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Control of bovine gastrointestinal nematode parasites using pellets of the nematode-trapping fungus *Monacrosporium thaumasium*

Controle de nematóides parasitos gastrintestinais de bovinos com *pellets* do fungo predador de nematóides *Monacrosporium thaumasium* **

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ABSTRACT

The viability of a formulation of the fungus ***Monacrosporium thaumasium*** *associated with ivermectin was evaluated for the biological control of bovine gastrointestinal nematode parasites. Four groups of five calves each were placed in pastures with a stocking rate of 1.6 animal/hectare. In group 1 (control), the calves did not receive any treatment. In group 2, each animal received 20g of pellets of* ***M. thaumasium*** *orally twice a week during a six-month period that began with the onset of the rainy season (October 23, 2000). In group 3, each animal received 20g of pellets of* ***M. thaumasium*** *orally twice a week during the same period as 2, as well as two strategic treatments with ivermectin (200 mcg/kg) on May 10, 2001 and July 5, 2001. In group 4, the animals were treated with ivermectin alone as described for group 3. EPG counts for group 1 were significantly greater (P< 0.01) than those for groups 2 and 3 and the difference at the end of the study period was near 100%. The EPGs of group 4 animals remained high until the first strategic treatment with ivermectin. Values for groups 1 and 4 differed significantly (P< 0.05) from those of groups 2 and 3 from December 2000 onwards. It was concluded that the use of this dose and periodicity of application of* ***M. thaumasium*** *pellets makes the application of anthelminthic treatments unnecessary.*

Key words: *biological control,* ***Monacrosporium thaumasium****, nematodes, nematophagous fungi, pellets, bovine.*

RESUMO

A viabilidade de uma formulação do fungo ***Monacrosporium thaumasium*** *associada com ivermectina foi avaliada no controle biológico de nematóides parasitos gastrintestinais de bovinos. Quatro grupos de cinco bezerros foram colocados em pastagens com uma taxa de lotação de 1,6 animal/hectare. No grupo 1 (controle), os bezerros não receberam nenhum tratamento. No grupo 2, cada animal recebeu 20 g de pellets de* ***M. thaumasium****, via oral, duas vezes por semana e durante um período de seis meses que começou na estação chuvosa (23 de outubro, 2000). No grupo 3, cada animal recebeu 20g de pellets de* ***M. thaumasium****, via oral, duas vezes por semana e durante o mesmo período do grupo 2, assim como dois tratamentos estratégicos com ivermectina (200 mcg/kg) em 10 de maio de 2001 e em 5 de julho de 2001. No grupo 4, os animais foram tratados somente com ivermectina como descrito no grupo 3. As contagens de OPG dos animais do grupo 1 foram significativamente maiores (P< 0,01) do que os animais dos grupos 2 e 3 e a diferença no final do experimento foi próxima de 100%. Os OPGs dos animais do grupo 4 permaneceram altos até o primeiro tratamento estratégico com ivermectina. Os resultados do grupo 1 e 4 diferiram significativamente (P< 0,05) daqueles dos grupos 2 e 3 a partir de dezembro de 2000. Concluiu-se que a aplicação de pellets de* ***M. thaumasium*** *nesta dosagem e periodicidade de aplicação tornaram os tratamentos anti-helmínticos desnecessários.*

Palavras-chave: *controle biológico,* ***Monacrosporium thaumasium****, nematóides, fungos nematófagos, pellets, bovinos.*

INTRODUCTION

Helminth infections are a major concern among the factors that interfere with the development of cattle raising. They cause reduction in animal growth, death and excessive handling expenses, leading to low herd productivity and high economic losses. In Brazil, this problem increases as pasture

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conditions worsen, mainly in the dry season, or when high stocking rates in certain areas increase infection rate. Generally, most animals present subclinical infections due to acquired immunity, making it more difficult to quantify the effects of such conditions. In Southeastern Brazil, ***Cooperia*** and ***Haemonchus*** are the most prevalent bovine gastrointestinal nematodes genera, based on eggs per gram of faeces (EPG) counts, followed by members of the genus ***Oesophagostomum*** (ARAÚJO et al., 1998).

