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Bioverm® (Duddingtonia flagrans) comparado à associação de Duddingtonia flagrans e Pochonia chlamydosporia para o controle biológico de nematóides bovinos no sudeste do Brasil

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Keywords: fungi; gastrointestinal parasites; ruminants; pasture.

Article

Bioverm® (*Duddingtonia flagrans*) Compared to the Association of *Duddingtonia flagrans* and *Pochonia chlamydosporia* for the Biological Control of Cattle Nematodes in Southeast Brazil

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Abstract: The use of bioproducts is an alternative to minimize the indiscriminate use of anthelmintics to control worms in livestock. This study evaluated the effects of oral administration of two commercial formulations, based on the fungus Duddingtonia flagrans (Bioverm®) and *D. flagrans* associated with *Pochonia chlamydosporia*, on the control of worms in naturally infected cattle on February pasture. to October 2021. Eighteen Holstein x Zebu cattle were divided into groups of six animals (group 1 Bioverm®, group 2 Association and control), separated into paddocks naturally infected with helminth larvae (L3). Collection of feces samples to determine parasite load and pasture samples to determine L3 infestation. The study demonstrated low recovery of L3 in the pasture due to low rainfall during the experiment. The EPG was lower in groups 1 and 2 compared to the control in April, May and July, while in March the values were lower only in treatment 1 compared to the control. The individual or combined administration of fungal products reduced the release of eggs and the presence of larvae in pastures in certain months of the year, indicating their effectiveness in the strategic control of gastrointestinal parasites in cattle.

Keywords: fungi; gastrointestinal parasites; ruminants; pasture

1. Introduction

Brazil has the largest commercial bovine population in the world, and the Southeast region has greatest representation, with emphasis on the state of Minas Gerais for dairy cattle breeding [1]. The current trends of ruminant breeding are increasingly demanding increased production while also assuring the welfare of animals. The control of helminths is an important factor related to both aspects [2,3].

Animals become infected with helminths principally through exposure in pastures [4]. The main form of combating these parasites is through treatment of animals with antiparasitic agents [5,6], but in many cases these are administered incorrectly, with excessive and indiscriminate use of therapeutic bases, increasing production costs without achieving effective control of infestations [7], besides leaving harmful residues in products of animal origin [8].

Control using bioproducts is an alternative for management of bovine nematodes in pastures [9]. The use of biological control methods involving nematophagous fungi has been shown to be a safe and viable alternative [9–11].

Promising results have been achieved with various species nematophagous fungi, in particular *D. flagrans* for control of helminth larvae and *P. chlamydosporia* with ovicidal action to control helminth eggs [12–14]. Therefore, the development of biological control of helminths is an increasingly attractive option, but requires the selection of the most effective fungal isolates or their associations.

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With this in mind, we evaluated the effects of two commercial bioproducts, one based on *Duddingtonia flagrans* (Bioverm®) and the other its association with *Pochonia chlamydosporia* for the reduction of eggs in animal feces and larval infestations in pastures.

2. Materials and Methods

The commercial formulations based on the fungus *Duddingtonia flagrans* (AC01 isolate) (Bioverm®) and on *D. flagrans* (AC01 isolate) associated with *Pochonia chlamydosporia* (VC04 isolate) were supplied by the company GhenVet Saúde Animal (Brazil).

The experiment was conducted on a farm located in the municipality of Abre Campo, state of Minas Gerais, southeastern Brazil, latitude 20º 18 '04 "S, longitude 42º 28' 39" W.

Initially, to monitor the animals, feces and pasture samples were collected and subjected to the egg count per gram of feces (EPG) according to the methods of Gordon and Whitlock [15] and Dennis, Stone and Swanson [16], while the pasture forage samples were analyzed by the technique described by Raynaud and Gruner [17] and the parasites were identified according to Keith [18].

