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Predatory activity of nematophagus fungus *Duddingtonia flagrans* in infective larvae after gastrointestinal transit: biological control in pasture areas and *in vitro*

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Research Paper

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Abstract

Biological control is a strategy to decrease parasitic populations, and the action takes place through natural antagonists in the environment. We studied the predatory activity of the fungus *Duddingtonia flagrans* in infective larvae (L3) of gastrointestinal nematodes after gastrointestinal transit. Ten heifers were divided into two groups: treated (animals received pellets containing fungus) and control (animals received pellets without fungus). Twelve hours after administration, faeces samples were collected for *in vitro* efficacy tests. The animals then remained for 7 h in the experimental pasture area. At the end of this period, 20 faecal pads (ten treated and ten control) were selected at random. Pasture, faecal pad and soil collections occurred with an interval of 7 days, totalling four assessments. *In vitro* activity demonstrated that fungi effectively preyed on L3, achieving a reduction percentage of 88%. In the faecal pad of the pasture area, there was a difference ($P < 0.05$) between collections 3 and 4 for both groups; in the treated group a reduction of 65% was obtained, while in the control group there was an increase of 217% in the number of L3. The recovery of L3 in the soil and in the pasture was similar in both groups. There was no influence ($P = 0.87$) of the passage time on the fungus predatory activity. *Duddingtonia flagrans* demonstrated the ability to survive gastrointestinal transit in the animals, reducing the number of L3 in the faeces, indicating that this biological control has great potential in the control of worm infections.

Introduction

Gastrointestinal nematode infections in dairy herds compromise the health and welfare of animals, leading to productive and economic losses (Geurden *et al.*, 2015; Ravinet *et al.*, 2016). Parasitism can negatively affect growing rate and puberty in heifers (Lacau-Mengido *et al.*, 2000), in addition to promoting losses in milk production (Antonello *et al.*, 2010). From an epidemiological point of view, only 5% of gastrointestinal nematodes are on the animals, while the remainder are on the pasture in the form of immature stages (Sagüés *et al.*, 2011). The biological cycle of these parasites occurs in pastures; when contaminated, the pasture is the main vehicle for the transmission of gastrointestinal nematodes to animals (Araújo *et al.*, 2006).

Control of worms is a major challenge in livestock systems; to control these infections, anthelmintic drugs have been used for almost 40 years (Geurden *et al.*, 2015). Nowadays, a new challenge facing livestock systems is parasitic resistance to available drugs, a fact reported by Baiak *et al.* (2018). The authors demonstrated through a meta-analysis, involving results from several countries, that the indiscriminate use of medications (overdose) in cattle resulted in the ineffectiveness of the different chemical groups of anthelmintics (Baiak *et al.*, 2018). On the other hand, in agroecological systems, the main difficulty faced is restrictions on the use of anthelmintic drugs. The sanitary management used in these systems is usually carried out with homoeopathics, herbal medicines and factors inherent to the system management (Almeida, 2013); however, new control alternatives are being sought to minimize infection by gastrointestinal nematodes.

Biological control through nematophagous fungus is a promising alternative to reduce the number of infective larvae (L3) in livestock farming (Peña *et al.*, 2002). Among the groups of nematophagous fungus (endoparasites, ovicides and predators), the group of predators is most commonly used in biological control. This group of fungi have the ability to slow the

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development of larvae in the environment without negative impacts on the host (Braga & Araújo, 2014). Among the most widely used species in this group, the following stand out: *Arthrobotrys robusta* and *Duddingtonia flagrans*. These species survive passage through the gastrointestinal tract of ruminants and germinate in the faecal pads, where they prey on the larvae (Campos *et al.*, 2008). Furthermore, they are easy to grow in the laboratory and demonstrate the potential to be developed for commercial formulations (Mota *et al.*, 2003).

