Vet. Res. 38 (2007) 141–150

DOI: 10.1051/vetres:2006050

Short note

Effect of nematophagous fungus *Duddingtonia flagrans* and energy supplementation on the epidemiology of naturally infected kids

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(Received 3 May 2006; accepted 15 September 2006)

Abstract – Gastrointestinal (GI) nematode infection is a major constraint for grazing livestock production. The increasing prevalence and severity of anthelmintic-resistant nematodes in many parts of the world has led to a search for non-chemical control options. Under experimental conditions, the nematophagous fungus *Duddingtonia flagrans* is emerging as an alternative to chemotherapy for the control of GI nematode infection in biological production systems. Also, recent information points to the role of energy nutrition to increase the immune response against GI nematode infection. In this study the effect of *D. flagrans* and energy supplementation on the epidemiology of GI nematode infections is explored on grazing kids. Four groups of 10, 4-month old goats were turned out on infected pasture in the early spring and allocated to four separate paddocks where they were rotationally grazed for 16 weeks. One of these groups (F) received 0.5×10^6 *D. flagrans* spores/kg BW/d. Another group (S) was supplemented with 100 g barley grain per day. A third group (F+S) received both nematophagous fungi and barley supplement treatments simultaneously while the fourth group (C) was used as a non-treated control. Both nematophagous fungi and barley supplement had a significant effect ($P < 0.01$) on reducing pasture infectivity, faecal egg excretion and worm burdens at slaughter that was particularly evident for *Trichostrongylus colubriformis*. The combination of both treatments showed a synergistic effect on the control of gastrointestinal nematode infections. At slaughter, the average total post-mortem worm count of the F+S group was reduced by 65% compared with the non-treated control. The results herein show that *D. flagrans* can act as an efficient biological control agent against kid GI nematode infections on pasture, which could further improve carcass characteristics. While small amounts of energy supplement can also reduce kid infection, the effect of *D. flagrans* as a biological control agent appeared clearly enhanced both in magnitude and duration by energy supplementation. This has clear implications for grazing animals and provides an efficient method for the practical control of parasitic nematodes in biological production systems.

***Duddingtonia flagrans* / nematode parasite / biological control / energy supplementation / goat**

1. INTRODUCTION

The increasing demand for goat dairy products and the farmers' interest in diversifying their agricultural products to meet market demands, have boosted the traditional goat production for meat or milk in Mediterranean countries. However, the widespread increase in the incidence of anthelmintic resistance in gastro-intestinal (GI) nematode parasites of goats in many parts of the world [6, 18], including

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Spain [15], now threatens the profitability of these production systems. Also, the society's environmental concerns and the increasing market demand for products that are free of chemicals, have led to the search for alternative methods of parasite control [25]. Amongst the methods of control available, two of the most promising options are the use of nematophagous fungi to minimise the pasture contamination made by animals and to maximise animal immunological response through optimising nutrition.

Recent studies have shown that the net trapping nematophagous fungus *Duddingtonia flagrans* is able to effectively control the developing GI infective larvae on pasture and subsequent levels of infection in various livestock species [5]. More recently, it has been proven that *D. flagrans* also has the ability to survive the passage through the GI tract of goats and subsequently reduce the numbers of parasitic nematodes in faecal cultures [10, 19], and on herbage over a short period of time [27].

While the potential of *D. flagrans* to control the free-living stages of nematode parasites of different ruminant species has been demonstrated, this effect has not always had consequences on the level of infection in young animals as the grazing season progressed. This has been discussed in terms of a differential immune response since immunity appears to develop faster in heavily infected than in fungus-treated young calves [26].

The nutritional status of the host has long been considered as an important factor in influencing the host-parasite relationship and the pathogenesis of parasite infections. While the mechanisms underlying this fact are not clear, energy balance and supply is increasingly considered to be an important factor in the immune response [7]. Indeed, recent reports have shown that an improvement in energy supply improved resistance to *Teladorsagia circumcincta* infection of growing lambs [22] and, a similar response to supplementary feeding was found to natural GI infections in Criollo kids [20].