Eighteen Holstein x Zebu crossbred cattle, aged between 12 and 15 months, with average initial weight of 150 kg, were previously treated with an anthelmintic suspension of 15% albendazole sulfoxide (Agebendazol®) by injection, in a single dose of 1 mL/44 kg of body weight. Twenty-one days after anthelmintic treatment and after confirmation of the absence of nematode eggs eliminated by feces in the EPG, a field test was conducted of the efficacy of products as described by the World Association for the Advancement of Veterinary Parasitology (WAAVP) [19]. The animals were randomly divided into three groups of six animals each and placed in three *Brachiaria brizantha* paddocks naturally infested with helminth larvae, having been previously used for grazing by young and adult animals. In treatment group 1, each animal was treated with 10 to 6 g/100 kg body weight containing the fungus *D. flagrans*, administered daily with corn bran, while group 2 received 10 to 6 g of each fungus/100 kg body weight containing *D. flagrans* and *P. chlamydosporia*, also administered daily along with corn bran. In the control group, each animal received daily feed of corn bran without fungus. The animals were monitored fortnightly and the dose of the products was maintained by the body score and based on previous studies conducted on the same farm, with the same animals, as described by Vieira and collaborators [14].

The experiment lasted nine months (February to October 2021), during which fecal and pasture vegetation samples were collected. The method was consistent with the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) and followed the determinations for assessing the efficacy of anthelmintics in cattle and sheep described by Powers *et al*. [19] and the second edition of Wood *et al*. [20] besides the requirements of Edict 48 from Brazil's Ministry of Agriculture and Supply [21]. The study was also approved by the Ethics Committee on Animal Use (CEUA) of Viçosa Federal University (UFV), under reference no. 37/2020.

Every 15 days after the animals were introduced to the pastures, the body score was checked, and fecal samples were collected from all animals in each group directly from the rectum. Egg count per gram of feces (EPG) was determined by the method of Gordon and Whitlock [15], with modifications suggested by Lima [22]. At the same time, coprocultures were produced using 20 g of feces mixed with vermiculite and placed in biochemical oxygen demand (BOD) chamber at 26 °C for 15 days to obtain infective larvae (L3), which were subsequently identified according to Keith [18].

Every 15 days from the start of the experiment, two samples of *Brachiaria brizantha* grass (0-20 cm and 20-40 cm distance from stools) were collected from the grazing areas of the treated and control groups at six different points according to Raynaud and Gruner [17]. Samples of 500 g of pasture forage were used to recover infective larvae (L3) following the method described by Lima [22]. The sediment was examined under an optical microscope and the larvae were counted and identified according to the criteria established by Keith [18]. The 500 g samples of grass that were used were placed in an oven at 100 °C to obtain dry matter. The data obtained were transformed into the number of larvae per kilogram of dry matter.

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The climatic data regarding minimum, average and maximum monthly temperatures and monthly precipitation were obtained from the Agricultural Meteorological Monitoring System (Agritempo), available at the website https://www.agritempo.gov.br/agritempo/index.jsp.

Mean egg counts per gram of feces (EPG), nematodes recovered in coproculture and L3 from pasture samples and weather data during the nine months of the experiment were converted into monthly values. Then, EPG and L3 data from pasture were log (x + 1) transformed, submitted to Levene's test of equality of variances, ANOVA and the Tukey test for EPG data, and the Chi-square test for L3 in pasture, in all cases at 5% significance levels. All were performed with the IBM SPSS Statistics 2.0 software.

3. Results

3.1. General Results

The results obtained by the initial evaluation, before starting to provide the products, verified the absence of trematode eggs according to the method of Dennis, Stone and Swanson [16]. However, it is important to mention that Cestoda eggs and unsporulated oocysts were verified, identified by morphology according to Araújo [23].

3.1.1. Egg Counting Techniques

The EPG results are presented in Table 1, which shows the monthly average EPG of feces in the three groups during the period from February to October 2021. In the first month of treatment, the low EPG value was due to the result of the anthelmintic treatment administered to the animals before the beginning of the experiment. This protocol is based on the recommendations of WAVVP [19,20] and MAPA [21].

Table 1. Monthly mean and standard error of the number of eggs/g of feces (EPG) in treatment 1, based on *Duddingtonia flagrans* (Bioverm®), and treatment 2, based on combination of *D. flagrans* and *Pochonia chlamydosporia* (Association) and the control group during the period from February to October 2021, in Abre Campo, Minas Gerais State, Brazil.

