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Short Communication

Efficacy of a commercial fungal formulation containing *Duddingtonia flagrans* (Bioverm®) for controlling bovine gastrointestinal nematodes

Eficácia de uma formulação comercial contendo *Duddingtonia flagrans* (Bioverm®) no controle de nematódeos gastrintestinais de bovinos

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Abstract

Bioverm® (*Duddingtonia flagrans*) is a fungal formulation indicated for controlling gastrointestinal nematodes in ruminants and horses, which has recently been authorized for commercialization in Brazil. The objective was to determine the efficiency of Bioverm® against larvae of gastrointestinal nematodes after passage through the gastrointestinal tract of cattle. Twelve animals were used, divided into two groups. In the treated group, a single dose of 1 g of Bioverm® per 10 kg of live weight (containing 10^5 chlamydospores of *D. flagrans*) was provided for each animal. Fecal samples were obtained from the animals in each group at 12, 24, 36, 48, 60 and 72 hours after administration. In assay A, 2 g of feces were added to Petri dishes containing 2% agar-water medium. In assay B, coprocultures were performed. In both assays, the peak of larval predation occurred within 48 hours after administration of Bioverm®. In assay A, a significant larval reduction ($P < 0.05$) was seen at 48 h (88.2%). In assay B, significant reductions ($P < 0.05$) were seen at 36 h (43.7%) and 48 h (82.3%). Bioverm® showed high predatory capacity after passage through the gastrointestinal tract of cattle and was effective for controlling gastrointestinal nematodes.

Keywords: Biological control, cattle, helminthiasis, nematophagous fungi.

Resumo

O Bioverm® (*Duddingtonia flagrans*) é uma formulação fúngica indicada para o controle de nematódeos gastrintestinais de ruminantes e equídeos, recentemente autorizado para a comercialização no Brasil. Objetivou-se determinar a eficiência do Bioverm® contra larvas de nematódeos gastrintestinais após a passagem pelo trato gastrintestinal de bovinos. Foram utilizados doze bovinos divididos em dois grupos. No grupo tratado, foi fornecida, por animal, a dose única de 1g (10^5 clamidósporos de *D. flagrans*) do Bioverm® para cada 10 kg de peso vivo. Foram obtidas amostras fecais dos animais de cada grupo a partir de 12, 24, 36, 48, 60 e 72 horas após a administração. No ensaio A, 2g de fezes foram adicionadas em placas de Petri contendo meio ágar-água 2%. No ensaio B, foram realizadas coproculturas. Em ambos os ensaios, o pico de predação larval ocorreu em 48 horas após a administração do Bioverm®. No ensaio A, houve redução larval significativa ($P<0,05$) em 48h (88,2%). No ensaio B, as reduções significativas ($P<0,05$) ocorreram em 36h (43,7%) e 48h (82,3%). O Bioverm® apresentou elevada capacidade predatória após a passagem pelo trato gastrintestinal de bovinos, sendo eficaz no controle dos nematódeos gastrintestinais.

Palavras-chave: Controle biológico, bovinos, helmintoses, fungos nematófagos.

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Use of nematophagous fungi for biological control over gastrointestinal helminths in animals has been considered to be a promising alternative method. These fungi prey on infective larvae in the environment, thereby reducing the contamination of pastures and reinfection of animals (Mota et al., 2003; Braga & Araújo, 2014; Vilela et al., 2018).

Oral administration of fungi is the most practical way of supplying them to animals, because they have the capacity to mix with the animals' feces, such that they are released into the environment together with the parasites' eggs (Fernandes et al., 2017; Rodrigues et al., 2020). The fungal species that form chlamydospores stand out due to their ability to survive passage through the animals' gastrointestinal tract (Chandrawathani et al., 2004; Luns et al., 2018; Costa et al., 2019). *Duddingtonia flagrans* is the species that has been most studied. It has been found to be effective for controlling gastrointestinal helminths in animals (Larsen et al., 1992; Maciel et al., 2006; Braga et al., 2010; Braga & Araújo, 2014; Vilela et al., 2020).

