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Haemonchosis Control in Sheep with *Duddingtonia flagrans* Fungi: Evaluation of the First Commercial Product in Brazil

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Abstract: The resistance of small ruminants to synthetic anthelmintics and helminthosis poses considerable challenges to global livestock production. Integrating biological control with nematophagous fungi, particularly *Duddingtonia flagrans*, is crucial in addressing worm infestations. Although effective in experiments, the absence of a commercial product has been a limitation. The introduction of Bioverm®, the first commercial product using *D. flagrans* in Brazil, marks a significant advancement. This study on a Brazilian sheep farm evaluated a 167-day Bioverm® treatment, following moxidectin application, focusing on eggs per gram of feces (EPG), FAMACHA score, and sheep weight (WEIGHT). Statistical results showed marked improvements in all parameters after 80 days with Bioverm®. EPG values gradually declined, demonstrating successful biological control, while FAMACHA increased steadily, stabilizing after 130 days. Minor weight changes indicated effective nutritional management. These outcomes suggest Bioverm® significantly reduces dependence on chemical anthelmintics and addresses resistance issues. *D. flagrans* thus emerges as a promising tool for managing nematode infestations without negatively impacting animal weight. This research enhances the understanding of Bioverm®'s role, confirming its practicality as a viable alternative for helminth control in varied environments, thereby reinforcing its strategic importance in livestock management.

Keywords: *Arthrobotrys flagrans*; *Duddingtonia flagrans*; anthelmintic resistance; sheep; goat

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1. Introduction

The breeding of small ruminants varies significantly across different regions worldwide. In countries like Australia and New Zealand, it is a large-scale practice and constitutes a primary economic activity on many farms. Conversely, in regions such as northeastern Brazil and countries across Africa and Asia, small ruminant breeding holds crucial social and economic importance, often serving as a subsistence activity [1–7].

Regardless of the breeding model or production purpose, helminthosis, particularly due to *Haemonchus contortus*, poses a major challenge globally [8]. This parasite contributes to economic losses amounting to millions of dollars as it develops resistance to anthelmintics worldwide. Numerous studies indicate that these parasites exhibit resistance to commercially available anthelmintics, highlighting the urgent need for effective alternatives for helminthosis control in goats and sheep [9–16].

One promising alternative is biological control using the nematophagous fungus *Duddingtonia flagrans* [17]. Although various experimental studies have demonstrated its effectiveness [18], the absence of a commercial product has been due to high production costs, difficulties in achieving a formulation with a long shelf life, and challenges in large-scale production at viable costs. Recently, a commercial product (Bioverm®, Ghenvet Biotecnologia Ltda, 13148-153, Paulínia, SP, Brazil) was launched in Brazil. This study marks the first experiment conducted in the country based on the commercial-scale production model of *D. flagrans*.

This study aims to evaluate the characteristics of eggs per gram of feces (EPG), parasite infestation indicator scores (FAMACHA), and weight (WEIGHT) in sheep subjected to a biological control regimen with the nematophagous fungus *D. flagrans* over 167 days, following the use of the active ingredient moxidectin.

2. Materials and Methods

The experiment was conducted at Fazenda Talisman, located in Itapira, SP, Brazil (22°28′40.1″ S 46°45′38.3″ W). The study was based on data collected from a commercial sheep production operation, which complies with all animal welfare requirements. Commercial sheep production is considered a zootechnical practice and is not regulated under Brazilian Federal Law no. 11,794/2008, as it does not pertain to teaching or scientific research activities [19]. Therefore, there is no requirement for approval from an Ethics Committee on the Use of Animals. The treatment and use of experimental animals complied fully with Brazilian laws, guidelines, and policies related to animal welfare.

Data were collected from 50 female sheep of the Dorper (n = 15), Santa Inês (n = 21), and White Dorper (n = 14) breeds. All animals were adults, part of a commercial flock, and were estimated to be between 1 and 4 years old. During the experimental period, the animals were fed using three distinct systems: Pasture, Pasture+Silage, or Stall+Hay+Feed. These systems were tailored to meet the specific needs of the animals during maintenance, early gestation, and late pregnancy/newborn lamb phases. As this was a commercial flock, the treatments varied according to the phase the animals were in.

