

Control of sheep gastrointestinal nematodes on pasture in the tropical semiarid region of Brazil, using Bioverm® (Duddingtonia flagrans)

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Research Article

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Abstract

We aimed to evaluate a Brazilian commercial formulation of the fungus *Duddingtonia flagrans* (Bioverm®) for controlling gastrointestinal nematodes in sheep kept on native pasture in the Caatinga biome, in the semiarid region of Brazil. Twenty ewes, aged between 12 and 18 months, were divided into two groups. In the treated group, each animal received 1 g of the Bioverm® product for each 10 kg of live weight, daily, together with commercial feed, for six months. In the control group, the animals received feed without Bioverm®. Each group remained throughout the experiment in a 1.2 ha paddock. Monthly counts of eggs per gram of feces (EPG), fecal cultures, packed cell volume (PCV), weight measurements and collection of leaf mass from the pasture were performed. There was greater reduction in EPG, greater weight gain and less infestation by infective larvae in the paddock of the Bioverm® group, compared with the control group ($p < 0.05$). There was no significant difference ($p \geq 0.05$) in the mean PCV percentage between the Bioverm® and control groups. In coprocultures, *Haemonchus* sp. was the most prevalent helminth. Bioverm® (*D. flagrans*) was efficient for biological control of sheep gastrointestinal nematodes in the semiarid region of Brazil.

1. Introduction

Gastrointestinal nematodes are the main problem that affects the health of sheep worldwide (Charlier et al., 2014). These diseases have been aggravated by high levels of parasite resistance, as a result of indiscriminate use of anthelmintics in attempts to control these parasites, thus resulting in treatment ineffectiveness (Salgado and Santos, 2016; Silva et al., 2018).

The development of anthelmintic resistance, along with the presence of chemical residues in food intended for human consumption, has imposed restrictions on the use of these drugs, as well as creating the need for an alternative means of control (Lanusse et al., 2018). Supplying nematophagous fungi to animals has been considered to be an efficient alternative for reducing the numbers of nematodes in free-life stages, thereby reducing environmental contamination (Mota et al., 2003; Araújo et al., 2021).

Duddingtonia flagrans stands out as the ideal biocontrol agent, proven to be effective in controlling domestic animals' gastrointestinal parasites (Buzatti et al., 2017; Vilela et al., 2018; Luns et al., 2018; Rodrigues et al., 2018; Vilela et al., 2020). This fungus produces an abundant amount of chlamydospores, which are highly resistant structures that when ingested resist digestion and pass through the gastrointestinal tract of animals (Larsen et al., 1992). After elimination with feces, they are capable of germinating and destroying the infecting larvae, thus interrupting the life cycle of the parasite in the environment (Araújo et al., 2021; Baiak et al. 2021).

In Brazil, there is a commercially available product based on the fungus *D. flagrans* (Bioverm®). The effectiveness (in vitro) of Bioverm® was reported against gastrointestinal nematodes of different animal species, such as horses (Fausto et al., 2021); sheep (Braga et al., 2020) and cattle (Rodrigues et al., 2021). Among cattle, the efficacy of the product for controlling verminosis in the field, in the central-

western region, has also been described (Oliveira et al., 2021). However, the in vivo efficacy of this product against helminthic infections of sheep under semiarid conditions has not yet been reported.

Therefore, the aim of this study was to evaluate the effectiveness of Bioverm® for controlling gastrointestinal helminthiasis in sheep kept on native pasture of the Caatinga biome, in the semiarid region of northeastern Brazil.

2. Material And Methods

2.1. Fungal formulation

Bioverm® (Ghenvet Animal Health, Paulínia, Brazil), presenting *D. flagrans* at a dosage of 10^5 /g chlamyospores.

2.2. Experimental assays

The experiment was conducted between April and September 2021, at the Federal Institute of Paraíba (IFPB), Sousa campus, Paraíba, northeastern Brazil, which is located at latitude 06° 50' 22" S, longitude 38° 17' 42" W. The region is characterized by a rainy season from January to May, during which 98.6% of the annual rainfall occurs; and a dry season, in the other months (INMET, 2010).

