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Capability of the nematode-trapping fungus *Duddingtonia flagrans* to reduce infective larvae of gastrointestinal nematodes in goat feces in the southeastern United States: dose titration and dose time interval studies

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Abstract

Infection with gastrointestinal nematodes, particularly *Haemonchus contortus*, is a major constraint to goat production in the southeastern United States. Non-anthelmintic control alternatives are needed due to increasing resistance of these nematodes to available anthelmintics. Two studies were completed in Central Georgia in August 1999, and April–May 2000, using Spanish does naturally infected with *Haemonchus contortus*, *Trichostongylus colubriformis*, and *Cooperia* spp. to evaluate effectiveness of nematode-trapping fungi as a biological control agent. In the first experiment, five levels of *Duddingtonia flagrans* spores were mixed with a complete diet and fed once daily to the does (three per treatment) in metabolism crates. The treatment concentrations were (1) 5×10^5, (2) 2.5×10^5, (3) 10^5, and (4) 5×10^4 spores per kilogram body weight (BW), and (5) no spores. Fungal spores were fed for the first 7 days of the 14-day trial, and fecal samples were collected daily from individual animals for analysis of fecal egg count and establishment of fecal cultures. Efficacy of the fungus at reducing development of infective larvae (L3) in the fecal

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these animals were excluded to avoid biasing data from healthy animals. Each doe was fed individually for 14 days (days 1–7: feed ration g + spore formulation; days 8–14: feed ration only). Feces were collected from each animal daily (days 1–14) to determine FEC (expressed as eggs per gram (EPG) of feces) and to establish fecal cultures for larval recoveries. Fecal cultures were made by crushing and mixing 12 g of each animal's feces with vermiculite and deionized water (to create a moist, friable appearance) in a 190 ml polystyrene cup, which was then covered with aluminium foil and incubated at 27 °C for 10 days (Mechanical convention incubator, Model 6LN, Precision Scientific, Chicago, IL). Cultures were stirred daily and additional water was added as needed to maintain an adequate level of moisture. After 10 days, infective larvae (L3) were recovered by the Baermann technique and then counted and identified to genus using a Swift microscope (Swift Instruments, San Jose, CA). Number of larvae were expressed as L3 per gram (LPG) of cultured feces.

Residual activity of the fungus was evaluated by collecting and processing feces for 7 days after the fungal spore feeding was discontinued. Each day of the trial, % relative reduction in yield of L3 recovered from cultures was calculated as the LPG/EPG ratio in the treatment group compared to this ratio in the control group; % relative reduction = 100 (treated group yield × 100/control group yield).

2.2.2. Time interval study

For 1 week prior to the study, the does received a daily supplement of approximately half kilogram of the feed ration described previously, in addition to being given access to fresh pasture (permanent grass, perennial warm-season, and annual cool-season species). After randomly selecting four animals per group on the basis of FEC and hematocrit, each group was randomly assigned to one of four treatments, including a no spore control and three spore dosed groups, 2.5×10^5 spores per kilogram BW fed daily, every second day, or every third day. The treatment dose of 2.5×10^5 spores per kilogram BW was chosen for the dose timing trial because of the relatively high reduction in infective larvae with this dose in the first trial (80.2%) and more favourable economics of using half the spore concentration compared with the highest dose level of 5×10^5 spores per kilogram BW. As with the first trial, the goats were allowed to consume the fungal spores mixed with approximately 30 g of feed each morning before receiving their remaining daily ration to assure that all spores were consumed. Over a 20-day period, feces were collected daily for estimate of FEC. Fungus feeding started on day 4 and lasted for 13 days.

The fecal culture cup technique described by Peña et al. (2000) was modified and adapted for this study. We conducted a series of experiments and determined that moistened, slightly crushed, whole pellets, when incubated at 27 °C for 9 and 13 days (Peña et al., 2000), provided a higher yield of L3 compared to breaking up feces and mixing with vermiculite (data not shown). Based on these data, we used this modified method for subsequent studies. Six small holes were made in the bottom of 200 ml flexible plastic cups, and the cups were cut in half. Each culture was established by weighing 10 g of feces into the 75 ml lower section of the cup, gently crushing the fecal pellets by means of a wooden spatula and adding distilled/deionized water until the material was moist, but not saturated. The lower cup section with feces was covered with cheesecloth, inverted, and placed into the upper cut section of the cup. Each culture cup was subsequently placed in an intact cup containing approximately 10 ml of distilled/deionized water and then incubated at 27 °C for 10 days.

This provided a continually moist and aerated environment for larval development without the daily stirring that was performed in the first experiment. After incubation, the water was discarded and the L3 were extracted from the culture material in the small cup using a modified Baermann technique (Peña et al., 2000). Harvested L3 were stained with iodine and counted using a mieroscope. Calculation of % relative reduction in L3 development for all fungal treatment groups for each day was performed as described previously.

