

Fungal endophyte effects on intake, health and liveweight gain of grazing cattle

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Abstract

The direct effects of fungal endophyte-produced alkaloids on dairy beef cattle were determined in early summer 1992 and in autumn of 1993 and 1994. Endophyte level (nil vs high) or strain (wild-type vs selected 187BB) did not affect liveweight gain (LWG), but in 1994 weaners on nil endophyte consumed more dry matter than those on either endophyte treatment. The presence of clover enhanced LWG, reflected in higher dry matter intake in autumn 1993. Under the severe endophyte challenge of 1994, clover reduced the severity of ryegrass staggers even though the daily intake of lolitrem B was higher in the presence of clover. Under the rotational grazing management contrast imposed in 1994, followers had lower LWG than leaders and tended to have higher daily intake of lolitrem B. Grazing management effects on ergovaline intake were inconsistent and differed between endophyte strain and clover level. Animal responses to endophyte strain under contrasting conditions during each measurement period were related to daily alkaloid intake when scaled to liveweight. It is concluded that under the environmental conditions experienced in the Manawatu, endophyte alkaloids have minimal direct effect on cattle performance, although the reduction in ryegrass staggers by clover presence and its elimination by using a selected endophyte have important practical effects. Indirect effects through pasture production, composition or persistence may be more important to animal performance.

Keywords: alkaloid intake, cattle, endophyte, ergovaline, herbage intake, liveweight gain, lolitrem B, peramine

Introduction

Ryegrass endophyte (*Acremonium lolii*, Latch, Christensen & Samuels) causes staggers and other disorders in cattle but effects have not been studied in detail, in contrast to sheep. A related endophyte (*Acremonium coenophialum*, Morgan-Jones & Cams) in tall fescue (*Festuca arundinacea* Schreb.) reduces

intake and liveweight gain (LWG) in grazing cattle (Chestnut *et al.* 1991; Fribourg *et al.* 1989) and sheep (Debessai *et al.* 1993; Hannah *et al.* 1990).

Endophyte effects on grazing cattle may be direct, through ingestion of toxins, or indirect, through effects on the growth or composition of pasture. Direct effects result from both the concentration of alkaloids in the herbage ingested and from the total alkaloid intake. Direct effects reported in sheep have included elevated body temperature (Fletcher 1993), ryegrass staggers, and reduced LWG. Recent results indicate inconsistent associations of visible effects in cattle with concentrations of alkaloids, mainly ergovaline, in the herbage on offer (H.S. Easton, pers. comm.). Trials with young dairy cattle (McCallum & Thomson 1994) or lactating cows (Thorn *et al.* 1994) have shown no effects of endophyte on animal performance; however, alkaloid intakes were not reported.

This paper reports results of measurements made over 1992/93 and 1993/94 within a larger trial, to examine the relationship between endophyte effects on animals and the dietary concentration and intake of causal toxins. Some preliminary results of the larger trial, evaluating the effects of wild-type and a selected endophyte strain (187BB) on pasture and animal performance, have been reported (Clark 1992; Cosgrove *et al.* 1993).

Materials and methods

Site and treatments

The experiment was located at the AgResearch Aorangi Lowland Research Station, Palmerston North. The main experiment was a 3 × 2 factorial laid out in a randomised complete block design with 3 replications. Ryegrass (*Lolium perenne* cv. Grasslands Nui) with 3 endophyte level and strain combinations (endophyte strain 187BB [98.3% tillers infected]; wild-type endophyte [95% tillers infected]; and nil endophyte [2.7% tillers infected]), were each sown with or without white clover (*Trifolium repens* cv. Grasslands Kopu) into a cultivated seedbed in March 1991. Paddocks not sown with clover received 250 kg N/ha/yr as urea, in 5 applications of 50 kg each in August, September, October, December and March. All treatments received maintenance superphosphate at 250 kg/ha.

