

Biological Control of Sheep Parasites using *Duddingtonia flagrans*: Trials on Commercial Farms in Sweden

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Waller P, Ljungström BL, Schwan O, Rudby Martin L, Morrison DA and Rydzik A: Biological Control of Sheep Parasites using *Duddingtonia flagrans*: Trials on Commercial Farms in Sweden. Acta vet. scand. 2006, 47, 23-32. –Trials were conducted on 3 commercial sheep farms in Sweden to assess the effect of administering spores of the nematode trapping fungus, *Duddingtonia flagrans*, together with supplementary feed to lactating ewes for the first 6 weeks from turn-out on pastures in spring. Also control groups of ewes, receiving only feed supplement, were established on all 3 farms. Groups were monitored by intensive parasitological investigation. The ewes and their lambs were moved in late June to saved pastures for summer grazing, the lambs receiving an anthelmintic treatment at this time. After approximately 6 weeks on summer pasture the lambs were weaned, treated a second time with anthelmintic, and returned to their original lambing pastures for finishing. Decisions as to when lambs were to be marketed were entirely at the discretion of the farmer co-operators. No difference in lamb performance was found between the two treatments on all three farms. This was attributed to the high levels of nutrition initially of the ewes limiting their post-partum rise in nematode faecal egg counts in spring, which in turn resulted in low levels of nematode infection on pastures throughout the autumn period. Additionally, pastures were of good quality for the lambs during the finishing period, so they grew at optimal rates as far as the farmers were concerned.

Nematode parasites / sheep / biological control / *Duddingtonia flagrans*

Introduction

The nematophagous microfungus, *Duddingtonia flagrans*, has been shown to survive passage through the gastro-intestinal tract of livestock, then germinate and spread on freshly deposited dung producing specialised nematode trapping structures. Thus, this fungus has the potential to break the life cycle of nematode parasites by capturing infective larval stages before they migrate from dung to pasture, where they would otherwise be acquired by grazing animals (Larsen 1999). What is now conventionally termed biological control, this non-chemical approach to the control of nematode parasites

of ruminant livestock has been exhaustively tested under laboratory and controlled field conditions (for recent reviews see: Larsen 2000, Waller 2003). However, it now needs to be evaluated under a range of commercial farming operations to take this from being a novel concept to possibly a novel practical alternative for parasite control. Towards this objective a three-year study was recently conducted on two sheep farms on the Swedish island of Gotland, where a feed supplement containing spores of *Duddingtonia flagrans* was compared with conventional parasite control practices, and the

outcome of this work proved to be favourable (Waller et al. 2004). These investigations were extended to study the effects of using the fungus in situations where no anthelmintic treatments were applied to ewes in the Gotland trials, and also to investigate the use of fungus on a farm situated on mainland Sweden, where *Haemonchus contortus* (absent from Gotland) is endemic.

Materials and Methods

Trials were conducted on two sheep farms on the Swedish island of Gotland and one farm located in the province of Skåne of southern mainland Sweden during the grazing season of 2004. The weather on Gotland tends to be warmer and drier during summer (late June – mid August) than in Skåne, and the nematode parasite *Haemonchus contortus* is considered to be absent on sheep farms in Gotland. The typical management of sheep flocks in Sweden is for lambing to occur in early – mid spring (late March – April). Around this time, the ewes and their young lambs are turned-out onto high quality pasture in the immediate proximity of the winter housing shed. This provides easy access to adequate shelter and facilitates management practices such as supplementary feeding of lactating ewes. Around late June / early July, farmers generally move ewes and lambs to reserved extensive grazing areas (especially on Gotland), or to aftermath grazing following hay or silage harvesting. Weaning occurs in early autumn (August), with the lambs usually being returned to the high quality pastures that were used for lambing early in the season, but which have been spelled during the summer.

The aim of this study was to investigate, in practical farming situations, the ability of the nematophagous fungus, *Duddingtonia flagrans*, to reduce the number of infective larvae on pasture in the latter part of the grazing season, when provided to the ewes by way of a feed

supplement during the immediate post-lambing period in spring – early summer.

