Use of *Duddingtonia flagrans* in the control of gastrointestinal nematodes of feedlot goats

Utilização de *Duddingtonia flagrans* no controle dos nematódeos gastrintestinais de caprinos mantidos em confinamento

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Highlights:
D. flagrans was effective on gastrointestinal helminthiases of feedlot goats.
D. flagrans group had better PCV values.
D. flagrans group had EPG reduction of 92.3%.
D. flagrans group showed a final mean weight gain of 8.8 kg.

Abstract

This study aimed to evaluate the use of a sodium alginate matrix-pelletized formulation of *Duddingtonia flagrans* for biological control of gastrointestinal nematodiasis in feedlot goats in the semiarid region of Northeastern Brazil. We used 20 Saanen female goats (age, 4 months; average weight, 12 kg) that did not receive anthelmintic treatment and had counting of eggs per gram of faeces (EPGs) $\geq$ 500. The animals were divided into two groups: in group 1 (*D. flagrans* group), each animal received 3 g of pellets (0.6 g of *D. flagrans* mycelium) per 10 kg of body weight, twice a week, over 4 months; and in group 2 (control group), each animal received 3 g of pellets without fungus per 10 kg of body weight, twice a week, over 4 months. Each group was maintained in a separate 15-m² stall. Larval cultures and measurements of weight, EPG, and packed cell volume (PCV) were performed every 15 days. We observed low EPG levels in the *D. flagrans* group throughout the experimental period, with a significant difference ($p < 0.05$) on day 30 and from day 60, having, at the end of the experiment, average OPG values of only 150, reduction of 92.3% when compared to control group. *Haemonchus* sp. was the most prevalent helminth in all larval cultures. The *D. flagrans* group showed a mean weight gain of 8.8 kg at the end of the experiment ($p < 0.05$), while the control group showed a mean weight gain of 4.8 kg. The best PCV results ($p < 0.05$) were also observed in the *D. flagrans* group from day 30. Thus, the use of *D. flagrans* pellets in a sodium alginate matrix was effective in controlling gastrointestinal nematodiasis of feedlot goats in the semiarid region of Northeastern Brazil.
Key words: Farming goat. Biological control. *Haemonchus* sp. Helminthiasis.

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Resumo

Objetivou-se avaliar a utilização da formulação peletizada em matriz de alginato de sódio de *Duddingtonia flagrans* no controle biológico das nematodeoses gastrintestinais de caprinos mantidos em confinamento no semiárido do Nordeste brasileiro. Foram utilizados 20 caprinos da raça Saanen, fêmeas, com quatro meses de idade, média de peso de 12 kg, sem tratamento anti-helmíntico prévio e com contagem de ovos por grama de fezes (OPG) ≥ 500. Os animais foram divididos em dois grupos: grupo 1, cada animal recebeu na ração 3 g de péletes (0,6 g de micélio fúngico de *D. flagrans*) para cada 10 kg de peso vivo, duas vezes por semana, durante quatro meses; grupo 2, cada animal recebeu na ração 3 g de péletes sem fungos para cada 10 kg de peso vivo, duas vezes por semana, durante quatro meses, servindo como grupo controle. Cada grupo permaneceu em uma baia de 15 m². Foram realizadas contagens de OPG, determinação de volume globular (VG), coproculturas e pesagem dos animais a cada 15 dias. Observaram-se baixos valores de OPG no grupo *D. flagrans* durante todo o experimento, com diferença significativa (p < 0,05) no dia 30 e a partir do dia 60, tendo, ao final do experimento, valores médios de OPG de apenas 150. No grupo Controle, a contagem final foi de 1950 OPG. *Haemonchus* sp. foi o gênero de helminto mais prevalente em todas as coproculturas. O grupo *D. flagrans* apresentou média de ganho de peso de 8,8 kg ao final do experimento e o grupo Controle, 4,8 kg (p < 0,05). Também foram observados os melhores índices de VG (p < 0,05) no grupo *D. flagrans* a partir do dia 30. Concluiu-se que a utilização de péletes em matriz de alginato de sódio de *D. flagrans* foi eficaz no controle das nematodeoses gastrintestinais de caprinos mantidos em confinamento no semiárido do Nordeste brasileiro.
Palavras-chave: Caprinocultura. Controle biológico. *Haemonchus* sp. Verminose.