Management

Paddocks of 0.5 ha were continuously stocked with yearling Friesian bulls at 6.0/ha from August to December in both years and with weaner bulls from January to early May in 1993. For autumn 1994 additional grazing treatments were added. On one half of each of the plots of the wild-type endophyte treatments, weaners were continuously stocked at 8.0/ha to provide a core treatment for comparison with previous years. For the other half of these plots, and the nil endophyte and strain 187BB treatment plots, weaners from each replicate were pooled into leaders and followers and grazed rotationally around the field replicates using a 10-day grazing duration (5 days by leaders, 5 days by followers) and 20-day spell sequence to allow comparison of leaders (high allowance, leafy pasture) vs followers (low allowance, less leaf and more stem), as a management to modify the exposure to endophyte-related toxins. Additional (put and take) grazing animals were used to keep endophyte and clover treatments at a common mean sward height, as measured with a rising-plate meter. This height varied slightly with season.

Measurements

Liveweight and liveweight gain: Animals were weighed monthly following a 5-hour fast and LWG calculated. To relate LW and LWG to intake measurements, LW at the end of the month and LWG for the month during which the intake measurement was conducted, were used in all calculations.

Animal health: Incidence of ryegrass staggers, scored on a 0-5 scale (0 = no visible symptoms; 5 = unable to be moved from paddock, Keogh 1973), was assessed weekly during February to April of each year. Body temperatures were determined on 10, 16 and 25 March 1993, and 23 February and 23 March 1994 using a digital rectal thermometer.

Daily herbage intake: Intake was estimated for periods in December 1992 (summer) and in March of 1993 and 1994 (autumn) using intra-ruminal controlled-release capsules (Captech New Zealand Ltd) containing a matrix of chromium sesquioxide (Cr_2O_3). Faecal grab samples were freeze dried, bulked across days on an equal dry weight basis and ground. Chromium concentration was assessed according to Lee *et al.* (1986) and adjusted for background chromium (of soil and herbage origin). Capsule release rate (RR) was determined from the drop-off in chromium concentration in end-point faecal samples taken from a subgroup of animals. Individual

faecal output (FO) was calculated as $\text{FO} = \text{RR}/[\text{Cr}]$. Group mean herbage intake (I) using an *in vitro* digestibility (D) estimate for each plot, was calculated as $I = \text{FO}/1-D$.

Diet quality and alkaloid concentrations: Hand-plucked samples of herbage were selected by two operators following visual observations of animal grazing behaviour to provide representative diet samples. Each operator plucked from at least 5 sites covering the plot and this herbage was bulked into one sample for the plot. This procedure was repeated on 2 days in week 1, and 2 days in week 2 for December 1992 and March 1993. In March 1994, samples were collected on 3 individual days over a period of 2 weeks. Samples from individual days were bulked, freeze dried, ground, and analysed for *in vitro* digestibility and concentrations of lolitrem B, ergovaline and peramine.

Statistical analysis

For December 1992 and March 1993, main effects and interactions were compared by ANOVA using rep interactions as error. For the modified management in March 1994 with replicate groups pooled, effects were tested against animal interactions.

The period from September 1992 to April 1993 was cooler than average, with a particularly wet December (Table 1). In contrast, 1994 was drier than average during January-March and slightly warmer than average during February.

Table 1 Weather conditions at the trial site during spring, summer and autumn of 1992/93 and 1993/94.

Month	Rainfall		20-year Mean	Mean daily maximum air temperature		20-year Mean
	1992/93	1993/94		1992/93	1993/94	
	mm			°C		
September	85.8	75.2	78.9	12.7	12.7	15.1
October	81.8	59.4	75.7	15.3	16.4	16.7
November	52.2	124.4	59.5	18.0	16.6	18.7
December	197.2	81.8	69.3	19.2	18.8	20.8
January	55.6	30.8	59.6	19.3	22.2	22.6
February	40.4	13.0	50.6	20.2	23.8	22.9
March	68.6	50.6	70.2	19.1	20.2	21.6
April	58.8	45.2	59.9	16.6	18.8	18.8

Results

Herbage intake and animal performance

Summer: Herbage DM intake and LW were similar for each endophyte and clover treatment. The overall mean intake of 8.4 kg DM/head/day for yearling bulls which averaged 409 kg LW represented a mean intake of 2.1% of LW (0.021 kg DM/kg LW/day). Endophyte

status did not affect LWG for the month during which intake was estimated but LWG was significantly higher for clover (1.21 kg/hd/day) than for nil clover (0.91 kg/hd/day) treatments.