Non-chemotherapeutic approaches to the control of nematode parasites of livestock are no longer largely of academic interest and alternatives or adjuncts to anthelminthic drugs are now considered to be necessary. Significant advances have recently been made in the development of ruminant vaccines against parasites (MEEUSEN, 1996), in breeding of animals for parasite resistance (WOOLASTON & BAKER, 1996) and in biological control, particularly by exploiting nematophagous fungi (ARAÚJO et al., 1998). The last of these alternatives appears to be highly promising (WALLER & LARSEN, 1993).

These fungi are the most widely studied of the organisms used in nematode control and almost all of them effectively reduce laboratory populations of the parasites. Their efficacy against nematodes on pastures has also been demonstreated (WALLER & LARSEN, 1993). Species of ***Monacrosporium*** (Hyphomycetales) can control phytonematodes, free-living nematodes and parasitic nematodes of cattle (ARAÚJO et al., 1992; GOMES et al., 1999).

The objective of the present study was to assess the viability of a formulation with the fungus ***Monacrosporium thaumasium*** associated with ivermectin in the biological control of bovine gastrointestinal nematode parasites. This fungus was selected based on previous tests involving passages through the gastrointestinal tract (ARAÚJO et al., 1999) for field control of bovine gastrointestinal nematodes.

MATERIALS AND METHODS

Organisms

Infective ***Cooperia punctata*** (Trichostrongylidae), ***Haemonchus placei*** (Trichostrongylidae) and ***Oesophagostomum radiatum*** (Cyathostomidae) larvae (L_3) were obtained from the faeces of calves naturally infected.

One isolate of nematode-trapping fungus ***M. thaumasium*** (NF 34a – isolate) was obtained from Brazilian soil (Viçosa – Minas Gerais state) and kept in small flasks containing 2% corn-meal-agar (2% CMA) at 4°C in "Departamento de Veterinária – Universidade Federal de Viçosa". Mycelium grown of the fungi was performed in liquid medium of KADO & HESKET (1970) after nine days of incubation at 25°C in the dark. Sodium alginate pellets were made as described by WALKER & CONNICK (1983) and modified by LACKEY et al. (1993).

Experimental design

The study was performed at an experimental farm of the Federal University of Viçosa, in the county of Viçosa, Minas Gerais State, Brazil, whose latitude is of 20° 45' 20" S, longitude of 42° 52' 40" W and is 649 m above sea level during the period of September 1, 2000 to August 31, 2001.

Twenty crossbred Holstein x Zebu calves of six months old were treated previously with two doses of ivermectin (200mcg kg^{-1}), with an interval of two weeks. The animals grazed on molasses grass pastures (***Melinis minutiflora***) on the September 11, 2000. These pastures were naturally infested by gastrointestinal helminth stages due to previous grazing by infected calves and adult cattle. After a 21-day adaptation period and the last anthelmintic treatment, each calf was experimentally infected orally with 5,000 L_3 each of ***C. punctata***, ***H. placei*** and ***O. radiatum***. Animals were randomly divided into four groups (1, 2, 3 and 4) of five calves each and placed on pastures at a stocking rate of 1.6 animal/hectare. In group 1 (control), the calves did not receive any treatment. In group 2, each animal received 20g of ***M. thaumasium*** pellets orally twice a week during a six-month period that began with the onset of the rainy season (October 23, 2000). In group 3, each animal received 20g of pellets of ***M. thaumasium*** orally twice a week during the same period as group 2, as well as two strategic treatments with ivermectin (200mcg kg^{-1}) on May 10, 2001 and July 5, 2001. In group 4, the animals were treated with ivermectin alone as described for group 3.

After the administration of L_3 to the calves, faecal samples were collected directly from the rectum of each animal of all groups every fourteen days. Eggs per gram of faeces (EPG) values were determined according to the technique of GORDON & WHITILOCK (1939) and larvae were also cultured. The latter involved mixing 20g of faeces with fragmented vegetal coal and incubating at 26ºC for eight days, in order to collect infective larvae of gastrointestinal nematode parasites. These larvae were identified according to the criteria established by KEITH (1953). Samples of approximately 500g of pasture were collected at random points in each paddock at 14-day intervals and L_3 recovered according to the technique described by LIMA et al. (1990). Five hundred grams of forage were dried in an oven at 100 ^oC for three days to calculate the dry matter content. The results obtained were then transformed into number of larvae per kg dry matter.

