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ORIGINAL ARTICLE

Efficiency of the *Bioverm*® (*Duddingtonia flagrans*) fungal formulation to control in vivo and in vitro of *Haemonchus contortus* and *Strongyloides papillosus* in sheep

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Abstract
The objective of the present work was to evaluate the efficiency of Bioverm® fungal formulation (*Duddingtonia flagrans*—AC001) in controlling *Haemonchus contortus* and *Strongyloides papillosus* in sheep. In vitro predation tests were carried out in Petri dishes containing agar culture medium 2%. Four experimental groups were formed, with five replicates each: Group 1: 1 g of *Bioverm*® and 1000 third-stage larvae (L_3) of *H. contortus*; Group 2: 1000 L_3 of *H. contortus*; Group 3: 1 g of *Bioverm*® and 1000 L_3 of *S. papillosus*; and Group 4: 1000 L_3 of *S. papillosus*. In the in vivo tests, twelve 11-month-old sheep males positive for *H. contortus* were used. The animals were sorted in two groups (treatment and control), based on fecal egg counts (eggs per gram, EPG). Each group comprised six animals: treatment group—each animal received orally 100 g of *Bioverm*®; and control group—each animal received orally 100 g of rice. Subsequently, feces from these animals were collected at 12, 24, 36, 48, 60, 72, 84, and 96 h after *Bioverm*® administration. In vitro results demonstrate that *D. flagrans* kept its predatory activity with 91.5% of mean reduction percentage of L_3. After the passage test, *Bioverm*® presented efficacy already after 12 h of its administration and kept similar results for 60 h. Bioverm® fungal formulation (*D. flagrans*—AC001) was efficient in reducing the population of *H. contortus* and *S. papillosus* under laboratory conditions in sheep feces. However, further studies are needed under natural conditions of ruminant grazing to prove the efficiency of this product.

Keywords Biological control · Commercial batch · *Duddingtonia flagrans* · Nematophagous fungi

Introduction

The biological control of nematodes, gastrointestinal parasites of animals, using nematophagous fungi has been shown to be a safe and feasible alternative in different parts of the world (Larsen et al. 1991; Araujo et al. 1993, 1994; Araujo and Salcedo 1995; Nansen et al. 1995; Mendoza-De Gives and Vazquez-Prats 1994; Mendoza-de Gives et al. 2018; Waller et al. 2001; Faedo et al. 2002; Fontenot et al. 2003; Chandrawathani et al. 2004; Braga et al. 2009, 2013; Silva et al. 2009; Luns et al. 2018; Vilela et al. 2018). These fungi act specifically on the fecal environment, where they are efficient in the decrease and in the recurrence of helminthic infections.

Literature shows that, among all nematophagous fungi isolates, the *Duddingtonia flagrans* species was the most evaluated and most efficient in the control of ruminants' nematodes (Mendoza-De Gives and Vazquez-Prats 1994; Silva et al. 2010; Fernandes et al. 2017; Sobral et al. 2019). The first studies in Brazil started at 1980 with the utilization of the isolate AC001 of *D. flagrans*, provided in the feed through sodium alginate pellets containing conidia and chlamydospores (Dias et al. 2007; Assis et al. 2013). The in vitro and in vivo experiments settled the scientific basis to the technological development of a commercial product.

Recently, in 2019, the AC001 isolate was used in *Bioverm*® production, permitted by the Ministry of Agriculture, Livestock and Supply under licensing number nº

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SP-10.261/2019. *Bioverm*® is indicated for prevention of helminthic gastrointestinal parasites in goat, sheep, and cattle. It is easy to administer and presents oral stability through salt or other food of animal consumption.

The present study aimed to evaluate first time the efficacy of the Bioverm®, in the control of sheep gastrointestinal parasitism.

Materials and methods

Product

The tested product was *Bioverm*® (Ghenvet Animal Health, Paulinia, SP, Brazil), containing 10^5 chlamydospores per gram of commercial product, as carrier of the *D. flagrans* (AC001) sample. The product is a solid thin powder that is sold in hermetically sealed, crystal-colored polypropylene bags.

Larvae

Third-stage infective larvae (L_3) of *Haemonchus contortus* and *Strongyloides papillosus* were obtained from fecal culture of naturally infected sheep (Guimarães 1971). Larvae have been recovered from fecal culture and quantified (Roberts and O'Sullivan 1950).

In vitro predation test

In vitro predation tests were carried out in 6-cm-diameter Petri dishes containing agar culture medium 2%. Four experimental groups were formed, with five replicates for each of them: Group 1: 1 g of *Bioverm*® and 1000 L_3 of *H. contortus*; Group 2: 1000 L_3 of *H. contortus*; Group 3: 1 g of *Bioverm*® and 1000 L_3 of *S. papillosus*; and Group 4: 1000 L_3 of *S. papillosus*.