These advances in knowledge have led to development of a Brazilian biological control product called Bioverm® (GhenVet Saúde Animal Ltda.), which has been licensed for commercialization by the Ministry of Agriculture, Livestock and Supply (Ministério da Agricultura, Pecuária e Abastecimento, MAPA) under no. SP-10.261/2019. The composition of Bioverm® includes chlamydospores of the fungus *D. flagrans*, and it is indicated for controlling gastrointestinal helminthiasis in ruminants and horses.

The first report on the effectiveness of Bioverm® was from Braga et al. (2020), who found that it caused high predation of infective larvae of *Haemonchus contortus* and *Strongyloides papillosus*, which are parasites of sheep. However, there are still no reports regarding the efficacy of this product on bovine gastrointestinal nematodes. Thus, the objective of the present study was to evaluate the effectiveness of Bioverm® (*D. flagrans*) against the larvae of gastrointestinal nematodes, after passage through the gastrointestinal tract of cattle.

The antiparasitic product Bioverm®, for veterinary use, containing 10^5 chlamydospores of *D. flagrans* per gram, was used. The fungal formulation comes in the form of fine-grained powder, packed in a hermetically sealed colorless polypropylene bag.

To obtain infectious larvae (L3), coprocultures were performed (Roberts & O'Sullivan, 1950) on fecal samples from eight naturally infected cattle, with a mean egg count per gram of feces (EPG) of 450 ± 150 (Gordon & Whitlock, 1939). The fecal cultures were kept for 15 days in a biochemical oxygen demand (BOD) incubator, adjusted to a temperature of 26 °C. After this period, the larvae were recovered using the Baermann technique (Willcox & Coura, 1989). The larvae were identified in accordance with the morphological criteria recommended by Keith (1953), as follows: *Haemonchus* spp. (76%), *Trichostrongylus* spp. (12%), *Oesophagostomum* sp. (11%) and *Strongyloides* sp. (1%). To quantify the larvae, 10 samples of 50 µL of the suspension containing the larvae were counted under an optical microscope, with 4x objective (40x magnification), thus establishing the average number of larvae in the suspension volume.

To evaluate the predatory capacity of the fungus after passage through the gastrointestinal tract of cattle, two experimental assays (A and B) were performed. Twelve male crossbred cattle aged between seven and nine months, with an average weight of 180 kg, were used. They were kept in individual stalls in the cattle sector of the Instituto Federal da Paraíba (IFPB), Sousa campus, state of Paraíba, during the entire experiment. Each stall had 12 m^2, concrete flooring, roofing with clay tiles, troughs, feeders and drinking fountains. 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*. To prevent infection by nematode larvae, the stalls underwent complete sanitation every day, during which the bed composed of wood shavings was completely replaced and the floors and walls were washed and disinfected with 1% sodium hypochlorite.

These animals were firstly treated with the anthelmintic levamisole hydrochloride (7.5%) (Ripercol® L; Zoetis Indústria de Produtos Veterinários Ltda.), at a dose of 1 mL/20 kg, subcutaneously. Ten days after anthelmintic treatment, three egg counts (in EPG) were performed on each animal. After confirmation of zero EPG, the animals were randomly divided into two groups (treated and control), with six animals in each.

In the treated group, a single dose of 1 g of the Bioverm® product per 10 kg of live weight (containing 10^5 chlamydospores of *D. flagrans*) was provided for each animal. This was administered together with 1 g of corn bran per 10 kg of live weight. In the control group, each animal received only 1 g of corn bran per 10 kg of live weight, which served as a placebo. Subsequently, fecal samples of approximately 50 g were obtained directly from the rectal ampoule of the animals, at 12, 24, 36, 48, 60 and 72 hours after administration of the treatments.

For assay A, three replicates were produced from each fecal sample, each consisting of 2 g of feces placed in a Petri dish of 5 cm in diameter, together with 2% agar-water medium (2% AA) and 1000 L3. This was an adaptation

from the methodology described by Costa et al. (2019). The Petri dishes were then placed in an incubator at 26 °C, in the dark. On the 10th day, the Petri dishes were examined to check for any presence of conidia and conidiophores that would be typical of *D. flagrans*. Afterwards, any L3 that had not undergone predatory action were recovered from the Petri dishes by means of the Baermann method.

