Top Dressing of Feed with Desiccated Chlamydospores of
*Duddingtonia flagrans* for Biological Control of the Pre-
Parasitic Stages of Ovine *Haemonchus contortus*

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**ABSTRACT**

Feeding trials were conducted with stall-fed sheep parasitized with *Haemonchus contortus.* For 10 days they were offered 250 g of a concentrate feed that had been top-dressed with desiccated chlamydospores of *Duddingtonia flagrans* at $1 \times 10^3$, $5 \times 10^3$, $1 \times 10^4$ or $2 \times 10^4$ chlamydospores/kg body weight. Pooled faeces from each group on day 7 of spore feeding were spread on different pasture plots. On day 28 after the start of spore feeding, further pooled faeces from each group were spread on the same plots. The larval burdens on the plots were monitored for 2 months and the larval harvest from *in vitro* faecal cultures were monitored regularly. The application of $1 \times 10^5$ or more spores/kg body weight virtually eliminated larvae from both the pasture and the faecal cultures. The application of as few as $1 \times 10^4$ spores/kg body weight had a profound impact on larval recovery. The effect persisted while the spores were being fed but not for more than 4 days following discontinuation of spore feeding. Top dressing supplementary feed with dried chlamydospores offers a potential way of using *D. flagrans* for biological control of the pre-parasitic stages of *H. contortus.*

**Keywords:** biological control, chlamydospores, *Duddingtonia flagrans*, *Haemonchus contortus*, infective larvae, nematophagous fungi, sheep

**Abbreviations:** CMA, corn meal agar; DF, degrees of freedom (source of variation); DFD, degrees of freedom (residual)

**INTRODUCTION**

In the endeavour to develop alternative strategies to chemotherapy for controlling nematode parasites in grazing livestock, work on biological control using nematode-trapping fungi – initially undertaken in Scandinavian countries (Gronvold *et al.*, 1993) – has also been undertaken in India. Earlier studies had shown that nematophagous fungi can be used to control worms by targeting larvae in faeces and on pasture. The important requirement is the presence of the fungi in the faecal pellets/pats where the development of the pre-parasitic larvae take place. Therefore, to be effective, the fungi should pass through the gastrointestinal tract of the host without loss of viability. The fungus *Duddingtonia flagrans*, which can be isolated from the fresh faeces of local
sheep, produces profuse thick-walled chlamydospores, the stage responsible for its survival during passage through the gut of ruminants following oral administration (Sanyal, 2000a). Successful gastrointestinal transit by the chlamydospores resulted in significantly reduced translation of the infective nematode larvae of both sheep and cattle under experimental conditions (Sanyal, 2000b, 2001).

On a laboratory scale, moistened barley grains serve as an ideal substrate for the production of fungal chlamydospores, which can easily be harvested in water by washing and sieving (Sanyal, 2000b). Initially, an attempt was made to use concentrate feed pellets to deliver the fungi to the animals. Dried chlamydospores of D. flagrans, obtained from barley grain culture, were incorporated into the feed ingredients at 4 x 10⁵ spores/kg and pelleted in a pilot-scale pelletizer. Randomly selected pellet samples were then cultured in corn meal agar (CMA) and baited with Haemonchus contortus larvae for fungal recovery. The feed pellets were also fed to sheep naturally infected with nematodes. Faecal cultures were made to enumerate the number of larvae recovered and the results were compared with data generated from sheep fed on concentrate feed pellets not incorporating fungal chlamydospores. No fungus could be recovered from the feed pellets from day 3 after manufacture and no significant reduction in larval recovery was observed in faecal cultures from sheep fed on these pellets (Anon., 2001). These results suggested that germination of chlamydospores had occurred in the feed pellets, so that the fungus was destroyed while passing through the gastrointestinal tract.

Desiccated chlamydospores were placed in sealed plastic packets, to prevent exposure to moisture, and stored at either room or refrigerator temperature. When their survival time was studied by sprinkling the stored chlamydospores on CMA plates every week, it was found that the dried spores could germinate even after 6 months of storage at either temperature (Anon., 2001).

Because of this prolonged viability, it was proposed (Anon., 2001) to investigate whether desiccated chlamydospores could be used as a daily top dressing on feed given to sheep experimentally infected with Haemonchus contortus, to control their pre-parasitic stages. It was also necessary to ascertain the number of chlamydospores required to be given to sheep to result in substantial reductions in the resultant numbers of infective larvae. The present study aimed to meet these objectives.

MATERIALS AND METHODS

Chlamydospores

Pure cultures of the previously isolated, naturally occurring D. flagrans were maintained on 2% CMA (Sigma, St Louis, MO, USA) plates (90 mm disposable and radiation-sterilized; Tarsons, Calcutta, India) containing 0.2% tetracycline (w/v), which was added after autoclaving the media to suppress bacterial growth. The fungus was then grown on barley grains to produce chlamydospores (Sanyal, 2000b). A piece of CMA medium containing pure fungal growth was put into a flask containing autoclaved barley grains and incubated at 25°C for 4 weeks, the flask being shaken