Farms

The prevailing weather conditions (temperature and rainfall) during 2004 were considered normal for the regions in which the three farms were located. All sheep used in the trials on Gotland (farms B and N) were Gotland pure-bred, whereas ewes on farm L located in Skåne were predominantly the Gotland breed, but some Texel and cross-bred ewes were included. On each farm, ewes that had lambed within a short interval (< 2 weeks) in mid April were selected for the trial. These were allocated to two treatment groups:

- *Fungus group*: This consisted of 20 ewes with twin lambs (1 male + 1 female) for the Gotland farms (B and N). The same number of ewes was used on farm L but there were 5 ewes with twins and 15 ewes with single lambs (equal sexes in both groups).
- *Control group*: The same number and breed of ewes and number of lambs of the ewes in the Fungus group was allocated to an area of comparable size, topography and pasture quality for each of the three farms.

Ewes were untreated on farms B and N, but on farm L all ewes received the recommended dose of anthelmintic (ivermectin: Ivomec®, Merial, New Jersey, USA; 200µg/kg) at housing in late November 2003. At turn-out the following year, all ewes received a daily supplement of concentrates (approx. 600 g / ewe / day) that was provided each morning in metal sheep feeding troughs large enough to allow each animal adequate space to feed. In addition, the Fungus group on each farm had a 40 g packet of *D. flagrans* spores (Chr. Hansen Biosystems, Hørsholm, Denmark), mixed each day with the feed supplement, which was calculated as the dose rate for 20 ewes with a mean weight of 80 kg, each receiving 250,000 *D. flagrans* spores /

kg body weight/day. When the lambs were approximately 8 weeks of age (early June), the dose rate of spores was doubled to account for the consumption of feed supplement (thus fungal spores) by the lambs. This increased dose rate was continued until the ewes and lambs were moved to summer grazing areas in late June.

At the time of the move to summer pastures, lambs were dosed with anthelmintic (ivermectin: Ivomec[®], Merial). In mid-August, weaned lambs were returned to their respective treatment paddocks following a second treatment with ivermectin.

Parasitological measurements

Ewe faecal egg counts

On each farm, at least ten ewes (50%) from each treatment group were sampled on 4 occasions on each farm. These samples were taken at approximately 3-weekly intervals from turnout until the move to summer pastures. Individual nematode faecal egg counts and pooled faecal cultures for larval differentiation were performed, following standard procedures (Lindqvist *et al.* 2001).

Lamb faecal egg counts

On each farm, twenty lambs (50%) from each treatment group were sampled on 4 occasions, commencing just prior to the move to summer pastures (late June – Gotland; early July - Skåne), then at the time they were re-introduced to the experimental plots (mid August), followed by the final two samplings approximately 4 weeks apart in autumn. It was not possible to collect samples from 8 lambs in the Control group on farm L on each of the last two sampling occasions.

Tracer tests

Sequential tracer tests were conducted on each farm for the duration of time that the sheep grazed the experimental pastures. Two tracer

lambs were used on each pasture for each test, with a new set of tracers being allocated at the time that existing tracers were removed from the treatment paddocks. The aim was to have tracer tests approximately every 3 weeks, however there was some variation in time between tests, due to logistical reasons. After removal from pasture, all tracers were held for 2 weeks indoors and pen fed before slaughter. In addition, on each farm four tracers were allocated to the flock when they grazed the summer pastures for each year.

Lambs born the previous year were used for the first three tracer tests and for the tests on the summer pastures. These had been previously rendered worm-free by several anthelmintic treatments with ivermectin and managed as a separate group on pasture that had been previously ungrazed by sheep. For each successive group of four tracers on each farm, the last anthelmintic treatment was given no later than 4 weeks prior to allocation to the paddocks. Lambs used in the latter four tracer tests in each year were current year born lambs.

Lambs were consigned to the local slaughterhouse and their viscera were collected and processed for worm recovery, speciation and enumeration by the methods described by Donald *et al.* (1978) and mucosal digestion procedures of the abomasums by the method described by Dobson *et al.* (1990).

Lamb performance

Lambs were weighed at birth and subsequently at the time when the sheep were gathered for faecal sampling. Decisions regarding the time of marketing of the lambs were made entirely by the farmers. Their criteria for selection were based on lamb condition and a liveweight of approximately 45 kg.

Statistical procedures

The log-transformed worm burdens for the

tracer lambs were analysed separately for each farm using a 3-factor mixed analysis of variance, with experimental treatment (Control/Fungus), grazing time (before/after summer grazing) and sample time (1–7 tracer tests, nested within grazing time) as the factors.

The analysis of data for lamb performance was complicated by lamb removal due to death and marketing, which made it difficult to directly compare the experimental groups through time. Thus, in order to standardize the data, growth curves were calculated separately for each lamb (based on an exponential saturation model of Landsberg (1977), which provided an estimate of the maximum weight achievable. The data were then analysed as percentages of the predicted maximum for each lamb.