Autumn 1993: As for yearling bulls in spring, endophyte did not affect DM intake of weaners (3.71 kg/hd/day), which was 1.8% of LW. However, DM intake (4.0 vs 3.4 kg/hd/day), LW (216 vs 190 kg) and LWG (0.57 vs 0.37 kg/hd/day) were each significantly ($P < 0.05$, $P < 0.01$ and $P < 0.01$, respectively) higher for clover than for nil clover treatments. Ryegrass staggers occurred only on wild-type endophyte plots. Incidence (50%) and mean severity (1.2; 0-5 scale) were low and did not differ between clover levels.

Autumn 1994: Under the rotational grazing management applied to all treatment combinations, endophyte status affected DM intake, weaners on nil endophyte (4.6 kg DM/hd/day) eating significantly more than those on either wild-type or 187BB endophytes (mean 3.7 kg DM/hd/day) (Table 3). This difference was reduced, but still significant when scaled to LW because weaners on nil endophyte (191 kg; 0.024 kg DM/kg LW/day) were heavier than those on wild-type (171.5 kg; 0.021 kg DM/kg LW/day) or 187BB (182.5 kg; 0.021 kg DM/kg LW/day). However, LWG was similar for all endophyte treatments, but higher for clover (0.51 kg/day) compared with nil clover treatments (0.13 kg/hd/day) and higher for leaders (0.76 kg/hd/day) than followers (0.24 kg/hd/day) on clover plots, but leaders and followers on nil clover treatments (0.13 kg/hd/day) did not differ (clover \times management interaction, $P < 0.01$). Ryegrass staggers peak incidence (100%) and peak severity (2.8) were higher in 1994 than in 1993. The peak severity was higher on nil clover (3.7) compared with clover (1.85) treatments, but grazing management had no effect.

Alkaloid concentrations and intakes

Low levels of lolitrem B detected in the nil endophyte and 187BB treatments reflect the low-level contamination by endophyte-infected plants in these plots. For completeness zeros are recorded in Table 3, and in these instances statistical significance refers to the comparison between wild-type and 187BB.

Summer: Diets obtained from 187BB treatments contained effectively zero lolitrem B, but were higher in ergovaline concentration (0.48 mg/kg) than wild-type (0.30 mg/kg) (Table 2). As a result, bulls grazing 187BB consumed more ergovaline (5.4 mg/day) than those grazing wild-type plots (2.9 mg/day). Peramine concentration, and therefore daily peramine intake, were

Table 2 Effect of endophyte strain on diet alkaloid concentrations and daily intake of alkaloids in summer 1992 (December) and autumn 1993 (March).

	Endophyte strain		Statistical significance
	Wild-type	187BB	
Summer 1992			
Lolitre B (mg/kg)	0.48	0.01	***1
Ergovaline (mg/kg)	0.30	0.48	NS
Peramine (mg/kg)	10.51	9.17	**
Lolitre B intake (mg/day)	4.45	0.85	***
Ergovaline intake (mg/day)	2.92	5.44	*
Peramine intake (mg/day)	99.50	107.70	**
Autumn 1993			
Lolitre B (mg/kg)	1.33	0.17	***
Ergovaline (mg/kg)	0.40	0.80	***
Peramine (mg/kg)	33.40	32.49	***
Lolitre B intake (mg/day)	5.27	0.63	***
Ergovaline intake (mg/day)	1.58	3.15	***
Peramine intake (mg/day)	132.70	129.60	***

1 NS = Not significant; ● *, **, • = significant at $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively

similar for both endophyte treatments. The presence or absence of clover did not significantly affect diet alkaloid concentration.

Autumn 1993: Alkaloid concentrations were higher in autumn 1993 than in summer 1992 (Table 2). Trends among endophyte treatments, however, were similar to summer, 187BB having only small concentrations of lolitrem B, but higher ergovaline (0.8 mg/kg) than wild-type (0.4 mg/kg) and similar concentration of peramine (32.95 mg/kg). Daily intake of ergovaline was higher on 187BB (3.15 mg/day) than on wild-type (1.59 mg/day).