The monthly averages for minimum, median and maximum air temperature, monthly rainfall and relative air humidity were recorded. The data were transformed into logarithmic x+1 values to correct the average distortions and compared by analysis of variance.

RESULTS

The monthly average values of the EPG counts are shown in table 1. During the experiment, EPG counts for group 1 were significantly greater than those for groups 2 and 3 (P< 0.01). At the end of the six-month study period this difference was almost 100%. The EPG of group 4 calves remained high until the first strategic treatment with ivermectin (May 10, 2001). The EPG curves in group 1 and 4 animals showed peaks in March and May. In the EPG of group 3 calves appeared a short peak in March.

The percentage compositions of L_3 larvae of different nematodes obtained after larval culture are presented in figure 1. The prevalence of ***Cooperia*** sp. was greater than that for the other nematodes in all groups, followed in descending order by ***Haemonchus*** sp., ***Oesophagostomum*** sp., ***Trichostrongylus*** sp. and ***Bunostomum*** sp.

The numbers of L_3 per kg of pasture dry matter is presented in figure 2. Values for groups 1 and 4 differed significantly (P<0.05) from those of groups 2 and 3 from December 2000 onwards. Members of the genus ***Cooperia*** were most prevalent in all pastures.

Temperature, rainfall and relative humidity data from September 2000 to August 2001 are shown in figure 3.

Table 1 - Mean montly count of nematode eggs of the super-family Strongyloidea per gram of faeces (EPG) of calves in groups 1, 2, 3 and 4 during the period from October 2000 to August 2001.

Months	Group 1	Group 2	Group 3	Group 4
October	180.0	180.0	140.0	100.0
November	250.0	240.0	160.0	90.0
December	220.0	60.0	90.0	170.0
January	253.0	20.0	57.3	293.0
February	300.0	0.0	100.0	500.0
March	1573.3	8.3	120.0	856.6
April	910.0	22.5	20.0	540.0
May	2215.0	22.5	14.0	760.0
June	1700.0	20.0	10.0	40.0
July	1860.0	40.0	10.0	10.0
August	1960.0	40.0	0.0	60.0

DISCUSSION

Based on the results of the present study, this ***M. thaumasium*** isolate appears to be a promising agent for the biological control of bovine nematode parasites. The oral administration of pellets to the animals resulted in a pasture infestation control close to a 100% in Groups 2 and 3 animals (Table 1) during the last six months of the experiment (February-August 2001). In Group 3 (treated with fungus and an anthelminthic drug), the anthelminthic applications of the latter in May and June 2001 became almost unnecessary since EPG levels were low due to the action of the fungus and the short peak appeared in March was not significant. In Groups 1 and 4, the peaks appered in March and May showed that the pasture conditions were favorable to the development of helminth eggs and larvae, consequently increasing pasture contamination.

The use of nematophagous fungi in the biological control of animal helminth parasites can reduce pasture contamination, acting directly in the environment. HASHMI & CONNAN (1989) and WOLSTRUP et al. (1994) employed this, mainly, in the very beginning of the infective period, when the pasture conditions were adequate to the animal grazing, and at the same time were favorable to the development of helminths eggs and larvae, consequently increasing pasture contamination. Besides, in the region where the present study was conducted, the seasons of larger rainfall are those of the best pasture conditions, and highest mean temperatures. The development of fungi in an environment would be favored by high temperatures. Generally, the optimum temperature for development of ***Monacrosporium*** sp. range between 20 and 30^oC, according to ARAÚJO et al. (2000). ARAÚJO et al. (1998) tested the viability of an isolate of ***Arthrobotrys robusta*** in the biological control of bovine gastrointestinal nematode parasites, in the same region as the present study. Calves were treated with two million conidia of this isolate, administered orally twice a week during four months, showed, in relation to the non-treated calves (control), 53.81% of reduction in the EPG (P<0.05) and a reduction of 70.45% (P< 0.05) on the number of worms recovered at necropsy of the tracer calves in the last three months