Previous studies show promising results in the application of biological control in laboratory tests and field conditions (Jobim *et al.*, 2008; Silva *et al.*, 2013). In this way, the fungus contributes to the prophylactic control of nematodes, reducing the number of L3 in pasture and minimizing the risk of reinfection (Knox *et al.*, 2002; Mota *et al.*, 2003). However, in field conditions, the environment is influenced by several factors (rain, solar radiation, air temperature and relative humidity) that can affect the dynamics of nematophagous fungus (Hasanzadeh *et al.*, 2012; Saumell *et al.*, 2015). Thus, the objective of this work was to evaluate the predatory activity of the nematophagous fungus *D. flagrans* on infective larvae of gastrointestinal nematodes from dairy heifers in pasture areas and *in vitro*, after gastrointestinal transit from faeces, soil and pasture.

Materials and methods

This study was approved by the Ethics Committee on Animal use at the Universidade Federal do Paraná under protocol number 083/2018, and was performed in accordance with the ethics of animal experimentation. This study was conducted between April and July of 2019. To meet our objectives two experiments were performed: (1) evaluation of the predatory activity of the nematophagous fungus *in vitro*; (2) evaluation of the predatory activity of the nematophagous fungus in a pasture area.

Evaluation of the predatory activity of the nematophagous fungus in vitro

The first experiment was carried out in order to confirm the passage of the fungus through the gastrointestinal system and its presence in faeces for further testing in a pasture area. The experiment was performed between May and July of 2019 at the Laboratório de Parasitologia Animal da Universidade Estadual de Ponta Grossa, Paraná, Brazil. Ten Jersey heifers were used in this study, with an average age of (mean ± SD) 11.7 ± 4.3 months, and an average weight of 236 ± 81 kg. The animals were divided into two homogeneous groups (five animals per group) according to the number of gastrointestinal nematode eggs per gram of faeces (EPG, fig. 1). An EPG test was performed according to the methodology of Gordon & Whitlock (1939), modified by Ueno & Gonçalves (1998).

Our treatments consisted of faeces obtained from animals treated with fungus (treated group) and faeces from animals that did not receive fungus (control group). For this purpose, we adopted the methodology proposed by Silva *et al.* (2013), in which the treated group received 150 g of pellets (single dose) containing a mycelial mass of the fungus *D. flagrans* (0.2 g of mycelium) mixed with 500 g of wheat bran, while the control group received 150 g of pellets (single dose) without the mycelial mass of *D. flagrans* fungus mixed with 500 g of wheat bran. The fungus isolate of *D. flagrans* (AC001) used was produced and provided by the Laboratório de Controle Biológico de Fitonematóides (Instituto de Biotecnologia Aplicada à Agropecuária – BIOAGRO) of the

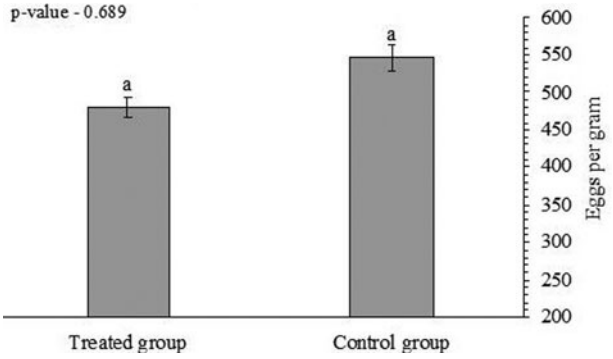

Fig. 1. Average values ± standard deviation (SD) of the EPG values from animals used in experiments 1 and 2. Average values followed by the same letter did not differ ($P < 0.05$).

Department of Phytopathology at the Universidade Federal de Viçosa (UFV), Minas Gerais, Brazil.

These isolates of the nematophagous fungus *D. flagrans* (AC001) were obtained from Brazilian agricultural soil in the municipality of Viçosa, in the Zona da Mata region of the state of Minas Gerais. They were collected using the soil-sprinkling method of Duddington (1955). To produce fungal mycelia of *D. flagrans* (AC001), culture discs approximately 4 mm in diameter, in 2% water agar (2% WA), were transferred to 250-ml Erlenmeyer flasks containing 150 ml of the liquid medium GPY (glucose, sodium peptone and yeast extract). The flasks were continually stirred at 120 rpm in the dark and at the temperature of 26°C for 10 days. After this period, the mycelia were removed, filtered and weighed on an analytical scale. All the procedures followed the methodology of Braga *et al.* (2009).