Results

Ewe faecal egg counts

For the two Gotland farms (B and N), the mean faecal egg counts of ewes at turnout were low (generally < 100 epg) with approximately 25% showing zero egg counts. Subsequently, the ewe faecal egg counts remained low at the second sampling, but showing a modest rise after 1 month on pasture. The final samples showed

that the egg counts fell to negligible levels, particularly on farm B (see Table 1). Infective larval differentials showed that the predominant species was *Teladorsagia circumcincta*, with some *Trichostrongylus* spp. on the Gotland farms. Pre-experimental sampling (6 May) of ewes on farm L, showed that the majority of ewes (9/16) had egg counts less than 50 epg, however 3 ewes had egg counts exceeding 1,000 epg, which were shown by larval differential to be predominantly *H. contortus*. Egg counts on 17 May showed that approximately 50% of ewes had a positive egg count with a mean of 200–250 epg. Subsequently, the egg counts of ewes in the Fungus group remained approximately the same, whereas those in the Control group fell to negligible levels, with many ewes showing zero egg (see Table 1). *H. contortus* was recorded in faecal sample cultures during May and June, but not at the final sampling in July. The predominant species throughout were *Teladorsagia/Trichostrongylus* spp on farm L.

Lamb faecal egg counts

At the first sampling occasion in late June (Farms B and N), or early July (Farm L), when

Table 1. Ewe nematode faecal egg counts (epg) on farms in Gotland (B & N) and Skåne (L).

Sampling Date	Farm B		Farm N		Farm L	
	Control	Fungus	Control	Fungus	Control	Fungus
3 May	65 (7/10) ⁺	65 (6/10) ⁺	130 (9/10) ⁺	65 (7/10) ⁺		
17 May					200 (10/20) ⁺	253 (8/19) ⁺
18 May	25 (5/10)	30 (5/10)	60 (6/10)	35 (5/10)		
2 June	280 (7/10)	230 (8/10)	215 (6/10)	115 (5/10)		
3 June					20 (1/10)	280 (2/10)
16 June					17 (2/10)	240 (8/10)
30 June*	5 (1/10)	20 (1/10)	85 (6/10)	60 (4/10)		
7 July*					9 (3/17)	125 (5/20)

⁺ () number of ewes with positive faecal egg counts

* Ewes and lambs moved off pasture

Table 2. Lamb nematode faecal egg counts (epg) on farms in Gotland (B & N) and Skåne (L)

Sampling Date	Farm B		Farm N		Farm L	
	Control	Fungus	Control	Fungus	Control	Fungus
30 June*	73 (15/20)	200 (11/20) 8 N (3/20)	160 (17/20)	160 (17/20)		
7 July*					138 (12/21)	153 (15/21) 12 N (5/21)
9 Aug#	280 (20/20) 5 N (2/20)	303 (20/20) 13 N (9/20)	81(10/21)	153 (8/21)		
24 Aug#					800 (23/24) 148 N (19/24)	1092 (21/25) 82 N (20/25)
6 Sept	158 (14/20)	160 (17/20)	81 (10/21)	153 (8/21)		
22 Sept					0	13 (3/24) 2 N (1/24)
13 Oct	155 (18/20)	128 (16/20)			88 (10/12)	90 (15/21)
21 Oct			316 (19/20)	380 (20/20)		
3 Nov					65 (10/12) 13 N (2/12)	61 (15/21) 19 N (7/21)

*Ewes and lambs moved off pasture. Lambs dosed with ivermectin

Lambs only moved back to pasture and dosed with ivermectin

they were approximately 10 weeks of age, the majority of lambs recorded positive egg counts. These were predominately *T. circumcincta*/*Trichostrongylus* spp, with low numbers of *Nematodirus* spp (Farm B) and *H. contortus* (Farm L). At this time the lambs were dosed with ivermectin and moved with their dams to summer grazing pastures. When they returned to their trial pastures approximately 6 weeks later, the majority of lambs on Farms B and N had relatively low faecal egg counts, however the lambs on Farm L had mean counts approximating 1,000 epg, which were comprised of a substantial proportion of *H. contortus*. All lambs were dosed with ivermectin again at this time and subsequently egg counts remained low for the remainder of the grazing season (see Table 2).

