Journal of Invertebrate Pathology 150 (2017) 1–5

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Nematophagous fungi combinations reduce free-living stages of sheep gastrointestinal nematodes in the field

Wendeo Ferreira da Silveira[a], Fabio Ribeiro Braga[c,1,*], Alexandre de Oliveira Tavela[d], Lucas Fernando dos Santos[b], Rafael Reis Domingues[b], Anderson Rocha Aguiar[c], Carolina Magri Ferraz[c], Lorendane Millena de Carvalho[b], Thiago de Hollanda Ayupe[b], Jose Cola Zanuncio[e], Jackson Victor de Araújo[b]

[a] Departamento de Biologia Geral, Universidade Federal de Viçosa, 36570-900 Viçosa, Minas Gerais, Brazil
[b] Departamento de Medicina Veterinária, Universidade Federal de Viçosa, 36570-900 Viçosa, Minas Gerais, Brazil
[c] Departamento de Medicina Veterinária, Universidade de Vila Velha, 29102-920 Vila Velha, Espirito Santo, Brazil
[d] Centro de Ciências Rurais, Universidade Federal de Santa Catarina, 89520-000 Curitibanos, Santa Catarina, Brazil
[e] Departamento de Entomologia/BIOAGRO, Universidade Federal de Viçosa, 36570-900 Viçosa, Minas Gerais, Brazil

ABSTRACT

Gastrointestinal nematodes (GIN) can reduce or limit sheep production. Currently there is a clear deficiency in the action of drugs for the control of these parasites. Nematophagous fungi are natural enemies of GIN. Fungal combinations have potential for reducing GIN populations. The aim of this study was to evaluate the efficiency combinations of nematophagous fungi in sodium alginate matrix pellets for the biological control agents of gastrointestinal sheep nematode parasites in the field. The nematophagous fungi (0.2 mg of fungus per kg of body weight), *Arthrobotrys conoides*, *A. robusta*, *Duddingtonia flagrans*, and *Monacrosporium thaumasium* were used. The treated groups were administered mycelium combinations in the following combinations: group 1 (*D. flagrans* + *A. robusta*); group 2 (*M. thaumasium* + *A. conoides*). The control group did not receive any fungal pellets. We used three groups with eight Santa Inês sheep each. Each animal was treated with approximately 1 g of pellet per 10 kg of live weight. During the experimental period, we evaluated: number of eggs per gram of feces (EPG), infective larvae (L_3) per kg of dry matter, larvae recovered from coprocultures, packed cell volume, total plasma protein concentration of sheep, and environmental conditions. Group 2 EPG (*M. thaumasium* + *A. conoides*) differed from the control group in September and October. The number of L3/kg of dry matter recovered from animals of groups 1 and 2 at distances of 0–20 and 20–40 cm from the fecal pats was lower than the control group. The packed cell volume and total plasma proteins of treated animals were similar to those of the control group. The combination of treatment groups (*D. flagrans* + *A. robusta* and *M. thaumasium* + *A. conoides*) reduced the number of L_3/kg of pasture. Therefore, treatment of nematophagous fungal combinations have the potential to manage free-living stages of GIN in sheep.

1. Introduction

Gastrointestinal nematodes (GIN) can have a negative impact on sheep production (Amarante, 2011; Charlier et al., 2014; Simpraga et al., 2015). In Brazil the main contributor to sheep nematodes are *Haemonchus* spp., *Trichostronhylus* spp., *Cooperia* spp. and *Oesophagostomum* spp. (Amarante, 2011). Control strategies and prevention of these diseases could be beneficial for sheep production (Falzon et al., 2014; Vadlejch et al., 2015). Gastrointestinal helminthes are controlled with anthelmintic compounds, but with the development of anthelmintic resistance in nematode populations (Torres-Acosta et al., 2012), new alternatives capable of controlling GIN has been widely encouraged (Nicola et al., 2014; Silveira et al., 2017).

Nematophagous fungi can be effective biological control agent of nematodes (Waller and Faedo, 1993; Braga and Araújo, 2014; Liu et al., 2015), reducing the number of infective larvae (L3) in pastures (Vilela

Abbreviations: AC001, *Duddingtonia flagrans*; I31, *Arthrobotrys robusta*; I40, *Arthrobotrys conoides*; NF34, *Monacrosporium thaumasium*; L_3, Infective larvae; GIN, Gastrointestinal nematodes; EPG, Eggs per gram of feces; GT, Gastrointestinal tract

* Corresponding author.
E-mail address: fabioribeirobraga@hotmail.com (F.R. Braga).