For the *in vitro* analyses, 12 h after pellet administration (with and without fungus), faeces samples were collected directly from the rectal ampoule of the animals ($n = 10$; 5 by group). The time of 12 h was chosen based on Silva *et al.* (2013) and Braga *et al.* (2020); these authors reported that *D. flagrans* activity did not differ among different time periods (range: 12–72 h). The experimental design to evaluate the *in vitro* predatory activity of the fungus *D. flagrans* was adapted from Mauad (2008), pooled for each group (treated and control) and used a 1-g faeces sample from each animal. At the end of this step, 2 g of each pooled sample was removed and placed in a 9-cm Petri dish, containing solid Agar and antibiotic (Chemitril 0.05 g/l). In total, we used four Petri dishes, two for each group. A suspension of approximately 3000 larvae (L3) of *Haemonchus* spp. (41.1%) and *Cooperia* spp. (58.9%), obtained through coprocultures according to the technique proposed by Roberts & O'Sullivan (1950), was added to the Petri dishes.

Samples for coprocultures were collected from the heifers before the beginning of the experiment. The amount of L3 present in the coprocultures was estimated by counting larvae in ten aliquots of 10 µl. The Petri dishes were sealed with plastic film and incubated in an oven (25°C) for 14 days. After this period, non-predated larvae (L3) were recovered by the Baermann method (see Staniland, 1954) and identified using the methodology proposed by Keith (1952). The effectiveness of the fungus *D. flagrans* in preying on infective larvae (L3) was evaluated through the percentage of larvae reduction (PR, %) according to equation (1):

$$\text{PR (\%)} = \frac{X - Y}{Y} \times 100 \quad (1)$$

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where PR is the percentage of larvae reduction, X is the number of larvae in the treated group and Y is the number of larvae in the control group.

Evaluation of the predatory activity of the nematophagous fungus in a pasture area

Experimental area and climate pattern

The second experiment was carried out at the Estação de Pesquisa Agroecológica – CPRA at the Instituto de Desenvolvimento Rural do Paraná, Paraná state, in Southern Brazil (25°26′41″S, 49° 11′33″W). This is an agroecological farm, where deworming of animals with anthelmintic drugs is not permitted; sanitary management is usually carried out with the use of homeopathic herbal medicines and factors inherent to management. The climate in this region is characterized as humid maritime temperate (Cfb) according to the Köppen's classification. The region has mild summers with an average annual temperature between 18 and 20°C (Alvares *et al.*, 2013).

At the farm, animals are raised permanently on pasture, mainly composed of plant species of *Paspulum* spp., *Arachis* spp., *Lolium* spp. and *Brachiaria* spp. The farm is divided into paddocks, under the Voisin's Rotational Grazing system (Voisin, 2001), in which mineral salt and water are offered *ad libitum*. In this system, the pasture area is divided into paddocks, and the herd enters a new paddock every day so that the plants have enough time to recover. Voisin's system is ecologically useful, sustainable and offers a key solution for the problems faced by climate change, since this system improves the structure and productivity of the soil due to biocenosis; reduces greenhouse gas emissions from the animals; decreases fertilizer and pesticide use; and improves the health of animals (Pinheiro Machado, 2010). Before the animals enter a new paddock, they receive supplementation (wheat bran) at the trough. The experimental area consisted of a paddock (900 m²), with a 60-day pasture rest period set before the start of the tests.

Animals and definition of treatments

The definitions and characteristics of the animals and treatments used have already been described in the methodology of the first experiment.

Definition of the experimental unit

Twelve hours after pellet administration (with and without fungus), animals were taken to the experimental area, where they remained for 7 h (7:00 to 14:00). Four observers were positioned, one on each corner of the paddock, to help to record the time that each animal defecated. All observers remained at a safe distance (approximately 5 m) so as not to interfere in the animal's behaviour, entering the paddock only to fix the pole. All faecal pads were identified with poles with colours representing each treatment (treated and control). After the animals left the paddock, 20 faecal pads (treated, $n = 10$ and control, $n = 10$) were selected at random and the diameter (cm) was measured. During the observational period (7:00 to 14:00), the time that each animal defecated was recorded so that the passage time corresponding to the time between intake of the pellets and defecation in the experimental areas could be calculated.

