

Resistance of different fungal structures of *Duddingtonia flagrans* to the digestive process and predatory ability on larvae of *Haemonchus contortus* and *Strongyloides papillosus* in goat feces

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Abstract The dynamics of the passage of conidia, chlamydospores, and mycelia of the fungus *Duddingtonia flagrans* through the digestive tracts of goats was evaluated. Four groups with five goats each were formed. In the group conidia, each animal received 1×10^6 *D. flagrans* conidia per kilogram of live weight. In the group chlamydospore, each animal received 1×10^6 chlamydospores per kilogram of live weight. In the group mycelia, each animal received 1 g of mycelium mass per kilogram of live weight. In the control group, the animals received no fungal structure. Feces were obtained 3 h before and 12, 24, 30, 36, 42, 48, 60, 72, 84, and 96 h after the inoculation. The feces were placed in Petri dishes containing water-agar. The Petri dishes were examined to detect the fungus and trapped nematodes. A second trial evaluated the effect of the fungal structures on the number of gastrointestinal larvae of *Haemonchus contortus* and *Strongyloides papillosus* har-

vested from the fecal cultures of the goats. The feces were obtained from the goats in the 12–24, 24–30, 30–36, 42–48, 60–72, 72–84, and 84–96 intervals after the inoculation. *D. flagrans* survived the digestive process of the goats and maintained its predatory activity, being observed from 12 to 96 h before inoculation in the animals that received chlamydospores and conidia.

Introduction

Gastrointestinal parasitism with nematodes is one of the main limiting factors in raising small ruminants. *Haemonchus contortus* is considered the most important gastrointestinal nematode parasite in small ruminants, causing large economic losses worldwide. The damages caused by this parasite are associated to their hematophagous habit, which leads to severe anemia a few days after infection, loss of weight and appetite, weakness, and death in some cases (Urquhart et al. 1996).

Strongyloides papillosus is an important gastrointestinal parasite in ruminants, mainly in tropical and subtropical regions of the world. This nematode can develop both parasitic and free-living reproductive life cycles. Females are responsible for the embryonic egg production that occurs by parthenogenesis during the parasitic phase (Chandrawathani et al. 1998; Urquhart et al. 1996). In some cases, the infection by *S. papillosus* may lead to diarrhea, loss of appetite, growth retardation, and sudden death (Taira et al. 1992; Pienaar et al. 1999).

Predatory nematophagous fungi have been shown as a viable alternative to the control of the pre-parasitic stage of several species of gastrointestinal nematodes in ruminants (Larsen 1999).

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Several studies have demonstrated the viability of *Duddingtonia flagrans* for this use. This species can produce not only mycelia and conidia but also thick-walled chlamydo spores that have been shown to survive passage through the gastrointestinal tract of ruminant and subsequently germinate in the voided feces to attack the developing larvae (Larsen 2000).

Although there is an agreement that the production of chlamydo spores is the ideal form of administration of the fungi as a biological control, there are drawbacks to the development of efficient methodologies for mass production. It is also necessary to determine if the chlamydo spore is the only stage responsible for the high fungal viability (Araújo et al. 2004a).

The objective of this work was to evaluate the predatory effect of *D. flagrans* (CG-722) isolates on *H. contortus* and *S. papillosus* L₃, after the passage of mycelia, conidia, and chlamydo spores of this species through the gastrointestinal tract of goats in confinement.

Materials and methods

The isolate of *D. flagrans* (CG-722) was supplied by CENARGEM, kept at the mycological collection of the Universidade Federal de Viçosa (Parasitology Laboratory, Veterinary Department) at 4°C in culture tubes containing corn meal agar (CMA, Difco) in the dark.

Chlamydo spore production

The *D. flagrans* fungal isolates were kept in culture tubes containing 2% CMA culture medium, and culture disks of isolate, 4 mm in diameter, containing 2% CMA, were transferred with a sterile platinum loop to 50×10 mm Petri dishes containing 2% water-agar (2% WA, Sigma) culture medium. After mycelial growth of this new culture, a 5-mm-diameter culture disk was transferred to a 90×15-mm Petri dish containing a 2% WA.

