in the levels of environmental infestation, such that it became significantly lower than the control group ($p < 0.05$) after five months of administration of pellets containing *D. flagrans*, to horses in southeastern Brazil. Graminha et al. (2005) also observed reductions in L3/kg of dry matter, in managing nematophagous fungi in sheep.

5. Conclusion

Coadministration of pellets containing *D. flagrans* and *M. thausasium* at the dosage of this experiment proved to be effective in controlling gastrointestinal helminths in young and adult sheep in the semiarid region of northeastern Brazil.

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