Introduction

Goat farming is an activity of great importance in Northeastern Brazil, mainly in the semiarid region, where goat meat is considered the main source of animal protein. Although numerical expressive, goat herds show low productive indexes because of several factors, including infections by gastrointestinal helminths (Salgado, & Santos, 2016). In the semiarid, there are a predominant goat production in small farms, where feedlot dairy goats are raised in confinement, in precarious facilities characterized by free housing in concrete flooring (Riet-Correa et al., 2013). Another frequent problem is food and water contamination with feces, due to the inadequate positioning of feeders and drinking fountains, which are not placed on the external side, favoring the occurrence of neonatal infections, coccidiosis and gastrointestinal helminthiasis (Patil, 2010).

Moreover, due to the producers' lack of technical information, the indiscriminate use of anthelmintics has resulted in the development of resistance to the various molecules available in the market, as ivermectin, moxidectin, levamisole, albendazole, closantel and monepantel, causing major problems in the control of helminthiasis in goats and sheep in the Brazilian semiarid region (Lima et al., 2010; Vieira et al., 2014; F. F. Silva, Bezerra, Feitosa, & Vilela, 2018).

In this context, multiple efforts have been made to identify alternative agents capable of controlling gastrointestinal helminths in small ruminants. The use of nematophagous fungi in sodium alginate-based formulations is a promising option for *in vitro* and *in vivo* control of parasites of various domestic animal species, including goats, by producing traps that capture and fix nematodes by destroying their internal organs (Paraud, Pors, & Chartier, 2007; Braga et al., 2009; A. R. Silva, Araújo, Braga, Alves, & Frassy, 2011; Vilela et al., 2013; Silveira et al., 2017). Fungi pelleted in sodium alginate can be kept in stock and are made from inert materials, allowing their use mixed in food supplement supplied to herds. The orally administered pellets are eliminated in the faeces for up to 120 hours (J.

V. Araújo, 2009). The decrease in the larvae caused by administration of nematophagous fungi prevents clinical parasitism, reducing reinfection of the animals in the pasture and allowing them to develop natural immunity against nematodes (J. V. Araújo, Gomes, & Guimarães, 1998).

Duddingtonia flagrans is the most studied and is considered the most promising species for the control of gastrointestinal helminths of domestic animals (Larsen, Faedo, Waller, & Hennessy, 1998; Faedo et al., 2002). In addition, it has been used successfully for field control of parasitic helminths of production animals (Braga & Araújo, 2014). Feedlot goats constantly require anthelmintic treatment because they are more susceptible to reinfections by larvae of gastrointestinal parasites, usually by direct and permanent contact with the feces. However, there are no studies evaluating the action of nematophagous fungi in controlling reinfection among confined animals in non-slatted floor.

The objective of this study was to evaluate the use of a pelleted formulation composed of *D. flagrans* in a sodium alginate matrix for the biological control of gastrointestinal nematodes of confined goats in the semiarid state of Paraíba, Northeast Brazil.

Materials and Methods

Fungi and production of mycelial mass

An isolate of the nematode predator fungus *D. flagrans* (AC001) was used for the experiment. This isolate was obtained from soils from the Zona da Mata region of the State of Minas Gerais, Brazil, and has been stored at the parasitology laboratory of the Universidade Federal de Viçosa, MG, Brazil.

The fungal mycelia were obtained by transferring culture disks (approximately 4 mm in diameter) of the fungal isolates (*D. flagrans*) in 2% agar-water (2% AA) to 250-ml Erlenmeyer flasks with 150 ml of GPY liquid medium (glucose, sodium peptone and yeast extract), followed by incubation

at 26 °C and 120 rpm in the dark for 10 days. After this period, the mycelia were removed, filtered, and weighed in a precision analytical balance. All procedures followed the methodology proposed by J. M. Araújo, Araújo, Braga and Carvalho (2010).

Experimental and animal testing

The experiment was carried out at a farm in the city of Patos, Paraíba, Northeast Brazil, latitude 7º1'28" S, longitude 37º16'48" W, from February to May 2015. To simulate the characteristic facilities of the semiarid region, as described by Riet-Correa et al. (2013), the experiment was carried out in a shed with two stalls of 15 m² each. The stalls had concrete flooring, roofing with clay tiles, troughs, and drinking fountains. They underwent complete sanitation every 15 days, during which the bed composed of wood shavings was completely replaced and the floors and walls were washed and disinfected with 1% sodium hypochlorite. An experimental group was allocated to each stall.

