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The relationship between staggers and diarrhoea in lambs grazing different components of endophyte-infected ryegrass.

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ABSTRACT

Lambs grazing ryegrass infected with endophyte (*Acremonium lolii*) may exhibit staggers or diarrhoea or both, with little correlation between these two conditions. Associated mycotoxins/toxins other than the staggers-inducing lolitrems are likely to be responsible for metabolic changes causing reduced intake, elevated temperature and diarrhoea. These mycotoxins are not necessarily confined to the base of the leaf sheath and stem as is the case for lolitrems. The distribution of mycotoxins to proximal portions of vegetative ryegrass and the effect of these plant components on grazing animals was investigated.

Following experimental confirmation in 1991 of nil correlation between staggers and diarrhoea, groups of lambs were grazed in autumn 1992 on 100% endophyte-infected hybrid ryegrass cv. Grasslands Marsden (*Lolium boucheanum* Kunth.), using a leader-follower system. Upper and lower leaves, and pseudostem were progressively removed by four groups of lambs on three day breaks, replicated twice.

Liveweight gain ranged from 164 g/day in lambs having first offer, to 28 g/day in those grazing the base of the plants ($P<0.01$). Rectal temperature averaged 40.2°C being slightly above normal in all treatments. Faecal moisture was high; ranging from 79-87% throughout the experiment with no significant differences due to treatment. Observed faecal soiling was greatest in lambs receiving high leaf component ($P<0.05$). Clinical staggers, barely detectable in lambs grazing leaf, became severe as the proportion of pseudostem in the diet increased ($P<0.01$).

In contrast to the clear association between endophyte and staggers in lambs grazing the base of infected ryegrass, consistently high faecal moisture, and consequent faecal soiling occurred regardless of the part of the plant consumed, but tended to be greatest in lambs grazing leaf. Analysis of plant components suggested that peramines may be associated with this problem.

Keywords: Endophyte, diarrhoea, faecal soiling, faecal moisture, mycotoxins, peramine.

INTRODUCTION

Perennial ryegrass (Lolium perenne L.) continues to be the dominant grass in New Zealand's developed pastures. Its persistence is substantially enhanced by infection with endophyte (*Acremonium lolii* Latch, Christesen and Samuels) (Prestidge *et al.*, 1982, Fletcher *et al.*, 1990). There are however a number of detrimental features affecting animal productivity associated with endophyte infected ryegrass.

The neuromuscular disorder ryegrass staggers is paramount and can be attributed to the endophyte mycotoxin - lolitrem B. (Gallagher *et al.*, 1984, Fletcher *et al.*, 1992). Among other effects, diarrhoea in animals consuming infected ryegrass is a major problem. Many of the diarrhoeic conditions observed in grazing ruminants, for which no common aetiology can be established, are nutritionally induced and due to mycotoxicosis (Familton, 1988). Largely unmeasured, the contribution by endophyte related mycotoxins to faecal soiling, represents a significant cost to the sheep production industry (Pownall, 1992).

There is an absence of published work on the association between diarrhoea and infected ryegrass. Results obtained by (Eerens *et al.*, 1992) established a clear link between faecal soiling and ryegrass containing endophyte. Unreported work by the authors has shown that in both pen-fed and grazing situations, animals exhibited highly significant differences in degree of staggers, faecal moisture and soiling when fed ryegrass either free of, or infected with endophyte. Individual animals receiving infected ryegrass varied widely in degree of staggers exhibited, indicating either an ability by some to select against parts of the plant high in lolitrems or genetic variation in susceptibility. Faecal moisture, although varying between individuals, was consistently higher in animals receiving endophyte-infected ryegrass. The degree of faecal soiling followed the same trend but was less consistent. An experiment conducted in autumn 1991 examining the possible correlation between ryegrass staggers, faecal moisture and faecal soiling is described in experiment 1.