Twenty female sheep, aged between 12 and 18 months, Santa Inês breed, were used. Before the beginning of the experiment, the animals were dewormed according to the protocol described by Vilela et al. (2012).

After confirmation that zero EPG had been achieved, the animals were randomly divided into two groups (treated and control), with ten animals each. Each group was placed in a 1.2 ha paddock, composed of native Caatinga pasture, predominantly *Senna obtusifolia* L., *Mimosa tenuiflora*, *Caesalpinia pyramidalis*, *Croton sonderianus*, *Malva* sp., *Zizyphus joazeiro*, *Digitaria horizontalis*, *Mimosa caesalpiniiifolia* and *Cnidocolus phyllacanthus*. The paddocks were naturally infested with helminth larvae, due to the previous grazing of four sheep over a 15-day period (1875 ± 900 EPG; *Haemonchus* sp. 86%; *Trichostrongylus* spp. 10% and *Oesophagostomum* sp. 4%). Throughout the experimental period, each group remained in its respective paddock.

The animals in each group received daily protein-energy commercial food in the proportion of 0.5% body weight, along with mineral salt and water ad libitum. In the treated group, each animal received 1 g of the Bioverm® product for each 10 kg of live weight, daily, together with the commercial feed. In the control group, the animals received the feed without Bioverm®. In order to avoid deaths among the animals, salvage treatments were applied, consisting of the anthelmintic 5% levamisole hydrochloride, in any situations in which the animals presented hematocrit lower than 16%.

Fecal samples were monthly collected from all the animals to measure egg counts per gram of feces (EPG - Gordon and Whitlock, 1939), and perform fecal cultures (Roberts and O'Sullivan, 1950). Infective

larvae were morphologically identified (Keith, 1953). Blood samples were monthly collected to determine the packed cell volume (PCV) percentage (Ferreira Neto et al., 1981). Animal weights were estimated on an analytical balance. Furthermore, to determine the levels of environmental infestation by infective larvae, five samples of approximately 500 g of leaf mass from the pasture were collected monthly from the paddocks of both groups, in different places of each paddock, in accordance to Vilela et al. (2018). The dry matter was weighed and the infective larvae were quantified, using an optical microscope, to determine the values of L3/kg of dry matter.

Data on maximum and minimum temperature, rainfall and relative humidity were recorded at a meteorological station in Sousa, Paraíba, Brazil.

2.3. Statistical analysis

Data were subjected to the Shapiro-Wilk normality test. All samples were found to be normally distributed and were then subjected to the t test for independent samples at the 5% probability level. The EPG values were analyzed using a log (x+1) transformation, but are presented as the untransformed values. The results were analyzed using the GraphPad Prism 9.0 software.

3. Results

The daily supply of Bioverm[®] was effective in reducing EPG and showed a significant difference ($p < 0.05$) from day 90 onwards (Figure 1). By the end of the experiment, a large reduction of EPG had occurred in the treated group, such that it reduced from an average of 1120 ± 300 on day 30 to an average of 260 ± 60 on day 180.

Over the course of the experiment, only one animal in the group treated with Bioverm[®] required salvage deworming ($PCV \leq 16\%$), which was done on day 60. On days 30, 60 and 90, the same animal needed salvage deworming in the control group.

In the fecal cultures, regardless of the group evaluated, there was higher frequency of the genus *Haemonchus* sp., followed by *Oesophagostomum* sp. and *Trichostrongylus* spp. (Table 1).

Table 1
 Percentage of infective *Haemonchus* sp. (H), *Trichostrongylus* spp. (T) and *Oesophagostomum* sp. (O) recovered from fecal cultures of sheep in the group treated with Bioverm® and in the control group, for 180 days, in the Brazilian semiarid region.

Groups		Day 0	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
	H	0	91	84	80	82	78	81
Bioverm®	T	0	5	9	7	8	6	8
	O	0	4	7	13	10	16	11
	H	0	93	89	88	90	91	89
Control	T	0	4	6	5	6	4	8
	O	0	3	5	7	4	5	3

Regarding the average weight gain, greater weight gain was observed among the animals treated with Bioverm® over the course of the experimental period. From day 120 onwards, there was a significant difference in weight between the groups ($p < 0.05$) (Figure 2). At the end of the experiment, on day 180, the animals in the treated group had an average weight of 35.1 kg, with an average weight gain of 9.6 kg, while the control group had an average weight of 31.6 kg and an average weight gain of 6.1 kg.