Prior to the biological control regimen, the flock was dewormed with moxidectin (0.2 mg/kg; orally). For the treatment with Bioverm®, as per product recommendations, it was mixed with mineral salt and provided continuously *ad libitum* at an estimated dose of 1 g of product for every 10 kg of live weight. The commercial product Bioverm®, containing the nematophagous fungus *D. flagrans*, was introduced to initiate the biological control system ten days post-deworming.

The variables assessed included eggs per gram of feces (EPG), FAMACHA scores indicating parasite infection (FAMACHA), and weight (WEIGHT). These were measured on days 1, 7, 14, 28, 43, 57, 78, 92, 139, and 167 following the start of Bioverm® treatment. For statistical analysis, EPG values below 50 were standardized to 50.

Fecal samples were collected directly from the rectum of each animal by the farm veterinarian, cooled to 8 °C, and transported to the laboratory. The McMaster technique was used for EPG determination [20]. Fecal sample analyses were conducted individually. The analyses involved 4 g of feces diluted in 56 mL of saturated NaCl solution, and eggs counted in both chambers of the McMaster slide were multiplied by a factor of 50 in order to obtain the EPG count. Only helminth eggs were counted, using the methodology described by Taylor et al. [21] as a reference. FAMACHA scores were assessed based on conjunctiva color [22,23]. Weights were measured using a scale designed for small ruminants (BL300Pro, Laboremus, Campina Grande, Brazil).

General mixed linear models were used to analyze the EPG, FAMACHA, and WEIGHT variables, incorporating the random effect of the animal simultaneously with the fixed

effects of breed and days of initiating biological control (included as covariate). We do not report the coefficient of determination (R^2) because, in methodologies based on mixed models, the emphasis is placed on the significance of the regression coefficients (individually and jointly) associated with the model adjusted with fixed and random effects. The quality of the model adjustment is assessed using the Akaike Information Criterion [24] and complemented by the graphical evaluation of the observed means (with error bars representing the standard errors of the mean) and the function adjusted by the mixed model. The adjusted model had a fixed breed and monitoring day included as a covariate, in addition to random components of animal and error. The animal effect was included as a random effect to account for repeated measures on the same experimental units over monitoring periods. The covariance between measurements of the same animals was considered, allowing the evaluation of different covariance structures using the AIC criteria [24]. Statistical significance was set at $p < 0.05$. All analyses were conducted using the SAS Program, version 9.2 (SAS Institute Inc., Cary, NC, USA).

3. Results

The number of observations (N) and estimates of means (MED), standard deviations (SD), coefficients of variation (CV), minimum (MIN), and maximum (MAX) for EPG, FAMACHA, and WEIGHT are presented in Table 1. Significant effects ($p < 0.05$) were detected in the analyses of variance regarding the "start days" of biological control with *D. flagrans* on EPG, FAMACHA, and WEIGHT. Regression analyses of these variables in relation to the introduction day of *D. flagrans* are shown in Figures 1–3. Each variable demonstrated a statistically significant quadratic model ($p < 0.05$). Table 2 provides the regression equations for predicting variable behavior based on the product's introduction day.