Autumn 1994: Diets obtained from 187BB treatments were higher in ergovaline, lower in lolitrem B, and similar in peramine concentration compared with wild-type, under rotational grazing (Table 3). Daily toxin intake reflected these trends in concentrations. Although clover or management did not significantly affect DM intake or alkaloid concentrations, clover \times management and endophyte \times clover interactions were detected in ergovaline intake. Leaders had a similar daily intake of ergovaline at both levels of clover (3.7 mg/day), but followers consumed more ergovaline in the absence of clover (5.7 mg/day) than in the presence (2.6 mg/day). The endophyte \times clover interaction resulted from the higher daily intake of ergovaline in the absence of clover (10.8 mg/day) compared with the presence (6.9 mg/day) on 187BB. Peramine intake was significantly higher for leaders (38.5 mg/day) than for followers (30.9 mg/day).

Table 3 Effect of endophyte level and strain, clover and grazing management on several indicators of animal performance, diet quality, diet alkaloid concentrations and daily intake of alkaloids in autumn 1994 (March), for rotationally grazed cattle.

Grazing management	Endophyte strain and infection level												Statistical significance ³						
	Wild-type						187BB						E ²	C	M	E × C	C × M	E × C × M	
	High			Nil			High			Nil									
	+ clover	- clover		+ clover	- clover		+ clover	- clover		+ clover	- clover		+ clover	- clover		P-value			
L	F		L	F		L	F		L	F		L	F						
Intake (kg DM/hd/day)	3.6			4.6			3.8						*	NS	NS	NS	NS	NS	
Liveweight (kg)	162	174	169	161	214	196	175	177	195	179	165	171		.	.	†	NS	NS	
Liveweight gain (kg/day)	0.56	0.41	0.22	0.06	0.94	0.16	-0.11	0.29	0.77	0.16	0.29	0.05		NS	.	.	NS	**	
Blood prolactin (ng/ml)	35.6	13.6	13.5	26.9	21.2	33.3	26.5	26.5	21.4	31.5	15.6	13.5		NS	NS	NS	NS	NS	
Diet digestibility (g/kg)	665	664	644	662	659	666	654	655	665	646	644	626		***	***	NS	***	NS	
Lolitre B (mg/kg)	1.95			0.0			0.08						.	≡	NS	NS	NS	NS	
Ergovaline (mg/kg)	0.63			0.0			2.33						.	NS	NS	NS	NS	NS	
Peramine (mg/kg)	13.35			0.0			14.80						
Lolitre B intake (mg/day)	7.16	9.20	5.69	5.09	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.13		***	†	†	***	NS	
Ergovaline intake (mg/day)	2.36	2.57	3.20	3.42	0.0	0.0	0.0	0.0	6.67	5.14	7.99	13.55		***	**	
Peramine intake (mg/day)	52.10	46.69	54.44	35.76	0.0	0.0	0.0	0.0	62.99	36.70	61.86	61.36		.	..	†	
Staggers peak score (0-5)	1.7	2.0	3.7	3.7										NA ⁴	.	=	NS	NA	NA
Peak incidence (%)	100	100	100	100															

¹ L, F = Leaders and followers in rotational grazing

² E, C, M = Endophyte, Clover and Management (leaders vs followers) main effects and interactions

³ ***, **, * and NS = Statistical significance at P<0.001, P<0.01 and P<0.05 and not significant, respectively

⁴ NA = Effect not applicable

Animal health

Endophyte and clover treatments had no effect on weaner rectal temperatures in 1993. In 1994, on a cool day (daily max: 17.8°C), weaners grazing 1877BB had slightly elevated (P<0.01) temperature (39°C) compared with wild-type or nil endophyte treatments (38.7°C). On a warmer day (23°C), earlier in the season prior to the peak incidence of staggers, followers on nil clover (39.1°C) had a higher temperature than leaders (38.1°C), but leaders and followers on clover did not differ (clover × management interaction, P<0.001).