For assay B, coprocultures were performed in triplicate for each sample. These consisted of 15 g of feces, with addition of expanded vermiculite. 1000 L3 of bovine gastrointestinal nematodes were added to each coproculture, following the methodology of Rodrigues et al. (2020). The fecal cultures were then incubated at 26 °C in a BOD incubator for 10 days. After this period, the L3 were recovered using the Baermann method.

The percentage of larval reduction in each treated group in relation to the control group was calculated using the following formula:

$$\text{Reduction } (\%) = \frac{\text{Mean L3 recovered from CG} - \text{Mean L3 recovered from TG}}{\text{Mean L3 recovered from CG}} \text{ x100} \quad (1)$$

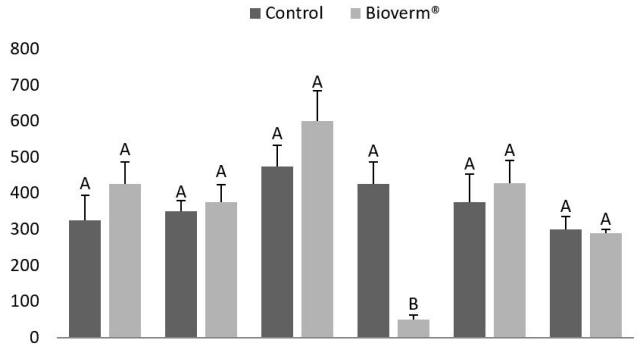
CG = control group; TG = treated group.

The data were subjected to Shapiro-Wilk normality test. All samples showed normal distribution and were subjected to T test for independent samples at the level of 5% probability, using the Biostat 5.0 software (Ayres et al., 2007).

All procedures performed in this study had previously been authorized by the IFPB Research Ethics Committee (protocol no. 23000.000665.2020-71).

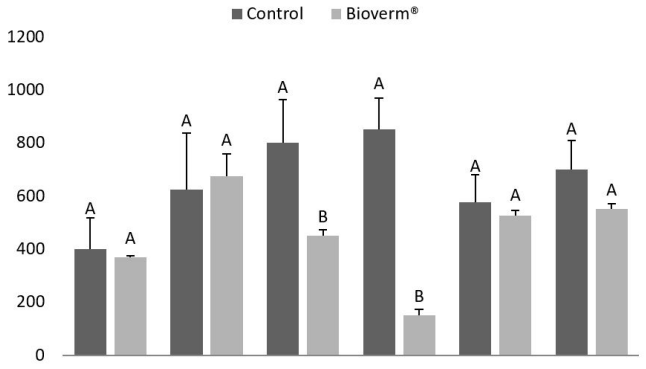
In assay A (Figure 1), there was a reduction of 88.2% ($P < 0.05$) in the number of larvae recovered from fecal samples within 48 hours after the product had been supplied orally, in comparison with the control group. Over the other time periods assessed, there were no significant reductions in the number of larvae. In assay B (Figure 2), significant reductions ($P < 0.05$) occurred over the first 36 and 48 hours, with percentages of 43.7% and 82.3%, respectively. Similar results were found by Silva et al. (2013), who found an 81.2% reduction in infective larvae, caused by the action of *D. flagrans* after passage through the gastrointestinal tract of cattle.

In both assays of the present study, the peak of larval predation occurred 48 hours after administration of Bioverm®. In cattle, the product tends to travel slowly through the gastrointestinal (Assis et al., 2012). In other species, such as sheep, the highest elimination of nematophagous fungi with feces occurs within 24 hours (Larsen et al., 1998; Oliveira et al., 2018).



	12 h	24 h	36 h	48 h	60 h	72 h
Control	325	350	475	425	375	300
Bioverm®	425	375	600	50	425	290
Reduction (%)	-30.7%	-7.1%	-26.3%	88.2%	-13.3%	3.3%

Figure 1. Total and percentage reduction in L3 recovery from bovine gastrointestinal nematodes in assay A. Different letters in the same time frame indicate statistical difference in the T test at 5% probability.



	12 h	24 h	36 h	48 h	60 h	72 h
Control	400	625	800	850	575	700
Bioverm®	370	675	450	150	525	550
Reduction (%)	7.5%	-8%	43.75%	82.3%	-8.7%	21.4%

Figure 2. Total and percentage reduction in L3 recovery from bovine gastrointestinal nematodes in assay B. Different letters in the same period of time indicate a statistical difference in the T test at 5% probability.