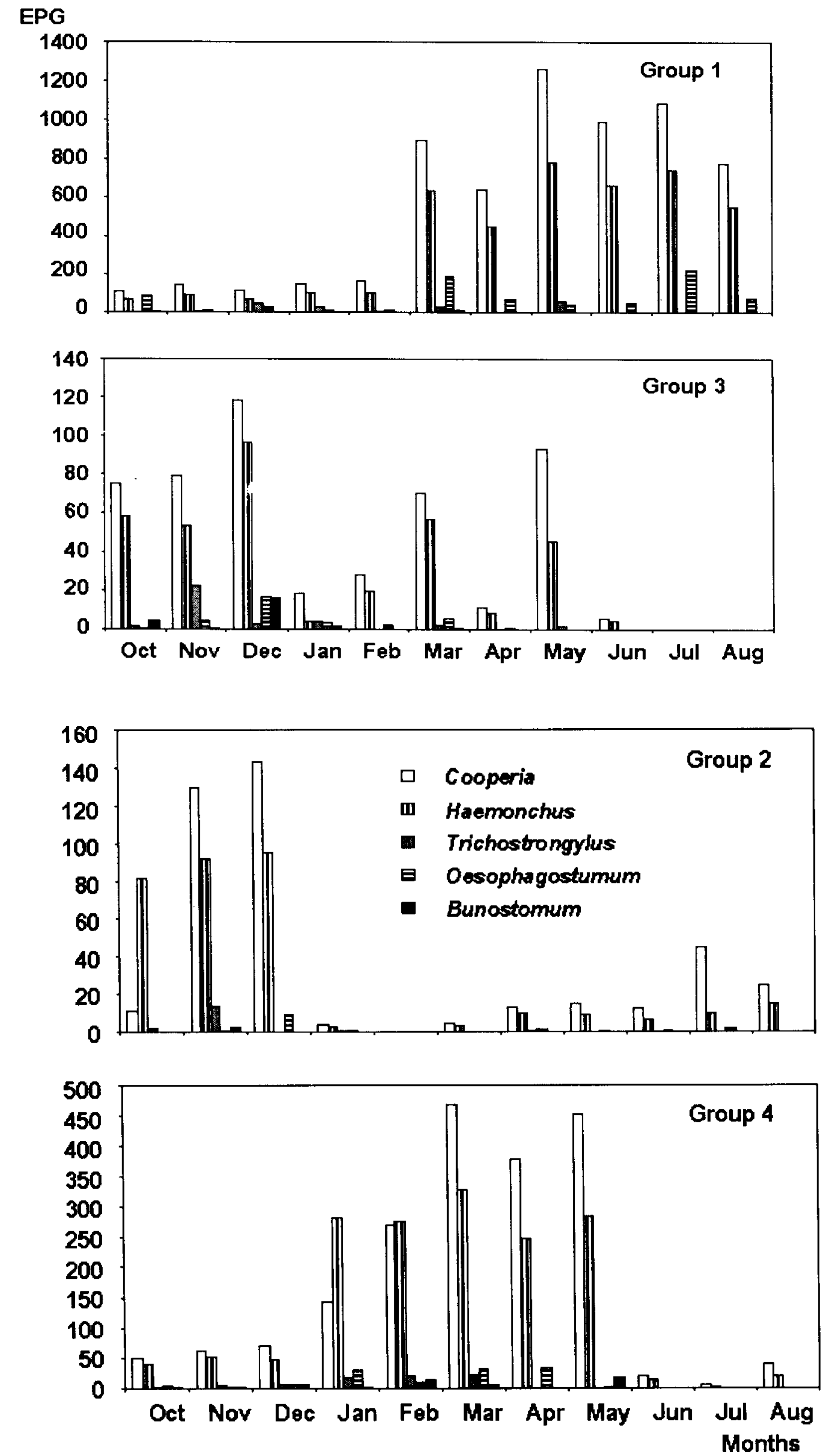

Figure 1 - Mean monthly count of nematodes eggs per gram of faeces (EPG) recovered from larval cultures, of the calves in groups 1, 2, 3 and 4 during the period from October 2000 to August 2001.