This nutrition-parasite interaction and the fact that long-term effect of *D. flagrans* on levels of infection in animals could be affected by a differential immune response, led us to explore to what extent an enhancement in the animals immune response through energy supplementation could improve the fungus efficacy as a practical biological control agent of parasitic nematodes in pastured ruminants. Hence, the aim of this trial was to evaluate the efficacy of *D. flagrans* and of energy supplement to control GI nematode infections of young goats on naturally infected pastures and to what extent the response of these two treatments can be improved when combined during the entire spring grazing season.

2. MATERIALS AND METHODS

2.1. Experimental animals and conditions

The study group was comprised of 40 Blanca Celtibérica female, 4-month old goats and weighing 23.06 ± 0.36 kg BW at the beginning of the experiment. Throughout the trial, kids were allowed to graze on an Italian ryegrass (*Lolium multiflorum* cv. Tetrone) pasture at a stocking density of 27 kids/ha. An area of 9636 m^2 of pasture that had been grazed during the previous autumn by an infected flock was fenced and divided into four plots of identical sizes. Inside fences were used to divide each experimental area into four paddocks, to allow a 21-day rotational grazing.

All animals were drenched with Ivermectin (200 μg/kg BW, Oramec® Merial, Barcelona, Spain) for two consecutive days. A week after the anthelmintic treatment, individual faecal samples were collected to ensure the animals were free from

parasites before being turned out on the experimental areas.

2.2. Experimental design

Kids were stratified on the basis of body weight (BW) and randomly allocated to four groups of ten animals each and allowed to graze for 16 weeks on the experimental areas.

After 2 weeks on pasture, the kids in one of these groups (F) received *D. flagrans* (0.5×10^6 chlamydospores per kg BW and day) mixed with 50 g of wheat bran. Another group (S) was supplemented daily with 100 g of course ground barley grain per head. A third group (F+S) received both nematophagous fungi and supplement treatments simultaneously while the fourth group (C) remained as a non-treated control. The *D. flagrans* troll A isolate of Danish origin (Chr. Hansen Biosystems A/S, Denmark) was used in this study. The animals were observed daily while feeding to ensure that all kids consumed the offered material. The pasture was cut to 10 cm in June to remove stems, which were left in the paddocks.

2.3. Measurements

Herbage allowance within each experimental area was estimated at fortnightly intervals from the amount of herbage cut at ground level within 3 quadrats of 0.25 m^2/paddock. Herbage intake was estimated by the difference between the amount of herbage before and after the animals were allowed to graze on it. The samples were collected separately, weighed and dried at 60 °C for 48 h. Chemical analysis of food for protein concentration gave an average value of 104.8 g crude protein (CP) per kg DM for herbage and 98.9 and 129.5 g CP/kg DM for the barley and wheat bran respectively. The estimated metabolisable (ME) content [9] of herbage, barley and wheat bran was 9.27, 13.14 and 9.34 MJ ME/kg DM respectively.

Pasture contamination was determined from three herbage samples collected at fortnightly intervals from a fixed paddock by the method of Taylor [17]. Each grass sample consisted of approximately 200 g of grass collected by hand, following a W-shaped route across the paddock. The results were expressed as infective larvae per kilogram of dry matter (L_3/kg DM).

Worm egg count on faecal samples (epg) collected directly from the rectum were performed at fortnightly intervals using 3 g of faeces according to the McMaster method modified by Raynaud [14]. Blood samples were also taken fortnightly on every other week and serum pepsinogen estimates were obtained following the technique proposed by Kerboeuf [3], with the results being expressed as milliunits of tyrosine (mU Tyr). The kids were weighed at fortnightly intervals and immediately before being slaughtered. Individual growth rates (GR) were calculated from regressions of body weight upon time.

Sixteen weeks after turn out, 7 kids/treatment chosen at random were slaughtered. The shoulder dissection [2] into fat muscle and bone were used to estimate carcass composition. Necropsy and worm count procedures were carried out following the method described by the Ministry of Agriculture, Fisheries and Food [8] with minor modifications [22]. Briefly, the abomasums and small intestines were separated, opened and washed in warm water. Subsequent washings were bulked to 2 L for the abomasums and 3 L for the small intestine. The washings were not filtered. Two aliquots (10% each) were taken from the respective gut segment washings and worms were counted. Worms recovered were classified as adult male, female and immature larval stages. The first 50 adult males recovered were examined for species identification.

