

Investigation on the influence of nematophagous fungi as feed additive on nematode infection risk of sheep and goats on pasture

Christian Epe*, Christine Holst*, Regine Koopmann**, Thomas Schnieder*, Michael Larsen*** and Georg von Samson-Himmelstjerna*

Abstract

Gastrointestinal nematodes in small ruminants cause high economic losses. Thus on most farms anthelmintic treatment is required. In response to increasing problems with anthelmintic resistance, biological control, for example the use of nematophagous fungi, has received significant attention. The aim of this study was to investigate the effect of *Duddingtonia flagrans* orally applied to small ruminants on natural infection with gastrointestinal nematodes in a field study in Northern Germany.

20 goats and 20 sheep were fed daily for 3 months with 5×10^5 spores of *D. flagrans* per kg bodyweight. Differences in body weight, faecal egg count and larval development in faeces and on pasture in comparison with same-sized control groups were analysed. After 3 months the control goats showed significantly higher mean faecal egg count than the fungus-fed group. No significant difference was found between the two sheep groups. The maximum in larval reduction in faeces was 81.3 % in the sheep groups and 67.9 % in the goat groups (not significant). At the end of the study the body weight gain in the fungus-treated groups was 1.7 kg higher in goats and 0.7 kg higher in sheep than in the control groups (not significant). Regarding the first-year-grazing goats only, the bodyweights revealed significant differences ($p < 0.05$). No statistically significant differences were observed in pasture larval counts.

In the study presented here, no clear effect of fungus could be observed. A modified feeding regimen, perhaps with permanent release boluses or feed blocks, may improve the efficacy. Furthermore, it seems that climatic conditions during the study period could have influenced the results and displayed how sensitive the fungus application may be on such parameters.

Keywords: biological control, *Duddingtonia flagrans*, sheep, goat, gastrointestinal nematodes

Zusammenfassung

Gastrointestinale Nematoden bei kleinen Wiederkäuern verursachen hohe wirtschaftliche Verluste, sodass eine Entwurmung auf den meisten Betrieben notwendig wird. Die zunehmende Verbreitung von Anthelminthikaresistenz lässt eine biologische Bekämpfung, wie z. B. mit nematophagen Mikropilzen, interessant werden. Dieser Feldversuch an kleinen Wiederkäuern in Norddeutschland befasste sich mit dem Einfluss der Zufütterung von *Duddingtonia flagrans* Sporen auf Infektionen mit Magen-Darm-Nematoden.

20 Ziegen und 20 Schafe wurden 3 Monate lang täglich mit 5×10^5 Sporen von *D. flagrans* pro kg Körpergewicht zugefüttert. Die Unterschiede zu den gleichgroßen Kontrollgruppen bzgl. Körpergewicht, Eizahl pro Gramm Kot und Larven in Kot- und Weidegrasproben wurden ermittelt. Nach 3 Monaten hatten die Ziegen der Kontrollgruppe signifikant höhere mittlere Eiausscheidungszahlen als die der Versuchsgruppe. Bei den Schafen gab es keine signifikanten Unterschiede. Maximal wurde die Larvenanzahl um 81,3 % bei den Schafen und um 67,9 % bei den Ziegen reduziert (nicht signifikant). Zum Ende war der Körpergewichtszuwachs bei den Versuchsziegen 1,7 kg und bei den Versuchsschafen 0,7 kg höher als in den Kontrollgruppen (nicht signifikant). Nur die Untergruppe der erstsömmerigen Ziegen erreichte signifikante Körpergewichtsunterschiede ($p < 0,05$). Keine signifikanten Unterschiede ergaben sich bei den Weidegraslarven. Witterungsbedingungen könnten die Resultate beeinflusst haben und damit gezeigt haben, wie empfindlich diese Methode gegen solche Parameter zu sein scheint.

Die vorliegende Studie zeigte keinen eindeutigen Effekt einer Zufütterung mit *D. flagrans*. Ein modifiziertes Fütterungsregime, z. B. mit Boli oder Lecksteinen, könnte die Wirksamkeit verbessern.

Schlüsselworte: biologische Kontrolle, *Duddingtonia flagrans*, Schafe, Ziegen, gastrointestinale Nematoden, Magen-Darm-Strongyliden

* Institute for Parasitology, Department of Infectious Diseases, University of Veterinary Medicine Hannover, Buenteweg 17, D-30559 Hannover, Germany, Email: Christian.Epe@tiho-hannover.de

** Institute of Organic Farming of Johann Heinrich von Thünen-Institut, Federal Research Institute for Rural Areas, Forestry and Fisheries, Trenthorst 32, D-23847, Westerau, Germany

*** Sanos Bioscience A/S, Gleeerupvej 2, DK-2610 Roedovre, Denmark

Introduction

Parasitism due to gastrointestinal (GI) nematodes is a major constraint to production of sheep and goats worldwide. The principal nematode species infecting both goats and sheep is *Haemonchus contortus*, a voracious blood-feeder that can cause reduced production at subclinical infection levels, and even death with severe infections (Rowe et al., 1988). The conventional method of gastrointestinal nematode control used by farmers is frequent administration of anthelmintics. Numerous cases of anthelmintic resistance have been documented in gastrointestinal nematodes of sheep and goats worldwide, in some areas already reaching alarming levels (Maingi et al., 1996; Borgsteede et al., 1997; Zajac and Gipson, 2000; Terrill et al., 2001; Mortensen et al., 2003; Schnyder et al., 2005). Alternative wormcontrol may be the challenge in the future.

Several species of microfungi are able to trap and kill the developing larval stages of parasitic nematodes in a faecal environment, but only one species, *Duddingtonia flagrans*, has been demonstrated to have a high degree of survival through the gastrointestinal tract (GIT) of ruminant animals (Larsen et al., 1992). After passing through the GIT, spores of this fungus germinate in faeces, forming specialized, three-dimensional networks that trap the parasite larvae (Larsen et al., 1997).

A good larval motility, sufficient humidity and temperature support good growth and trapping-efficacy in vitro and faecal pad studies on pasture proved the outdoor-efficiency (Larsen et al., 1994; Grønvold et al., 1999; Fernandez et al., 1999c; Fernandez et al., 1999d; Faedo et al., 2002; Paraud and Chartier, 2003; Waghorn et al., 2003).

Research on *D. flagrans* with cattle, (Grønvold et al., 1993; Fernández et al., 1999b; Sarkunas et al., 2000), horses (Larsen et al., 1996; Fernández et al., 1997; Baudena et al., 2000), pigs (Nansen et al., 1996), sheep (Githigia et al., 1997; Faedo et al., 1998; Peña et al., 2002; Chandrawathani et al., 2002) and goats (Chartier and Pors, 2003) has demonstrated the potential of this organism as a biological control agent against the free-living stages of parasitic nematodes in livestock, under both experimental and natural conditions. The number of recovered pasture larvae was reduced while spores were fed.

Paraud and Chartier (2003) showed that *D. flagrans* is able to significantly reduce the number of *Teladorsagia circumcincta*, but not *Muellerius capillaris* larvae, in faecal cultures of goats given 5×10^5 chlamyospores per kilogram body weight daily. All of this work has shown that biological control of parasitic nematodes in goats might be possible by daily dosing animals with spores of *D. flagrans*. However, the question of the optimal dosing level and dosing interval has not been adequately addressed so far.