In the mean PCV percentages between the Bioverm® and control group, there was no significant difference ($p \geq 0.05$) (Figure 3).

In Figure 4, over the course of the experiment it was observed that the values of L3/kg DM were lower in the paddock of the Bioverm® group. A significant difference ($p < 0.05$) was observed from day 90.

The average temperatures recorded ranged from 24.9°C in May (day 60) to 29.8°C in September (day 180) of 2021. Also, as the months went by, it was noticed that there was a reduction in the relative humidity of the air and low rainfall from June onwards (day 90) (Figure 5).

4. Discussion

This was the first study using Bioverm® as a controller of gastrointestinal nematodes in sheep under grazing conditions in the Brazilian semiarid region.

The animals treated with Bioverm® remained with lower EPG levels throughout the experiment ($p < 0.05$), and presented a 74.6% lower parasite load at the end of the study, compared with the control group. Similar results were demonstrated by Vilela et al. (2016), also in the semiarid region of northeastern Brazil, who found a reduction in EPG in sheep treated with pelleted formulations of the fungi *Monacrosporium thaumasium* and *D. flagrans* and, reaching a reduction in EPG of 76%. Silva et al. (2009) administered *D. flagrans* pellets to sheep and observed a 71.6% reduction in EPG, in southeastern Brazil. These studies corroborate the results from the present study, in which a reduction in the quantity

of infective larvae present in feces was demonstrated through use of Bioverm®, thus preventing recurrence of infection in these animals.

In the fecal cultures, *Haemonchus* sp. was the most frequent helminth. Similar results were found by Vieira et al. (2014), who demonstrated that the most prevalent sheep gastrointestinal nematode in the Brazilian semiarid region was *Haemonchus* sp. According to Taylor et al. (2017), *Haemonchus contortus* is the most prevalent nematode with a high intensity of infection, and is responsible for severe anemia that negatively impacts animal production.

There was greater weight gain in the animals that received Bioverm®, with an average weight gain of 9.6 kg by the end of the experiment. In other studies on small ruminants, in the semiarid region of Paraíba, there was greater weight gain among animals treated with nematophagous fungus pellets (Vilela et al., 2012; 2016; 2020). In the present study, a lower parasite load was observed in the treated group. According to Mingueto et al. (2021), reduced recurrence of infections can increase the animals' weight gain.

There was no statistical difference ($p < 0.05$) in the PCV percentages. Although, Vilela et al. (2016) observed that young animals that received the nematophagous fungi had higher PCV levels than animals that did not receive it. This difference was explained through the present study, in which adult sheep were used, which tend to have smaller PCV variations due to verminosis than do young animals.

The paddock of animals treated with Bioverm® showed lower rates of infective larvae recovered from the pasture throughout the study, with a reduction of 89.3% in L3/kg/D.M. Oliveira et al. (2021) also observed a reduction in the level of infestation in the paddock of cattle that received Bioverm®, reaching a reduction of 82.9% on day 180, in central-western Brazil.

In the present study, despite the increase in temperature and lower rainfall and humidity during the experiment, was observed a high L3 infestation in the paddock of the control group. This demonstrates that L3 can survive on pasture and cause infections in animals throughout the year, even under the unfavorable conditions of the dry period. Thus, the use of Bioverm® proved to be effective and necessary.

5. Conclusion

This commercial formulation of the fungus *D. flagrans* (Bioverm®) was effective for the control of gastrointestinal nematodes in sheep kept on native Caatinga pasture, in the Brazilian semiarid region.

Declarations

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Conflict of interest

The authors declare that they did not have any conflict of interest relevant to the content of this article.