The plates were kept on a Biochemical Oxygen Demand (BOD) incubator in the absence of light for 7 days at 25 °C. The plates were retrieved daily from the BOD incubator to evaluate whether or not there was formation of traps or destruction of the L_3 by the fungi. After this period, the larvae were recovered by Baermann funnel technique and was counted (Staniland 1954; Braga et al. 2011).

In vivo passage test

Twelve sheep with 11 months from Saanen breed naturally infected by *H. contortus* were used. These animals are originally from the Federal University of Viçosa, Minas Gerais, Brazil (UFV) and were kept in cement bays at the UFV Veterinary Department during the experimental period, fed with chopped elephant grass (*Pennisetum purpureum*), Tifton hay (*Cynodon dactylon*) and water ad libitum.

The animals were randomly sorted into two groups (treated and control) based on the quantity of egg found per gram of feces (EPG). Each group was composed of six animals: treated group—each animal received orally 100 g of *Bioverm*® through the feed; and control group—each animal received orally 100 g of type "1" rice autoclaved at 121 °C for 15 min (Araujo et al. 2007; Campos et al. 2008).

Then, animals of each experimental group had fecal samples collected with the aid of collection bags at 12, 24, 36, 48, 60, 72, 84 and 96 h after feeding. The fecal samples were homogenized and a 20 g sample from each animal was destined to vermiculite fecal cultures at 26 °C carried out in a BOD type incubator in the dark. After 10 days of incubation, L_3 recovery was performed by Baermann's method according to the description of Silva et al. (2010).

To determine the animals' EPG (Gordon and Whitlock, 1939) during the experimental period, three fecal samples were collected daily (at 8:00 a.m., 2:00 p.m. and 6:00 p.m.) directly from the animals' rectum following the protocol of Campos et al. (2008).

To discard fungal presence in animals' feces before the experiment and to assess the presence of *D. flagrans* from *Bioverm*® after treatment administration, fecal samples were collected in plastic bags directly from the animals' rectum 3 h before and 12, 24, 36, 48, 60, 72, 84, and 96 h after *Bioverm*® administration for the treatment group, and the autoclaved rice for the control group. Fecal samples of 2 g were macerated with a glass rod and spread on 9-cm-diameter Petri dishes containing agar culture medium 2%, 5 replicates for each collection period. Subsequently, 3000 larvae of *H. contortus* were poured to stimulate fungal growth and trap development. These plates were sealed with Parafilm (SIGMA), incubated at 25 °C and inspected daily for 14 days on a microscope to look for traps, conidia and chlamydospores typical of *D. flagrans*, and also preyed nematodes.

Statistical analysis

In vitro fungal predation rate for each nematode species was computed using the following formula: reduction % = (mean number of larvae retrieved from control group—mean number of larvae retrieved from treatment group/mean number of larvae retrieved from control group) * 100.

In the in vivo passage test, based on EPG means and number of larvae obtained in the fecal cultures, the larval development percentage was determined (ld %) for each collection period by applying the following equation: ld % = (larvae number by gram of feces retrieved in fecal cultures/number of eggs by gram of feces used for the fecal cultures) * 100.

The larval development reduction percentage (ldr %) for the treatment group was computed applying the following equation: ldr % = (mean percentage of larval development in control group—mean percentage of larval development in treatment group/mean percentage of larval development in control group) * 100.

Data regarding the larval development percentage were transformed ($\log X + 1$), submitted to analysis of variance, and the mean values were compared by Turkey test at the significance level of 0.05 (Ayres et al. 2003).

Results and discussion

In the in vitro predation test, predation structures like tridimensional adhesive nets and L_3 predation were observed 24 h after the addition of nematodes to the plates. Table 1 shows the mean number of L_3 of *H. contortus* and *S. papillosus* retrieved after 7 days of interaction in treatment and control groups. *D. flagrans* contained in *Bioverm*® presented activity upon *H. contortus* and *S. papillosus* larvae, with greater predatory activity upon *S. papillosus*.

These results agree with those of other studies that used the fungus *D. flagrans* grown in agar culture medium or solution containing conidia/chlamydospores (Dias et al. 2007; Braga et al. 2013). In such studies, the authors used the isolate AC001 in multiplying laboratory conditions. We herein demonstrate that the fungus *D. flagrans* from the commercial product *Bioverm*® kept its predatory activity with an average decrease percentage of 91.5% on the L_3.

Araujo et al. (2007) highlighted that the conditions present in ruminants' digestive tract could destroy and/or impair the passage of fungal isolates through it, reason why many researchers have been searching for an ideal period for the administration of the fungus via feeding (Vilela et al. 2018). Most of them suggest that the dosage of *D. flagrans* (AC001) twice a week would be ideal for the control of recurrent infections by gastrointestinal nematode parasites.