Here, under a typical extensive pasture situation in North-

ern Germany, we investigated the efficacy of a 3-month feeding period with daily 5×10^5 spores of *D. flagrans* in sheep and goats.

Materials and Methods

Animals

Forty female goats of the breed „ German Improved Fawn “, 14 second year grazing animals of 18 months age and 26 first year grazing animals of 6 months age, all naturally infected with GI nematodes, were used for the study. All animals were not kept on the study pasture before the study started and with turnout they were split into control and treatment groups equally (13 first and 7 second year grazers for each group, respectively).

In a second grouping forty female sheep of the breed „East Friesian Milk sheep, var. black“ (n=37) and „Merino“ (n=3) were used. 26 second year grazing animals with approx. 18 months of age and 14 first-year grazing animals of 6 months of age, also naturally infected with GI nematodes, were distributed to the groups equally (13 second and 7 first year grazers for each group, respectively). These animals, too, were not kept on the study pasture before the study period started.

Experimental design

Between May and October in 2002, 40 goats and 40 sheep were kept on pastures of the Institute of Organic Farming in Trenthorst, Germany. For each species animals were allocated to control and treatment groups according to body weight (BW). The treatment group of each animal species received spores of *D. flagrans* at a dose rate of 5×10^5 spores/kg BW/day mixed with approximately 100 g of feed every morning from the day of turnout (May 07) for 3 months (until July 31). Chr. Hansen Ltd., Hørsholm, Denmark, provided the fungal spore product. The animals were observed to ensure that they consumed the fungus-feed mixture and then were given the remainder of their daily feed ration. The control group animals received placebo feeding only, in the same amount of feeding (100 g of oat-barley-grist per animal and day). In a 14-day interval the animals were weighted and coproscopically examined for the presence of helminth stages. Gastrointestinal larval pasture contamination was monitored by testing 2 grass samples per pasture in 2 week intervals. Four blood samples were collected from all animals for Packed Cell Volume (PCV) and pepsinogen examination. In the middle and at the end of the grazing season 2 tracer animals per pasture were implemented and after 3 weeks of grazing and another 3 weeks of in-house feeding necropsied for identification and differentiation of helminths. The trial ended

after 5 month grazing season end of October 2002.

Experimental procedures

Faecal egg counts (FEC) were performed on all animals using a modified McMaster technique with a sensitivity of 33 eggs per gram (Schmidt, 1971). Faeces were collected from each animal every 14 days throughout the pasture season to determine the number of eggs per gram (epg) and to establish faecal cultures for larval recoveries.

Faecal cultures were performed qualitatively according to the method of Roberts and O'Sullivan (1950) with pooled samples of 3-5 animals. Larvae were identified microscopically according to the key of Buerger and Stoye (1968) after reisolation with the Baermann technique (Wetzel, 1930) and adding lugol solution. Additionally, a quantitative culture was performed of individual animal samples according to Henriksen and Korsholm (1983) with 4 g of faeces. Infective third stage larvae (L3) were recovered by the Baermann technique and then counted and identified to the genus level. Numbers of larvae were expressed as L3 per gram (LPG) of cultured faeces. For each sampling date the reduction of larvae in samples of fungus-fed compared with control animals samples were calculated according to the formula used by Fernandez et al. (1999a) and Pena et al. (2002).

Clinical examinations were done at each visit and sampling date. Only non physiological observations have been documented.

On four selected dates (at turnout, and on days 56, 112 and 168 after turnout) blood samples were taken for the examination of serum pepsinogen according to (Berghren et al., 1987) and PCV using the micro method.

Grass samples were collected and examined according to the method of Sievers Prekehr (1973) and recorded as number of larvae per 100 g dry matter of grass as pasture mean, indicating the contamination of the pasture with L3 stages of GI nematodes.

Two previously worm-free (6–9 months) tracer lambs were allocated to each group at mid and towards end of the grazing season. These animals were of the same breed as the experimental sheep (milk sheep) and goats (German Improved Fawn). After a 3 week grazing period the tracer lambs were housed for 3 weeks prior to slaughter to allow the development of adult stages prior to necropsy. A total of 16 tracer animals were used during the course of this study. Parasite identification was performed according to the keys of Barth and Visser (1991).

Lungs were examined for adult lungworm stages using the perfusion method of Inderbitzin (1976).

Statistical analyses

Data were analysed using t-Test and Mann Whitney Rank Sum Test (SigmaStat 2.0 and SigmaPlot, Jandel Scientific).

Results

Body weight

At turnout the differences of mean body weight between the fungus-fed group and the control group was 0.1 kg in sheep and zero in goats. The development of body weight is presented in Table 1. At no time statistically significant differences between groups ($p > 0.05$) were observed. However, for the subpopulation of first-year grazing goats, which followed the global development of the complete group as described above, single time points with statistically significant differences ($p < 0.05$) in body weight could be observed, whereas the first-year grazing sheep did not show significant differences (Figure 1a and 1b).

Table1:
Body weight development

Group n = 20	Day of study			
	0	56	112	182
Control Sheep				
Mean (kg)	37.3	43.8	48.0	55.7
SD (kg)	12.8	11.7	11.7	11.6
Treatment Sheep				
Mean (kg)	37.4	43.8	47.2	56.4
SD (kg)	13.3	13.0	11.7	11.5
Control Goats				
Mean (kg)	26.0	29.4	29.9	37.9
SD (kg)	10.7	10.4	11.1	10.4
Treatment Goats				
Mean (kg)	25.9	29.4	31.8	39.6
SD (kg)	11.2	10.8	9.7	9.2

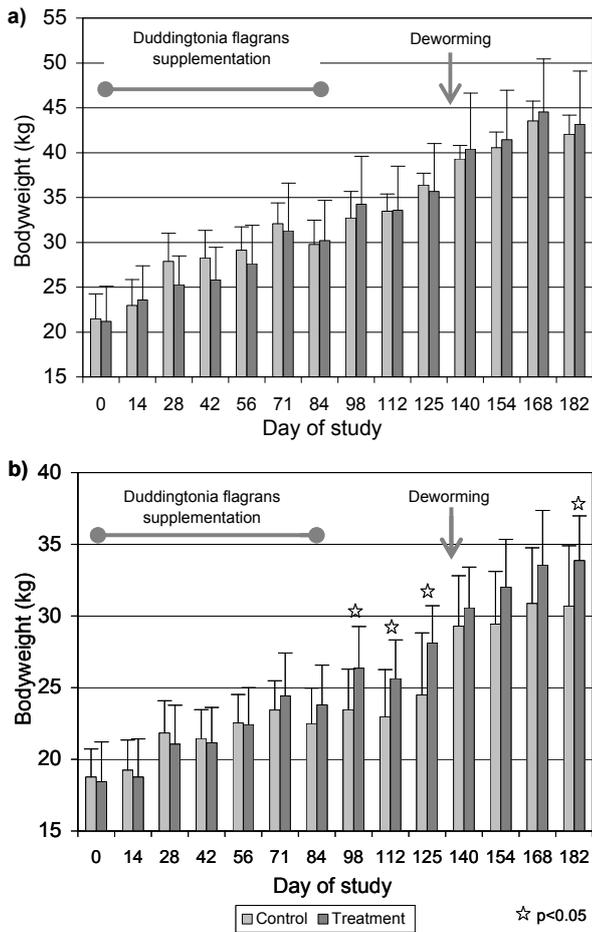


Figure 1: Mean body weights of the subpopulation of first-year grazing sheep (1a) (n=7) and goats (1b) (n=13). Asterisks indicate statistically significant differences (t-test, $p < 0,05$) between control and fungus-fed group. Error bars represent Standard Deviation.