Month	Control	Treatment 1	Treatment 2
Feb	0,00a ± 0,00	8,33a ± 20,41	83,33a ± 204,12
Mar	66,67a ± 66,46	0,00b ± 0,00	37,50ab ± 49,37
Apr	95,83a ± 74,86	25,00b ± 22,36	8,33b ± 20,41
May	79,17a ± 48,52	20,83b ± 24,58	16,67b ± 20,41
Jun	37,50a ± 37,91	33,33a ± 25,82	20,83a ± 29,23
Jul	62,50a ± 41,08	8,33b ± 12,91	12,50b ± 20,92
Aug	70,83a ± 82,79	8,33a ± 12,91	8,33a ± 12,91
Sep	16,67a ± 25,82	33,33a ± 51,64	8,33a ± 20,41
Oct	16,67a ± 40,82	25,00a ± 61,24	25,00a ± 61,24

[1] Data submitted to ANOVA and the Tukey test, with a significance level of 0.05. Means with different letters in the same row indicate statistical differences, followed by the standard deviation.

EPG values were lower in treatment group 1 than in the control in March, while in April, May and July, the values were lower in both treatment groups compared to the control. In June, September and October there were no statistical differences in the mean EPG values in any of the groups, although numerically the values were lower in August and September.

3.1.2. Larva Recovery Techniques

Figure 1 shows the values of the L3 genera recovered from the stool cultures of animals in treatment 1, based on *Duddingtonia flagrans* (Bioverm®), and treatment 2, based on combination of *D. flagrans* and *Pochonia chlamydosporia* (Association) and the control group.

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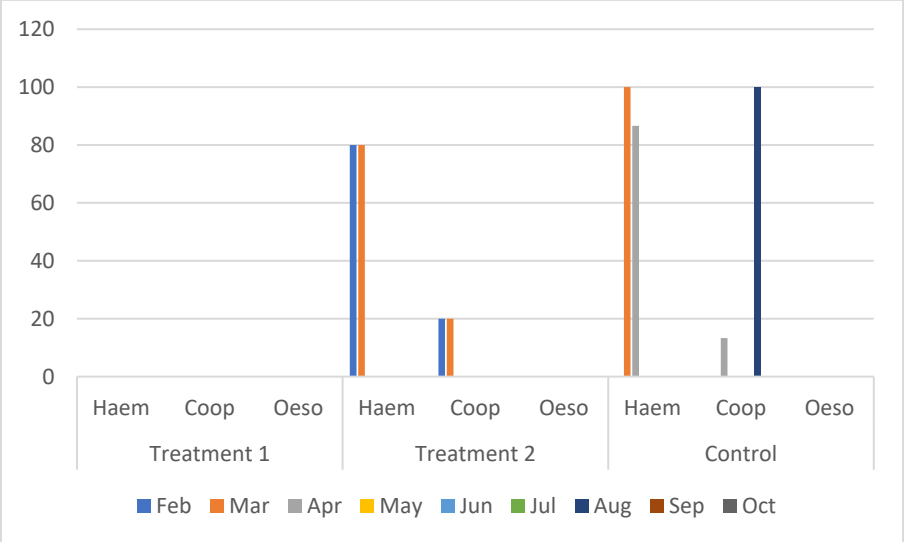

Figure 1. Mean values of the percentages of infective larvae of the genera *Haemonchus* (Haem), *Cooperia* (Coop) and *Oesophagostomum* (Oeso) recovered from coprocultures of groups of animals, treatment 1, with *Duddingtonia flagrans* (Bioverm®), and treatment 2, combination of *Duddingtonia flagrans* and *Pochonia chlamydosporia* (Association) and the control group during the period from February to October 2021, in Abre Campo, Minas Gerais State, Brazil.

The genus *Haemonchus* was predominant, followed by *Cooperiai,* in treatment 2 and control groups. No genera were observed in group 1 after the start of the treatment protocol.

The means of larvae recovered at both distances from the fecal masses in treatment 1, treatment 2 and control groups differed significantly ($p \leq 0.05$), as shown in Table 2. Like in the coprocultures, the agents recovered in the pasture predominantly belonged to the genera *Haemonchus* and *Cooperia* in early stages.

Table 2. Mean values and standard errors of infective larvae recovered per kilogram of dry matter in the forage samples of group 1, treated with *Duddingtonia flagrans* (Bioverm®), and group 2, treated with combination of *D. flagrans* and *Pochonia chlamydosporia* (Association) and the control group during the period from February to October 2021, in Abre Campo, Minas Gerais State, Brazil.

