INFLUENCE OF STORAGE TIME OF *Monacrosporium thaumasium* PELLETS ON THE PREDATION OF INFECTIVE LARVAE OF SHEEP GASTROINTESTINAL NEMATODES

INFLUÊNCIA DO TEMPO DE ARMAZENAMENTO DE PELETES DE *Monacrosporuim thaumasium* NA PREDIÇÃO DE LARVAS INFECTANTES DE NEMATODES GASTRINTESTINAIS DE OVINOS

ABSTRACT

The objective of this study was to evaluate the influence of the storage time of *Monacrosporium thaumasium* pellets on the predation of infective larvae of sheep gastrointestinal nematodes in the semi-arid area of Paraíba, Northeast Brazil. 16 sheep with zero in the count of eggs per gram of faeces – EPG, were divided into four experimental groups: Group I, 3 g/10 kg live weight *M. thaumasium* pellets - 36 months of storage, single dose; Group II, 3 g/10 kg live weight *M. thaumasium* newly produced, single dose; Group III, 3 g/10 kg live weight pellets without fungi; and Group IV, control. Every 24 h, up to 120 h, the faeces of the animals were collected and submitted to the laboratory for analysis. Fifteen grams of faeces were weighed from each animal and five grams of expanded vermiculite were added to produce the coprocultures. Subsequently, 1000 larvae (L3) sheep trichostrongilide were added, and larval recovery was performed after 7 days. Predation of larvae in Group I (*M. thaumasium* - 36 months) did not differ statistically ($p > 0.01$) from Group II (*M. thaumasium* - recent), with reductions of 75% and 79%. Both groups reached peak predation to larvae at 72 h. The helminth genus most recovered in the coprocultures was *Haemonchus* sp. The data indicate that the 36-month stocking period of *M. thaumasium* pellets in alginate matrix did not influence the efficacy of

predation of infective larvae of sheep gastrointestinal nematodes, with fungal activity in the faeces up to 96 hours after administration to the animals.

Key-words: Sodium alginate; biological control; nematophagous fungi.

RESUMO

O objetivo deste trabalho foi avaliar a influência do tempo de armazenamento de péletes de *Monacrosporium thaumasium* na predação de larvas infectantes de nematódeos gastrintestinais de ovinos no semiárido da Paraíba, Nordeste do Brasil. 16 ovelhas, com contagem de ovos por grama de fezes – OPG, negativo, foram divididas em quatro grupos experimentais: Grupo I, 3 g / 10 kg de peso vivo, péletes de *M. thaumasium* - 36 meses de armazenamento, dose única; Grupo II, 3 g / 10 kg de peso vivo *M. thaumasium* recém-produzido, dose única; Grupo III, 3 g / 10 kg de peso vivo sem fungos; e Grupo IV, controle. A cada 24 h, até 120 h, as fezes dos animais foram coletadas e submetidas ao laboratório para análise. Quinze gramas de fezes foram pesadas de cada animal e cinco gramas de vermiculita expandida foram adicionados para produzir as coproculturas. Subsequentemente, 1000 larvas (L3) de tricostrongilide de ovinos foram adicionadas e a recuperação larval foi realizada após 7 dias. A predação de larvas no Grupo I (M. thaumasium - 36 meses) não diferiu estatisticamente ($p > 0,01$) do Grupo II (M. thaumasium - recente), com reduções de 75% e 79%. Ambos os grupos atingiram o pico de predação para larvas em 72 h. O gênero helminto mais recuperado nas coproculturas foi *Haemonchus sp*. Os dados indicam que o período de estocagem de 36 meses de péletes de *M. thaumasium* na matriz de alginato não influenciou a eficácia da predação de larvas infectantes de nematódeos gastrintestinais de ovinos, com atividade fúngica nas fezes até 96 horas após a administração aos animais.

Palavras-chave: Alginato de sódio; controle biológico; fungos nematófagos.

INTRODUCTION

Parasitic diseases are a long-standing problem and a serious health and economic barrier for small ruminant producers. In view of nematode resistance to conventional anti-helmintics, the use of biological agents acting on eggs and larvae of trichostrongylide nematodes has been explored as an alternative for the hygiene of pastures and intensified in recent years (BRAGA and ARAÚJO, 2014). Nematophagous fungi are the most studied microorganisms for this purpose (CAMPOS et al., 2009).

Araújo et al., (2000) emphasized that *Monacrosporium thaumasium* sedimented in a matrix of sodium alginate can survive passage through the gastrointestinal tract of ruminants without losing its predatory activity.

Pellets containing *M. thaumasium* reduced helminth infections in sheep (SILVA et al., 2009) and goats (VILELA et al., 2013) under field conditions, during 6 months of administration. However, there are no descriptions of the predatory viability of pelleted nematophagous fungi in sodium alginate matrix after storage.

The objective of the present study was to evaluate the effect of 3-year storage time of *M. thaumasium* pellets on the predation efficacy on gastrointestinal nematode larvae of sheep.