[1] Scholarship CNPq.

http://dx.doi.org/10.1016/j.jip.2017.08.013
Received 24 March 2017; Received in revised form 23 August 2017; Accepted 25 August 2017
Available online 30 August 2017
0022-2011/

et al., 2012; Ahmed et al., 2015). Fungal structures (conidia and chlamydospores) from nematophagous fungi pass through the animal's gastrointestinal tract (GT) without undergoing morphological changes, develop and colonize the fecal pats, and form traps to capture free-living stage GIN as food (Yang et al., 2011). Nematophagous fungi of the genera *Arthrobotrys*, *Duddingtonia* and *Monacrosporium* have been tested individually against ruminant GIN (Rocha et al., 2007; Silva et al., 2010; Vilela et al., 2012).

Nematophagous fungi used in combinations can show positive outcomes. Combination of *Duddingtonia flagrans* + *Monacrosporium thaumasium* passed throughhorse GTs and killed L3 cyathostome in the animal's feces (Tavela et al., 2012). The combinations of *Duddingtonia flagrans* + *Arthrobotrys robusta* and *Monacrosporium thaumasium* + *Arthrobotrys conoides* reduced the number of L_3 of GIN *in vitro* and conidia and chlamydospores passed through the GT of goats without viability losses (Silveira et al., 2017).

The aim of this study was to test isolates from the nematophagous fungi *Arthrobotrys conoides*, *Arthrobotrys robusta*, *Duddingtonia flagrans*, and *Monacrosporium thaumasium* combined in a sodium alginate matrix to control free-living stages of sheep GIN in the field.

2. Materials and methods

2.1. Organisms

Isolates from the nematophagous fungi *A. conoides* (I40), *A. robusta* (I31), *D. flagrans* (AC001), and *M. thaumasium* (NF34) were used. These fungi were sourced from the mycology collection of the Parasitology Laboratory of the Veterinary Department of Medicine in the Universidade Federal de Viçosa (UFV) in Viçosa, Minas Gerais, Brazil; the cultures had been stored in culture medium corn meal agar (2% CMA) in the dark.

2.2. Mycelial mass production

Fungal cultures (approximately 5 mm) were transferred to 250 ml Erlenmeyer flasks with 150 ml of liquid medium (glucose yeast extract peptone) with pH 6.5, stirred at 10 × *g*, and stored in the dark in a Biochemical Oxygen Demand chamber (BOD) at 26 °C for ten days to induce mycelia mass formation. The mycelia obtained were filtered and weighed to make sodium alginate matrix pellets (Walker and Connick, 1983 modified by Lackey et al., 1993).

2.3. Pellet production

Mycelia masses (8.5 g) of the fungi *A. conoides* isolate I40, *A. robusta* isolate I31, *D. flagrans* isolate AC001, and *M. thaumasium* isolate NF34 were obtained. The combined masses (17 g of each fungal combination) were mixed in a solution composed by sodium alginate (12 g/L) and bentonite (50 g/L), as described by Walker and Connick (1983) and modified by Lackey et al. (1993). This mixture was dripped into $CaCl_2$ 0.25 M solution for pellet formation with the following two combination groups: AC001 + I31, and NF34 + I40.

2.4. Location of the experiment and animals

The experiment was conducted at a farm in the region of Coimbra, Minas Gerais state, Brazil (Latitude: 20°49′49″ longitude: 42°49′05″). Twenty-four Santa Ines sheep, aged between two and three years, were used. These animals were orally dewormed for three consecutive days with Farmazole®-Fagra Brazil (Albendazole 1.9%; 2 ml/10 kg live weight). The number of nematode eggs per gram of feces (EPG) per animal was counted seven days after the first deworming to evaluate its antiparasitic efficacy. The experiment began on the fifteenth day after the first deworming.

2.5. Experimental assay

Sheep were assigned to one of three treatment groups, balanced for weight and age. These animals were distributed in *Brachiaria decumbens* paddocks naturally contaminated by GIN. The stocking rate was one animal per hectare. Treatment of the groups was done with 1 g/10 kg pellets per live weight of sheep, containing combinations of the fungi isolates AC001 + I31 (group 1), NF34 + I40 (group 2) and pellets without fungi (control) twice a week for 26 weeks from June to November 2012. The animals received daily commercial sheep feed at the rate 0.7% of live weight, salt, and water *ad libitum*.