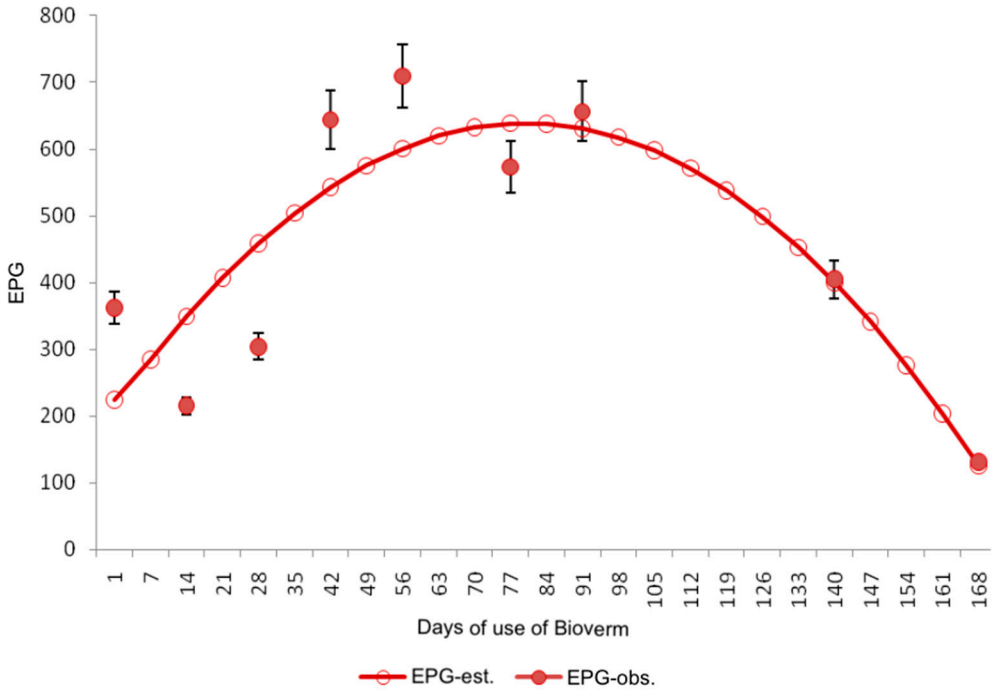

Figure 1. Behavior of the EPG variable in relation to the start days of biological control with Bioverme® containing the nematophagous fungus *D. flagrans*.

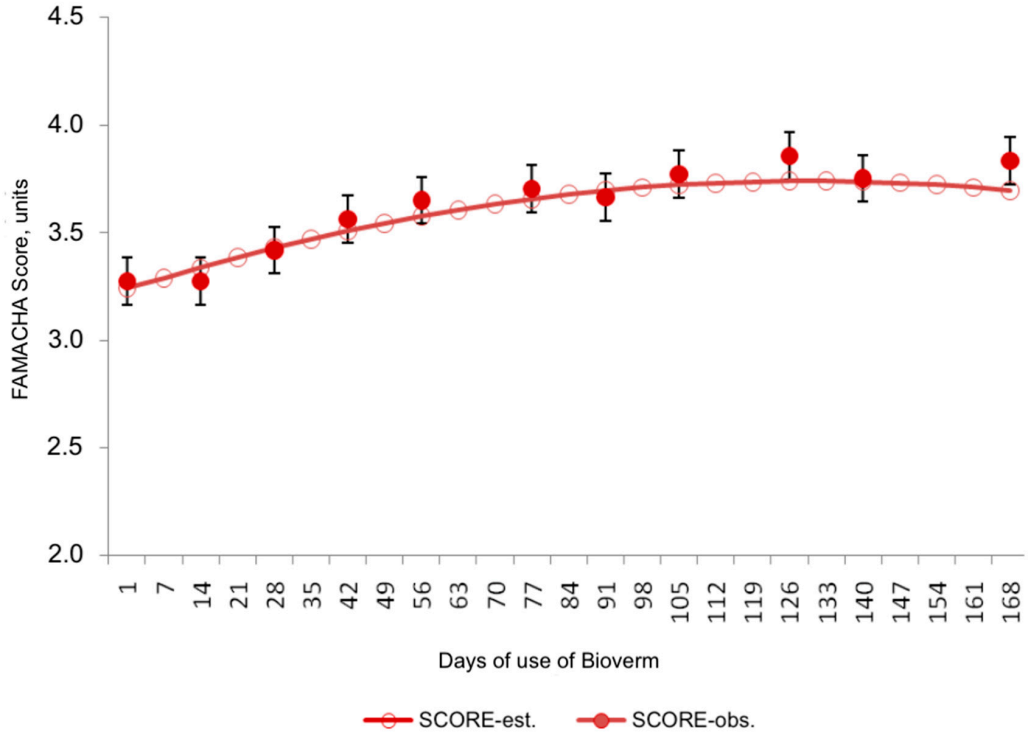

Figure 2. Behavior of the FAMACHA variable in relation to the start days of biological control with Bioverme® containing the nematophagous fungus *D. flagrans*.