Tracer lamb worm burdens

Results of the worm burdens acquired by tracers on the experimental treatment plots are par-

tioned into collective estimates of the trichostrongylid nematodes in which eggs hatch to release the free-living larval stages in dung and on pasture (ie. *Teladorsagia*, *Haemonchus*, *Trichostrongylus*, *Cooperia* spp), shown in Table 3, and those for *Nematodirus* spp. for which development through to the infective larval stage occurs within the egg (Table 4). The former group of parasites is considered to be the best source of prey for *D. flagrans*, because of the obligatory larval stages in the same environment as the fungus. For the two farms on Gotland (B and N), the great majority of parasites were *Teladorsagia circumcincta*, with small numbers of *Trichostrongylus vitrinus* recorded during tests 4 – 7. No *H. contortus* were recorded in any lamb on these two farms. Similarly on Farm L, the great majority of parasites recovered from tracers were *T. circumcincta*, with very few *H. contortus* and *T. vitrinus*. All farms showed statistically significant

variation in worm burdens between tracer tests ($p < 0.005$ in all cases). The pastures were infective at the time of turn-out with levels of larval pick-up remaining relatively constant from turn-out until the move of ewes and lambs to summer pastures. On farm B levels of infectivity were the greatest at the time that the lambs returned to their experimental pastures in mid August (test 4), but subsequently the infection levels steadily declined for the remainder of the grazing season. A similar pattern was observed on farm N. Levels of infection on the experimental pastures from the time that the lambs were re-introduced in mid August until the ter-

mination of the trial, were exceedingly low. There was no indication of any difference between the larval pickup on the Control and Fungus treatment pastures for any of the three farms ($p = 0.112, 0.487, 0.494$ for farms B, N and L respectively: see Table 3). Pick-up of *Nematodirus* spp was very low throughout the trial for each of the three farms and no statistically significant patterns were detected (see Table 4). Mean worm burdens of tracer lambs that grazed with the experimental sheep on the summer pastures are shown in Table 5. All lambs acquired infections during this period, predominantly *T. circumcincta* on Farms B and N, but

Table 3. Mean Trichostrongylid (excluding *Nematodirus* spp.) worm burdens ($n = 2$) of tracer lambs for control and fungus treatments on sheep farms on Gotland (B and N) and Skåne (L)

Tracer Test	Farm B		Farm N		Farm L	
	Control	Fungus	Control	Fungus	Control	Fungus
1	7700	5150	8550	3100	6250	10200
2	4350	3250	3050	500	4200	2550
3	2900	1100	1900	1000	5150	3200
$\Sigma 1 - 3$	14950	9500	13500	4600	15600	15950
Summer Grazing						
4	19250	12800	3450	1850	100	200
5	4050	5550	1700	2550	50	0
6	2450	2100	3900	1350	0	0
7	1050	750	0	50	450	0
$\Sigma 4 - 7$	27800	21200	9050	5800	600	200

Table 4. Mean *Nematodirus* spp. worm burdens ($n = 2$) of tracer lambs for control and fungus treatments on sheep farms on Gotland (B and N) and Skåne (L)

Tracer Test	Farm B		Farm N		Farm L	
	Control	Fungus	Control	Fungus	Control	Fungus
1	0	0	0	0	300	200
2	50	100	50	50	200	50
3	150	0	50	0	2200	650
$\Sigma 1 - 3$	200	100	100	50	2700	900
Summer Grazing						
4	100	100	50	50	150	50
5	100	200	150	0	50	150
6	600	250	250	100	350	450
7	900	800	250	50	800	350
$\Sigma 4 - 7$	1700	1350	700	200	1350	1000

Table 5. Mean worm burdens (n = 4) of tracers grazing on summer pastures of farms in Gotland (B and N) and Skåne (L)

Parasite spp.	Farm B	Farm N	Farm L
<i>H. contortus</i>	0	0	2500
<i>T. circum.</i>	975	2525	2233
<i>T. axei</i>	0	225	233
Intestinal <i>Trich</i>	0	200	1500
<i>Nematodirus spp.</i>	975	700	1267
<i>S. papillosus</i>	0	0	1567
Total	1950	3650	9300

Table 6. Lamb turn-off rate from sheep farms on Gotland and Skåne during 2004.