Fecal samples were collected weekly from the rectum of treated animals and from the control group to count the number of eggs per gram of feces (EPG) (Gordon and Whitlock, 1939). Coprocultures were produced from the samples and held in a BOD for 12 days, at 26 °C. After this period, L3 GIN were collected using the Baermann funnel technique and identified to the genus level as described by Ueno and Gonçalves (1998).

Every 15 days, herbage samples (approximately 500 g) were collected at distances of 0–20 and 20–40 cm from the fecal pats, for the three sheep groups, in a zigzag pattern from several alternated points, covering the whole paddock (Amarante et al., 1996). Herbage samples were placed in plastic buckets with 10L of water at 40 °C and decanted L_3 nematodes were collected and identified (Ueno and Gonçalves, 1998). The grazing samples were immediately placed in an oven at 60 °C for three days, dried and weighed to obtain dry matter. Average L3 recovered at the two distances in the grazing areas of the three treatment groups was calculated and the data converted to number of L_3 per kilogram of dry matter.

Blood samples were collected from all animals every 30 days via jugular venipuncture of the animals with placement into Vacutainer® tubes containing EDTA. The packed cell volume (PCV) and the concentration of total plasma proteins (TPP) were analyzed (Neto et al., 1981).

The percentage of GIN larval reduction in EPG and Baermann funnel collections was determined by the formula: Reduction (%) = (XC – XT)/XC × 100, where XT = treated group, XC = control group (Mendoza-de-Gives et al., 1999).

The maximum temperature, monthly average, and minimum relative humidity and rainfall during the experiment period were recorded daily at a weather station in the municipality of Viçosa, Minas Gerais state, Brazil.

2.6. Data analysis

Data from EPG, L_3 gastrointestinal nematode parasites recovered from coprocultures and pastures were transformed (log x + 1) and subjected to analysis of variance (ANOVA) and Tukey's test at 1 and 5% probability. The data of PCV and PPT were not transformed (Ayres et al., 2003).

3. Results

The number of L_3/kg of dry matter collected at distances of 0–20 cm and 20–40 cm from the fecal pats of animals treated with AC001 + I31 and NF34 + I40 was lower ($P < 0.05$) than in the control group (Fig. 1). AC001 + I31 and I40 + NF34 combinations reduced free-living stages of GIN in the pastures.

The EPG was lower with the NF34 + I40 combination in August in relation the AC001 + I31 combination ($P < 0.05$), and with the AC001 + I31 combination and control September and October ($P < 0.05$ and $P < 0.01$, respectively) (Fig. 2). The NF34 + I40 combination was more effective in reducing EPG in relation to AC001 + I31.

The rate of recovery of L_3 among species of the superfamily *Strongyloidea* in coproculture was similar between the treated and

