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

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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

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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