Date	Gotland		Farm N		Skåne Farm L	
	Farm B Fungus	Control	Fungus	Control	Fungus	Control
9/8	10 (45kg*)	6 (45kg)				
24/8			4 (44kg)	2 (43kg)		
6/9	5 (46kg)	8 (46kg)				
20/9			21 (48kg)	22 (48kg)		
11/10					4 (52kg)	12 (48kg)
12/10			13 (43kg)	12 (44kg)		
20/10	21 (47kg)	22 (46kg)				
2/11					21 (44kg)	12 (43kg)

*(x) average weight of the lambs consigned to market

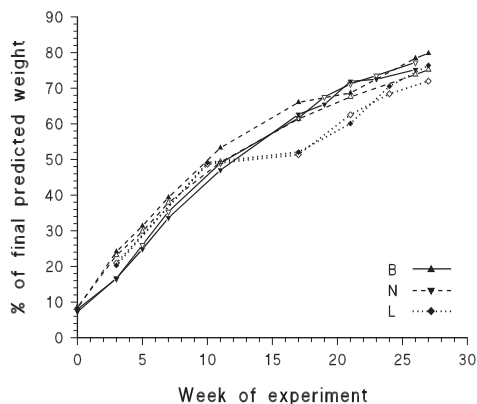


Figure 1. Weight gain trajectories for each of the six experimental groups. The data are the averages at each sample time of the percentage of the final predicted weight of each lamb (the prediction coming from the exponential saturation growth curve fitted to the data for each lamb individually). Open symbols: Control treatment; filled symbols: Fungus treatment.

tracers used on farm L showed *H. contortus* was present in equal numbers to *T. circumcincta*.

Lamb Performance

Weight gain trajectories of lambs grazing on the Control and Fungus treatments and the times, number and the mean weight in which lambs were removed from the trial (marketed or sold as flock replacements), for each of the three farms are shown in Figure 1 and Table 6, respectively. There was no consistent difference in the pattern of lamb weight gain between the two experimental groups on any of the farms, nor was there a notable difference in the averages of the maximum observed weight per lamb (for Fungus and Control, respectively: B = 46.7 and 45.4 kg; N = 47.4 and 46.5 kg; L = 46.3 and 44.7 kg).

Discussion

These trials showed that there was no significant benefit in performance of lambs in the fungal treatment for all three farms used in this trial. Whilst disappointing, it is important to reflect on the possible reasons for this before concluding that biological control used under the conditions of this trial was an outright failure.

Firstly, the numbers of infective larvae that the lambs were exposed to throughout the entire grazing season were generally low for all three farms. Although the levels of over-wintered infection (shown by the first two tracer tests for all 3 farms) were moderate, this was during a time when the lambs were generally less than 2 months of age, obtaining most of their nourishment from their dams and thus unlikely to be acquiring these infections. Infective larval numbers in the latter 3 weeks on the lambing pastures before the move in late June (tracer test 3), tended to show the normal decrease commonly observed in over-wintered populations of ruminant nematodes from turn-out onwards in Sweden (*Dimander et al.* 1999). However any early infections acquired by the lambs prior to the move would not have an effect, due to the biological lag of at least 2 months between larval ingestion and any adverse expression on weight gain of young lambs (*Waller et al.* 1987). This is indicated by the fact that there was no check in performance of the lambs up to the time that they were treated with ivermectin and moved to summer pastures.

Secondly and most importantly, the contamination by the ewes from turn-out until the mid-summer move was very low. It is a well established fact throughout Europe, that contamination by peri-parturient ewes in the first 6-8 weeks of spring is the major source of contamination that determines the magnitude of pasture larval numbers in autumn (*Urquhart et al.* 1996). The low egg counts of ewes was particularly surprising for Farms B and N on Got-

land, where they were not de-wormed during the housing period, and thus a peri-parturient rise in faecal egg counts around the time of turn-out would be expected. Thus it seems that parasite control measures implemented on these two farms in previous years (for description, see *Waller et al.* 2004) has been extremely successful in reducing the overall parasite loads to a situation where optimal production occurred without the need for any intervention – at least for this year of study. Ewes on farm L had been dosed in late November 2003 and pre-experimental sampling prior to turn-out confirmed the expectation that the majority of ewes had negligible faecal egg counts, although there were a few animals with high egg counts. This latter observation was attributed to mis-dosing rather than the presence of ivermectin resistance. Subsequently, egg counts of ewes on this farm remained relatively low, with a large proportion remaining zero. It is pertinent to mention that the ewes on all three farms were in very good condition at the time of lambing. This, plus the fact that all ewes were being supplementary fed from turn-out is likely to have had a mitigating effect against the development of a post-partum rise in their faecal egg counts (*Kahn* 2003). Therefore, with the ewe faecal egg counts being so low during the first 6 weeks following turn-out, the feeding of fungal material at this time (with the aim of reducing the number of infective larvae originating from this contamination in the latter part of the grazing season) could, in retrospect, reasonably be considered to have been of limited value. This is verified by the tracer tests during the autumn period, where apart from tracer tests 4 and 5 on Farm B, acquisition of parasites was low on all farms, especially for the whole autumn grazing on Farm L and the last tracer test for Farms B and N.