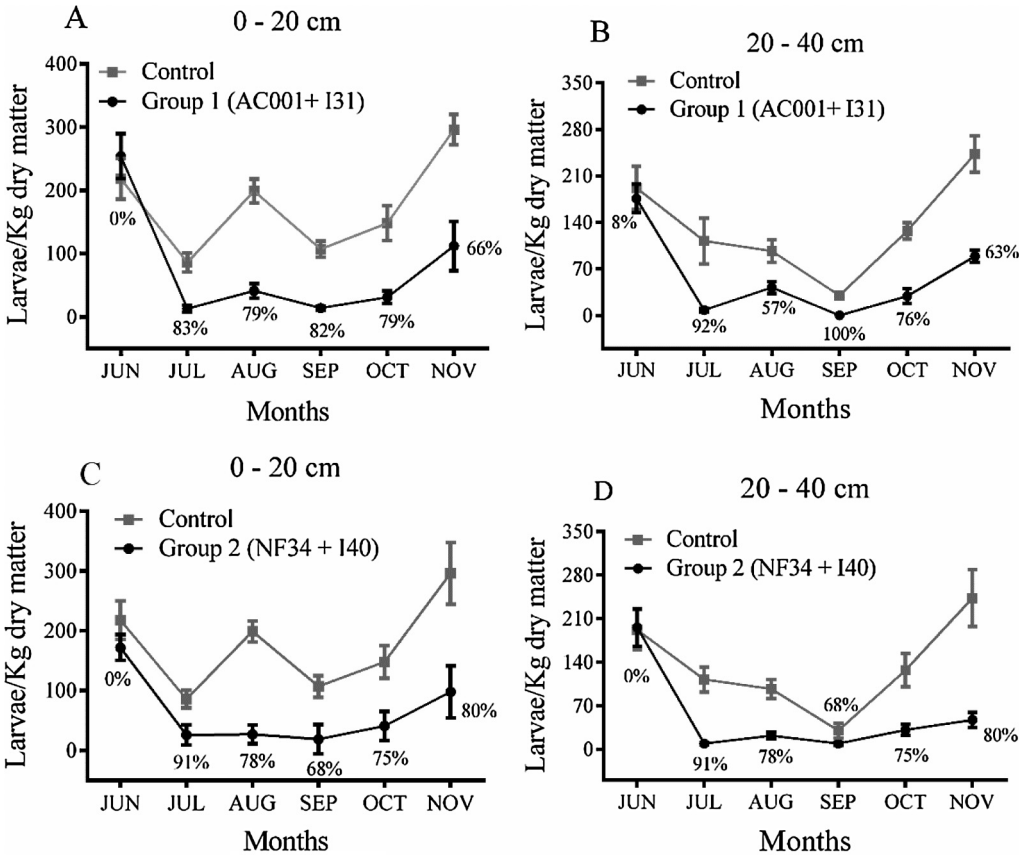

Fig. 1. Monthly average and reduction (%) of infective larvae (L_3) of gastrointestinal nematodes collected per kilogram of dry matter. Groups treated with the fungal combinations AC001 + I31 (*Duddingtonia flagrans* + *Arthrobotrys robusta*), A and B; NF34 + I40 (*Monacrosporium thaumasium* + *Arthrobotrys conoides*), C and D and the control at distances 0–20 cm and 20–40 cm from fecal pats.

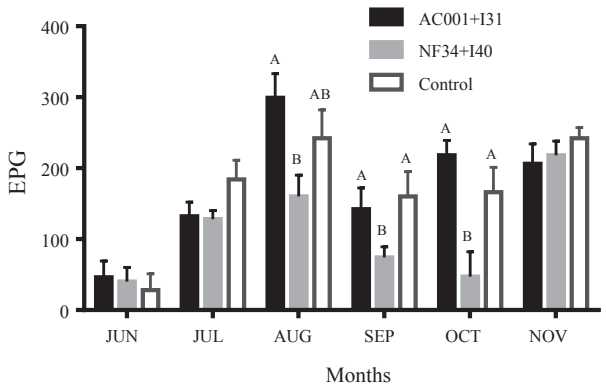

Fig. 2. Number of eggs per gram of feces (EPG) of gastrointestinal nematodes of animals treated with the fungal combinations *Duddingtonia flagrans* + *Arthrobotrys conoides* (AC001 + I31) and *Monacrosporium thaumasium* (NF34 + I40) and control (mean and standard deviation) from June to November. Means followed by the same capital letter A or B per column do not differ at 5% probability according to ANOVA and Tukey's test.

control groups. The prevalence of L3 *Haemonchus* spp. was highest followed by *Cooperia* spp., *Trichostrongylus* spp., and *Oesophagostomum* spp. (Table 1).

The packed cell volume (PCV) of the animals from the treated groups was similar to those of the control group ($P > 0.05$), 32% for groups treated with AC001 + I31 and I40 + NF34 and 33% in the control. Total plasma protein (TPP) of the groups treated with both combinations and in the control group was almost consistent with an average of 8 g/dl for the animals in the treated groups (AC001 + I31 and I40 + NF34) and 7.2 g/dL in the control group.

Average temperatures were low during the first three months of the dry season increasing in September and stabilizing during the rainy season. The relative humidity had a mean of 79% during the dry season

Table 1
Reduction (%) of the number of eggs per gram of feces (EPG) of gastrointestinal nematodes of the animal groups treated with treated with the fungi combinations AC001 + I31, NF34 + and control group.

Months	% Reduction (EPG)	
	AC001 + I31	NF34 + I40
June	–	–
July	29	30
August	–	34
September	–	53
October	–	72
November	15	10

AC001 + I31 (*Duddingtonia flagrans* + *Arthrobotrys robutsa*, NF34 + I40 (*Monacrosporium thaumasium* + *Arthrobotrys conoides*).

and 75.5% during rainy season. The average rainfall was very low during the dry season, and increasing during the rainy season (October, 89 mm and November 235 mm) (Fig. 3).