In assay A (treated group), intense spontaneous production of traps was observed in the Petri dishes within 48 hours. These consisted mainly of adhesive hyphae and constrictor rings, with the presence of L3 that had undergone predatory action by *D. flagrans* (Figure 3). The predatory capacity of *D. flagrans* isolates has also been reported by Larsen et al. (1992), in an evaluation on these isolates grown in barley grains. They observed that *D. flagrans* retained viability after transiting through the gastrointestinal system of cattle, and that it had the ability to reduce the development of larvae.

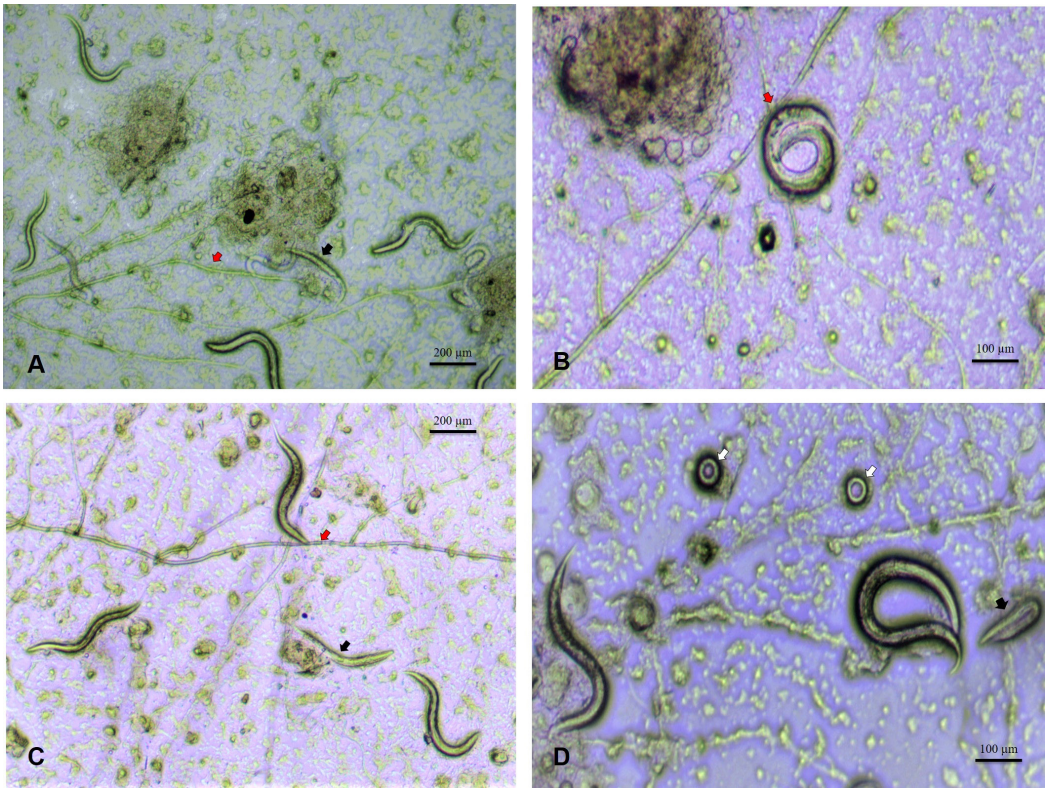


Figure 3. A-D. *Duddingtonia flagrans* (Bioverm®) trap production and interaction with nematode infective larvae after passage through the gastrointestinal tract of cattle. Assay A, agar-water 2% medium. Adhesive hyphae (red arrows), constriction ring (white arrows) and predatory action on infective larvae (black arrows). Optical microscopy (100x and 200x).

Survival of a fungal formulation after passage through the gastrointestinal tract of animals is a characteristic of fundamental importance, given that oral administration is the most practical way to supply the medication to the animals (Braga & Araújo, 2014). In this context, the present study demonstrated that Bioverm® resisted the adverse conditions of passage through the digestive tract of cattle. After release in the feces, *D. flagrans* developed and was effective in preying on infective larvae. Thus, *D. flagrans* was confirmed to be an efficient alternative for biological control over bovine gastrointestinal nematodes.

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