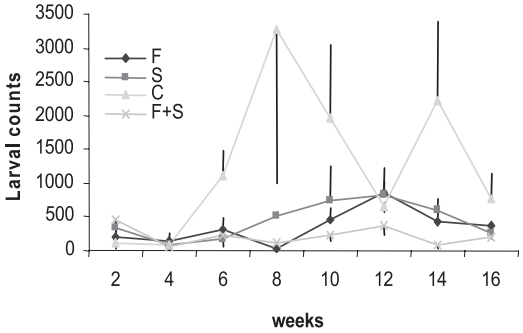

Figure 1. Pasture larval counts (L_3/kg DM) in paddocks grazed by goats fed the *D. flagrans* (F), barley supplement (S), both fungal and barley supplement (F+S) and control group (C). Bars show standard error of the mean. (A color version of this figure is available at www.edpsciences.org/vetres.)

2.4. Statistics

Repeated measures analysis of variance were performed using the general linear models procedure [16] to assess the significance of fungus (with and without), barley supplement (with and without) and their interactions (Fun × Sup) for variables such as L_3/kg DM, faecal egg counts and plasma pepsinogen. For the purpose of normalising the data, a logarithmic transformation was performed. The transformed data were used for statistical analysis; however, the results are reported as actual mean values. Variables not subjected to repeated measurements such as feed intake, growth rate, carcass composition and worm counts were analysed by analysis of variance. When these analyses indicated appropriateness, differences between particular comparisons were tested against the appropriate standard errors.

3. RESULTS

3.1. Pasture contamination

The analyses of pasture larval counts through repeated measurements showed a significant effect of both fungus ($P < 0.001$) and barley supplement ($P < 0.01$) in reducing pasture infectivity from week 4 onwards but no significant interaction between these two factors was detected. The time courses of pasture contamination showed that pasture larval counts start to diverge from the first grazing cycle showing the maximum differences by the end of the 2nd grazing cycle (Fig. 1). The average contamination of pasture grazed by C kids (1267 L_3/kg DM) was about 4 times higher than their fungi-treated equivalents (F) (350 L_3/kg DM). The mean number of L_3/kg DM recovered from the experimental areas grazed by barley supplemented kids (S) (438 L_3/kg DM) was between C and F values while pasture larval counts from F+S paddocks always showed the lowest values (216 L_3/kg DM).

3.2. Serum pepsinogen levels and faecal egg counts

While the level of pepsinogen in blood samples was not significantly affected by either fungus or barley treatments, it showed an abrupt increase in all groups of animals 2 weeks after the animals had been on pasture, reaching a mean value of around 900 mU Tyr and then increasing steadily from week 11 onwards.

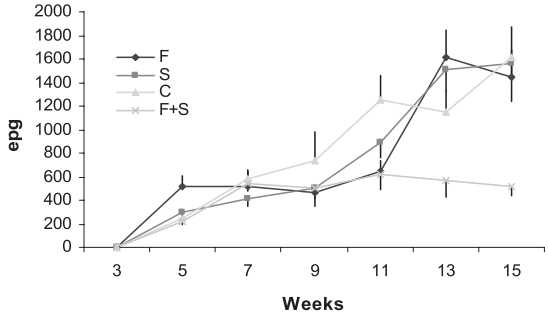

Figure 2. Faecal egg excretion (epg) of goats fed the *D. flagrans* (F), barley supplement (S), both fungal and barley supplement (F+S) and control group (C). Bars show standard error of the mean. (A color version of this figure is available at www.edpsciences.org/vetres.)

The effect of *D. flagrans* and barley supply on pasture contamination had consequences on faecal egg counts (Fig. 2) which appeared significantly reduced in both fungi-treated ($P < 0.01$) and barley supplemented kids ($P < 0.01$) from the 2nd grazing cycle onwards. Faecal egg counts (FEC) increased rapidly from a mean value of 300 epg by the end of the 2nd grazing cycle up to 1500 epg for F, S and C groups by the end of the trial while F+S goats showed a very constant mean FEC always remaining below 500 epg. An interaction ($P < 0.07$) was also detected between fungus and supplemented treatments showing that the effect of nematophagous fungi tended to be more noticeable when a barley supplement was offered to the animals.