Clinical examinations

Clinical symptoms as diarrhoea or pharyngeal oedema were observed mainly in the control group animals: 10 control goats vs. 3 fungus-fed goats and 6 control sheep vs. 4 fungus-fed sheep. Due to the course of clinical disease all animals had to be treated anthelmintically in September on study day 134.

GI Nematodes Egg Counts

Figure 2 shows the course of mean GI nematode egg counts throughout the pasture season for sheep (Figure 2a) and goats (Figure 2b). With the first increase of egg counts in sheep for the fungus-fed group and for the control group at day 28 after turnout the sheep groups did not show statistically significant differences between the epg courses throughout the study. The goat groups showed a similar pattern. After the end of the fungus feeding period

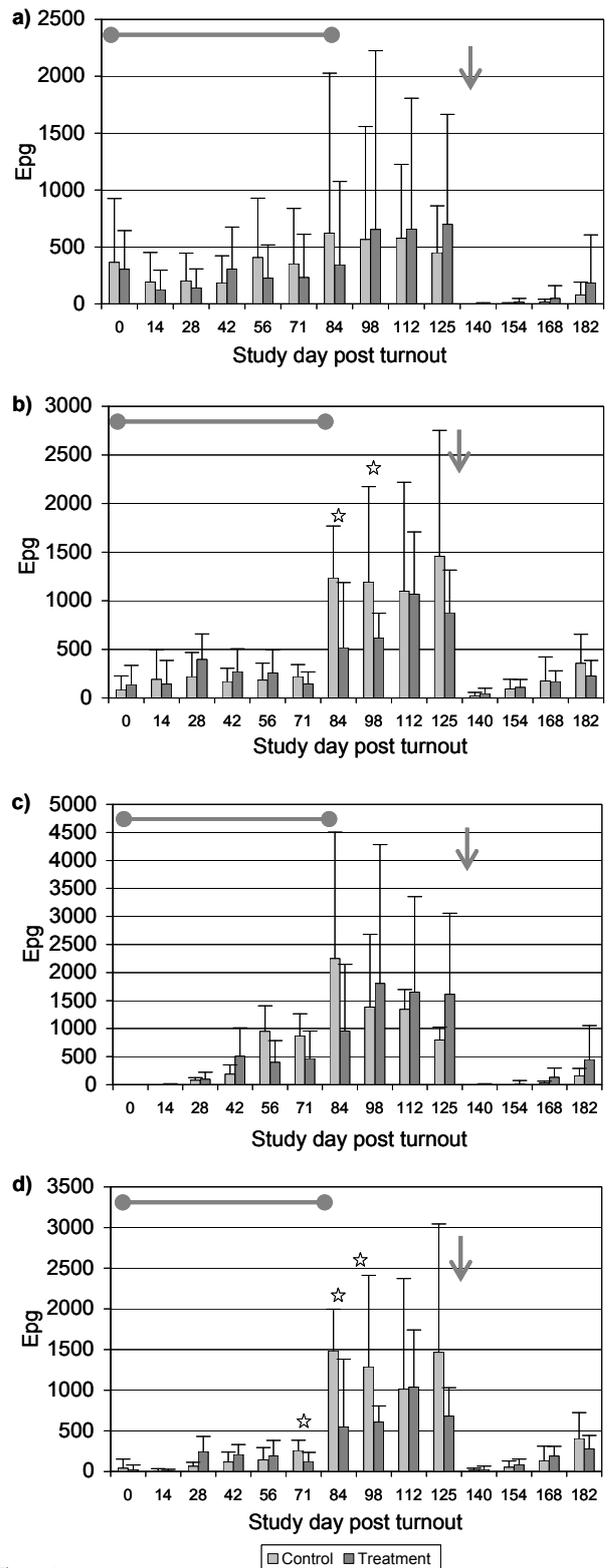


Figure 2: Mean gastrointestinal nematode egg counts/g faeces of sheep (2a) and goats (2b) and first year grazing sheep (2c) and goats (2d), respectively. Anthelmintic treatment (arrow) was performed on study day 134 due to clinical diseases. The *Duddingtonia flagrans* feeding period is marked by a horizontal bar. Asterisks indicate statistically significant differences (Mann-Whitney, $p < 0,05$)

the egg values peaked for both groups. Statistically significant differences could only be observed between the goat groups for two examination dates ($p < 0.05$) on day 84 and 98 after turnout, respectively (Figure 2b) and for 3 sampling dates in the first-year grazer goat group (Figure 2d). Even in the treatment groups, the seasonal raise of FEC could not be prevented. A curative anthelmintic treatment became necessary in September after 134 days on pasture (arrow) due to severe clinical symptoms and to avoid further damage to the flock. Accordingly, a subsequent decrease was observed in all groups with again an increased shedding of eggs in the last weeks on pasture.

No lungworm stages were detected throughout the whole study period.

Faecal cultures - quantitative

The quantitative larval cultures were performed 42 and 71 days after turnout and than bi-weekly until the end of the study (Figure 3).

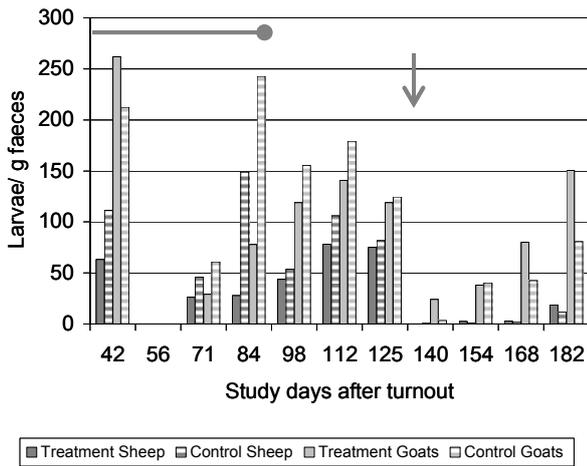


Figure 3: Mean larval counts in g/faeces of all groups. The *Duddingtonia flagrans* feeding period is marked by a horizontal bar

On day 42 the sheep control group mean larval counts were 110 (± 50) compared with 63 (± 17) for the fungus-fed group. Six weeks later the difference between these groups increased with 149 (± 193) and 28 (± 4) mean larvae counts in the control and fungus-fed group, respectively, leading to a 81.4 % reduction in the latter group. In the course of the study the mean values of both groups converged. In the fungus-fed goat group the larval cultures showed a 23.6 % higher larval count at the first sampling date compared with the control group. However, at the end of the feeding period (study day 84) a reduction of 67.9 % (243 L3 (± 138) vs. 78 (± 34)/g faeces) compared with the control group was observed.

This difference decreased during the following sampling dates. All differences in larval counts were not statistically significant.