Ethics approval

The activities involved in this research were approved by our institution's Ethics Committee for Animal Use (CEUA/IFPB), under protocol number 23000.000665.2020-71.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Jossiara A. Rodrigues, Francisco L. Roque, Brendo A. Lima, Geraldo M. Silva Filho, Clarisse Silva M. Oliveira, Luana C. Sousa, Ana Luzia P. Silva, Estefany F Lima and Vinícius Longo R. Vilela. The first draft of the manuscript was written by Jossiara A. Rodrigues, Thais F. Feitosa, Fabio R. Braga, Jackson V. Araújo and Vinícius Longo R. Vilela; and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

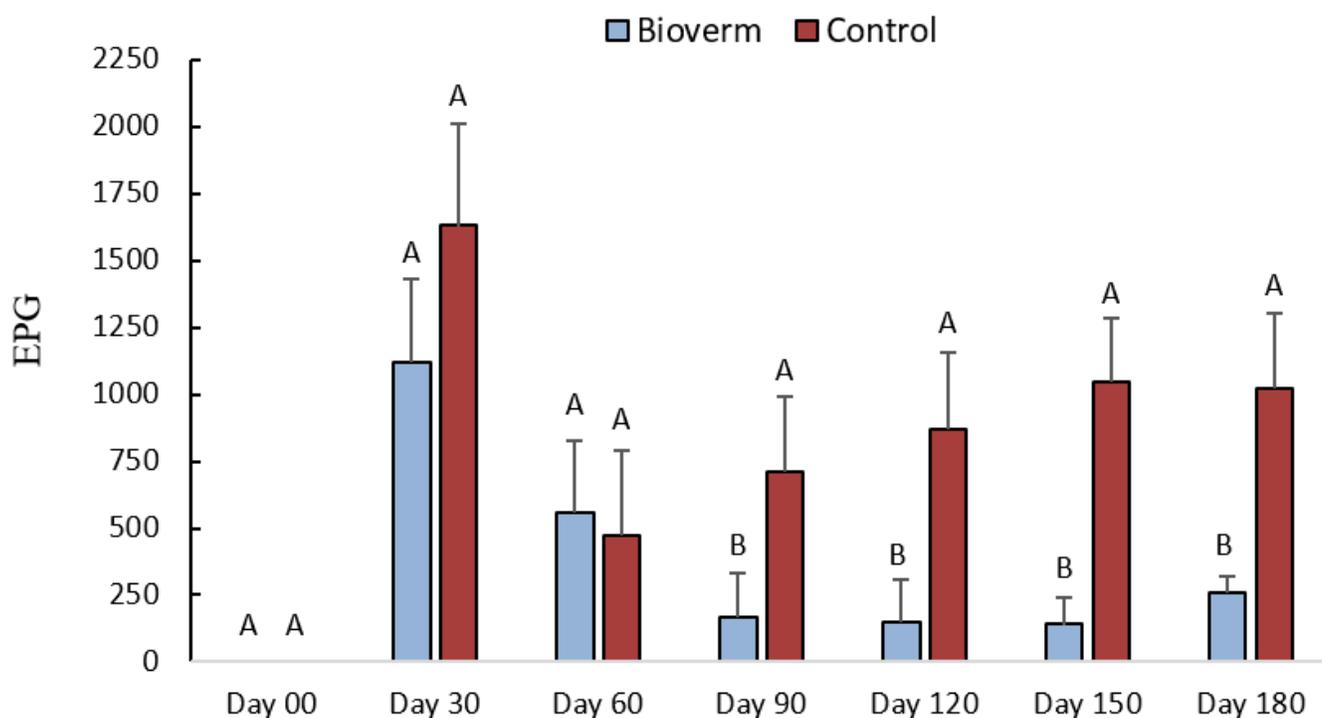


Figure 1

Egg counts per gram of feces (EPG) of sheep in the Bioverm[®] treated group and in the control group, for 180 days, in the Brazilian semiarid region. Same letters did not statistically differ (Tukey's test, $p < 0.05$).