3.3. Worm burden

T. circumcincta and *Trichostrongylus colubriformis* were the most frequent parasites found in the alimentary tract of the slaughtered kids, representing respectively 42.3% and 53% of the whole worm population in the control animals, whereas *Haemonchus contortus* and *Nematodirus* spp. represented less than 5% of the total worm counts (Tab. I). Total worm burden appeared significantly reduced both in fungi-treated ($P < 0.001$) and barley supplemented ($P < 0.01$) kids, which seem to be mainly due to the treatment effect on *T. colubriformis*. A similar tendency was observed on *T. circumcincta* although the treatment effects were not significant. While *Nematodirus* spp. counts were not significantly affected by any of the treatments, *H. contortus* appeared significantly reduced ($P < 0.05$) in barley-supplemented treatments. A significant interaction ($P < 0.05$) between fungus and nutrition treatment on worm burden was detected which showed that the effect of *D. flagrans* was more pronounced when a barley supplement was offered to the animals. The total worm count in the alimentary tract of the F+S group was reduced by 65% compared with the non-treated control group C, while the worm burden of the F group was reduced by 25%.

The number of immature stages was significantly lower in the small intestine (196; $P < 0.001$) and in the abomasums (563; $P < 0.07$) of the fungi-treated kids compared to the non-treated ones (1021 vs. 1043 respectively). However, no significant effect of the treatment on the proportion of immature to adult worms was found for any of the parasite species.

Table I. Effect of *D. flagrans* and barley supplement on worm counts recovered from goats after 16 weeks grazing. Logarithmic transformed mean values are given in parentheses.

Fungus	With		Without		RSD*	Effects		
Supplement	With	Without	With	Without		Fun	Sup	Fun × Sup
T. circumcincta	3215	4005	4142	4707				
	(3.465)	(3.563)	(3.600)	(3.641)	(0.1924)	NS	NS	NS
H. contortus	0	63	46	140				
	0	(1.524)	(0.832)	(0.707)	(0.8916)	NS	0.048	0.022
Abomasum	3215	4068	4188	4847				
	(3.465)	(3.570)	(3.604)	(3.650)	(0.1949)	NS	NS	NS
T. colubriformis	332	3940	4839	5907				
	(2.472)	(3.588)	(3.674)	(3.731)	(0.1975)	0.000	0.000	0.000
Nematodirus	327	287	285	374				
	(2.185)	(1.727)	(2.089)	(2.180)	(1.0540)	NS	NS	NS
Small intestine	660	4227	5124	6281				
	(2.797)	(3.614)	(3.657)	(3.754)	(0.1789)	0.000	0.000	0.000
Total burden	3875	8296	9312	11128				
	(3.557)	(3.899)	(3.940)	(4.012)	(0.1703)	0.000	0.003	0.047

* Residual standard deviation; NS: non significant.

Table II. Effect of *D. flagrans* (F), barley supplement (S), both fungal and barley supplement (F+S) and untreated control (C) on feed intake and goat performance.

Treatment	F+S	F	S	C	s.e.d.
Herbage intake (g/kg LW/d)	49.4[a]	47.9[a]	40.7[a]	33.8[a]	13.24
DM intake (g/kg LW/d)	55.4[a]	49.9[a]	44.8[a]	33.8[a]	13.25
ME intake (MJ/d)	12.9[a]	11.4[a]	10.4[a]	7.1[a]	2.98
ME/DM intake (MJ/kgDM/d)	9.67[a]	9.28[b]	9.73[a]	9.27[b]	0.194
GR (g/d)	34[a]	29[a]	30[a]	27[a]	9.85

Different superscript letters indicate $P < 0.001$ differences within a line.

3.4. Feed intake and kid performance

The herbage mass present on the paddocks before being grazed was reasonably constant throughout the trial showing an average herbage availability of 2521 ± 147.7 kg DM/ha.

Mean herbage intake ranged from a maximum of 49.4 g/kg BW/d for kids receiving (F+S) treatment to a minimum of 33.8 g/kg BW/d for kids in the control group that showed an average intake 20% lower than their barley-supplemented (S) equivalents. However, a comparison did not show significant differences in herbage intake among treatment groups (Tab. II). The estimated 770.1 g DM intake of herbage per day (group C) supplies adequate energy and provides a protein supply of 60.8 g MP that largely covered the animals' GR requirements.