Thirdly, all lambs were treated a second time with ivermectin prior to their return from sum-

mer grazing. Previous studies have shown that a summer grazing pasture, even if it has not been grazed early in the same season (ie. saved pasture, or aftermath), becomes infective during the latter part of the 6-8 weeks of grazing in summer. This is almost certainly due to the infection arising from contamination deposited by the ewes immediately following the move, as they are untreated (Waller *et al.* 2004). This was confirmed in this trial where all tracers acquired infections, which may indicate a potentially detrimental effect on the performance of lambs (particularly Farm L where a weight check of all lambs was recorded between weeks 15 – 20: see Figure 1), if they were returned to their original, prepared pastures in mid August without treatment. It is worth noting that the summer pastures used on Gotland (Farms B and N) were aftermath, whereas summer pastures used on Farm L, had not been grazed nor harvested during early summer, so the pasture growth was long and lush creating an ideal environment for translation of nematode eggs to infective larvae. Although the lambs on Farm L were rendered worm-free again in mid August, prior to re-allocation to their original lambing pastures, they were exposed to considerable pick-up of infection on the summer pastures.

The summer period for all three farms was favorable for re-growth on the saved pastures, and good quality feed was available throughout the remainder of the year. This resulted in very good weight gains by the lambs meeting (Farms B and L), or exceeding (Farm N), all three farmers' expectations with lamb turn-off times earlier, and numbers greater, than they normally experience. It is a common experience that good nutrition can mitigate any detrimental effects of moderate levels of parasitism in young sheep (for review, see Knox *et al.* 2003) and therefore the lambs on all three farms, which showed no difference between the two treatments, could also be a reflection of good

nutrition during the lamb-finishing period.

Biological control is one of the more promising non-chemotherapeutic approaches to parasite control of livestock (which also includes worm vaccines, bioactive forages, immuno-nutrition) that collectively represent a paradigm shift away from the traditional chemical approach to parasite control. Rather than focus on maximum parasite kill within the host, these approaches are more aimed at prophylaxis rather than a curative effect and particularly maintaining parasite infections below an economic threshold. A set of minimal performance requirements for these novel control methods has been proposed (Ketzis *et al.* 2005), namely: a significant level of efficacy when used alone in controlled laboratory studies; efficacy needs to be confirmed in on-farm trials in different environments; the efficacy achieved should result in an economic benefit on a herd or flock basis. With regards to biological control using *D. flagrans*, the first criterion has been adequately addressed, and the challenge is now to provide sound data for the latter two requirements. With regards to the results of these studies, there was no clearly demonstrable benefit from biological control. However, one has to be mindful that the level of parasitism on all three farms in these trials was very low, and that even the most effective form of parasite control imposed under such circumstances (say: suppressive treatment with an highly effective anthelmintic) most likely would have failed to improve upon this result.

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Sammanfattning

Biologisk kontroll av fårparasiter med hjälp av Duddingtonia flagrans: Försök i tre svenska färbesättningar.

Försöken genomfördes i tre svenska färbesättningar för att fastställa effekten av sporer av rosvampen *Duddingtonia flagrans*, givna tillsammans med kraftfoder till lakterande tackor under de första sex veckorna efter betessläppet på våren. Även kontrollgrupper av tackor, vilka fick enbart kraftfoder, etablerades i de tre besättningarna. Grupperna provtogs och undersöktes med hjälp av intensiva parasitundersökningar. Lammen avmaskades och tackorna och deras lamm flyttades i slutet av juni till sommarbeten. Efter ungefär sex veckor på sommarbetet avvandes lammen, avmaskades en andra gång och flyttades tillbaka till de ursprungliga vårfällorna för slutgödning. Beslut angående slakttidpunkt överlämnades helt till de medverkande färgarna. Ingen skillnad kunde ses mellan de två avmaskningstillfällena i de tre besättningarna. Detta tillskrevs tackornas initialt allmänt höga utfodringsstatus, vilket begränsade deras "post parturient rise" av antalet nematodägg avgivna på våren, vilket i sin tur resulterade i låga nivåer av nematodinfekterade beten under hela den kommande hösten. Dessutom var betena av god kvalitet för lammen under slutgödningsfasen, så att de växte optimalt enligt färgarnas uppfattning.

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