2.2.3. Statistical analyses

Larval reduction data for both the dose titration and dose time interval studies were analyzed as repeated measures analysis (SAS, 1992). When the treatment effect was significant at $P < 0.05$, means were separated using LS Means procedure. For the dose titration study, data from days 2–8 (spore saturation period) and days 9–14 (spore clearing period) were analyzed separately. For the dose time interval study, data from days 1–4 (pre-spore feeding), 5–17 (spore saturation), and 18–20 (spore clearing) were analyzed separately.

3. Results

3.1. Dose titration study

The nematode species present in the does used in this study, based on percent L3 recoveries ± S.D., were *H. contortus* (31.8 ± 21.8), *T. colubriformis* (3.7 ± 5.6), and *Cooperia* spp. (64.2 ± 23.5). This ratio was similar between control and fungal spore treatment animals, indicating that *D flagrans* reduced L3 development for these different nematode species at the same rate.

Average pre-treatment FEC ranged from 1913 to 6188 EPG for the five experimental groups (Table 1). After an initial drop, FEC for each group remained fairly stable throughout the trial, averaging 896, 2952, 1745, 2277, and 2015 EPG for the control animals and the four fungal spore groups (highest to lowest), respectively. Lower mean EPG for the control group was due to removal of the animal with highest EPG in this group because of clinical haemonchosis. Mean number of L3 recovered from fecal cultures on day 1, when there were no spores in the feces, and days 13 and 14, when there was no further evidence of fungal spore activity for any of the treatments, averaged 545, 1037, 1517, 1274, and 1268 LPG for the five groups, respectively. Mean % L3 yield (LPG/EPG) for the groups on these days were 34.3, 37.4, 59.3, and 48.3%, respectively (Table 2).

One day after spore feeding started (day 2), development of L3 in fecal cultures was reduced by 70.3–93.2% for all treatment levels when calculated relative to the untreated control (Table 2). The mean L3 reduction from day 2 of the treatment period until the day after treatment stopped (days 2–8) was 93.6, 80.2, 84.1, and 60.8% for animals given the highest to lowest spore doses, respectively. This time interval represents the time when *D. flagrans* chlamydospores are expected to be excreted in the feces after the gastrointestinal system has been 'saturated' with spores and also the period before the level of fungus excretion is expected to drop again. There was a dose treatment effect over this period, with lower ($P < 0.05$) reduction values at the 5×10^4 spores per kilogram BW dose compared with the three higher dose levels. The time effect was not significant over this period,

Table 1
Mean number of parasite eggs per gram (EPG) and infective third stage larvae per gram (LPG) of feces from nematode-infected goats fed with five different concentrations of the nematode-trapping fungi *D. flagrans*

Day[a]	Treatment group[b]									
	Control ($n = 2$)**		5×10^5 ($n = 4$)		2.5×10^5 ($n = 3$)		10^5 ($n = 2$)*		5×10^4 ($n = 3$)	
	EPG	LPG	EPG	LPG	EPG	LPG	EPG	LPG	EPG	LPG
1	1913 ± 38	1366 ± 103	6188 ± 1749	1502 ± 743	3767 ± 542	2036 ± 281	3713 ± 288	3133 ± 1178	2983 ± 1070	2804 ± 1277
2	725 ± 125	767 ± 116	2600 ± 835	253 ± 67	1883 ± 292	151 ± 66	2750 ± 1500	131 ± 48	1883 ± 767	576 ± 217
3	1225 ± 25	163 ± 52	2563 ± 228	32 ± 16	1558 ± 200	58 ± 46	2625 ± 1425	57 ± 41	2417 ± 1162	83 ± 34
4	650 ± 100	226 ± 131	1463 ± 279	19 ± 10	1217 ± 83	131 ± 40	2025 ± 1175	101 ± 18	2200 ± 929	180 ± 50
5	850 ± 600	335 ± 193	2650 ± 516	45 ± 16	1233 ± 209	86 ± 36	2250 ± 1000	19 ± 11	1317 ± 744	218 ± 158
6	1200 ± 150	400 ± 20	2838 ± 542	18 ± 2	1767 ± 557	72 ± 39	2450 ± 1150	113 ± 60	2400 ± 881	129 ± 31
7	800 ± 200	434 ± 125	2713 ± 637	51 ± 18	1633 ± 273	100 ± 34	2450 ± 750	349 ± 41	1983 ± 1092	160 ± 46
8	575 ± 175	312 ± 74	2500 ± 506	95 ± 38	1683 ± 233	303 ± 158	2363 ± 838	186 ± 17	1767 ± 766	384 ± 127
9	275 ± 125	438 ± 106	2088 ± 606	338 ± 91	1567 ± 567	455 ± 88	2375 ± 225	458 ± 352	850 ± 419	481 ± 230
10	800 ± 150	191 ± 67	3313 ± 672	309 ± 137	1467 ± 242	429 ± 121	2800 ± 550	242 ± 49	2272 ± 755	245 ± 136
11	925 ± 125	130 ± 6	4000 ± 690	373 ± 124	1875 ± 809	562 ± 141	2050 ± 800	437 ± 61	2533 ± 1189	199 ± 186
12	763 ± 13	90 ± 24	3300 ± 91	439 ± 69	2100 ± 1056	553 ± 124	1925 ± 825	243 ± 63	1717 ± 703	125 ± 77
13	1038 ± 213	33 ± 30	2975 ± 312	799 ± 177	1205 ± 267	412 ± 80	1250 ± 150	393 ± 114	1817 ± 1001	256 ± 160
14	800 ± 250	236 ± 124	2138 ± 219	811 ± 91	1417 ± 196	320 ± 114	1775 ± 625	1024 ± 277	2067 ± 865	744 ± 532