The decrease in voluntary herbage intake shown by the control group (C) was more evident for both total DM and

Table III. Effect of *D. flagrans* (F), barley supplement (S), both fungal and barley supplement (F+S) and untreated control (C) on goat thoracic limb components (g).

Treatment	F+S	F	S	C	s.e.d.
Thoracic limb	1020[b]	977[ab]	1023[b]	901[a]	41.40
Bone	239[a]	236[a]	237[a]	225[a]	7.86
Muscle	662[b]	626[ab]	676[b]	582[a]	28.44
Subcutaneous fat	28[a]	27[a]	26[a]	23[a]	5.06
Intermuscular fat	54[b]	59[b]	57[b]	39[a]	8.14
Total fat	82[b]	86[b]	83[b]	62[a]	9.44

Different superscript letters indicate $P < 0.05$ differences within a line.

energy intakes, though differences between groups were not significant. While the total amount of barley offered did not show a significant effect on the total energy intake of young goats grazing high quality pastures, the estimated energy concentration of diet consumed by the barley supplemented groups was significantly higher ($P < 0.001$) than for their non supplemented equivalents.

No significant differences between treatment groups were found in kid growth rate (GR) that ranged from 34 (F+S) to 27 g/d (C) according to energy intakes (Tab. II). However, the analysis of thoracic limb dissection showed that the treatment had had a significant effect on thoracic limb weights and most of their tissue components. Thoracic limb joint, muscle, intermuscular and total fat stored by animals in the control group (C) were significantly lower ($P < 0.05$) than by the other supplemented treatments that did not show significant differences amongst them (Tab. III). However, the fungi-treated (F) kids showed a similar thoracic limb weight and composition than the barley supplemented groups.

4. DISCUSSION

4.1. Goat performance

No significant differences in herbage voluntary intake were found among the treatment groups suggesting that the amount of barley grain supply was not high enough to cause a substitution effect. Moreover, kids in treatment C that did not receive any kind of supplement consumed 20% less grass than those supplemented with barley grain (S). The evolution of pasture contamination from the 1st grazing cycle, as well as the worm counts after 16-week grazing, suggest that the level of infection of kids in treatment C could be responsible for this decline in herbage intake. Indeed, voluntary feed intake depression has generally been recognised as a major feature of the pathogenesis of GI infections and reductions of intake between 10 and 30% have been commonly found [13, 24].

The depression in voluntary feed intake shown by the control kids had consequences in the estimated ME intake though no significant differences were found among treatment groups, since they were not in the animal weight gain. However, the animal body composition appeared significantly affected by the treatment. The efficiency with which ME available for production is utilised for body gain and energy storage is highly dependent on the diet energy concentration [9]. The fact that barley supplemented diets showed a significantly higher energy concentration than the non-supplemented ones could also have affected their carcass characteristics, particularly with regards to the control group

that showed significantly lower ($P < 0.05$) thoracic limb joint, muscle, intermuscular and total fat mean weights. Moreover, the similar thoracic limb composition of fungi-treated (F) and barley supplemented goats suggests that *D. flagrans* supply might improve carcass characteristics since it reduced the level of infection and feed intake was maintained close to their potential. While no specific reports exist on this effect on goats, a number of works have been reported showing that infection noticeably affected body composition of other ruminant species [13]. Recently, while no significant differences were found in body weight gain between infected and their pair-fed uninfected controls, significant differences in the total quantities of thoracic limb joint, fat and muscle were found in growing lambs infected with *T. circumcincta* [22].

4.2. Effect of nematophagous fungi and energy supplement on parasite population dynamics

Daily dosing of grazing kids with the fungus *D. flagrans* had a significant effect ($P < 0.001$) on the development of free-living stages of GI nematodes on pasture, thereby reducing pasture infectivity. This reduction in pasture larval counts had consequences on faecal egg counts collected from the fungi-treated kids ($P < 0.01$) that was confirmed by significantly lower worm burdens ($P < 0.001$) at slaughter. Previous reports have shown that *D. flagrans* was effective in reducing the development of infective larvae in goat faeces [10, 19] and on pasture over a short period during the spring-early summer [27]. The results herein showed an effect of biocontrol on kids naturally infected with GI nematodes.