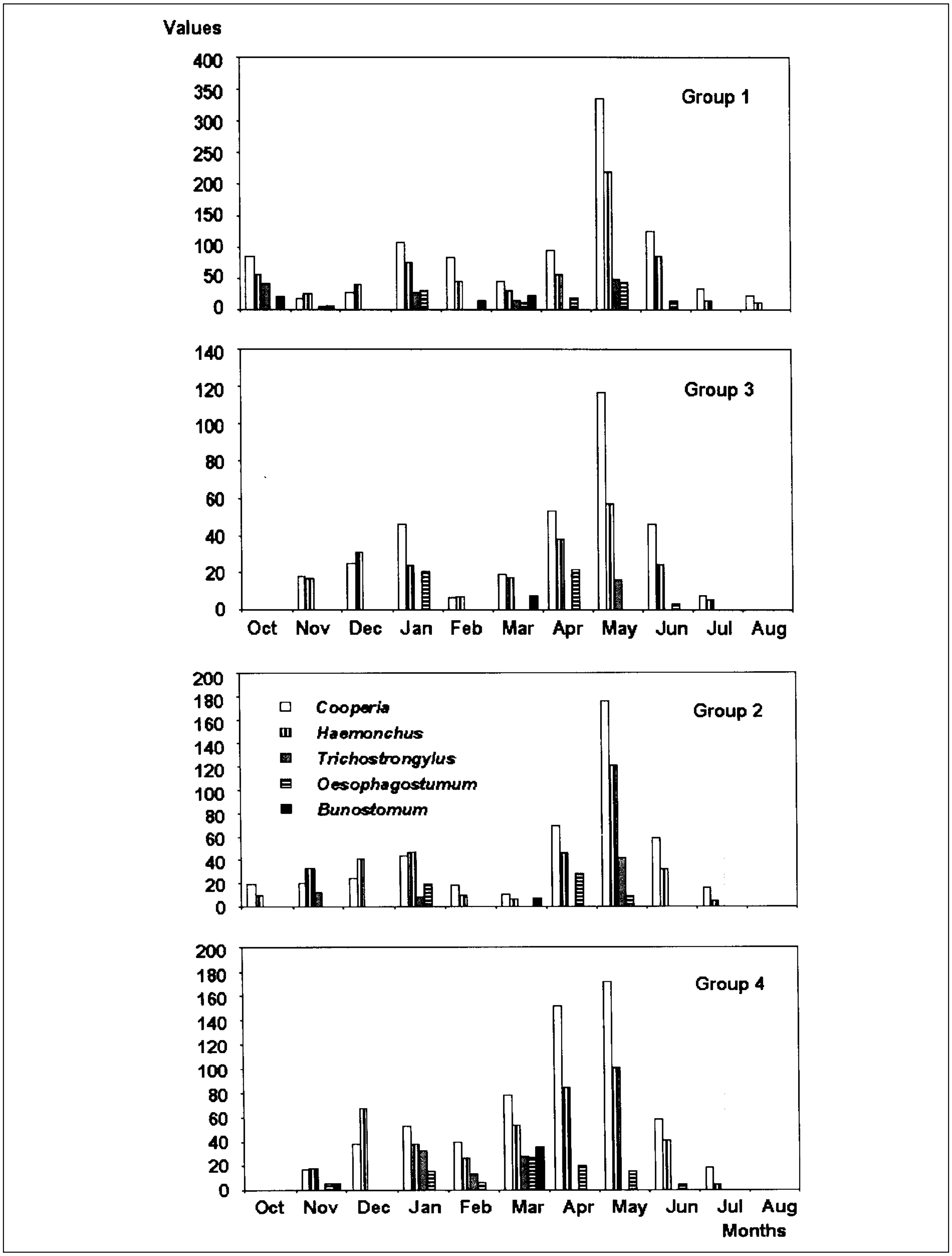

Figure 2 - Counting of infective larvae per kg of dry pasture collected in pastures with calves of the groups 1, 2, 3 and 4 in Viçosa, Minas Gerais State, Brazil, during the period from October 2000 to August 2001.