These plates were incubated in a biochemical oxygen demand (BOD) chamber at 25°C in the dark for 5 days. Agar disks of 4 mm in diameter were scraped with a sterile platinum loop from the edges of the fungal colonies, free from contamination, and inoculated onto 30 Petri dishes containing 20 ml of yeast-phosphate-sulfate-sucrose-agar (YPSSA) culture medium (yeast extract, 4 g; K₂HPO₄, 1 g; MgSO₄, 0,5 g; soluble starch, 20 g; agar, 20 g; water for 1 L solution). Next, the plates were incubated in BOD chamber at 25°C for 28 days in the dark. After this period, the plate surfaces were washed with 10 ml of sterile distilled water using a paintbrush. The suspension in the plates was sieved through a drum sieve coupled to a plastic container to eliminate mycelium fragments. The recovered

spores were counted ten times in a Neubauer chamber and classified (conidia or chlamydo spores) in three 10-μl aliquots. After counting and characterizing the spores, the dilution was prepared to the desired concentration and administered to each animal.

Conidial production

CMA disks (4 mm in diameter) containing *D. flagrans* were inoculated on 30 Petri dishes (9-cm diameter) with YPSSA medium. The plates were placed in a BOD chamber at 25°C for 7 days. Following incubation, the plate surfaces were washed with 10 ml of water and a paintbrush. Spore counting and characterization were carried out as described above, followed by dilution to the desired concentration and administration to each animal.

Mycelial mass production

Five 4-mm-diameter WA disks containing *D. flagrans* mycelia and spores were inoculated in 250 ml Erlenmeyer flasks containing 150 ml of glucose-peptone-yeast medium (15 g glucose, 2 g peptone, and 5 g yeast extract). The flasks were incubated for 10 days in a BOD chamber at 25°C in the dark on a rotary shaker (120 rpm). Following incubation, the mycelial mass was harvested by filtration on filter paper, separated from the culture medium, and washed three times with sterile distilled water. The excess moisture was removed by hand pressing between filter paper three times, and the mycelial mass was weighed in a precision scale. After weighing, five samples were collected from the total mycelial mass, mounted on a glass slide overlaid with a cover glass, and examined at ×100 and ×400 magnifications to eliminate the possibility of formation of conidia and chlamydo spores in the liquid medium.

Nematodes

Infective larvae (L₃) of *H. contortus* and *S. papillosus* were recovered by coproculture of 20 g feces from goats presenting monospecific infection and hospitalized in the clinic of the Veterinary Department of the Universidade Federal de Viçosa.

The larvae were recovered from the coprocultures by the Baermann method, washed five times by centrifugation (1,500 rpm) for 5 min, and the supernatant discarded. The L₃ were filtered in Kimwipes (Kimberly-Clark) antistatic film according to the methodology described by Barçante et al. (2003) to eliminate residues and bacteria and kept in 0.05% (w/v) streptomycin sulfate and 0.05% (p/v) chloramphenicol solution for 1 h. Then, the previous washing procedure was repeated.

Animals

Twenty-eight-month-old females of Saanem goats infected with *H. contortus* and *S. papillosus* were used in the experiment. The animals were purchased from the goat-breeding sector of the Animal Science Department of the Federal University of Viçosa. The animals were kept in concrete floor stalls at the Veterinary Department of the Federal University of Viçosa. During the experimental period, the goats were fed on chopped elephant grass (*Pennisetum purpureum*), Tifton hay (*Cynodon dactylon*), and water ad libitum.

Experimental procedure

In vitro predatory activity of *D. flagrans* on infective larvae of *H. contortus* and *S. papillosus*

Predation tests were carried out in Petri dishes (6 cm in diameter) containing 2% WA. Four groups were formed with five repetitions each:

- Group 1: fungal colony grown for 7 days at 25°C and approximately 10^3 L₃ of *H. contortus*.
- Group 2: 10^3 L₃ of *H. contortus*.
- Group 3: fungal colony grown for 7 days at 25°C and approximately 10^3 L₃ of *S. papillosus*.
- Group 4: 10^3 L₃ of *S. papillosus*.

The plates were incubated in a BOD chamber for 7 days at 25°C in the dark. Over the incubation time, the plates were removed from the chamber daily so that trap formation and capture of L₃ by fungi could be observed. After 7 days, the agar was removed from the plates with a metal spatula, and predation-free L₃ were recovered in hemolysis tubes by the Baermann method for 12 h. The content in the hemolysis tubes was transferred to centrifuge tubes and were centrifuged for 10 min at 1,500 rpm. After centrifugation, the volume was adjusted to 1 ml with a Pasteur pipette attached to a vacuum pump. The L₃ number estimate was calculated as the average of five 50- μ l aliquots and extrapolated to 1 ml in the tubes.