Surprisingly, energy supplement was also found to have a significant effect on the epidemiology of gastrointestinal nematodes. Since no significant differences were found on pasture larval counts in experimental areas, it is reasonable to assume that all animals received a similar level of infection at the beginning of the trial. However, from week 4 onwards pasture contamination was significantly lower in energy-supplemented treatments than in the non-supplemented equivalents. Interestingly, pasture larval counts from paddocks grazed by S kids were always below those shown by the control group (C) despite the fact that none of these two groups received any fungus supply that could explain the differences in pasture contamination. However, barley supplemented kids (S) showed significantly higher total fat depots than the control ones, suggesting that an immunological response associated to nutritional treatment could have taken place, thus affecting pasture contamination. Recent studies on the role of fat reserves on the immune response to GI infection in sheep [23] have shown that fat mass stored by ewes was related to the response of immunity to GI parasites and this commenced at an early stage of infection affecting the establishment and development of incoming larvae, thereby reducing faecal egg excretion and affecting pasture infectivity. In this respect, it is noteworthy that Australian studies on the efficiency of *D. flagrans* with naturally infected sheep [4] observed considerable differences both in BW gain as in FEC between replicate groups resulting from variations in consumption of barley supplement and concluded that the "best" consumers showed the greater effects of treatment.

However, the differential response on FEC and worm counts between fungi-treated groups, that showed a similar level of fat reserves at slaughter, appears to be in line with the fact that the nutritional effects of controlling the number of worms established was found to be more effective at lower than at higher levels of infection [22]. The interaction detected

between fungus and barley supplement in epg ($P < 0.07$) and total worm burden ($P < 0.02$) showed that the combination of both treatments had a greater effect as a method of parasite control than when applied separately.

Previous results have demonstrated that the nematode destroying effects of fungi on Trichostrongylidae parasites in faecal cultures [1, 11, 19] may be reflected in reduced pasture contamination under field grazing conditions [27]. However, the results herein showed that GI nematode species were affected differently by treatment. While *T. colubriformis* populations were significantly reduced by treatment, *T. circumcincta* populations were not significantly affected either by *D. flagrans* or by barley supplement though it followed the same tendency. On the contrary, Wright et al. [27] found a significant reduction of *T. circumcincta* worms recovered from tracer kids grazing a pasture previously grazed by Saanen goats fed with *D. flagrans* in the early spring but no effect was found on *T. colubriformis*. Previous studies carried out on sheep in our conditions [21] showed that *T. circumcincta* and *T. colubriformis* were the parasites most frequently found though the latter species appeared later in the season. The fact that treatments were applied after the animals have spent 2 weeks on pasture in the early spring together with the similar serum pepsinogen time courses suggest that the similar level of infection reached in all groups at the beginning of the trial was caused predominantly by the overwintering of the *T. circumcincta* larvae population. Thus, further treatment effect on *T. circumcincta* worm burden could have been masked by the already established larvae population. However, the methods of parasite control tested showed a greater effect on the subsequent *T. colubriformis* and *H. contortus* emerging populations that also appeared to be the most efficiently trapped by the fungus in goat faecal cultures [12]. These results suggest that treatment efficacy is highly related to the opportunity with which treatments are applied in relation to the evolution curve of the predominant species. The results herein show that *D. flagrans* may act as an efficient biological control agent on goat GI nematode infections on pasture that could further improve carcass characteristics. While small amounts of energy supplement can also reduce goat infection, the effect of *D. flagrans* as a biological control agent appeared clearly enhanced both in magnitude and duration by energy supplementation. This has clear implications for grazing animals and provides an efficient method for the control of parasites in biological production systems.

ACKNOWLEDGEMENTS

The authors wish to thank Christian Hansen's BioSystems A/S (Denmark) for providing *D. flagrans* chlamydospores and to Mrs. A. Guillén and Mr. J.A. Tanco for their technical assistance. This work has been partly supported by DGA Grupo consolidado A-11 and by the European Community through the QLK5-CT 2001-1843 project.

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