4. Discussion

Free-living stages of GIN migrate from fecal pats to the surrounding pasture, and subsequently the GIN can then be ingested during grazing (Bolajoko et al., 2015). The distances crossed by L_3 vary according to environmental factors such as temperature, humidity, and light (Tariq, 2015). Thus, it is important to develop ways of combatting and eliminating free-living stage GIN to reduce recurrent infections, improve animal welfare, and reduce treatment costs (Cabaret et al., 2009).

The L3/kg reduction in the dry matter collected at 0–20 and 20–40 cm from the fecal pats is related to predatory fungal action, suggesting that their combination was effective in the biological control of L_3 GIN. The combined action of group 1 (AC001 + I31) and 2

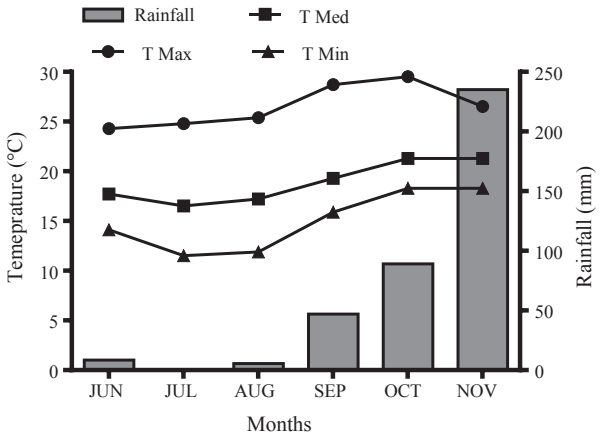

Fig. 3. Maximum, average, and minimum monthly temperatures (°C) and rainfall (mm) from June to November.

(NF34 + I40) fungi in the fecal pats showed that they captured and eliminated free-living stage GIN, in a manner similar to that recorded for the L3 capture of the superfamily *Strongyloidea* by *D. flagrans* and *M. thaumasium* (Campos et al., 2009; Tavela et al., 2012; Assis et al., 2013, 2015). The presence of animals prior to this study, parasitized by GIN in the area used by groups treated with AC001 + I31, I40 + NF34 explains the large number of L_3 recovered at 0–20 and 20–40 cm from the fecal pats in June. The numbers of L_3/kg of dry matter in the grasslands with animals treated with AC001 + I31 and NF34 + I40 combinations were lower than in the control group, from July to November at different distances. This shows a possible synergism between fungal combinations and their effectiveness in capturing and eliminating L3. L3 reduction in pastures at 20–40 cm distance from fecal pats was higher than the 33.3% reported in a study which examined AC001 and NF34 separately, to manage sheep GIN in southeastern Brazil (Silva et al., 2009). The AC001 + NF34 combination reduced the L_3 GIN number in pastures of the semiarid region of northeastern Brazil by 91.6% (Vilela et al., 2016). The lowest number of L_3/kg of dry matter collected at distances of 20–40 cm from the fecal pats suggests that predatory fungus activity reduced L_3 migration in the pasture as reported in other researches (Assis et al., 2012, 2015).

The lower EPG of animals treated with the NF34 + I40 at a concentration of 1 g/kg live weight showed the benefits of this combination. In the months of September and October, the EPG reduction of GIN was 53 and 72% respectively. Similar results were reported with the independent use of NF34, reducing GIN in sheep (61.1%) (Silva et al., 2009) and cattle (64%) EPG (Assis et al., 2015). Combinations of *Duddingtonia flagrans* + *Arthrobotrys robusta* (1 g pellets/kg live weight) showed unsatisfactory results in EPG reduction animals. *Duddingtonia flagrans*, used alone in the same concentration also not reduce sheep EPG in the field in southeastern Brazil (Rocha et al., 2007) or in northeast Switzerland (Faessler et al., 2007). The pellets concentration of 3 g/kg live weight EPG sheep was reduced by 83%, indicating that the combined dosage formulations nematophagous fungi is important to their success in controlling GIN (Vilela et al., 2012, 2016). In addition, *D. flagrans* combined with *A. robusta* did not present antagonism in antibiosis tests, and did not inhibit the growth one another, indicating that they can be used in a combined fashion to control GIN (Ayupe et al., 2016).

The higher prevalence of the GIN *Haemonchus* spp. in coprocultures and pastures constituted an important epidemiological factor because these nematodes can cause severe anemia and hypoproteinemia in animals (Domke et al., 2013; Tariq, 2015). Symptoms that are most apparent are distended abdomen, diarrhea, pale mucous membranes, and submandibular edema (Hussain et al., 2014; Olah et al., 2015). *Cooperia* spp., *Haemonchus* spp., *Oesophagostomun* spp. and *Trichostrongylus* spp. are nematodes of tropical climate, most commonly found in Brazilian ruminants (Silva et al., 2009, 2010; Vilela et al., 2016). Mixed infections with these parasites increases their pathogenicity and can cause animal death (Tariq, 2015).