MATERIALS AND METHODS

This research was submitted to the Ethics Committee Federal University of Campina Grande and obtained protocol number 022.2017.

An isolate of *M. thaumasium* fungus (NF43a) was maintained at 4 °C in the dark and in test tubes containing 2% Corn-Meal agar (2 % CMA). The isolate was obtained from soil in

Viçosa region, Minas Gerais state, Brazil, using a soil spreading method described by Duddington (1955) and modified by Santos et al., (1991).

Mycelia were obtained by transferring culture discs (approximately 4 mm in diameter) of the fungal isolate kept in 2% water agar (2% WA) to 250 mL Erlenmeyer flasks with 150 mL GPY liquid medium (sodium peptone, glucose and yeast extract) medium and incubated with agitation at 120 rpm in the dark at 26°C for 10 days. The mycelia were removed, filtered and weighed. All procedures followed those described by Walker and Connick (1983).

The experiment was carried out at the Department of Veterinary Medicine of the Instituto Federal da Paraíba (IFPB), Sousa - PB. Pellets of NF34a that were freshly prepared or stored in a sodium alginate matrix stored for 3 years were used. During storage, the pellets remained in sealed plastic bags at temperatures ranging from 2-8ºC, relative humidity exceeding 80%, in the dark.

16, 6-month-old, 30 kg body weight, female sheep of the Santa Inês breed from the IFPB herd were used. The sheep had zero in the counting of eggs per gram of faeces (EPG), as determined using a previously described method (GORDON and WHITLOCK 1939). The sheep were divided into four experimental groups containing four animals each. Group I, received a single 3 g/10 kg live weight dose of *M. thaumasium* pellets that had been stored for 36 months. Group II, received newly produced *M. thaumasium* pellets using the same dose and single administration. Group III received newly produced *M. thaumasium* pellets without fungi using the same dose and single administration. Group IV (control group) did not receive pellets. Coprocultures (ROBERTS and O'SULLIVAN, 1950) of other sheep of the IFPB herd, with an EPG > 1000, were carried out to obtain larvae.

Faeces were collected and sent to the Veterinary Parasitology Laboratory of IFPB. To perform coprocultures, 15 g of faeces were weighed from each sample and mixed with 5 g of

expanded vermiculite. Infective larvae (L3, n = 1000) of sheep gastrointestinal nematodes were added. Examinations were performed in triplicate at 24, 48, 72, 96, and 120 h after treatment.

Subsequently, the coprocultures were incubated in Biochemical Oxygen Demand (BOD) for 7 days at 28ºC. The L3 of the coprocultures were recovered by the Baermann method (WILLCOX and COURA 1989), quantified, and then identified by optical microscopy at 100× magnification, as previously described (UENO and GONÇALVES, 1994).

Percentage reduction of the mean number of L3 was calculated according to the following equation:

$$\text{Reduction \%} = \frac{(\text{Mean of L3 recovered from control group} - \text{Mean of L3 recovered from treatment groups}) \times 100}{\text{Mean of L3 recovered from control group}}$$

Data were subjected to analysis of variance (F test) and the results were compared using the Tukey test at the 1% level of probability using Bioestat 3.0 software.

The experiment was approved by the Ethics Committee of the Universidade Federal de Campina Grande – UFCG, Patos-PB, Brazil, on May 23, 2016, under protocol 035-2016.

RESULTS

There were no significant differences in the predation of L3 of sheep gastrointestinal nematodes between Group I and II throughout the evaluated period (larval reduction 75% and 79%, respectively; $p > 0.01$; Figure 1). In group I, larval reduction were 75% and in Group II, 79%.

The larval predation was statistically significant ($p \leq 0.01$) when Groups I and II were compared with Groups III and IV from 24 to 96 hours. The peak activity was at 72 h in Groups

I and II, with respective reductions of 75% and 79% (Fig. 1). Predatory activity on larvae was no observed up to 96 hours after pellet administration.

The recovery of larvae in Group III did not differ statistically ($p > 0.01$) from Group IV during the experiment. Pellet composition did not interfere with fungal activity.

In the coprocultures, there was a higher prevalence of *Haemonchus*, followed by *Trichostrongylus*, *Oesophagostomum*, and *Strongyloides* (Table 1).

DISCUSSION

This study is the first to describe the predatory activity of a nematophagous fungus pelleted in a matrix of sodium alginate after a long period of storage (36 months). Larval reductions reached 75% in Group I and 79% in Group II. Mota et al., (2002) observed reduction of *H. contortus* larvae by *Arthrobotrys robusta* (I-31) and *M. thaumasium* (N3F4a) stored on silica gel for 18 months, with reductions of 68.83% and 73.83%, respectively, compared to the number of larvae in the control group. Braga et al., (2014) observed that *A. robusta* fungus stored in silica gel for 7 years showed reduction of *H. contortus* larvae in 73.84%.