Each faecal pad was delimited to identify the experimental unit ($n = 20$) and divided into four equal parts. To demarcate the collection areas, transects that exceeded 10 cm from the largest radius of the faeces were used (fig. 2). During the collections, a minimum distance of 100 cm and a maximum distance of 350 cm between the faecal pads were respected; in this way interaction between faecal pads was not possible. This methodology was adopted because it is known that approximately 90% of infective larvae (L3) do not migrate more than 10 cm away from faecal pads (Skinner & Todd, 1980).

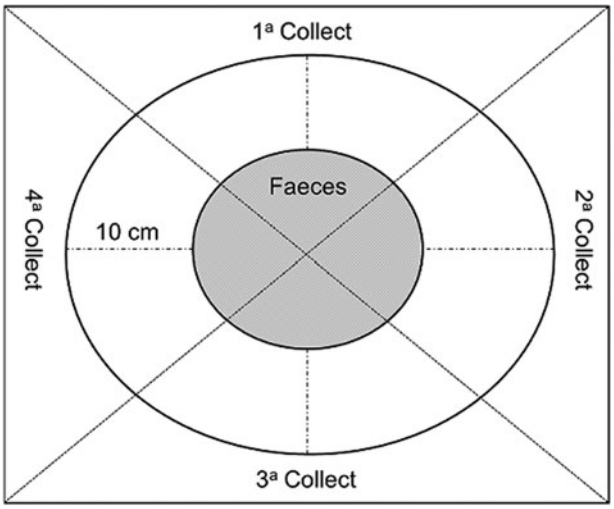

Fig. 2. Schematic representation of the collection areas.

Data collection

Pasture, faecal pad and soil collections occurred at CPRA in April of 2019 with an interval of 7 days, totalling four assessments. The weekly collection was always performed in the morning at the same time (beginning 8:00; ending 11:00) and by the same person. During this period the paddock remained free of animals. For the recovery of infective larvae (L3) from the pasture, samples were collected within 10 cm of a faecal pad at two heights: 50% of the upper pasture (corresponding to the aerial part of the pasture) and 50% of the lower pasture (corresponding to the pasture stalk). To evaluate the infective larvae in the faecal pad, all the matter of a part was collected, as well as the soil sample under the faecal pad to a depth of 10 cm. All samples were placed in thermal boxes and sent to the Laboratório de Parasitologia Animal at the Universidade Estadual de Ponta Grossa, where the laboratory analyses were performed.

Laboratory analysis – recovery of infective larvae

For recovery of infective larvae (L3) from pasture, samples were placed in a sedimentation calyx. These samples were wrapped in gauze and fixed in the upper part of the sedimentation calyx, where they remained submerged in mineral water for 24 h. At the end of this stage, the pasture samples were removed from the sedimentation calyx and conditioned in a forced ventilation oven (55°C) for 72 h to determine the dry matter. Meanwhile, the sedimentation calyx supernatant was discarded and the sediment (1.5 ml) transferred to a graduated conical tube with a lid. The sediment was examined under a microscope and the infective larvae were quantified according to the methodology proposed by

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Keith (1952); the results were expressed in number of L3 per kg of dry matter (L3/kg DM).

For recovery of infective larvae (L3) from the faecal pad and soil, the samples were conditioned on a tissue, inside a sieve and placed in a sedimentation calyx. Mineral water was added to the sedimentation calyx to cover the samples, where they remained for 24 h. Sediment collection as well as quantification and identification of larvae were carried out in the same way as described for pasture. After the larvae were recovered, both samples (faecal pad and soil) were transferred to paper packages of known weight and conditioned in a forced ventilation oven (55°C) for 72 h to determine the dry matter. The larvae genera were identified according to their characteristics described by Keith (1952).