Discussion

In general, endophyte infection level and strain had minimal effects on economic performance of dairy beef animals, consistent with results obtained with dairy cattle in other North Island regions (McCallum & Thomson 1994; Thorn *et al.* 1994), but different from results reported for sheep in Canterbury (Fletcher *et al.* 1990). Our results describe LWG and health in relation to both daily DM intake and toxic alkaloid diet concentrations and intake. Estimates of DM intake for each of the three measurement periods are consistent with those predicted from standard feeding tables (NRC 1984), indicating that the technique provided reliable estimates. Although

absolute intake differed between yearlings and weaners, when adjusted for LW to allow comparison across seasons, DM intakes ranged from 1.8 to 2.2% of LW. This level of intake is below what could have been expected with *ad libitum* intake. This reflects the relatively short pasture height maintained to ensure animal exposure to endophyte-related alkaloids, particularly those concentrated towards the base of tillers (e.g., lolitre B). The high intake of weaners on the nil endophyte treatment in 1994 was not reflected in higher LWG and cannot be explained.

Measurements in summer 1992 provide a benchmark for animal intake and diet composition in the absence of clinical effects of endophyte. In contrast, measurements in March were timed to coincide with the peak incidence of staggers. Diet concentrations of lolitre B, ergovaline and peramine were higher in autumn than summer. However, calculation of alkaloid intake per unit of LW shows that ergovaline intakes were approximately similar in summer and autumn, for each endophyte (0.0075 mg/kg LW/day and 0.0144 mg/kg LW/day for wild-type and 187BB, respectively) because of the slightly higher DM intake in summer (0.021 kg/kg LW/day) than autumn (0.018 kg/kg LW/day). This is consistent with the lack of any ergovaline-related symptoms in either summer or

autumn. For lolitrem B, which caused a low severity of staggers in autumn 1993 but none in summer, intake per unit LW more than doubled from summer (0.011 mg lolitrem B/kg LW/day) to autumn (0.026 mg/kg LW/day) and was even higher in autumn 1994 (0.041 mg/kg LW/day). Based on similar calculations, a comparison of autumn 1993 with autumn 1994 shows a large increase in ergovaline intake on both wild-type (0.008-0.017 mg/kg LW/day) and 187BB (0.016-0.048 mg/kg/day), consistent with the detection of elevated body temperatures in 1994. Thus, while the calculations of alkaloid intakes cannot be used as dose responses, they indicate that intakes of lolitrem B at or above 0.026 mg/kg LW/day are required for moderate staggers severity, and similarly, intakes of ergovaline at 0.048 mg/kg LW/day for ergovaline-related effects in cattle, at least under the environmental temperatures experienced in Manawatu. In contrast, Debessai et al. (1993) reported reduced feed intake and reduced LWG in lambs at daily ergovaline intakes as low as 0.026 mg/kg LW/day.

Within a measurement period, alkaloid intakes reflect concentrations in the diet sample because of the similarity among treatments in DM intake. However, between periods, prediction of animal responses (i.e., staggers) is most reliable when alkaloid intakes are related to LW. Predictions based on pasture assessment only (pasture on offer or a diet sample) are unlikely to be reliable when treatment affects intake.

While endophyte affected body temperature, heat stress was not observed. The elevated temperature can be related to the higher ergovaline intake on 187BB compared with wild-type endophyte, and for weaners grazed as followers on nil clover plots. Thus the potential for heat stress exists, but for visible symptoms, higher ambient temperatures may be required.

The grazing management contrast between leaders and followers resulted in predictable effects on LWG. However, effects on the diet were less clear. The dry conditions during summer 1994 severely reduced pasture growth rate. The contrast in pasture mass and composition offered to leaders and followers diminished with each successive rotation, compared with the contrast when the experiment began in January, such that each group was grazing drought-affected pasture with a high proportion of dead material.

Conclusions

Direct effects of endophyte alkaloids on cattle intake and liveweight gain were small and influenced by season. Where effects on cattle are likely, use of selected lolitrem B-free endophyte, encouragement of high clover proportion in pastures and avoiding grazing to low residuals will reduce the severity of staggers.

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