[a] Fungal chlamydospores fed to goats on days 1–7.
[b] Chlamydospores per kilogram body weight.
* Initially three animals, one died during the trial, data excluded from the study.
** One animal became sick during the trial, data excluded from the study.

Table 2
Effect of feeding four different chlamydospore dose levels of *D. flagrans* to goats on yield and % relative reduction of developing nematode larvae in fecal cultures

Day[a]	Treatment group[b]								
	Control ($n = 2$)*	5×10^5 ($n = 4$)		2.5×10^5 ($n = 3$)		10^5 ($n = 2$)**		5×10^4 ($n = 3$)	
	Yield[c]	Yield	% Reduction[d]	Yield	% Reduction	Yield	% Reduction	Yield	% Reduction
1	71.6	35.7	50.0	56.7	20.6	87.4	0.0	99.8	0.0
2	100.0	12.3	88.3	7.2	93.2	8.1	92.3	31.4	70.3
3	13.4	1.3	90.1	3.4	74.1	1.9	86.1	5.3	60.5
4	32.4	1.8	94.7	10.8	69.0	6.8	80.5	12.1	65.3
5	46.6	1.6	96.0	7.4	81.1	0.8	98.0	37.4	37.4
6	33.6	0.7	97.7	5.2	84.5	7.4	77.8	9.1	72.7
7	53.7	1.9	96.5	5.8	89.3	16.3	70.0	18.2	66.4
8	55.5	4.5	91.8	16.3	69.9	8.7	83.9	25.6	52.8
9	100.0	17.7	88.9	32.3	79.7	18.0	88.7	53.2	66.6
10	23.1	10.7	55.3	28.2	0.0	9.4	60.8	11.6	51.4
11	14.4	9.1	35.0	55.9	0.0	23.8	0.0	8.9	36.8
12	11.8	13.3	0.0	35.2	0.0	13.7	0.0	10.4	11.7
13	3.9	26.9	0.0	32.6	0.0	30.8	0.0	16.9	0.0
14	27.3	40.3	0.0	22.8	22.6	59.6	0.0	25.4	13.9

[a] Fungal chlamydospores fed to goats on days 1–7.
[b] Chlamydospores per kilogram body weight.
[c] Yield = (LPG/EPG) × 100.
[d] % Reduction = 100 − (treatment yield × 100/control yield).
* Initially three animals, one became clinically ill during the trial and data from that animal was excluded.
** One animal died during the trial, data excluded from the study.

indicating that the response to including different dose levels of *D. Flagrans* spores in the diet was consistent during the spore saturation period. There was no more reduction in L3 development for all treatment groups within 3–6 days after spore feeding was stopped. This effect was consistent in all four groups, as there was no treatment differences in percent L3 reduction for days 9–14, but the time effect was significant ($P < 0.05$).

3.2. Time interval study

Pre-treatment FEC for the dose time interval study animals (average for days 1–4) was 2622, 2625, 2947, and 4138 EPG for the control goats, and animals fed with fungal spores daily, every 2 days, and every 3 days, respectively (Table 3). Mean FEC for the whole trial was 2390, 2170, 2228, and 3519 EPG, respectively, for these groups. Number of L3 recovered from fecal cultures on days when the fungus was not present in the feces (days 1–4 and 18–20) averaged 1669, 1125, 1166, and 2171 LPG for the four groups, respectively. Larval yield as a percentage of eggs present in feces for these days was 56.3, 58.8, 58.1, and 47.5%, respectively, for the control and fungus fed every day, every second day, and every third day groups, respectively (Table 4). There appeared to be a distinct difference between the results of the first (days 4–9) and second week (days 10–16) of fungal spore