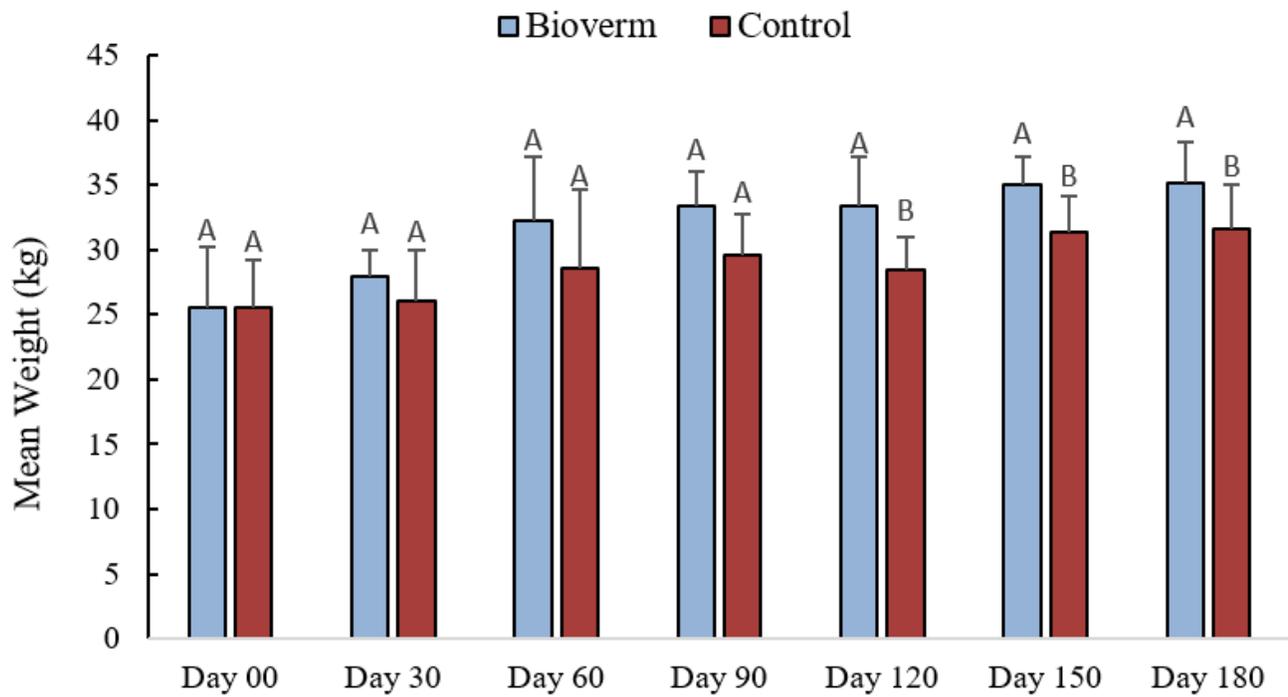


Figure 2

Body weight (kg) measures of sheep in the group treated with Bioverm® and in the control group, for 180 days, in the Brazilian semiarid region. Same letters did not statistically differ (Tukey's test, $p < 0.05$).