Faecal cultures - qualitative

All groups showed the presence of the following GI nematode genera: *Strongyloides*, *Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia* and *Oesophagostomum* in sheep (Figure 4a) and goats (Figure 4b). A maximum of 10 % of the larvae could not be identified.

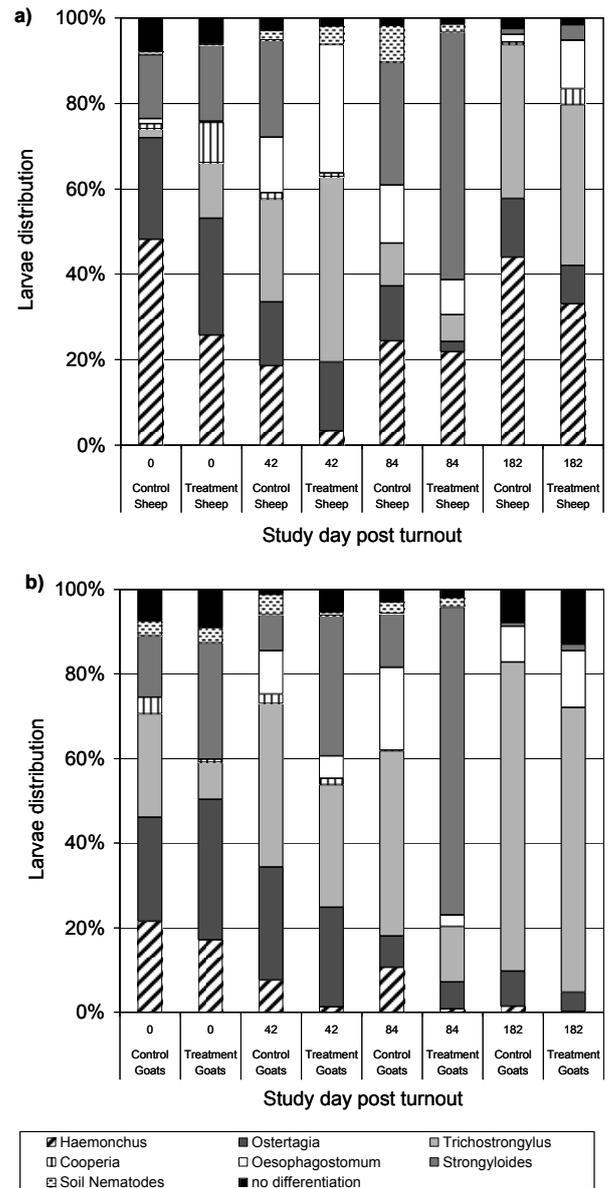


Figure 4: Relative nematode species composition found in faecal larval cultures. Results in sheep (4a) and goats (4b)

The sheep groups showed a decreasing percentage of *Haemonchus* spp. during the grazing season (47.5 % to 18.5 %) as well as for *Ostertagia* and *Trichostrongylus* in the control group. No statistically significant differences between control and treated groups were observed. The goat groups showed *Haemonchus*, *Trichostrongylus* and *Ostertagia* as dominating genus in the control group at turnout and additionally *Strongyloides* in the treated group. Towards the end of study the dominating genera were in both groups *Trichostrongylus*, *Oesophagostomum* and *Ostertagia* but with no statistically significant differences.

Grass samples

The sheep groups showed insignificant differences of nematode larval numbers in dry mass of grass (DM). On day 98 after turnout on average 9 infective larvae more were found in pasture-grass samples from the control group. But later on day 154 after turnout the treated group had 370 L₃/100g DM and control group only 135 L₃/100g DM. The goat groups showed similar larval counts per DM with an increasing difference between the groups with higher counts in the control group from day 70 on pasture onwards up to day 154 (77 Larvae in the treated group and 243 Larvae in the control group). But no statistical significances were detected throughout the study (Figure 5).

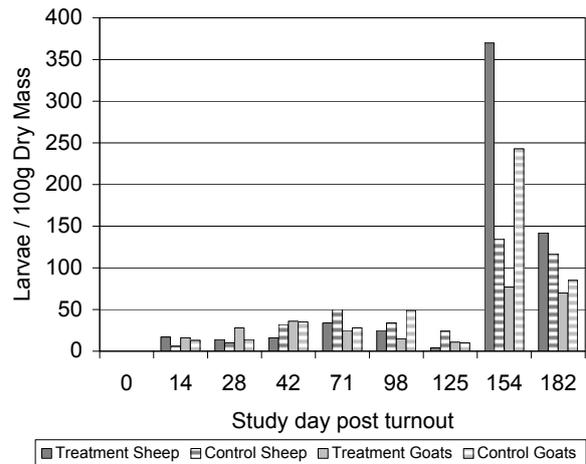


Figure 5: Quantitative larval cultures from Grass Samples: Results in sheep and goats

PCV

Most PCV values remained in the physiological range of 28-40 % for sheep and goats during the course of the study, with no significant differences between the groups (data not shown).

Serum pepsinogen

Fungus-fed goats showed lower pepsinogen levels, sheep revealed no differences. Most results are not significant (data not shown).

Table 2: Total worm burden and distribution of species in Tracer animals I. turnout at study day 86

Animal ID	Epg on day of nekropsy	Total worm burden	Percentage genus in %						
			<i>H. contortus</i>	<i>Tel. circum.</i>	<i>Tr. axei</i>	<i>Str. papill.</i>	<i>N. battus</i>	<i>Oes. venul.</i>	<i>Tr. ovis</i>
Treatment Sheep 01	2700	813	19.93	52.40	17.22	8.36	2.09	0.00	0.00
Treatment Sheep 02	3567	1083	4.89	23.92	9.79	60.39	1.02	0.00	0.00
Control Sheep 03	10933	1726	17.56	43.16	25.72	10.43	1.74	1.33	0.06
Control Sheep 04	13333	618	29.94	9.87	41.10	15.53	1.62	1.94	0.00
Treatment Goat 39	500	876	1.03	55.94	9.59	30.94	2.40	0.00	0.11
Treatment Goat 40	400	811	0.99	68.19	7.89	21.33	1.36	0.12	0.12
Control Goat 37	2533	870	4.14	49.66	27.59	12.99	3.22	2.41	0.00
Control Goat 38	1600	770	7.14	51.82	18.96	17.92	2.99	1.17	0.00

Tracer

Prior to turnout all tracers used were diagnosed as parasite-naïve according to faecal sample examination. The first set of tracers was turned out to pasture at day 86 at the end of the feeding period and after 3 week pasture period they turned in. The last examination showed clear differences between the sheep fungus-fed and the control faecal egg counts of 3133 and 12133 epg, respectively. Similarly, also in the samples of the goat tracer animals, kept on the fungus-fed group pasture, a mean of 450 epg was observed while the control tracers showed a mean epg of 2067. Table 2 shows the parasitological findings of the tracer pairs in the first set demonstrating the presence of the following species: *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus axei*, *Nematodirus battus*, *Strongyloides papillosus*, *Oesophagostomum venulosum* and *Trichuris ovis*. The total mean worm burden of the tracer animals were 1172 and 948 in sheep, and 820 and 844 in goats, control and treatment groups, respectively (Table 2).