In this study, the peak of larval predation occurred at 72 h in Groups I and II. Araújo et al., (2010) reported that these time intervals were ideal, because there was greater fungal passage through the gastrointestinal tract. Tavela et al., (2013) reported a reduction in the number of cyatostomine larvae recovered from equine coprocultures treated with different doses of the combination of *D. flagrans* (AC001) and *M. thaumasium* (NF34a), where all time intervals (12 to 72 h) showed a reduction rate of L3 that exceeded 80%.

Presently, reduction of larvae was observed up to 96 h. After their administration, the pellets become mixed with food in the digestive tract of the animals and tend to be released

gradually, starting at 24 h and continuing until 96 h. After 120 h, no more release of fungal pellets through the faeces was apparent.

The recovery larvae from the Group III did not differ statistically ($p > 0.01$) from the control group, reinforcing the view that the use of sodium alginate in the composition of the pellets did not interfere with larval predation. The use of sodium alginate pellet formulations has been successful under laboratory and field conditions (VILELA et al., 2013). Araújo et al. (2000) reported that pelleting of the mycelium did not interfere with fungal predation. This may be an important method in the biological control of nematodes.

In the studied area, it is common for helminthic fauna of small ruminants are commonly composed of *Haemonchus* sp., *Trichostrongylus* spp., *Oesophagostomum* sp., and *Strongyloides* sp. (VILELA et al., 2012; VIEIRA et al., 2014). Vilela et al., (2016) observed the same helminth composition in sheep coprocultures in the semiarid environment of Paraíba, Brazil, with a higher prevalence of *Haemonchus* sp. Probably, this nematode acquires faster resistance due to high biotic potential, broad genetic variability, and the presence of an allele that decreases drug susceptibility (BLACKHALL et al., 1998).

The present results strengthen the prospects for the commercialization of sodium alginate matrix pellets of *M. thaumasium* stored at temperatures between 2ºC and 8ºC, since they can be stored for long periods without loss of predatory efficacy.

In conclusion, 36-month storage of *M. thaumasium* pellets in the alginate matrix did not influence the efficacy of predation of L3 sheep gastrointestinal nematodes. Larval recovery was markedly diminished and fungal activity was apparent in faeces up to 96 h after administration to sheep.

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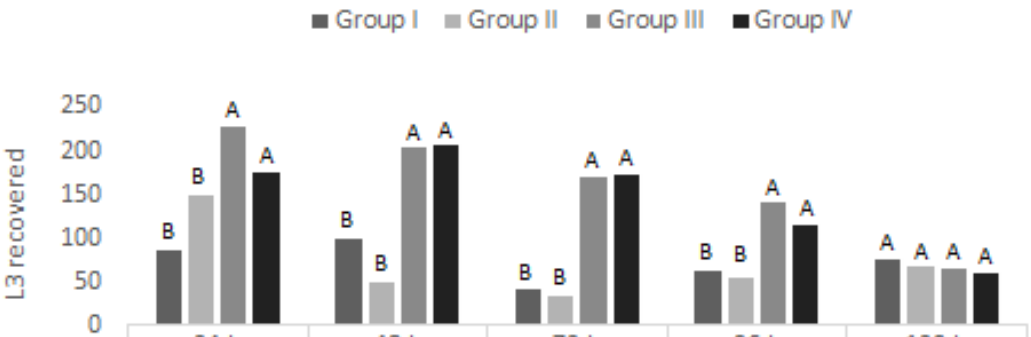
Figure 1. Means and L3 reduction percentage of sheep gastrointestinal nematodes recovered from coprocultures. Group I - *M. thaumasium* - 36 months of storage; Group II - *M. thaumasium* - newly produced; Group III - pellets without fungi; and Group IV - control. Different letters in the same time interval indicate statistical difference determined by the Tukey's test at 1% probability.



	24 h	48 h	72 h	96 h	120 h
% Reduction GI	51	52	75	46	-25
% Reduction GII	14	76	79	52	-13

336 **Table 1.** Percentage of L3 sheep gastrointestinal nematodes recovered from coprocultures

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		0 h	24 h	48 h	72 h	96 h	120 h
Group I	H	64	62	72	70	55	63
	T	24	38	24	20	40	10
	O	12	0	4	10	5	27
	S	0	0	0	0	0	0
Group II	H	72	56	70	90	70	80
	T	18	40	30	10	20	10
	O	10	4	0	0	10	10
	S	0	0	0	0	0	0
Group III	H	69	75	70	82	70	90
	T	23	22	30	18	10	6
	O	8	3	0	0	10	4
	S	0	0	0	0	10	0
Group IV	H	90	68	94	86	74	80
	T	10	25	6	14	15	14
	O	0	7	0	0	5	6
	S	0	0	0	0	6	0

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339 Group I - *M. thaumasium* - 36 months of storage; Group II - *M. thaumasium* - newly produced;

340 Group III - pellets without fungi; and Group IV - control

341 H. *Haemonchus* sp.; T.- *Trichostrongylus* spp.; O. – *Oesophagostomum*.; S. – *Strongyloides*