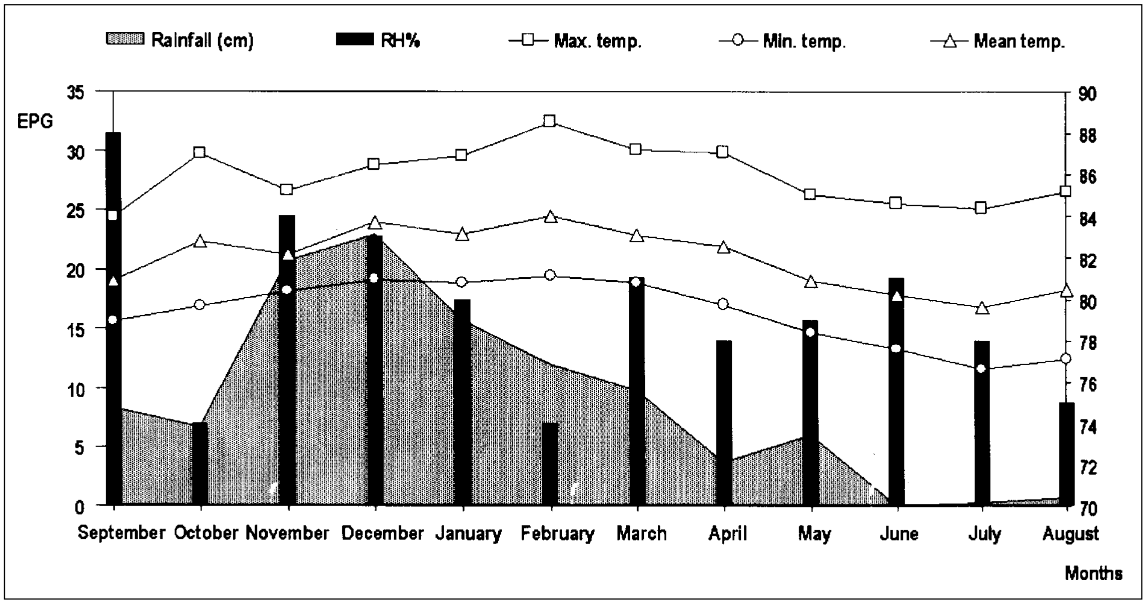

Figure 3 - Average minimum (min. temp), medium (mean temp) and maximum temperatures (max. temp) (^{o}C); monthly rainfall (cm) and relative humidity (RH) (%) in Viçosa, Minas Gerais State, Brazil, during the period from September 2000 to August 2001.

of the experiment. The results showed that this isolate of ***A. robusta*** is a promising agent to be used in the biological control of bovine gastrointestinal nematode parasites. ARAÚJO et al. (1999) were able to get this ***M. thaumasium*** isolate through the gastrointestinal tract of calves without loss of viability to prey infective ***H. placei*** larvae. ARAÚJO et al. (2000) performed experiments to determine whether ***M. thaumasium*** could survive encapsulation in sodium alginate and the effects of different temperatures and mineral salt. Pellets of sodium alginate were treated with paraffin, mineral salt or without these elements. They were put in Erlenmeyers flasks of 250ml at 4°C, room temperature, 25°C, 30°C and 35°C. Once a week, during 16 weeks, one pellet was put in the center of 8.5cm Petri dish containing 20ml of 2% potato dextrose agar and the radial growth was followed during nine days. The fungal pellets without paraffin, at 4°C, appeared to be the best treatment (P<0.01) and remained viable up to 16 weeks of storage. The pellets without paraffin induced higher growth than pellets with paraffin at room temperature as well as the pellets without mineral salt in all temperatures (P<0.01).

According to WALLER & LARSEN (1993), the application of fungi in nematode biocontrol helps the chemical control and should be administered not only when there is a prediction of greater pasture infestation by helminths eggs and larva, but also, when there would be better conditions for the fungi growth at the environment, this way preventing the clinical parasitism and the productivity losses, already supplying a sufficient number of larva to allow these animals to develop a naturally acquired immunity.

As demonstrated in the present study, the use of nematophagous fungi for biological control in the rainy season would prepare pastures for the dry season, the critical period for infection of calves in this region (FURLONG et al., 1985). At this time of year large numbers of larvae are available in the pastures while forage is most limited.

The ***Cooperia*** sp. predominance in relation to the other helminth genera was found in the counting of infective larvae per kg of pasture dry matter and in the faeces of the animals (Figures 3 and 4). These results probably reflect the greater resistance of the ***Cooperia*** sp. free life stages to the climatic variations and dry conditions, and the greater migratory capacity of these larva (REINECKE, 1960), besides the smaller requirements of pluvial precipitation in relation to ***Haemonchus*** sp. and ***Oesophagostomum*** sp. larvae (ROBERTS et al., 1952).

The results were analysed based on the overall experimental period and we conclude that this dose and periodicity of application of ***M. thaumasium*** pellets makes the use of other anthelminthic treatments unnecessary. The fungal formulation used in the present study proved to be a powerful tool for biological control of gastrointestinal nematodes of grazing calves under natural conditions.

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