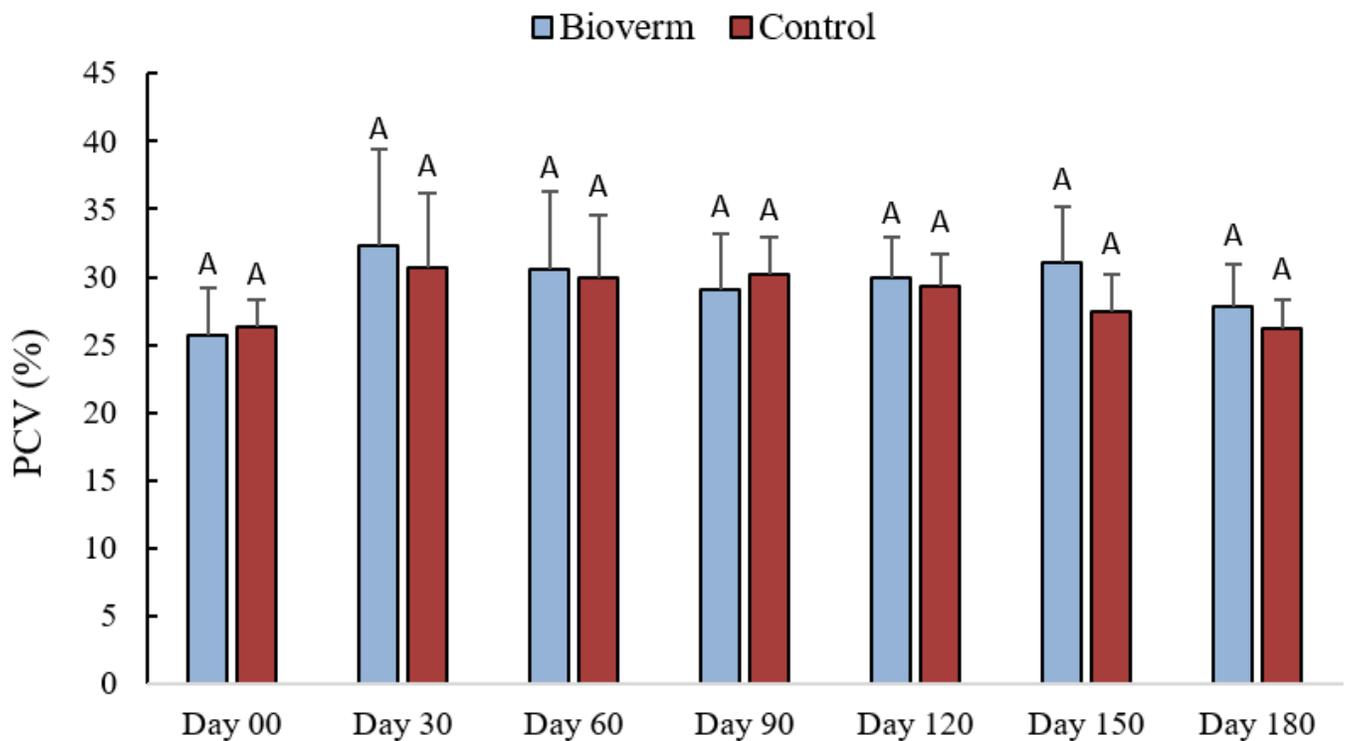


Figure 3

Packed cell volume (PCV) measures of sheep in the group treated with Bioverm® and in the control group, for 180 days, in the Brazilian semiarid region. Same letters did not statistically differ (Tukey's test, $p < 0.05$).