Table 1 Mean number of *Haemonchus contortus* and *Strongyloides papillosus* infecting larvae (L_3) recovered by the Baermann method on the plates of the group treated with *Bioverm*®, containing 10^5 chlamydospores of *Duddingtonia flagrans* per gram, and control group without fungal supplementation, after 7 days of interaction

Experimental groups	Mean recovered L_3 from the plates	Reduction %
Control with L_3 from *S. papillosus*	860.66 ± 101.99[a]	
Control with L_3 from *H. contortus*	802.66 ± 131.99[a]	
Bioverm® + L_3 from *S. papillosus*	6.66 ± 2.03[b]	99.33
Bioverm® + L_3 from *H. contortus*	124 ± 20.99[b]	84.55

Different letters are statistically different ($p < 0.05$)

The literature is rich in studies showing *D. flagrans* (AC001) viability after its passage through the digestive tract of ruminants, being recovered in feces, germinated in laboratory, and still viable maintaining its predation (Assis and Araujo 2003; Dias et al. 2007; Campos et al. 2008). *D. flagrans* from *Bioverm*® presented efficacy upon passage through the animals' digestive tract already after 12 h of its administration, germinating and maintaining its viability in all plates from the treatment group up to 60 h after administration. Fungal recovery dropped only after 72 h of *Bioverm*® administration to the treatment group. However, recovery was observed until the maximum tested period, i.e., 96 h (Table 2).

Table 3 illustrates that the larval development of *H. contortus* on the fecal culture tests from the animals treated with *Bioverm*® was inferior to the control group values. What it suggested is that, at natural conditions, there will possibly be a reduced development of the L_3 and, consequently, less recurrence of infections. This development was compromised as the eggs were neither viable nor presented normal development. These observations meet those of Morgan-Jones et al. (1983), who mentioned that nematophagous predatory fungi could impair egg development. Supporting this premise, Braga et al. (2008) demonstrated that the AC001 isolate of *D. flagrans* displayed predatory effect over eggs of the helminth *Fasciola hepatica*.

Mota et al. (2003) state that one of the major challenges in implementing nematophagous fungi in biological control programs would be the development of economically viable and easily applicable fungal formulations. In this sense, the present study brought evidence to light that *Bioverm*® (*D. flagrans*) was efficient in reducing the population of *H. contortus* and *S. papillosus* in sheep feces. Nevertheless,

Table 2 *Duddingtonia flagrans* occurrence before and after *Bioverm*® administration to sheep of the Saanen breed (100 g per animal), and control group without fungi supplementation, on the 5 plates containing feces from the respective collection period

Collection time	Experimental groups	
	Control	Treatment
3 h BB	− − − − −	− − − − −
12 h AB	− − − − −	+ + + + +
24 h AB	− − − − −	+ + + + +
30 h AB	− − − − −	+ + + +
36 h AB	− − − − −	+ + + + +
48 h AB	− − − − −	+ + + + +
60 h AB	− − − − −	+ + + + +
72 h AB	− − − − −	+ + + − −
84 h AB	− − − − −	+ + + − −
96 h AB	− − − − −	+ + − − −

BB—before the beginning; AB—after the beginning; (−) non-occurrence of fungus and (+) occurrence of fungus

Table 3 Means percentage of larval development of *Haemonchus contortus* in fecal culture tests after 10 days, and feces collection at 12, 24, 36, 48, 60, 72, 84, and 96 h after treatment with *Bioverm*® and control

Collection time	Experimental groups	
	Control	Treatment
12 h	2.40 Aa	0.69 Bb
24 h	4.65 Aa	0.60 Bb
36 h	3.64 Aa	0.57 Bb
48 h	4.35 Aa	1.57 Bb
60 h	3.42 Aa	1.30 Bb
72 h	3.08 Aa	1.80 Bb
84 h	2.07 Aa	1.50 Bb
96 h	3.09 Aa	2.03 Bb
ld %	3.338%	1.257%
ldr %	0.624%	

ld %—mean percentage of larval development; ldr %—percentage of larval development reduction

Means followed by the same uppercase letters within a column and lowercase letters in the same row do not differ ($p < 0.05$)

further studies should be performed with these ruminants to prove the efficiency of this product under natural grazing conditions.

Authors' contributions Conceived and designed the experiments: FRB and JVA. Performed the experiments: FRB, CMF, ENP, and JVA. Analysed the data: FRB, ENS, and JVA. Contributed reagents/materials/analysis tools: FRB and JVA. Wrote the paper: FRB, CMF, ENP, and JVA. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statements The research developed in this work has been carried out following recommendations of Ethics Commission on the Use of Animals from Universidade Federal de Viçosa, Brazil.

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