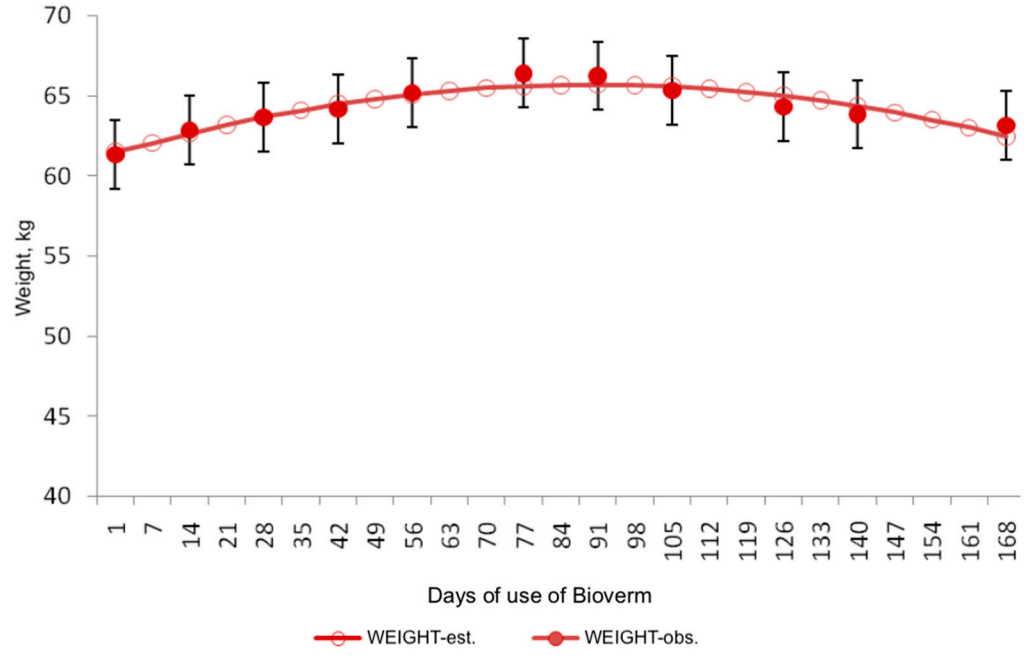

Figure 3. Behavior of the WEIGHT variable in relation to the days of initiation of biological control with Bioverme® containing the nematophagous fungus *D. flagrans*.

Table 1. Descriptive statistics for the EPG, FAMACHA, and WEIGHT variables.

Variable	N	MED	SD	CV	MIN	MAX
EPG	431	408.4	1181.4	289.3	50.0	10,200.0
FAMACHA	532	3.6	0.8	21.7	2.0	5.0
WEIGHT	533	61.1	16.0	26.2	40.0	125.0

N = Observations; MED = Estimates of means; SD = Standard deviations; CV = Coefficients of variation; MIN = Minimum; MAX = Maximum.

Table 2. Regression equations, points of.

Variable	Regression Equation	X Max.	Y Max.
EPG	Y = 213.5796 + 10.6346X − 0.06642X2	80.05 days	639.26 EPG
FAMACHA	Y = 3.2351 + 0.00778X − 0.00003X2	129.72 days	3.74 un
WEIGHT	Y = 5.1033 + 0.00871X − 0.000052X2	89.88 days	65.69 kg

4. Discussion

Haemonchus contortus is globally recognized as the most significant parasite affecting sheep farming, primarily due to its notable resistance to synthetic anthelmintics [8]. The development of resistant populations has been exacerbated by the indiscriminate use of these chemical agents.

Biological control presents an effective alternative for reducing parasite populations, utilizing natural antagonists such as nematode-predatory fungi [15,17]. These fungi produce structures like constricting and non-constricting rings, hyphae, buds, and three-dimensional adhesive networks along their mycelium. The process involves trapping the nematode, after which hyphae penetrate the cuticle, leading to hyphal development and digestion of the nematode's internal contents [25]. Among these fungi, *Duddingtonia flagrans* is notable for its ability to pass through the ruminant digestive tract intact, significantly reducing the population of infective larvae in pastures and thereby lowering the risk of animal recontamination.

In this study, the efficacy of *D. flagrans* in its commercial form was assessed by analyzing EPG, FAMACHA, and WEIGHT variables. Our results show that the biological control effect on the EPG variable becomes apparent after approximately 80 days, attributed to the time required for fungal dissemination across pastures. This effect is characterized by a gradual decline in EPG values post the 80th day of *D. flagrans* application, as demonstrated in Figure 1, alongside a reduction in the variation of observed EPG, evidenced by decreased standard error estimates.

For the FAMACHA variable, the data reveal a steady increase in scores until they stabilize. Initial mean scores were 3.24 following deworming, peaking at 3.74 around 130 days later. These findings suggest a stronger correlation between the FAMACHA and other hemoparasite infestation indicators compared to EPG.