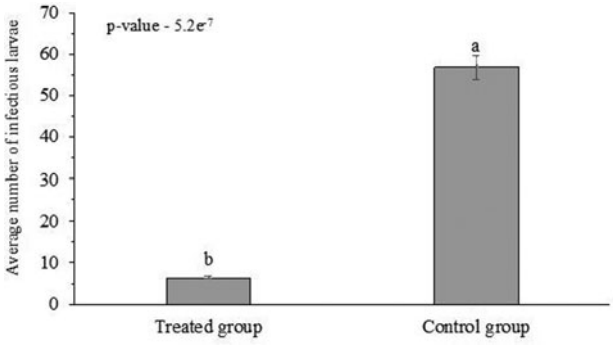

Fig. 3. Average values of infective larvae (L3) of *Cooperia* spp. and *Haemonchus* spp. recovered. Average values followed by the same letter did not differ ($P < 0.05$).

Statistical analysis

In order to confirm there was no significant difference in eggs per gram (EPG) between the groups (treated and control), the data of the EPG test were submitted to a simple generalized linear model with Poisson distribution, logarithmic bonding function, and 95% confidence interval. The experimental design of the *in vitro* biological control was composed of two treatments (faecal pad with fungus and faecal pad without fungus) and four experimental units (two Petri dishes from the treated group and two Petri dishes from the control group). For analysis, a simple generalized linear model with Poisson distribution, logarithmic bonding function and 95% confidence interval was used.

The experimental design of the pasture area was composed of two treatments (faecal pad with fungus and faecal pad without fungus), four replications (parts of faecal pad) and 20 experimental units (ten faecal pads from treated group and ten faecal pads from control group). For analysis a mixed model (generalized linear model – GLM) with Poisson distribution was used and a 95% confidence interval. Animals, diameter of faecal pad (DF) and EPG were defined as random effects following the model

$$Y_{ij} = \alpha_{ijk} + A_i + \beta_{ij} + e_{ij'}$$

where Y_{ij} is the non-predated infective larvae (L3/Kg DM); α_{ijk} is the fixed effect (i corresponds to group (treated and control), j corresponds to collections, k corresponds to passage time); A_i is the random effect of animals; β_{ij} is the random effect (i corresponds to DF, j corresponds to EPG); and e_{ij} is the residual effect.

The analyses were performed separately, and for each site of collection (pasture (upper and lower), faecal pad and soil) a GLM model was obtained. The model was adjusted by statistical package lme4 (Bates *et al.*, 2015). The confidence intervals were estimated using Type II Wald chi-square tests and the fit of the model was given by a likelihood test. The normality of the random facts was given by quartile plot means with 95% confidence interval. In order to evaluate fungus predatory activity in the faeces collections, the data were submitted to confirmatory analysis by a paired samples Wilcoxon test at the confidence level of 95%.

The environment data were used for a descriptive analysis of the climate during the experimental period. All analyses (descriptive, influence and confirmatory) were performed using statistical software R (R Core Team, 2017).

Results

In experiment 1 (*in vitro*) there was a difference ($P < 0.05$) in the number of non-predated infective larvae (L3) between the groups (treated and control). There was a difference ($P < 0.05$) in L3 recovered between the groups (fig. 3). The highest number of L3 was obtained in the control group (454), while in the treated group, only 52 infective larvae were non-predated. After gastrointestinal transit of dairy heifers, a percentage reduction of 88% *in vitro* was obtained.

Monthly average temperature during the experimental period is shown in table 1. The average maximum temperature throughout the experimental period was 23.6°C, the average minimum temperature was 15.2°C and the average precipitation was 0.38 mm.

The predominance of gastrointestinal nematode genera recovered during the experimental period from pasture (upper and lower), faecal pad and soil by group is shown in table 2. Regarding genera, there was a predominance of *Cooperia* spp. and *Haemonchus* spp. in both groups.

In experiment 2, there was no influence ($P = 0.87$) of the passage time (treated group range: 473 min to 835 min) on the fungus predatory activity. There was a difference ($P < 0.05$) in the amount of non-predated larvae in the faecal pad (range: 0.00–86,294) and upper pasture (range: 0.00–37,419) between treatments. In addition, the collections influenced ($P < 0.05$) the

Table 1. Average values ± standard deviation (SD) during experimental period for air temperature (AT, °C), relative humidity (RH, %) and rainfall (RF, mm).