The packed cell volume (PCV) and the total plasma protein concentration (PPT) were similar to their reference values (Neto et al., 1981), although the animals were infected during the experiment. This result disagrees with the findings of Vilela et al. (2012). However, the lack of PVC change in animals treated with fungal combinations was also reported for sheep treated with *D. flagrans* and *A. conoides* isolates (Silva et al., 2010).

Our experiment took place during part of the dry (June to September) and the rainy (October-November) seasons. The mild average temperatures (17.7 °C, 16.5 °C, 17.2 °C, and 19.3 °C) in the dry period, favored L3 survival in the fecal pats and in the pasture (Tariq, 2015). High temperatures dehydrate fecal pats, hinder nematode larval development and reduce longevity in environment. The average temperature in the rainy season was 21.3 °C, which is adequate for egg hatching (O'Connor et al., 2006; Assis et al., 2012). During the dry season (June to September), rainfall rates were lower than 50 mm, which is considered an adequate level for L_3 development in pastures (Boom and Sheath, 2008). However, precipitation, prior to the beginning of the experiment, during April and May was 47.3 and 104 mm, respectively (data not shown), which may have contributed to the development of larvae in the pasture.

The reduction of L_3 GIN in the pastures was due to the predatory action of nematophagous fungi combinations (*D. flagrans* + *A. robusta* and *M. thaumasium* + *A. conoides*). The predatory action of fungi on the fecal pats prevents the migration of nematode larvae across the pasture and interrupts biological cycle of GIN. This contributes to pasture hygiene, improvements in animal welfare, increased productivity and a reduction of costs due to treatment with chemical controls.

Acknowledgements

We acknowledge "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)", "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)", Fundação de Amparo a Pesquisa do Espirito Santo (FAPES), and Fapemig (Fundação de Amparo a Pesquisa de Minas Gerais). Dr. Phillip Villani revised and corrected the English language used in this manuscript.

Conflict of interest

None.

References

Ahmed, M., Laing, M.D., Nsahlai, I., 2015. Use of *Clonostachys rosea* against sheep nematodes developing in pastures. Biocon. Sci. Technol. 24, 389–398.

Amarante, F.T., 2011. Why is it important to correctly identify *Haemonchus* species? Rev. Bras. Parasitol. Vet. 20, 263–268.

Amarante, A.F.T., Padovani, C.R., Barbosa, M.A., 1996. Contaminação de pastagens por larvas de nematóides gastrintestinais parasitos de bovinos e ovinos em Botucatu-SP. Rev. Bras. Parasitol. Vet. 5, 25–73.

Assis, R.C.L., Luns, F.D., Araújo, J.V., Braga, F.R., 2012. Biological control of trichostrongyles in beef cattle by the fungus *Duddingtonia flagrans* in alginate pellets formulation under natural grazing conditions in Brazil. Exp. Parasitol. 132, 373–377.

Assis, R.C.L., Luns, F.D., Araújo, J.V., Braga, F.R., Assis, R.L., Marcelino, J.L., Freitas, P.C., Andrade, M.A.S., 2013. Comparison between the action of nematode predatory fungi *Duddingtonia flagrans* and *Monacrosporium thaumasium* in the biological control of bovine gastrointestinal nematodiasis in tropical southeastern. Brazil. Vet. Parasitol. 193, 134–140.

Assis, R.C.L., Luns, F.D., Araújo, J.V., Braga, F.R., 2015. An isolate of the nematophagous fungus *Monacrosporium thaumasium* for the control of cattle trichostrongyles in southeastern. Brazil. J. Helminthol. 89, 244–249.

Ayupe, T.H., Monteiro, T.S.A., Braga, F.R., Soares, F.E.F., Mello, I.K., Araújo, J.V., 2016. Assessment of compatibility between the nematophagous fungi *Arthrobotrys robusta* and *Duddingtonia flagrans* under laboratory conditions. Rev. Iberoam. Micol. 33, 129–130.