The fungus predation rate for each nematode species was calculated using the following formula: [(mean number of larvae recovered in the control group – mean number of larvae recovered in the treated group)/mean number of larvae recovered in the control group].

Predatory ability on larvae of H. contortus and S. papillosus in goat feces following the oral administration of conidia, chlamydo spores, and mycelia

The animals were randomly assigned to four groups on the basis of eggs per gram feces (EPG), with five animals each:

Conidia: Each animal received 120 ml of oral suspension containing approximately 1×10^6 spores (95.28% conidia and 4.72% chlamydo spores) of *D. flagrans*.

Chlamydo spores: Each animal received 120 ml of oral suspension containing approximately 1×10^6 spores (93.37% chlamydo spores and 6.63% conidia) of *D. flagrans* per kilogram of live weight.

Mycelia: Each animal received 120 ml of oral suspension containing 1 g of fresh *D. flagrans* mycelial mass per kilogram of live weight.

Control: Each animal received 120 ml of sterile distilled water.

Fecal collections were carried out in cotton bags, coated internally with plastic tape to facilitate cleaning between collections, in intervals of 12–24, 24–30, 30–36, 42–48, 60–72, 72–84, and 84–96 h after administration of the fungal material. The collected feces were homogenized, and 20 g samples from each animal of the four experimental groups were removed and mixed to 10 g of vermiculite and 25 ml of water, for coproculture, in 200 ml polyvinyl chloride (PVC) glasses incubated in a BOD chamber at 26°C. Coprocultures were visually examined over the incubation time every 3 days. After 18 days of incubation and starting from the time of sample collection, the L₃ were recovered in hemolysis tubes by the Baermann method with water at 42°C for 12 h. The predation-free larvae were counted using an optical microscope ($\times 100$) in five 100- μ l aliquots taken from the initial sample and then extrapolated to the total volume in the hemolysis tubes.

For EPG determination, the feces were collected from the animals' rectum three times a day at 08:00, 14:00, and 18:00 hours during the experimental period. The EPG counting was performed following each fecal collection (Gordon and Whitlock 1939).

The percentage of larval development was determined for each group and each collection interval using the formula (mean lpg/mean epg) $\times 100$ described by Paraud et al. (2004).

The percentage of reduction in the larval development in the groups treated with conidia, chlamydo spores, and mycelia was calculated with the formula: [(mean lpg of the control group/mean epg of the control group) – (mean lpg of the fungus group/mean epg of the fungus group)] / (mean lpg of the control group/mean epg of the control group) $\times 100$.

Dynamics of conidium, chlamydo spore, and mycelium elimination in the goat feces after passing through the gastrointestinal tract of goats

To eliminate the possibility of fungal presence in the animal's feces before the experiment and to evaluate the

fungal presence after treatment, feces samples were collected in plastic bags directly from the animal's rectum 3 h before and 12, 24, 30, 36, 42, 48, 60, 72, 84, and 96 h after the administration of fungal structures. Two grams of these samples were crushed with a glass rod and inoculated on 9-cm Petri dishes with 2% WA. Three thousand *H. contortus* larvae were dropped onto the agar to stimulate fungal growth and trap development. The plates were sealed with Parafilm (Sigma®) and incubated at 25°C in the dark. The plates were examined daily for 30 days using a stereoscopic microscope to visualize traps, conidia, and chlamydospores typical of the isolate and nematodes trapped on plate surface.

Statistical analysis

The experiment was arranged in a split plot design, with the treatments (chlamydospore, conidia, mycelia, and control) as the main plots and the collection intervals (12–24, 24–30, 30–36, 36–48, 48–60, 60–72, 72–84, and 84–96 h) as the sub-plots in a complete randomized design with 20 repetitions (five animals per treatment). The data of the percentage of larval development were transformed (Arcsin), examined by analysis of variance, and the means were compared using the Tukey test at 5% probability level using the SAEG statistical package.

Results and discussion

Conidia, chlamydospores, and mycelia production

Chlamydospores (93.37%) and conidia (6.63%) were recovered from the plates used for the production of chlamydospores. In the plates used for the production of conidia, 95.28% of the collected spores were conidia, and 4.72% were chlamydospores. Mycelium fragments were observed in suspensions of conidia and chlamydospores, even after successive passages through the sieve.