	1st collection		2nd collection		3rd collection		4th collection	
Variables	Average	Range	Average	Range	Average	Range	Average	Range
AT (°C)	18.75 ± 0.71	16.50–21.00	18.58 ± 1.17	13.67–23.50	19.71 ± 1.18	14.57–24.86	20.00 ± 1.10	16.86–23.14
RH (%)	80.72 ± 9.05	71.00–92.25	80.21 ± 8.72	65.75–90.25	78.89 ± 5.83	72.50–86.75	85.05 ± 5.51	73.00–91.00
RF (mm)	2.92	–	0.00	–	0.02	–	0.34	–

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Table 2. Recovery of infective larvae by pasture samples (upper and lower), faecal pad and soil at the four collections by groups.

	Treated group				Control group			
Collection	*Haemonchus* spp.	*Trichostrongylus* spp.	*Ostertagia* spp.	*Cooperia* spp.	*Haemonchus* spp.	*Trichostrongylus* spp.	*Ostertagia* spp.	*Cooperia* spp.
Upper pasture								
1	2	0	0	0	5	0	0	0
2	3	0	0	0	0	0	0	23
3	0	0	0	1	14	0	0	31
4	2	0	0	0	5	0	0	0
Lower pasture								
1	6	0	0	4	1	0	0	0
2	1	0	1	5	4	0	0	24
3	0	0	0	1	5	0	0	40
4	3	0	0	0	6	0	0	0
Faecal pad								
1	2	0	0	8	1	0	0	8
2	0	0	0	0	0	0	0	38
3	11	0	2	66	92	7	15	86
4	3	0	0	5	3	0	0	20
Soil								
1	1	0	2	0	2	0	1	4
2	0	0	0	2	0	0	0	0
3	4	0	0	11	9	0	0	4
4	1	0	2	0	2	0	1	4

Table 3. Average values and coefficient of variation (CV, %) of non-predated infective larvae (L3/kg DM) from pasture (upper and lower), faecal pad and soil at the four collections by treated and control groups.

	Treated group		Control group		
Collection	Average	CV	Average	CV	*P*-value
Upper pasture					
1	223.61	1.80	0.00	0.00	0.93
2	191.43	1.59	75.32	2.62	<0.05
3	785.86	1.36	1,294.48	1.66	<0.05
4	6,069.92	1.48	7,348.50	1.74	<0.05
Lower pasture					
1	134.54	2.79	0.00	0.00	0.94
2	88.29	1.10	56.56	1.73	<0.05
3	1,367.52	1.96	809.36	1.38	<0.05
4	2,145.56	1.91	1,776.98	1.97	<0.05
Faecal pad					
1	0.00	0.00	0.00	0.00	1
2	0.00	0.00	0.00	0.00	1
3	20,544.84	1.53	3,864.51	1.95	<0.05
4	7,070.02	0.88	12,286.07	1.04	<0.05
Soil					
1	0.00	0.00	0.00	0.00	1
2	0.00	0.00	0.00	0.00	1
3	0.00	0.00	48.17	2.33	0.96
4	210.29	1.05	610.75	1.92	<0.05

Averages followed by $P < 0.05$ in the line had the influence of treatment by the generalized linear model.

number of non-predated infective larvae in the evaluated sites. The third and fourth collections showed the highest mean values of L3/kg DM (table 3).

When analysing the number of non-predated infective larvae (L3) in the faecal pads from the pasture area, there was a difference ($P < 0.05$) between collections 3 and 4 for both groups (treated and control); in the treated group a reduction of 65% was obtained, while in the control group there was an increase of 217% in the number of L3.

Discussion

This is the first study to report the predatory activity of the fungus *D. flagrans* in faecal pads divided into parts and evaluated over time. The nematophagous fungus *D. flagrans* stands out due to its large production of chlamydospores that are highly resistant to adverse conditions (Silveira *et al.*, 2017). Its predatory activity has been widely studied *in vitro* (Braga *et al.*, 2011; Silva *et al.*, 2013; Silveira *et al.*, 2017) since research in controlled environments (*in vitro*) is important to find fungi with the greatest potential for biological control. However, our study demonstrates that even after gastrointestinal transit, the fungus *D. Flagrans* is an efficient biological control not only *in vitro*, but also for pasture areas. Regarding the genera of larvae recovered from the samples, there was a predominance of groups *Cooperia* spp. and *Haemonchus* spp. This fact has already been reported in other studies (Assis *et al.*, 2013; Fernandes *et al.*, 2017), demonstrating that in tropical regions, these genera are common in cattle. However, the fungus *D. flagrans* is not selective for specific genera of parasites, which demonstrates that the genera of the parasite does not affect the predatory activity of the fungus (Araújo *et al.*, 2006).