Ayres, M., Ayres, J.M., Ayres, D.L., Santos, A.S., 2003. Aplicações estatísticas nas áreas de

ciências biomédicas. Sociedade Civil Maniraua, Belém.
Bolajoko, M.B., Rose, H., Musella, V., Bosco, A., Rinaldi, L., Van Dijk, J., Cringoli, G., Morgan, E.R., 2015. The basic reproduction quotient (Q0) as a potential spatial predictor of the seasonality of ovine haemonchosis. Geo. Health. 9, 333–350.
Boom, C.J., Sheath, G.W., 2008. Migration of gastrointestinal nematode larvae from cattle faecal pats onto grazable herbage. Vet. Parasitol. 157, 260–266.
Braga, F.R., Araújo, J.V., 2014. Nematophagous fungi for biological control of gastrointestinal nematodes in domestic animals. Appl. Microbiol. Biotechnol. 98, 71–82.
Cabaret, J., Benoit, M., Laignel, G., Nicourt, C., 2009. Current management of farms and internal parasites by conventional and organic meat sheep French farmers and acceptance of targeted selective treatments. Vet. Parasitol. 164, 21–29.
Campos, A.K., Araújo, J.V., Guimarães, M.P., Dias, A.S., 2009. Resistance of different fungal structures of *Duddingtonia flagrans* to the digestive processes and predatory ability on larvae of *Haemonchus contortus* and *Strongyloides papillosus* in goat feces. Parasitol. Res. 105, 913–919.
Charlier, J., Van Der Voort, M., Kenyon, F., Skuce, P., Vercruysse, J., 2014. Chasing helminths and their economic impact on farmed ruminants. Trends. Parasitol. 30, 361–367.
Domke, A.V.M., Chartier, C., Gjerde, B., 2013. Prevalence of gastrointestinal helminths, lung worms and liver fluke in sheep and goats in Norway. Vet. Parasitol. 194, 40–48.
Faessler, H., Torgerson, P.R., Hertzberg, H., 2007. Failure of *Duddingtonia flagrans* to reduce gastrointestinal nematode infections in dairy ewes. Vet. Parasitol. 147, 96–102.
Falzon, L.C., O'Neill, T.J., Menzies, P.I., 2014. A systematic review and meta-analysis of factors associated with anthelmintic resistance in sheep. Prev. Vet. Med. 117, 388–402.
Gordon, H.M., Whitlock, H.V., 1939. A new technique for counting nematode eggs in sheep faeces. J. Council Sci. Indust. Res. 12, 50–52.
Hussain, T., Periasamy, K., Nadeem, A., Babar, M.E., Pichler, R., Diallo, A., 2014. Sympatric species distribution, genetic diversity and population structure of *Haemonchus* isolates from domestic ruminants in Pakistan. Vet. Parasitol. 206, 188–199.
Lackey, B.A., Muldoon, A.E., Jaffe, B.A., 1993. Alginate pellet formulation of *Hirsutella rossiliensis* for biological control of plant-parasitic nematodes. Biol. Cont. 3, 155–160.
Liu, W., Han, Y., Wang, B.B., Sun, L.J., Chen, M.Y., Cai, K.Z., Li, X., Zhao, M.W., Xu, C.L., Xu, Q., Yi, L.X., Wang, H., 2015. Isolation, identification, and characterization of the nematophagous fungus *Monacrosporium salinum* from China. J. Basic. Microbiol. 55, 992–1001.
Mendoza-De-Gives, P., Davies, K.G., Clarck, S.J., Behnke, J.M., 1999. Predatory behaviour of trapping fungi against srf mutants of *Caenorhabditis elegans* and different plant and animal parasitic nematodes. Parasitology 119, 95–104.
Neto, J.F., Viana, E.S., Magalhães, L.M., 1981. Patologia clínica veterinária. Rab. Bras. 79.
Nicola, L., Tosi, S., Savini, D., 2014. *In vitro* evaluation of nematophagous activity of fungal isolates. J. Bas. Microbiol. 54, 1–5.
O'Connor, L.J., Walkden-Brown, S.W., Kahn, L.P., 2006. Ecology of the free-living stages of major trichostrongylid parasites of sheep. Vet. Parasitol. 142, 1–15.
Olah, S., Van Wyk, J.A., Wall, R., Morgan, E.R., 2015. A potential tool for targeted selective treatment of chronic fasciolosis in sheep. Vet. Parasitol. 15, 188–192.