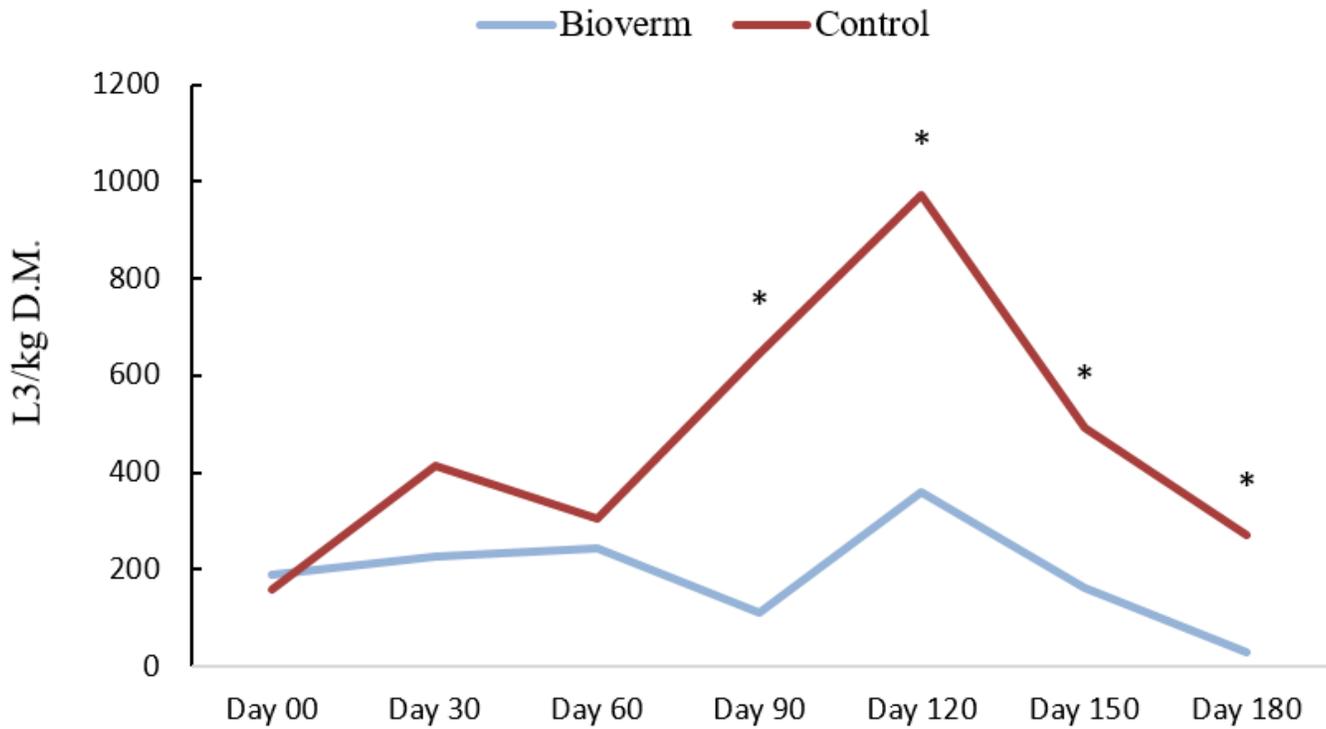


Figure 4

Counts of infecting larvae/kg dry matter (L3/kg D.M.) recovered from the paddocks of sheep in the group treated with Bioverm® and in the control group, for 180 days, in the Brazilian semiarid region. Asterisks indicate a significant difference (Tukey's test, $p < 0.05$).

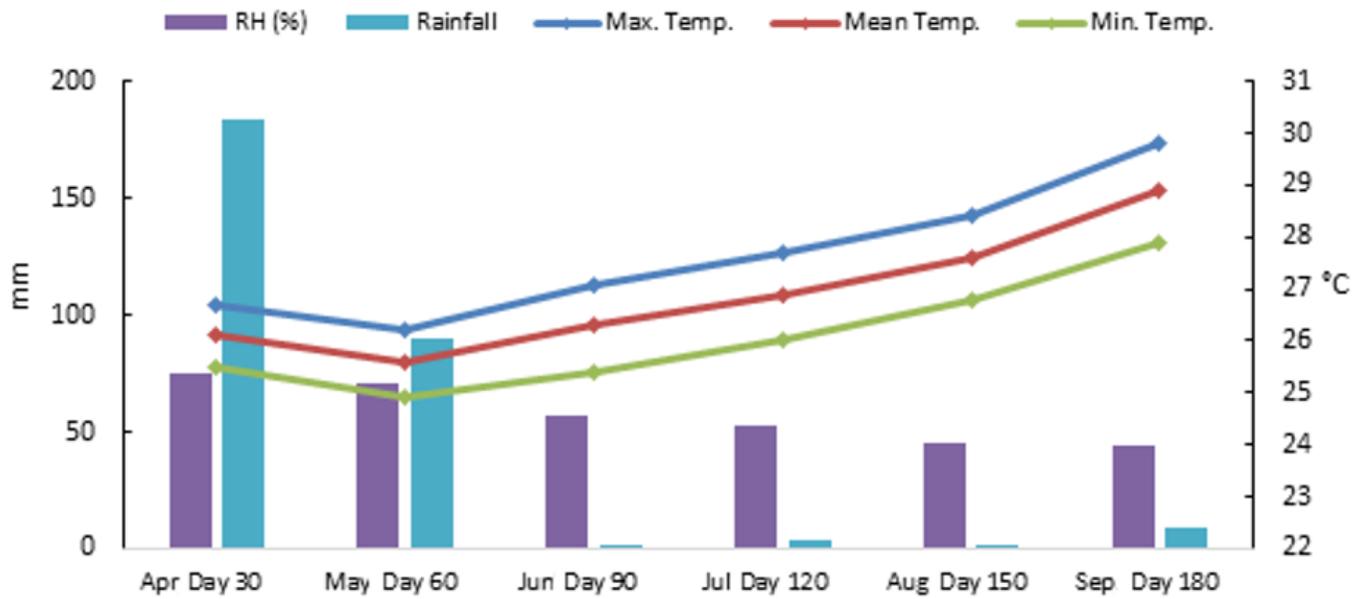


Figure 5

Meteorological data collected from April to September 2021, Sousa, Paraíba State, Brazil.

Supplementary Files

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