The different times of passage of the fungus through the gastrointestinal system of animals did not influence the predatory action of the fungus evaluated in the pasture area. Under controlled conditions (*in vitro*), it is possible to observe the viability and capacity of the fungus to prey on infective larvae after 72 h of gastrointestinal transit (Silva *et al.*, 2013). However, with our results, we suggest that the fungus can remain viable after 12–19 h of gastrointestinal transit. For *in vitro* experiments, the faecal pads were collected 12 h after the fungus was offered; and collection of faecal pads in the pasture areas was carried out after 19 h (12 h after offered + 7 h animals remained at the paddock). This information is important since environmental conditions can influence the predatory action of the fungus in the faecal pad (Saumell *et al.*, 2015).

No infective larvae were collected in the soil and faecal pads in the first and second collections in either group (treated and control). This may be because of development of the larvae, which in favourable conditions (air temperature ≥25°C) takes around 7 days (Amarante, 2014). In our study, the average air temperature was 23.6°C, which may lead to later development of infective larvae. In the third collection, the increase in the number of infective larvae recovered may be related to the increase in air

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temperature. With this effect, it is suggested that the fungus started to act in the third collection (14 days after deposition of faecal pad) since they form their traps (three-dimensional network) quickly when they are stimulated by free-living nematodes or parasites. Generally, traps are formed in the first 4 h of contact of the fungus with the larvae (Nansen *et al.*, 1986; Charles *et al.*, 1993), with predation of the larvae occurring up to approximately 20 h (Nansen *et al.*, 1986).

In the fourth faecal pad collection, there was an increase in non-predated infective larvae in the control group; whereas in the treated group, there was a reduction in larvae from collection 3 to collection 4. Previous studies also showed a reduction (range: 60–90%) in the number of infective larvae present in faecal pads of cattle that received *D. flagrans* (Saumell *et al.*, 2015; Bilotto *et al.*, 2018; Healey *et al.*, 2018).

However, the infective larvae recovery in the soil and in the different heights of the pasture (upper and lower) increased in both groups (treated and control) from collection 3 to 4. This result suggests that the non-predated infective larvae in the faecal pad continued with their growth and development, resulting in a greater number of non-predated infective larvae in the final evaluation, as a consequence of the amount of eggs that were eliminated in the animals' faeces. This occurred because the fungus *D. flagrans* has little growth beyond the faecal pad and in the surrounding soil (Faedo *et al.*, 2002). In addition, the migration of infective larvae is controlled by environmental factors (e.g. solar radiation, air temperature and relative humidity), with relative humidity being the main factor that promotes migration, since the larvae need a film of water for their movement (Sciacca *et al.*, 2002). In uncontrolled conditions (pasture areas), the difficulties in studying biological control are greater than in laboratory conditions, due to the direct action of environmental factors. Thus, further research should be carried out to deepen knowledge of this biological control technique in pasture areas. Biological control has growth potential, mainly due to popular pressure for safer food, in addition to not requiring a grace period for the consumption of products of animal origin.

In conclusion, the fungus *D. flagrans* was demonstrated to withstand the gastrointestinal transit of animals by reducing the number of infective larvae in the *in vitro* test and in faecal pads in the field test. The results of this study demonstrate that biological control of gastrointestinal nematodes with nematophagous fungi is a promising alternative; however, due to the complex influence of the environment on the predatory activity of the fungus, there is a need for more research on different types of grazing over time. In this way, knowledge of the development characteristics of nematophagous fungi will enable better decision making in the use of fungi in the environment. This, in turn, will allow researchers to improve current knowledge in order to apply nematophagous fungi to livestock systems.

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