In vitro predatory activity of *D. flagrans* on *H. contortus* and *S. papillosus* infective larvae

The structures used for predation were three-dimensional adhesive nets, which started to be produced 10 h after the addition of nematodes to the plates. The first trapped nematodes were observed 12 h after plate incubation.

Table 1 shows the mean number of *H. contortus* and *S. papillosus* infective larvae recovered after 7 days in plates with *D. flagrans* (CG-722) and in control plates.

D. flagrans showed higher predatory activity on *S. papillosus*. Experiments with other *D. flagrans* isolates had already demonstrated the high predatory effectiveness of

Table 1 Mean number of *Haemonchus contortus* and *Strongyloides papillosus* infective larvae (L₃) recovered by the Baermann method from 6-cm diameter Petri dishes containing 2% water-agar with *Duddingtonia flagrans* (CG-722) after 20 days of interaction at 25°C in the dark

Group	MNL	Percent reduction
Control <i>Strongyloides papillosus</i>	956.66 ± 139.99	–
Control <i>Haemonchus contortus</i>	842.66 ± 161.98	–
<i>Duddingtonia flagrans</i> + L ₃ <i>S. papillosus</i>	4.66 ± 3.01	99.51% ± 0.35%
<i>Duddingtonia flagrans</i> + L ₃ <i>H. contortus</i>	148 ± 30.98	81.52% ± 0.45%

MNL mean number of larvae recovered from the Petri dishes

this species on *H. contortus* L₃ (Mendoza-de-Gives et al. 1998; Chandrawathani et al. 2002).

No reports have been found in the literature concerning the use of *D. flagrans* against *S. papillosus*. The predation rate observed in the present work is higher than the rate reported by Gonzalez-Cruz et al. (1998), who found that the percentage of predation of *S. papillosus* L₃ by *Monacrosporium gehryopagum* was 93.1% and by *Arthrobotrys robusta* was 32.3%.

Studies have demonstrated that variations in fungal predatory activity on nematodes are common (Mendoza-de-Gives et al. 1994; Larsen 2000), independently of the fungus or nematode species used in the experiments. These variations can be related to different factors such as the experimental design, losses of viability during storage in laboratory, and genetic factors inherent to the assessed fungal strains as reported by (Gronvold et al. 1996).

In vitro experiments do not expose the fungi to the adverse conditions imposed by the gastrointestinal tract of the animal and environmental biotic and abiotic factors such as temperature variation, humidity, pH, and competition with other organisms of the coprophagic fauna and flora. Therefore, the results obtained in this kind of test cannot be extrapolated for in vivo experiments. On the other hand, we can confirm that the fungi-nematode interaction is occurring, which is important for the initial stages of the microorganism selection for biological control programs.

Considering that the production of chlamydospores is desirable in fungi used in biological control programs, it is important to select isolates with higher chlamydospore production associated with high predatory capacity.

Dynamics of conidium, chlamydospore, and mycelium elimination after passing through the gastrointestinal tract of goats

D. flagrans was not found in any of the fecal samples collected before the administration of the fungal structures

or in the plates from the control group during the entire experimental period (Table 1). Chlamydo spores, conidia, and trapped larvae were seen on the surface of the plates inoculated with feces collected from the animals of the groups treated with chlamydo spores, conidia, and mycelia. However, there were variations among treatments and among times (Table 1). The fungus was detected in 84.44% of the plates inoculated with feces from the animals treated with chlamydo spores, in 68.88% of the plates from the group treated with conidia, and in 40.0% of the plates from the group treated with mycelia.

The results obtained for the animals treated with chlamydo spores confirm the passage of chlamydo spores of the CG-722 isolate and confirm the findings of Larsen et al. (1998), who isolated fungi from sheep feces 12 h after oral administration of *D. flagrans* chlamydo spores.

This is the first report of the dynamics of *D. flagrans* conidia and mycelia through the gastrointestinal tract of goats.

Comparing the results of this work with other studies, we found that the time for fungus elimination after the oral administration of conidia and chlamydo spores can vary significantly. Previous studies reported activity of *D. flagrans* from 14–116 h in sheep and goat feces (Llerandi-Juárez and Mendoza-de-Gives 1998; Ojeda-Robertos et al. 2005).