LARVAE / KG Dry Matter (0-20 cm)			
Month	**Control**	**Treatment 1**	**Treatment 2**
Feb	2,00	0,00	0,00
Mar	6,00	0,00	3,50
Apr	1,00	0,50	0,00
May	3,00	0,00	0,00
Jun	1,50	0,50	0,50
Jul	0,00	0,00	1,00
Aug	8,00	3,00	0,00
Sep	0,00	5,00	1,00
Oct	0,00	0,00	4,00
Mean	**2,38a**	**1,0 b**	**1,11 c**
LARVAE / KG Dry Matter (20-40 cm)			
Month	**Control**	**Treatment 1**	**Treatment 2**
Feb	1,00	050	0,00
Mar	2,00	0,50	0,00
Apr	1,50	12,00	1,50
May	0,50	0,00	0,00
Jun	3,00	0,00	0,00
Jul	1,00	1,50	0,00
Aug	2,50	0,00	0,00
Sep	0,00	0,00	2,00

Oct	7,00	0,00	0,00
Mean	**2,05d**	**1,61b**	**0,38e**

* Different letters in the same row indicate significant differences between the data ($p<0.05$) by the Chi-squared test.

3.1.3. Climate Determinations

The presentation of the minimum, average and maximum monthly temperatures, as well as the monthly precipitation during the experimental period in Abre Campo, Minas Gerais State, Brazil, are shown in Table 3. The lowest temperatures recorded were in the months of May and July 2021. The average monthly temperatures ranged from 19.26 °C in July to 25.57 °C in March 2021. The highest precipitation rates were recorded in October, with 6.60 L/m², while in other months the rainfall ranged from zero in July to 1.46 L/m²in March.

Table 3. Evaluation of minimum (T Min), average (T Average), maximum (T Max) and total precipitation (mm³/month) from February to October 2021 in the region of Abre Campo, Minas Gerais State, Brazil.

Month	T min	T mean	T max	Precipitation
Feb	20,87	24,63	28,38	1,25
Mar	20,04	25,57	31,09	1,46
Apr	17,46	23,37	29,28	0,83
May	12,24	19,26	26,29	0,00
Jun	13,96	20,22	26,47	0,28
Jul	12,24	19,26	26,29	0,00
Aug	14,20	20,86	27,52	0,31
Sep	18,24	24,82	31,39	0,56
Oct	20,36	24,59	28,81	6,60

3.1.4. Other Findings

However, it is important to emphasize that Cestoda eggs and unsporulated oocysts were verified, identified by morphology according to Araújo [23]. The Cestoda eggs were found in the treatment group 1 in the months of May and October, and oocysts were found from the pilot phase in all groups in February, March and April.

4. Discussion

Ruminant breeding must evolve to adapt to more stringent global food management standards. Parasitosis is a factor that must be considered, and the main control measure is based on administration of anthelmintic chemicals. However, these chemicals can cause negative environmental effects as well as development of resistance of nematodes due to indiscriminate use and overdosing. These drawbacks have attracted interest in the use of alternative methods, in particular the use of nematophagous fungi for biological control of ruminant helminths. Various studies have shown the effectiveness of biological control of gastrointestinal nematodes in pastures.

According to Li *et al*. [24], for efficient worm control, it is essential to interfere in their entire life cycle, i.e. to reduce the number of nematodes in animals and in pastures at the same time. Our study is in this line, investigating the environmental action of nematophagous fungi. Many tests have been performed to determine the fungal action against gastrointestinal nematodes of ruminants. We analyzed the use of two commercial bioproducts (Bioverm® and Association), with different functional characteristics. According to Kaplan [6], the effect of anthelmintics eliminates the adult stages and eggs in the feces, which was noted in the analysis before the start of the experiment. However, the subsequent period of natural exposure to *Brachiaria brizantha* forage without chemical

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control, natural reinfection by the most common helminths was observed in this environment, especially in the control group (Table 1).

Parasitic agents are found in about 95% of pastures according to epidemiological studies conducted by Kenyon *et al*. [25] and Franco *et al*. [26]. This can be reduced by the use of nematophagous fungi for control, either singly or combined. The use of the fungus *D. flagrans* (Bioverm®) or the combined use of *D. flagrans* and *P. chlamydosporia* (Association) significantly reduced the EPG counts compared to the control group in some periods of the experiment. Similar results were found in a previous study by the same research group performed on the same farm, using different fungal bases [14].

Oral administration of fungi is both practical and most effective, because the fungus or fungi have the ability to bind to the feces of the animals, and are released along with the eggs [24,27].