The second set of tracers was turned out to pasture on day 140, stayed on pasture for 3 weeks and was necropsied after a 3 week indoor period. These animals became positive 2-4 weeks after turnout confirming the uptake of nematodes. The expected differences of epg's between the tracers of the fungus-fed group and the control group of both species were not observed. The epg's, the necropsy results and the total worm burdens are shown in Table 3 revealing the following species: *Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus axei*, *Nemato-*

dirus battus, *Strongyloides papillosus* and *Oesophagostomum venulosum*.

Climatic Conditions

The nearest German Weather Service Station (Lübeck) provided the weather data for the trial site. Monthly mean rainfall is shown in Figure 6, compared to a 30years-period mean (1961-1991). Particularly the rainfall in July and August (between days 70 and 112 after turnout) exceeded the long-term mean remarkably (Figure 6).

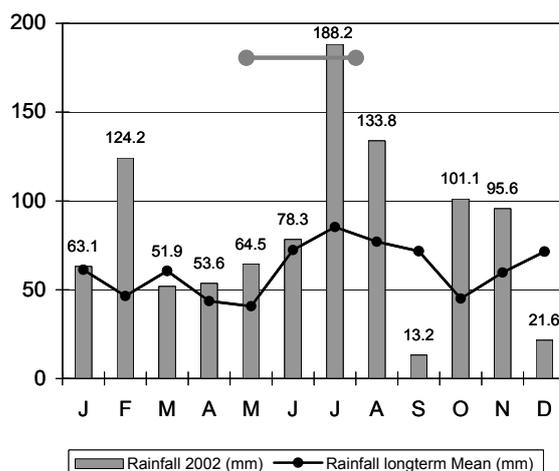


Figure 6: Monthly Rainfall Documentation of the study pastures in 2002. Data from German Weather Service (DWD), Station Airport Lübeck-Blankensee, 13,5 km distance to the study location. The horizontal bar demonstrates the feeding period

Table 3: Total worm burden and distribution of species in Tracer animals II. turnout at study day 140

Animal ID	Epg on day of necropsy	Total worm burden	Percentage genus in %					
			<i>H. contortus</i>	<i>Tel. circumcinc.</i>	<i>Tr. axei</i>	<i>Str. papill.</i>	<i>N. battus</i>	<i>Oes. venul.</i>
Treatment Sheep 05	3333	893	3.58	2.91	79.51	0.11	12.99	0.90
Treatment Sheep 21	1267	1788	0.17	1.06	86.07	1.90	10.79	0.00
Control Sheep 20	933	2013	0.05	0.00	94.59	1.14	4.22	0.00
Control Sheep 07	1700	3008	0.70	0.40	85.37	0.70	12.73	0.10
Treatment Goat 92	2333	2552	0.04	1.80	68.14	25.82	4.11	0.08
Treatment Goat 94	4900	3372	0.09	3.05	87.25	5.52	3.26	0.83
Control Goat 91	867	1613	0.06	4.84	77.31	11.22	6.45	0.12
Control Goat 93	1467	2509	0.04	3.43	66.96	23.48	5.06	1.04

Discussion

Helminth infection of small ruminants are of great importance worldwide, causing economical losses by direct and indirect effects such as reduced appetite, maldigestion and wool lesions (Hiepe and Zimmermann, 1966; Steel et al., 1982; McLeod, 1995). Therefore, an anthelmintic treatment with chemotherapeutics, often as strategic treatment, is necessary and justified. Reports of anthelmintic resistance in the last decades, particularly of trichostrongyle populations, against one or more classes of compounds are increasing in number (van Wyk et al., 1999; Bartley et al., 2004; Kaplan et al., 2004). In parallel, alternative control approaches have been developed, especially aiming at sustainable and ecological, so-called organic production systems to supplement the chemoprophylaxis (Larsen, 2000). Nematophagous fungi offer abilities to reduce pasture contamination and therefore the infection pressure of pasture animals. This study aimed to describe the effect of *D. flagrans* on helminth infection of small ruminants during a pasture season in an organic production system.

The fungus dose of 5×10^5 spores/kg BW/day used in this study was described to be effective using in vitro and in vivo trials in small ruminants (Faedo et al., 1998; Knox et al., 2001; Paraud and Chartier, 2003; Peña et al., 2002; Sanyal, 2001). However, no report was available on the titration of fungus concentration and duration of feeding prior to the present study. Due to the limited number of animals available on the research farm, the previously published dosage regime was applied here (Waghorn et al., 2003; Fontenot et al., 2003; Paraud et al., 2004; Paraud et al., 2005). Previously, in other studies, e.g. 1.000.000 spores/kg BW in a study with lambs for 3-5 months (Githigia et al., 1997) were used, or more recently with unsatisfying results 250,000 spores /kg BW (Chartier and Pors, 2003).

Generally, the course of GI nematode egg counts of sheep and goats observed in the present investigation reflects the expected epidemiological situation in Central Europe. Trichostrongyles were shown to be present on the pastures used for the study. Typically, first year grazers are infected by stages shed from older animals in previous season or in course of the same season (Gibbs, 1979; Armour, 1980). Towards the end of the fungus feeding the control group animals shed slightly more eggs than the fungus-fed group animals, but being statistically significant only between the two goat groups. Following the end of the feeding period both groups showed similar faecal egg counts. Clinical symptoms like diarrhoea or pharyngeal oedema were observed mainly in the control group animals. However, due to the course of clinical disease all animals had to be treated anthelmintically in September, which dimin-

ished the differences between egg courses of the respective groups even more, also resulting in a decrease of the mean egg values down to 400 in goats and 200 in sheep for both groups, respectively.

It is known that *D. flagrans* does not have any influence on egg output (Fernandez et al., 1999a; Larsen et al., 1995; Larsen et al., 1996; Paraud and Chartier, 2003; Sanyal, 2001) since nematode stages already present at the begin of the fungus application are not affected by the fungus (Dimander et al., 2003; Githigia et al., 1997; Sarkunas et al., 2000). The fungus only starts having an effect on the larvae when it was shed with the faeces, leading to reduced pasture contamination following close co-development with the motile nematode larvae (Nansen et al., 1988; Grønvold et al., 1996a and b; Faedo et al., 2002). In the present study this was only partly and indirectly observed by the goat group egg courses (Figure 2b) and the effect was transient. Possibly, a longer feeding period may lead to a stronger reduction of new pasture contamination with nematode stages (Dimander et al., 2003, Knox et al., 2001).

The larval counts per g faeces were seen as primary parameter to evaluate the fungus effect, since larvae are trapped by the fungus' chlamydo spores. The larval counts in the quantitative coproculture results reflect the course of the egg values in coproscopical examination of the animals. Therefore, for the first weeks of the study period the number of larvae of the sheep control group was slightly higher than that of the fungus-fed group (Figure 3). Until the end of the feeding period both host species showed higher mean larvae in coproculture larval counts in control than in fungus-fed group, but this difference was diminished within 6 weeks after the end of the feeding period showing no long-term effect of the fungus feeding.

The calculated reduction in the quantitative larval culture results of the fungus-fed groups at the end of feeding period were 81.4 % for sheep and 67.9 % for goats which is in accordance with other observations of 82-99 % (Peña et al., 2002).

The qualitative faecal larval cultures revealed different genera (Figure 4), which are commonly seen in Central Europe as reported by various authors (e.g. Benesch, 1993; Rehbein et al., 1996), but no differences between the groups.