Other factors related to the diet used during the experiments may have influenced the retention time of the digest in the gastrointestinal tract of the animals. This may also be influenced by the content and quality of fiber, food particle size, amount of ingested water, and occasional variations in the digestive physiology.

Studying the elimination of fungal material in animals treated with mycelia, conidia, and chlamydo spores, we found that in up to 36 h post-administration of fungal structures, the results were similar. Starting from this time, there was a clear reduction in fungus recovery in the animals treated with mycelia (Table 2).

During the transit through the gastrointestinal tract of ruminants, the ingesta are subjected to mechanical, microbial, and chemical actions (Ribeiro 1997) that are adverse to fungus survival. Thus, the longer the retention time in the digestive tract, the greater is the possibility of changes in the fungal structures. The structural composition of conidia and chlamydo spores possibly allowed them to remain intact, thus facilitating their isolation until the last collection. On the other hand, mycelium fragments may have been damaged and turned unviable.

Predatory ability on larvae of *H. contortus* and *S. papillosus* in goat feces following the administration of conidia, chlamydo spores, and mycelia

The percentage means of larval development of *H. contortus* and *S. papillosus* in coproculture are shown in Tables 3 and 4.

Table 2 Occurrence of *Duddingtonia flagrans* fungus before and after the administration of chlamydo spores, conidia, and mycelia to goats in plates containing water-agar inoculated with 2 g of goat feces and 3,000 *Haemonchus contortus* larvae

CT (h)	Group			
	Control	Chlamydo spore	Conidia	Mycelia
3 BI	-----	-----	-----	-----
12 AI	-----	++++ -	+++ --	++++ -
24 AI	-----	+++++	+++++	+++ --
30 AI	-----	+++++	+++++	++++ -
36 AI	-----	+++++	++++ -	++++ -
48 AI	-----	+++++	+++ --	+ ----
60 AI	-----	+++++	+++++	+ ----
72 AI	-----	+++ --	++ ---	+ ----
84 AI	-----	++++ -	++ ---	-----
96 AI	-----	++ ---	++ ---	-----

CT feces collection time, CTBI time of collection before inoculation with fungal structures, CTAI time of collection after inoculation with fungal structures, + plates with fungus, - plates without fungus

On the average of the collection intervals, the percentage of larval development of *H. contortus* for the groups control, conidia, chlamydo spores, and mycelia were 3.486%, 2.653%, 1.327%, and 2.306%, respectively; and the percentage of reduction in larval development for the conidia, chlamydo spores, and mycelia groups were 23.89%, 61.23%, and 33.84%, respectively.

The percentage of larval development obtained for *H. contortus* is similar to the percentage found by Ojeda-Robertos et al. (2005; 0.9–11.1%) and lower than the results of Terril et al. (2004; 3.9–100%) in goat coprocultures.

The mean percentage of larval development of *S. papillosus* at the end of the experiment for the control, conidia, chlamydo spores, and mycelia groups were 15.95%, 13.417%, 4.97%, and 12.82%, respectively; and the percentage of reduction in larval development for the groups treated with conidia, chlamydo spores, and mycelia were 15.88%, 68.84%, and 19.62%, respectively.

The results of the percentage of larval development for *H. contortus* and *S. papillosus* show that the treatment with chlamydo spores was the most effective in controlling the two species. Except for the first interval of collection of *S. papillosus* and the sixth interval for *H. contortus*, the reductions followed the dynamics of the chlamydo spore elimination described in Table 2.

In the group of animals treated with conidia, the percentage of larval development for *H. contortus* was significantly lower ($p < 0.05$) than the percentage for the control group and similar to the groups treated with chlamydo spores and mycelia (up to 36 h post-

Table 3 Means for the percentage of larval development of *Haemonchus contortus* in goat coprocultures in the intervals of 12–24, 24–30, 30–36, 36–48, 48–60, 60–72, 72–84, and 84–96 h after treatment with 1.0×10^6 conidia/kg live weight, 1.0×10^6 chlamydo-spores/kg live weight, 1 g *Duddingtonia flagrans* mycelia/kg live weight, and control without treatment