The fungal species *Duddingtonia flagrans* forms chlamydospores and stands out for its ability to survive and spread through the gastrointestinal tract of animals [9,14,24,28,29]. It has been shown to be an excellent way to control helminth larvae in live animals. This is the case of the commercial product based on chlamydospores of *D. flagrans* (Bioverm®), licensed and marketed in Brazil [27,30].

In addition, there are also ovicidal fungi, such as *P. chlamydosporia*, which form dictyochlamydospores, with selective action against gastrointestinal helminth eggs, used for control of helminthiases [30,31].

The most frequent parasite genera in our coprocultures were *Haemonchus* sp. and *Cooperia* sp. (Figure 1), corroborating previous studies [12–14]. Species of these genera are prevalent in Brazil and cause economic losses due to problems such as anemia, appropriation of nutrients and even death of parasitized animals [32,33].

The life cycle of gastrointestinal parasites occurs inside the animal and in the environment (in pastures in extensive grazing systems) [34]. In the present study, fungal control led to significant reductions of environmental contamination by larvae (Table 2) as well as of EPG counts. This can be explained by the different recovery techniques and the action of different fungi on the life stages of the helminths. A previous study by our group, performed in a short interval using other control fungi, also reduced these parameters, with the residual action in the pasture, contributing to the low levels of environmental contamination and release of eggs [14].

The EPG examinations or sedimentation methods of the animals in the present study did not indicate the presence of trematodes and other helminths such as ascarids. The can be explained by the action of the nematophagous fungus *P. chlamydosporia*, since helminths of the genera *Haemonchus* and *Cooperia*, have rapid life-stage transitions, and their egg phase is brief before hatching to form the first larval stage (L1). Hence, the direct action of this fungus may have been interrupted. However, Cestoda eggs (*Moniezia* spp.) were found in the Bioverm® group but not in the Association group, which suggests ovicidal action of *P. chlamydosporia*.

The grass height of the pasture varied from 15 cm to 80 cm. This variation probably destabilized the microclimate favorable to the development and survival of the larvae, in addition to interfering with the protection of the feces and their rapid degradation [35]. Climatic conditions are important factors that influence the growth of pastures and the maintenance of infective larvae [36]. Another factor that influenced the distribution of L3 was the average temperature recorded during the experimental period.

According to Heckler and Borges [33], temperature variations between 13 °C and 26 °C are adequate to maintain the free-living stages of the *Haemonchus* and *Cooperia* genera. The maximum temperature was above that level (Table 3). The high temperature associated with the lack of rain during the experimental phase, mainly in the months of May, June, July and August, contributed to reduce the pasture areas, and consequently increased the degradation of eggs and reduced the load of larvae in the pastures in all groups, as indicated by the EPG and pasture results in the final months of the experiment. The presence of water is essential for the migration of L3 from feces to pasture [36,37].

In this sense, our study showed that climatic factors were involved in the reduction of both factors (OPG counts and pasture parasite loads), but there was a significant action of the fungi D.

flagrans and P. chlamydosporia, alone or in combination, reducing the number of infective larvae in pastures and the parasitic load of cattle by counting with reduction of eggs from animals raised in pastures.

Extensive grazing of ruminants is the most widespread and productive method in Brazil, therefore the availability of bioproducts to control parasites will contribute immensely to minimize the negative impacts resulting from helminth infections and the indiscriminate use of drugs in animal production systems [30].

5. Conclusions

Knowledge about sustainable products is important for greater adherence to the use of alternative practices by livestock breeders. In our study, we showed the effects of exposure of naturally infected cattle to the use of the nematophagous fungus *D. flagrans* alone (Bioverm®) or combined with *P. chlamydosporia* (Association), which reduced the release of eggs and the presence of larvae in pastures. Therefore, employment of these bioproducts is a promising method for the integrated control of helminths in ruminant livestock.

Author Contributions: Maria Larissa and Jackson Victor, de Araújo conceived and designed the study. Maria Larissa performed the data collection. Maria Larissa and Italo performed statistical analyses. Maria Larissa, Ítalo, Samuel, Lorena, André and Jackson Victor wrote the article.

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Informed Consent Statement: Not applicable.

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Conflicts of Interest: The authors declare that they have no competing financial interests or personal relationships that may have influenced the work reported in this article.

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