Rocha, R.A., Araújo, J.V., Amarante, A.F.T., 2007. Efficacy of the nematode-trapping fungus *Duddingtonia flagrans* against infections by *Haemonchus* and *Trichostrongylus* species in lambs at pasture. J. Helminthol. 81, 387–392.
Simpraga, M., Ljubicic, I., Hlede, J.P., Vugrovečki, A.S., Marinculić, A., Tkalčić, S., 2015. Alternative approaches for the control of gastrointestinal nematodes in sheep farming. Berl. Munch. Tierarztl. Wochenschr. 128, 257–270.
Silva, A.R., Araújo, J.V., Braga, F.R., Frassy, L.N., Tavela, A.O., Carvalho, R.O., Castejon, F.V., 2009. Biological control of sheep gastrointestinal nematodiosis in a tropical region of the southeast of Brazil with the nematode predatory fungi *Duddingtonia flagrans* and *Monacrosporium thaumasium*. Parasitol. Res. 105, 1707–1713.
Silva, B.F., Mauad, J.R.C., Braga, F.R., Araújo, J.V., Campos, A.K., Amarante, A.F.T., 2010. Efficacy of *Duddingtonia flagrans* and *Arthrobotrys robusta* in controlling sheep parasitic gastroenteritis. Parasitol. Res. 106, 1346–1350.
Silveira, W.F., Oliveira, G.D., Braga, F.R., Carvalho, L.M., Domingues, R.R., Zanuncio, J.C., Araújo, J.V., 2017. Predation rate of nematophagous fungi after passing through the gastrointestinal tract of goats. Small Ruminant Res. 147, 101–105.
Tariq, A.K., 2015. Review of the epidemiology and control of gastrointestinal nematode infections of small ruminants. Proc. Natl. Acad. Sci. 85, 693–703.
Tavela, A.O., Araújo, J.V., Braga, F.R., Silveira, W.F., Silva, V.H.D., Júnior, M.C., Borges, L.A., Araujo, J.M., Benjamin, L.A., Carvalho, G.R., Paula, A.T., 2012. Coadministration of sodium alginate pellets containing the fungi *Duddingtonia flagrans* and *Monacrosporium thaumasium* on cyathostomin infective larvae after passing through the gastrointestinal tract of horses. Res. Vet. Sci. 94, 568–572.
Torres-Acosta, J.F.J., Mendoza-De-Gives, P., Aguilar-Caballero, A.J., Cuéllar-Ordaz, J.A., 2012. Anthelmintic resistance in sheep farms: update of the situation in the American continent. Vet. Parasitol. 189, 89–96.
Ueno, H., Gonçalves, P.C., 1998. Manual para diagnóstico das helmintoses de ruminantes. Jpn. Int. Cooper. Agency 140.
Vadlejch, J., Kotrba, R., Čadková, Z., Růžičková, A., Langrová, I., 2015. Effects of age, sex, lactation and social dominance on faecal egg count patterns of gastrointestinal nematodes in farmed eland (*Taurotragus oryx*). Prev. Vet. Med. 121, 265–272.
Vilela, V.L.R., Feitosa, T.F., Braga, F.R., Araújo, J.V., Souto, D.V.O., Santos, H.E.S., 2012. Biological control of goat gastrointestinal helminthiasis by *Duddingtonia flagrans* in a semi-arid region of the northeastern Brazil. Vet. Parasitol. 188, 127–133.
Vilela, V.L.R., Feitosa, T.F., Braga, F.R., Araújo, J.V., Santos, A., Morais, D.F., Souto, D.V., Athayde, A.C.R., 2016. Coadministration of nematophagous fungi for biological control over gastrointestinal helminths in sheep in the semiarid region of northeastern Brazil. Vet. Parasitol. 221, 139–143.
Waller, P.J., Faedo, M., 1993. The potential of nematophagous fungi to control the freeliving stages of nematodes parasites of sheep: screening studies. Vet. Parasitol. 49, 285–297.
Walker, H.L., Connick Jr., W.J., 1983. Sodium alginate for production and formulation of mycoherbicides. Weed. Sci. 31, 333–338.
Yang, J., Wang, L., Xinglai, J., Feng, Y., Li, X., Zou, C., Xu, J., Ren, Y., Mi, Q., Wu, J., Liu, S., Liu, Y., Huang, X., Wang, H., Niu, X., Li, J., Liang, L., Luo, Y., Ji, K., Zhou, W., Yu, Z., Li, G., Liu, Y., Li, L., Qiao, M., Feng, L., Zhang, K.O., 2011. Genomic and proteomic analyses of the fungus *Arthrobotrys oligospora* provide insights into nematode-trap formation. PLoS. Pathog. 7, 100–179.