Regarding the WEIGHT variable, minimal fluctuations were observed throughout the experiment. The average animal weight started at 61.50 kg, peaked at 65.70 kg approximately 90 days after commencing product administration, and then slightly decreased to 62.46 kg. This minor fluctuation suggests that the nutritional management implemented was suitable for reproductive animals, where weight gain is not a primary objective.

The commercial product Bioverm® demonstrated encouraging outcomes, validating its legal registration in Brazil. The application of Bioverm® not only reduced the need for chemical anthelmintics but also decreased resistance to these agents, enabling the reuse of previously ineffective treatments. The farm continued using Bioverm® for several years post-experiment, deworming solely animals with clinical symptoms. Notably, the farm maintained a high stocking density, with over 500 animals on 15 hectares of pasture. Further studies corroborated the product's efficacy across various species, including small ruminants [26,27], domestic buffalos [28], cattle [29,30], pigs [31], and horses [32,33]. Also, with cage-free layers (unpublished data).

Concerns about *H. contortus* extend beyond livestock production to include zoos and natural reserves, underscoring the resistance to synthetic anthelmintics [34]. This resistance poses a threat to a broader range of animals, especially those involved in One Conservation programs [35]. The long-term viability of species within these programs can

be compromised by the introduction of resistant parasites, as seen in European bison (*Bison bonasus*) [34].

Research has shown *D. flagrans*' efficacy in inhibiting larvae growth and viability in feces of various captive wild animals, including wapitis (*Cervus canadensis*) [36], Reticulated and Masai giraffes (*Giraffa camelopardalis reticulata* and *G. c. tippelskirchi*), scimitar-horned oryx (*Oryx dammah*), roan antelope (*Hippotragus equinus*), sable antelope (*H. niger*), gerenuk (*Litocranius walleri*) [37,38], grivet monkey (*Chlorocebus aethiops*) [39], plains zebra (*Equus guagga*) [39,40], African wild ass (*Equus africanus*) [40], blackbuck (*Antilope cervicapra*), Cuvier's gazelle (*Gazella cuvieri*), American bison (*Bison bison*), sitatunga (*Tragelaphus spekii*), European mouflon (*Ovis orientalis musimon*) [41], and ostriches (*Struthio camelus*) [42]. The use of chemical parasiticides, common in livestock, appears to be the sole solution for parasites affecting ex situ wild animals [43]. However, integrating nematophagous fungi into the feed offers a promising, sustainable approach for reducing infective stages of roundworms in wildlife, contributing to sustainable parasitic infection management in zoos [44]. The use of nematophagous fungi in wild animal helminth management is safe, as the fungi act exclusively in the fecal matter without systemic effects. Therefore, the use of Bioverm® in captive wild animals, although off-label, should be considered a viable management strategy.

This study holds significant implications for animal production and health. By integrating *D. flagrans*, biological control reduces reliance on chemical treatments, cuts costs, and mitigates parasite resistance. This benefits breeders preserves herd health and positively impacts the environment by reducing the dependency on chemical agents. Its successful application may promote similar practices globally, advocating for sustainable methods in various animal breeding sectors.

5. Conclusions

The implementation of biological control using the nematophagous fungus *Duddingtonia flagrans* resulted in a gradual and significant reduction in EPG values after 80 days, alongside decreased variation in EPG among animals. Additionally, FAMACHA was inversely related to observed EPG values, indicating a more reliable correlation with hemoparasite infestation levels. Despite minimal variations in animal weight throughout the study, there was no indication that the dietary administration of *D. flagrans* negatively impacted the animals' weight, supporting its efficacy as a biological control agent without adverse effects on animal growth. In conclusion, the biological control of nematodes using *D. flagrans* emerges as a strategic tool for managing parasitic worm infestations, allowing for the secure application of the product to prevent losses due to these parasites. Bioverm® shows potential for effective nematode management in small ruminants and beyond.

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Informed Consent Statement: Not applicable.

Data Availability Statement: This work utilized descriptive statistics and included all relevant data within the manuscript.

Conflicts of Interest: None of the authors have any conflicts of interest to declare.

Abbreviations

The following abbreviations are used in this manuscript:

EPG	Eggs per gram of feces
FAMACHA	parasite infestation indicator scores

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