IC (h)	Control	Conidia	Chlamydo-spore	Mycelia
12–24	2.60 Aa	0.26 Cb	0.73 Bb	0.65 Cb
24–30	5.66 Aa	1.54 BCb	0.80 Bb	1.08 BCb
30–36	3.58 Aa	1.46 BCb	0.76 Bb	2.18 ABCab
36–48	3.25 Aa	1.81 ABab	1.1 Bb	2.25 ABCab
48–60	3.32 Aa	3.47 ABa	1.16 Bb	2.78 ABab
60–72	2.77Aa	2.77 ABa	1.69 ABa	2.78 ABa
72–84	3.94 Aa	3.40 ABa	0.46 Bb	3.00 ABa
84–96	2.77 Aa	4.71 Aa	3.92 Aa	3.50 Aa
Percent mdl ^a	3.486%	2.653%	1.327%	2.306%
Percent rdl ^b	–	23.89%	61.93%	33.84%

Means followed by the same capital letter in the column and small letter in the row were not significantly different by the Tukey test at 5% probability. Statistical significant was based on Arcsin transformed data

^a Mean percentage of larval development

^b Percentage of reduction in larval development

administration). However, the reductions were not correlated with the dynamics of conidium elimination, showing that damage may have occurred during the passage through the animal gastrointestinal tract, which did not harm the fungus germination capacity, but did harm its predatory activity. The results obtained in this group for *S. papillosus* corroborate this hypothesis because no significant differences ($p < 0.05$) were found among the percentage means of larval development when compared with the control group.

The mycelium treatment significantly reduced ($p < 0.05$) the percentage of larval development of *H. contortus* only in the first two collection intervals, but it was not possible to demonstrate its effect on *S. papillosus* larvae.

These results demonstrated that the administration of chlamydo-spores from the Brazilian isolate of *D. flagrans* CG-722 is effective in controlling *H. contortus* and *S. papillosus* larvae in goats.

Further studies are recommended to evaluate the effectiveness of this isolate in the field, in different environmental conditions, and after the passage through the gastrointestinal tract of another animal species.

The administration of conidia and mycelia to the animals resulted in a short period of efficacy. The use of formulations that can protect these structures from the

Table 4 Means for the percentage of larval development of *Strongyloides papillosus* in goat coprocultures in the intervals of 12–24, 24–30, 30–36, 36–48, 48–60, 60–72, 72–84, and 84–96 h after treatment with 1.0×10^6 conidia/kg live weight, 1.0×10^6 chlamydo-spores/kg live weight, 1 g of *Duddingtonia flagrans* mycelia/kg live weight, and control without treatment

IC (h)	Control group	Conidia	Chlamydo-spore	Mycelia
12–24	9.93 Ba	3.31 Ca	3.07 Ba	4.27 Ca
24–30	9.41 Ba	3.93 Cab	1.17 Bb	5.66 BCab
30–36	13.02 ABa	13.15 ABCa	0.92 Bb	12.68 ABCa
36–48	19.04 ABa	7.23 BCab	2.80 Bb	16.45 ABa
48–60	15.16 ABa	15.8 ABa	1.77 Bb	8.88 BCa
60–72	20.93 ABa	20.93 ABCab	5.67 Bb	13.24 ABCab
72–84	13.91 ABab	16.72 ABa	6.52 Bb	17.55 ABab
84–96	26.27 Aa	26.27 Aa	17.84 Aa	23.13 Aa
Percent mdl ^a	15.95%	13.417%	4.97%	12.82%
Percent rdl ^b	–	15.88%	68.84%	19.62%

Means followed by the same capital letter in the column and small letter in the row were not significantly different by the Tukey test at 5% probability. Statistical significant was based on Arcsin transformed data

^a Mean percentage of larval development

^b Percentage of reduction in larval development

adverse conditions in the gastrointestinal tract of animals is a possibility. Alves et al. (2003), Araújo et al. (2004b), and Assis et al. (2005) obtained significant reductions of infective larvae of gastrointestinal parasites in bovines after the administration of fungal mycelia of *Monacrosporium* isolates encapsulated in sodium alginate to bovines and goats.

Although chlamydo-spores are very resistant to the passage through the gastrointestinal tract of animals and can maintain their predatory activity, their production requires a longer period of time when compared with the production of mycelia and conidia.

The use of nematophagous fungi in the biological control of nematode parasites of ruminants will only be possible after the involvement of industries in the development of fungal formulations. It will be then necessary to study the cost/benefit relationship for the industrial-scale production of fungal structures and the development of methodologies to improve production.

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