In order to select the anthelmintic to be used, 18 goats were divided into three groups and underwent the faecal egg count reduction (FECR) test according to the method described by Coles et al. (1992). The anthelmintics orally used were moxidectin 0.2% - Cydectin oral®/Fort Dodge (0.5 mg/kg body weight), ivermectin 0.08% - Ivomec ovino®/Merial (0.2 mg/kg body weight), and levamisole hydrochloride 5% - Ripercol®/Fort Dodge (5 mg/kg body weight), which showed a higher FECR (91%) and was chosen for the study.

Twenty female Saanen goats (age, 4 months; mean weight, 12 ± 1.93 kg) that had not received anthelmintic treatment previously and had egg counts per gram of faeces (EPGs) ≥ 500 were used. The animals were divided into two groups: group 1 (*D. flagrans* group), in which each animal received 3 g of pellets containing 0.6 g of *D. flagrans* mycelia per 10 kg of body weight twice a week for four months; and group 2 (control group), in which each animal received 3 g of fungus-free pellets per 10 kg

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of body weight twice a week for four months (A. R. Silva et al., 2011; Silveira et al., 2017).

The animals received a complete diet based on corn and soybean meal, amount equivalent to 1.5% of live weight, as well as hay of Tifton (*Cynodon dactylon*) grass and a complete mineral mixture and water *ad libitum*, in order to meet the nutritional requirements for sheep according to National Research Council [NRC] (1985).

To prevent mortality, salvage anthelmintic treatments were performed individually when the animals had a packed cell volume (PCV) of less than 16%; the dewormer used was levamisole hydrochloride (5 mg/kg body weight).

Faecal and blood collection were performed every 15 days for EPG analyses (Gordon & Whitlock, 1939), coprocultures (Roberts & O'Sullivan, 1950), and PCV measurements (Ferreira, Viana, & Magalhães, 1981). Every 15 days, the animals were also weighed to monitor development.

The specialized station of the Federal University of Campina Grande, Patos-PB campus, supplied the meteorological data such as temperature, relative humidity and rainfall. During the experiment, the temperature varied from 26 °C to 36 ºC, the relative air humidity from 51% to 88% and the monthly rainfall was 70 mm in June 2015 and 45 mm in July-September 2015.

The study was approved by the Animal Care and Ethics Committee, Federal University of Campina Grande, Patos, Paraíba, Brazil (Approval number: 23000,000525,2015/13).

Statistical analysis

Data were assessed with one-way analysis of variance (ANOVA) and test t 5% probability. The EPG values were analysed using log (x + 1) logarithmic transformation; however, they are presented in the figures as arithmetic means of the untransformed values. The analyses were performed using BioEstat 5.0 Software (Ayres, Ayres, Ayres, & Santos, 2003).

Results

The *D. flagrans* group showed low EPG values during the entire experiment, with a statistically significant difference ($p < 0.05$) in comparison with the control group on day 30 and from day 60 (Figure 1). At the end of the experiment, the EPG value in the *D. flagrans* group was only 150 ± 135, whereas that in the control group was 1950 ± 955.

No animals of the *D. flagrans* group required salvage anthelmintic treatment. However, in the control group, four goats were dewormed: two on day 45 and two on day 60.

The most prevalent helminth in all coprocultures was *Haemonchus* sp., followed by *Trichostrongylus* spp., *Strongyloides* sp., and *Oesophagostomum* sp. (Table 1).