The sheep groups did not show any fungus effect in the field, since the larval counts on pastures at which the fungus-fed animals were kept were even higher at the end of the grazing season than those of the control animal pastures. The goats showed a lower number of larvae in the fungus-group pasture at that time (Figure 5).

At the beginning of the study period the larval counts from grass sample washings revealed low levels of larvae on all pastures. The counts increased in September, showing

largest differences between groups in October (Figure 5). It was shown previously, that the numbers of larvae depend on the stocking rate of animals (Armour, 1980; Thamsborg et al., 1996), and since the average liveweight/ha in this study was rather low with 625 kg/ha in sheep and 415 kg/ha in goats, respectively, a slower development of the nematode population on pasture is considered to have occurred. This could have contributed to an insufficient fungus trapping structure stimulus, which was found to be correlated positively with a high abundance of nematode larvae. But also climatic conditions influence the larval development, like sufficiently warm temperatures and humidity both stimulating development (Armour, 1980; Eckert and Buerger, 1979; Eysker et al., 1997; Goldenstein, 1978). In the study period the spring showed warm but dry weather. In contrast, the summer had more than average rainfall with flooding of pasture, which definitely will have had negative influence on the larval concentration within the faeces and the dung pad structure.

The larval counts of sheep are in contrast to several other reports of experimental contamination trials as well as of field trials which all described a clear reduction of larvae on pasture at which fungus-fed animals were kept (Fernandez et al., 1997; Faedo et al., 1998; Wolstrup et al., 1994; Nansen et al., 1995; Sarkunas et al., 2000). However, Dimander et al. (2003) could not find effects during the first year of grazing. They only found effects in the second grazing period during a long-term study. Finally, the use of different larval culture and counting methods in previously published studies makes the exact comparison of the present results with those of other investigations difficult.

A difference in the body weight development due to the benefits resulting from a *D. flagrans* effect on the nematode pasture burden, as reported previously (Dimander et al., 2003; Knox et al., 2001; Fernandez et al., 1997; Larsen et al., 1995), was not found in the present study apart from the sub-group of the first-year-grazing goats.

Tracer animals were used for the identification and differentiation of the nematode genera present in the flock. Due to the limited number of tracer animals, no statistical evaluation could be performed. First tracers in August showed higher epg values in control group animals than in fungus-fed group animals, the overall worm counts, however, did not show clear differences (Table 2). The second set of tracer animals showed a different picture with higher epg values in fungus-fed group animals than in control group animals (Table 3). Thus the results in tracer's wormburden support the assumption that the 3 month's feeding period was too short to have a long standing effect. The nematode species detected are in accordance with the reported spectrum in small ruminants in Germany (Benesch, 1993; Rehbein et al., 1996). These results reflect again the situation observed for

the faecal larval counts in the trial groups. Similarly, in a comparable sheep field trial Knox et al. (2001) also did not see differences in tracer animal worm counts.

The serum pepsinogen values correlate with the intensity of abomasal helminth infection (Berghen et al., 1993). Since the fungus-fed sheep showed higher values than the control, no fungus effect was observed concerning this parameter, which is in accordance with previous findings (Nansen et al., 1995). Others, however, described a positive correlation of fungus feeding and lower pepsinogen levels (Dimander et al., 2003; Larsen et al., 1995; Sarkunas et al., 2000; Wolstrup et al., 1994). The latter was reflected in goat pepsinogen courses, but not being statistically significant.

During this investigation an indication of a *D. flagrans* effect has only been observed in goats with respect to a transient reduction in mean GI-nematode eggs, but no effect was found in sheep. Reasons for this can be the low input and/or the insufficiently short period of feeding. Feeding was done manually in a trough with all animals at the same time. Therefore, a confirmation of sufficient intake for each animal was difficult. For any homogenous distribution on pasture this is crucial for the fungus (Bird et al., 1995; Peña et al., 2002; Waller et al., 2001a; Knox et al., 2001). In future, a longer feeding period or automated feeding could help to overcome this problem. A different and more convenient application such as a bolus could also be of advantage. First experiences in packing *D. flagrans* spores in bolus-like device are reported (Waller et al., 2001a, b). Here, the dosage used was 5×10^5 spores/kg BW/day, since it was previously reported to be effective in various in vitro and in vivo studies in small ruminants (Faedo et al., 1997; Knox et al., 2001; Paraud and Chartier, 2003; Peña et al., 2002; Sanyal 2001). However, in the present study the animal density was more extensive, which might have led to low nematode larvae dung pad infestations and thus a slower spreading and development of trapping structures in the fungus. Additionally, after a dry and warm spring a plus-average rainfall in summer needs to be considered as another factor of influence which complicated the monitoring of fungus effect. The rainfall peak occurred during the last month of fungus feeding and at the same time as increasing epg values were observed. Consecutively, the increase of larvae found in grass samples followed approximately three weeks later, indicating the favourable conditions. Accordingly, the climatic influence on larval development is reported repeatedly in the past (Dimander et al., 2003; Fernandez et al., 1997; Larsen et al., 1995).

Conclusions

Future studies should include a modified feeding regimen, perhaps with permanent release boluses or feed blocks, probably combined with different dosages; whereas environmental parameters like weather can always influence the presence of fungus on pasture. Perhaps an isolate, adapted to northern climatic options, could improve results in trapping parasitic larvae.

Therefore, currently this fascinating system of biological control might be regarded as a supplemental tool for parasite control rather than a realistic alternative to the classical chemoprophylactic control.

The study was performed at the experimental farm of the Institute of Organic Farming of the former Federal Agricultural Research Centre, Trenthorst, Germany.

We thank Chr. Hansen Ltd. for providing *Duddingtonia flagrans* chlamydo-spores.