There was a significant statistical difference ($p < 0.05$) between the groups in relation to the weight gain from day 90 (Figure 2). The *D. flagrans* group had a mean weight gain of 8.8 ± 1.46 kg throughout the experiment. In the control group, the mean weight gain was 4.8 ± 0.91 kg.
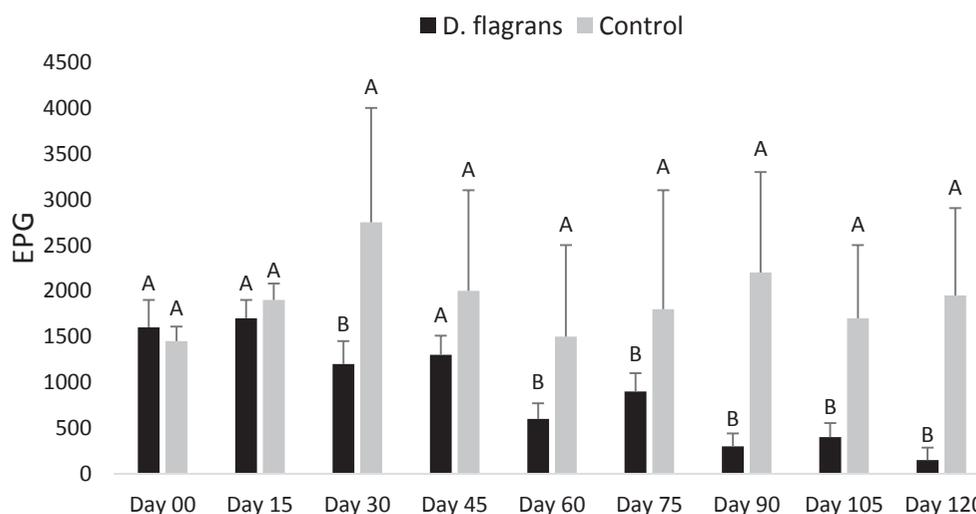
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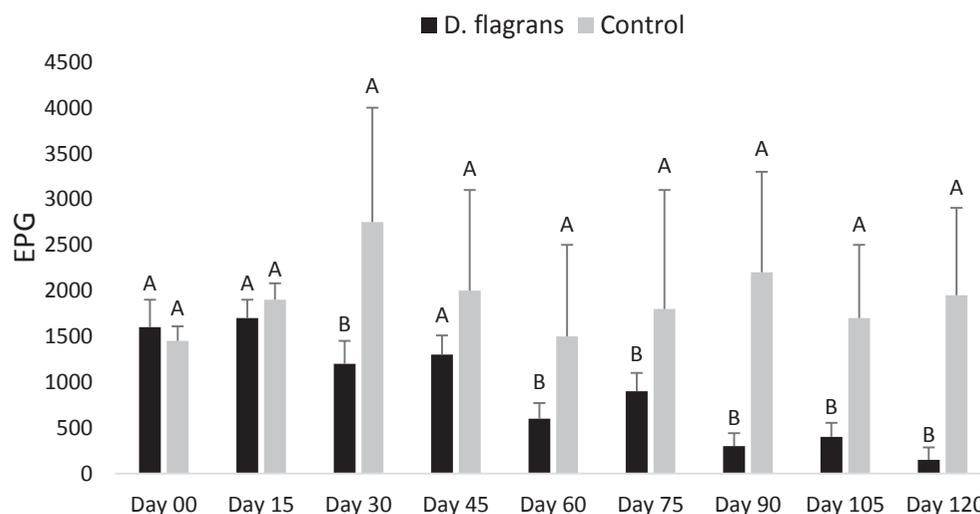
Figure 1. Monthly means and standard deviations of egg counts per gram of faeces (EPG) of goats of the *D. flagrans* (0.6 g/10 kg body weight, twice a week) and control groups kept in confinement for 120 days in the Northeastern semiarid region. The values followed by equal letters are statistically similar ($p > 0.05$)–Tukey's test at 5%.

Table 1
Percentage of infecting larvae of *Haemonchus* sp. (H), *Trichostrongylus* spp. (T), *Strongyloides* sp. (S), and *Oesophagostomum* sp. (O) in coprocultures of goats treated with *D. flagrans* and control group, kept in confinement for 120 days in the Semiarid region of Northeastern Brazil

Groups		Days								
		00	15	30	45	60	75	90	105	120
	H	89	89	92	88	90	81	76	88	91
D. flagrans	T	10	6	4	8	6	12	16	7	8
	S	1	5	3	2	4	3	5	4	1
	O	-	-	1	2	-	4	3	1	-
	H	88	93	92	88	90	86	89	83	82
Control	T	11	4	5	6	7	10	9	11	12
	S	-	3	2	4	3	3	2	4	3
	O	1	-	1	2	-	1	-	2	3

Figure 2. Monthly means and standard deviations of the weight (kg) of goats in the *D. flagrans* (0.6 g/10 kg of live weight, twice a week) and control groups kept in confinement for 120 days in the Northeastern semiarid region. The values followed by equal letters are statistically similar ($p > 0.05$) - Tukey's test at 5%.