References

- Armour J (1980) The epidemiology of helminth disease in farm animals. *Vet Parasitol* 6:7-46
- Barth D, Visser M (1991) Magen-Darmnematoden des Rindes. Stuttgart : Enke, 104 p
- Bartley DJ, Jackson F, Jackson E, Sargison N (2004) Characterisation of two triple resistant field isolates of *Teladorsagia* from Scottish lowland sheep farms. *Vet Parasitol* 123:189-99
- Baudena MA, Chapman MR, Larsen M, Klei TR (2000) Efficacy of the nematophagous fungus *Duddingtonia flagrans* in reducing equine cyathostome larvae on pasture in south Louisiana. *Vet Parasitol* 89:219-230
- Benesch C (1993) Parasiten des Magen-Darm-Traktes von Schafen in Hessen : eine Sektionsstudie. Gießen : Univ, 145 p
- Berghen P, Dorny P, Vercruysee J (1987) Evaluation of a simplified blood pepsinogen assay. *Am J Vet Res* 48:664-669
- Berghen P, Hilderson H, Vercruysee J, Dorny P (1993) Evaluation of pepsinogen, gastrin antibody response in diagnosing ostertagiasis. *Vet Parasitol* 46:175-195
- Bird J, Herd RP (1995) In vitro assessment of two species of nematophagous fungi (*Arthrobotrys oligospora* and *Arthrobotrys flagrans*) to control the development of infective cyathostome larvae from naturally infected horses. *Vet Parasitol* 56:181-187
- Borgsteede FHM, Pekelder JJ, Dercksen DP, Sol J, Vellema P, Gaasenbeek CPH, Linden van der JN (1997) A survey of anthelmintic resistance in nematodes of sheep in the Netherlands. *Vet Q* 19:167-171
- Buerger HJ, Stoye M (1968) Parasitologische Diagnostik : Teil 2, Eizählung und Larvendifferenzierung. *Therapogen Praxisdienst* (3)1-22
- Chandrawathani P, Jamnah O, Waller PJ, Høglund J, Larsen M, Zahari WM (2002) Nematophagous fungi as a biological control agent for nematode parasites of small ruminants in Malaysia : a special emphasis on *Duddingtonia flagrans*. *Vet Res* 33:685-696
- Chartier C, Pors I (2003) Effect of the nematophagous fungus, *Duddingtonia flagrans*, on the larval development of goat parasitic nematodes : a plot study. *Vet Res* 34:221-230
- Dimander SO, Høglund J, Uggla A, Sprondly E, Waller PJ (2003) Evaluation of gastro-intestinal nematode parasite control strategies for first-season grazing cattle in Sweden. *Vet Parasitol* 111:193-209
- Eckert J, Buerger HJ (1979) Die parasitäre Gastroenteritis des Rindes. *Berl Münch Tierärztl Wschr* 92:449-464
- Eysker M (1997) Some aspects of inhibited development of trichostrongylids in ruminants. *Vet Parasitol* 72:265-283
- Faedo M, Barnes EH, Dobson RJ, Waller PJ (1998) The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep : pasture plot study with *Duddingtonia flagrans*. *Vet Parasitol* 76:129-135
- Faedo M, Larsen M, Groenvold J (2002) Predacious activity of *Duddingtonia flagrans* within the cattle faecal pat. *J Helminthol* 76:395-302
- Fernandez AS, Larsen M, Nansen P, Groenvold J, Henriksen SA, Wolstrup J (1997) Effect of the nematode-trapping fungus *Duddingtonia flagrans* on the free-living stages of horse parasitic nematodes: a plot study. *Vet Parasitol* 73:257-266
- Fernandez AS, Henningsen E, Larsen M, Nansen P, Groenvold J, Sondergaard J (1999a) A new isolate of the nematophagous fungus *Duddingtonia flagrans* as biological control agent against free-living larvae of horse strongyles. *Equine Vet J* 31:488-491
- Fernandez AS, Larsen M, Nansen P, Groenvold J, Henriksen SA, Bjorn H, Wolstrup J (1999b) The efficacy of two isolates of the nematode-trapping fungus *Duddingtonia flagrans* against *Dictyocaulus viviparus* larvae in faeces. *Vet Parasitol* 85:289-304
- Fernandez AS, Larsen M, Nansen P, Henningsen E, Groenvold J, Wolstrup J, Henriksen SA, Bjorn H (1999c) The ability of the nematode-trapping fungus *Duddingtonia flagrans* to reduce the transmission of infective *Ostertagia ostertagi* larvae from faeces to herbage. *J Helminthol* 73:115-122
- Fernandez AS, Larsen M, Wolstrup J, Groenvold J, Nansen P, Bjorn H (1999d) Growth rate and trapping efficacy of nematode-trapping fungi under constant and fluctuating temperatures. *Parasitol Res* 85:661-668
- Fontenot ME, Miller JE, Peña MT, Larsen M, Gillespie A (2003) Efficiency of feeding *Duddingtonia flagrans* chlamydo-spores to grazing ewes on reducing availability of parasitic nematode larvae on pasture. *Vet Parasitol* 118:203-213
- Gibbs HC (1979) Relative importance of winter survival of larval nematodes in pasture and infected carrier calves in a study of parasitic gastroenteritis in calves. *Am J Vet Res* 40:227-231
- Gibson TE (1971) Epidemiology of parasitic gastroenteritis in sheep. *Vet Record* 2:151-152
- Githigia SM, Thamsborg SM, Larsen M, Kyvsgaard NC, Nansen P (1997) The preventive effect of the fungus *Duddingtonia flagrans* on trichostrongyle infections of lambs on pasture. *Int J Parasitol* 27:931-939
- Goldenstein N, Brunke V, Buerger HJ (1978) Ökologische und epizootologische Untersuchungen über Magen-Darm-Strongylyden beim Schaf. *Berl Münch Tierärztl Wschr* 91:286-289
- Grønvold J, Wolstrup J, Nansen P, Henriksen SA, Larsen M, Bresciani J (1993) Biological control of nematode parasites in cattle with nematode-trapping fungi : a survey of Danish studies. *Vet Parasitol* 48:311-325
- Grønvold J, Henriksen SA, Larsen M, Nansen P, Wolstrup J (1996a) Biological control : aspects of biological control-with special reference to arthropods, protozoans and helminths of domesticated animals. *Vet Parasitol* 64:47-64
- Grønvold J, Nansen P, Henriksen SA, Larsen M, Wolstrup J, Bresciani J, Rawat H, Friberg L (1996b) Induction of traps by *Ostertagia ostertagi* larvae, chlamydo-spore production and growth rate in the nematode-trapping fungus *Duddingtonia flagrans*. *J Helminthol* 70:291-297
- Grønvold J, Wolstrup J, Nansen P, Larsen M, Henriksen SA, Bjorn H, Kirchheiner K, Lassen K, Rawat H, Kristiansen HL (1999) Biotic and abiotic factors influencing growth rate and production of traps by the nematode-trapping fungus *Duddingtonia flagrans* when induced by *Cooperia oncophora* larvae. *J Helminthol* 73:129-136
- Henriksen SA, Korsholm H (1983) A method for culture and recovery of gastrointestinal strongyle larvae. *Nord Vet Med* 35:429-30
- Hiepe T, Zimmermann H (1966) Beeinflussung der Wollqualität beim Merinofleischschaf durch experimentelle Trichostrongylen-Infektionen. *Monatsh Veterinärmed* 17:641-645
- Inderbitzin F (1976) Experimentell erzeugte Entwicklungshemmung von *Dictyocaulus viviparus* des Rindes. Zürich : Univ, 45 p
- Kaplan RM, Burke JM, Terrill TH, Miller JE, Getz WR, Mobini S, Valencia E, Williams MJ, Williamson LH, Larsen M, Vatta AF (2004) Validation of the FA-MACHA eye color chart for detecting clinical anemia in sheep and goats on