The groups differed significantly ($p < 0.05$) in relation to the percentage of PCV on days 30, 45, 75, 105, and 120. The best PCV indices were observed in *D. flagrans* group (Figure 3).
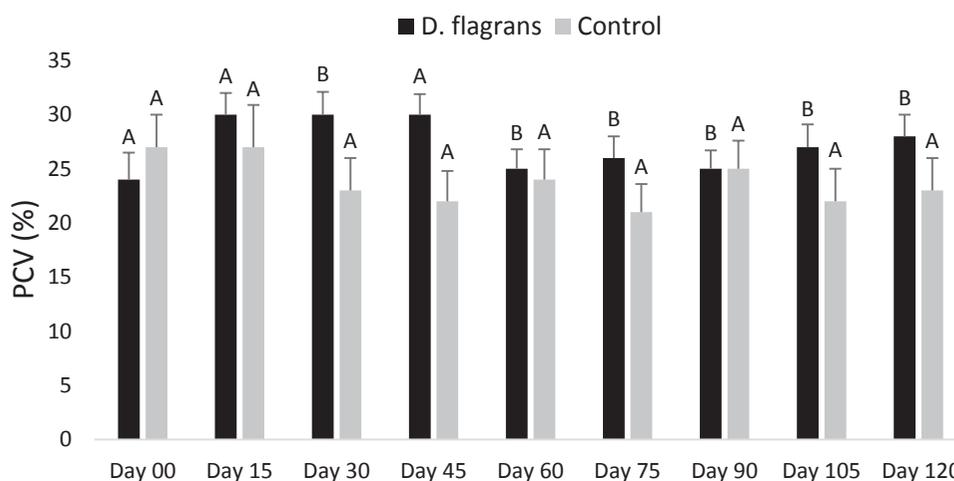
Figure 3. Monthly means and standard deviations of the packed cell volume (PCV) of goats in *D. flagrans* (0.6 g/ 10 kg of live weight, twice a week) and control groups kept in confinement for 120 days in the Northeastern semiarid region. The values followed by equal letters are statistically similar ($p > 0.05$) -Tukey's test at 5%.

Discussion

The *D. flagrans* group showed low EPG values throughout the experiment ($p < 0.05$), with the parasite load on day 120 being 92% lower than that in the control group. A reduction in small ruminant EPG, although lower than that noted in the present study, was observed by Vilela et al. (2012), Vilela et al. (2013), Vilela et al. (2016) and Vilela et al. (2018) after the use of pellets of nematophagous fungi in

the Northeastern semiarid region. However, these animals were kept in an extensive regime and fed on pasture native to the Caatinga, which increased reinfection rates.

Since the parasitic load of the *D. flagrans* group remained low throughout the experiment, no salvage anthelmintic treatment was required for these animals. On the other hand, four animals in the control group required anthelmintic treatment when they showed PCV ≤ 16%.

Haemonchus sp. was the most frequently observed helminth genus in the coprocultures. According to Vieira et al. (2014), who studied the prevalence of goat gastrointestinal helminths in the Paraíba semiarid region, the genus *Haemonchus* sp., represents 83.2% of the helminth fauna of the animals. In tropical countries, *Haemonchus contortus* is the most prevalent nematode in herds, causing severe losses due to the high pathogenic pressure it exerts through hematophagy (Kassai, 1999).

From day 90, the *D. flagrans* group showed better weight gain, with a mean gain of 8.8 kg at the end of the experiment. In the control group, the mean weight gain was 4.8 kg. Studies by J. V. Araújo, Rodrigues, Silva and Vieira (2007), Vilela et al. (2012), and Vilela et al. (2016) also reported higher weight gain in groups of small ruminants receiving pelleted formulations of sodium alginate matrix containing nematophagous fungi in the Brazilian semiarid region. Due to the reduced reinfection rates in the animals, they were able to overcome preexisting helminth infections, thereby increasing weight gain.

The *D. flagrans* group showed better PCV indices from day 30. Similar results were observed by Vilela et al. (2012), who reported better PCV percentages 60, 90, and 120 days after administration of *D. flagrans* pellets to goats. However, B. F. Silva et al. (2010) did not find a significant statistical difference in PCV in sheep that received *D. flagrans* pellets in Southeastern Brazil.

Animals kept in confinement in poorly sanitized stalls with concrete flooring constantly require anthelmintic treatment, even when they receive pasture that is free of L_3 gastrointestinal nematodes, since these conditions favour the addition of moisture in the faeces through the urine of the animals, which facilitates the development of L_3 parasites and allows reinfection.

The use of the pelleted formulation of *D. flagrans* in a sodium alginate matrix could reduce reinfection by predation of L_3 parasites in the environment, which in this case was the stall of the *D. flagrans* group. This approach reduced the parasitic load to the point where no salvage anthelmintic treatment was needed, which directly led to better PCV indices and weight gain.

Conclusion

The findings show that the sodium alginate matrix-pelleted formulation of *D. flagrans* was effective in achieving biological control of gastrointestinal nematodes in goats kept in confinement in the Brazilian Northeast semiarid region.

Acknowledgment

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