- farms in the southern United States. *Vet Parasitol* 123:105-120
- Knox MR, Faedo M (2001) Biological control of field infections of nematode parasites of young sheep with *Duddingtonia flagrans* and effects of spore intake on efficacy. *Vet Parasitol* 101:155-160
- Larsen M, Wolstrup J, Henriksen SA, Groenvold J, Nansen P (1992) *In vivo* passage through calves of nematophagous fungi selected for biocontrol of parasitic nematodes. *J Helminthol* 66:137-141
- Larsen M, Faedo M, Waller PJ (1994) The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep : survey for the presence of fungi in fresh feces of grazing livestock in Australia. *Vet Parasitol* 53:275-281
- Larsen M, Nansen P, Wolstrup J, Groenvold J, Henriksen SA, Zorn A (1995) Biological control of trichostrongyles in calves by the fungus *Duddingtonia flagrans* fed to animals under natural grazing conditions. *Vet Parasitol* 60:321-330
- Larsen M, Nansen P, Grondahl C, Thamsborg SM, Groenvold J, Wolstrup J, Henriksen SA, Monrad J (1996) The capacity of the fungus *Duddingtonia flagrans* to prevent strongyle infections in foals on pasture. *Parasitol* 113:1-6
- Larsen M, Nansen P, Groenvold J, Wolstrup J, Henriksen SA (1997) **Biological control of gastro-intestinal nematodes – facts, future, or fiction?** *Vet Parasitol* 72:479-485
- Larsen M (2000) Prospects for controlling animal parasitic nematodes by predacious microfungi. *Parasitol* 120(Suppl):121-131
- Maingis N, Bjorn H, Thamsborg SM, Bogh HO, Nansen P (1996) Anthelmintic resistance in nematode parasites of sheep in Denmark. *Small Ruminant Res* 23:171-181
- McLeod RS (1995) Costs of major parasites to the Australian livestock industries. *Int J Parasitol* 25:1363-1367
- Mortensen LL, Williamson LH, Terrill TH, Kircher RA, Larsen M, Kaplan RM (2003) Evaluation of prevalence and clinical implications of anthelmintic resistance in gastrointestinal nematodes in goats. *J Am Vet Med Assoc* 223:495-500
- Nansen P, Groenvold J, Henriksen SA, Wolstrup J (1988) Interactions between the predacious fungus *Arthrobotrys oligospora* and third-stage larvae of a series of animal-parasitic nematodes. *Vet Parasitol* 26:329-337
- Nansen P, Larsen M, Groenvold J, Wolstrup J, Zorn A, Henriksen SA (1995) Prevention of clinical trichostrongylidosis in calves by strategic feeding with the predacious fungus *Duddingtonia flagrans*. *Parasitol Res* 91:371-374
- Nansen P, Larsen M, Groenvold J, Wolstrup J, Zorn A, Henriksen SA (1996) Control of *Oesophagostomum dentatum* and *Hyostrongylus rubidus* in outdoor-reared pigs by daily feeding with the microfungus *Duddingtonia flagrans*. *Parasitol Res* 82:580-584
- Paraud C, Chartier C (2003) Biological control of infective larvae of a gastrointestinal nematode (*Teladorsagia circumcincta*) and a small lungworm (*Muellerius capillaris*) by *Duddingtonia flagrans* in goat faeces. *Parasitol Res* 89:102-106
- Paraud C, Pors I, Chartier C (2004) Activity of *Duddingtonia flagrans* on *Trichostrongylus colubriformis* larvae in goat faeces and interaction with a benzimidazole treatment. *Small Ruminant Res* 55:199-207
- Paraud C, Hoste H, Lefrileux Y, Pommaret A, Paolini V, Pors I, Chartier C (2005) Administration of *Duddingtonia flagrans* chlamyospores to goats to control gastro-intestinal nematodes : dose trials. *Vet Res* 36:157-166
- Peña MT, Miller JE, Fontenot ME, Gillespie A, Larsen M (2002) Evaluation of *Duddingtonia flagrans* in reducing infective larvae of *Haemonchus contortus* in faeces of sheep. *Vet Parasitol* 103:259-265
- Rehbein S, Kollmannsberger M, Vossler M, Winter R (1996) Untersuchungen zum Helminthenbefall von Schlachtschafen in Oberbayern : 1. Mitt: Artenspektrum, Befallsextenstität und Befallsintensität. *Berl Münch Tierärztl Wschr* 109:161-167
- Roberts FHS, O'Sullivan PJ (1950) Methods for egg counts and larval cultures for strongyles infesting the gastrointestinal tract of cattle. *Austr J Agric Res* 1:99-102
- Rowe JB, Nolan JV, De Chaneet G, Teleni E, Holmes PH (1988) The effect of haemonchosis and blood loss into the abomasum on digestion in sheep. *Brit J Nutr* 59:125-139
- Sanyal PK (2001) Biological control of *Haemonchus contortus* in sheep by nematophagous fungus, *Duddingtonia flagrans*. *Indian Vet J* 78:23-26
- Sarkunas M, Larsen M, Nansen P, Hansen JW (2000) Biological control of trichostrongylid infections in calves on pasture in Lithuania using *Duddingtonia flagrans* : a nematode-trapping fungus. *J Helminthol* 74:355-359
- Schmidt U (1971) Vergleichende Untersuchungen verschiedener Anreicherungsverfahren zum Nachweis von Wurmeiern. München : Univ, 85 p
- Schnyder M, Torgerson PR, Schonmann M, Kohler L, Hertzberg H (2005) Multiple anthelmintic resistance in *Haemonchus contortus* isolated from south African Boer goats in Switzerland. *Vet Parasitol* 128:285-290
- Sievers Prekehr GH (1973) Methode zur Gewinnung von 3. Strongylienlarven aus dem Weidegras. Hannover : Tierärztl Hochsch, 58 p
- Steel JW, Jones WO, Symons LEA (1982) Effects of a concurrent infection of *Trichostrongylus colubriformis* on the productivity and physiological and metabolic responses of lambs infected with *Ostertagia circumcincta*. *Aust J Agric Res* 33:131-140
- Terrill TH, Kaplan RM, Larsen M, Samples OM, Miller JE, Gelaye S (2001) Anthelmintic resistance on goat farms in Georgia: efficacy of anthelmintics against gastrointestinal nematodes in two selected goat herds. *Vet Parasitol* 97:261-268
- Thamsborg SM, Joergensen RJ, Waller PJ, Nansen P (1996) The influence of stocking rate on gastrointestinal nematode infections of sheep over a 2-year grazing period. *Vet Parasitol* 67:207-224
- van Wyk JA, Stenson MO, Van der Merwe JS, Vorster RJ, Viljoen PG (1999) Anthelmintic resistance in South Africa : surveys indicate an extremely serious situation in sheep and goat farming. *Onderstepoort J Vet Res* 66:273-284
- Waghorn TS, Leathwick DM, Chen LY, Skipp RA (2003) Efficacy of the nematode-trapping fungus *Duddingtonia flagrans* against three species of gastro-intestinal nematodes in laboratory faecal cultures from sheep and goats. *Vet Parasitol* 118:227-234
- Waller PJ, Faedo M, Ellis K (2001a) The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep : towards the development of a fungal controlled release device. *Vet Parasitol* 102:299-308
- Waller PJ, Knox MR, Faedo M (2001b) The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep : feeding and block studies with *Duddingtonia flagrans*. *Vet Parasitol* 102:321-330
- Wetzel R (1930) Zur Diagnose der Lungenwurminvasionen bei Rind und Schaf. *Dtsch tierärztl Wschr* 38:49-50
- Wolstrup J, Groenvold J, Henriksen SA, Nansen P, Larsen M, Bogh HO, Ilsoe UB (1994) An attempt to implement the nematode-trapping fungus *Duddingtonia flagrans* in biological control of trichostrongyle infection of 1st year grazing calves. *J Helminthol* 68:175-180
- Zajac AM, Gipson TA (2000) Multiple anthelmintic resistance in a goat herd. *Vet Parasitol* 87:163-72