Lolitrem B is known to be confined to the leaf sheath and reproductive portions of the plant (Fletcher *et al.*, 1990). Other mycotoxins, in particular peramine, appear to be more widely distributed. A second experiment to examine the effects of progressive removal of plant components on the animal, while monitoring concentrations of mycotoxins, was carried out in autumn 1992 and is described in experiment 2.

These experiments were conducted in the absence of gastrointestinal parasitism.

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MATERIALS AND METHODS

Experiment 1:

Two groups of 32 lambs rotationally grazed ryegrass/ white clover pastures containing cv. Grasslands Nui (*Lolium perenne* L.) which was either nil or 80% endophyte infected. Coopworth ram lambs were given weekly breaks in eight sub-paddocks of each pasture type for thirteen weeks in autumn 1991. Pasture allowance was limited to near maintenance, animals being required to graze to low residuals.

Pasture mass on offer, residual and disappearance for each treatment was assessed visually, correlated with pasture height measurements and confirmed by cuts before and after each grazing. Endophyte activity was monitored by assessment of hyphal filament numbers from dissected leaf sheath using the method described by Harvey *et al.* (1982).

Animals were weighed, sampled for faecal moisture and assessed for degree of staggers and faecal soiling every two weeks. Anthelmintic drench was routinely administered and faecal egg and pasture larval counts monitored.

Experiment 2:

Eight groups of eight animals grazed 100% endophyte infected pure cv. Grasslands Marsden hybrid ryegrass, (*Lolium boucheanum* Kunth.), to four residual pasture heights in two replicates. Coopworth ewe lambs were grouped by liveweight, and randomly allocated to treatment flocks. These flocks entered the four pasture treatments in each replicate progressively over ten days using a grazer-follower system. Each successive treatment continued receiving the residual pasture left by the preceding treatment. The experiment ran for 38 days in March-April 1992.

Pasture mass on offer increased from 3500 to 6000 kg DM/ha during the period and consequently the size of the grazing breaks was decreased over time from 0.043ha. to 0.027ha. Break size was largely determined by the ability of sheep in the initial treatment to remove the upper pasture horizon to the required level in three to four days. Pasture mass on offer, residual and disappearance for each treatment group was assessed visually and confirmed by pasture cuts.

Endophyte activity was monitored each week by assessing dissected leaf sheath for numbers of hyphal filaments (Harvey *et al.,* 1982). Pasture height measurements were taken in all treatments, as were samples for dissection into plant components, to monitor pasture horizons and the material consumed in each treatment. Dissected newest, second and oldest leaves, upper pseudostem, lower pseudostem and dead sheath were sampled at the start of the experiment and towards the end. Samples of each of these parameters were freeze dried and ground for alkaloid analysis (Davies *et al.* 1993).

Animal measurements included fasted liveweights at the start and finish of the experiment and weekly monitoring of rectal temperature, faecal moisture and degree of staggers. Anthelmintic drench was routinely administered and faecal egg counts monitored.

RESULTS

Experiment 1:

Average pasture mass at the start of each weekly break, residual and calculated disappearance figures for each treatment are shown in Table 1, together with liveweight gain, staggers incidence and averages for faecal moisture and degree of soiling.

Endophyte activity, or frequency of occurrence of hyphal filaments per dissected leaf sheath, peaked near the start of the experiment at 2.4 and gradually declined to 0.6 toward the end. While there was a gradual recovery by animals exhibiting ryegrass staggers, there was no reduction in faecal moisture percentage over time, with animals receiving high endophyte pasture having 8.6% higher faecal moisture than those on nil endophyte, throughout the experiment.

There was no correlation between staggers and faecal soiling. Linear regression of (i) incidence of staggers against faecal moisture percentage and (ii) incidence of staggers against degree of faecal soiling accounted for less than 1% of the variation in each case. Regression of faecal moisture percentage against degree of faecal soiling accounted for 15% of the variation.

Experiment 2:

Table 2 shows that as pasture mass and height reduced with each subsequent grazing treatment, pasture disappearance and resulting liveweight gain were reduced ($P<0.01$).

The grazing treatment effect on incidence and severity of ryegrass staggers ($P<0.01$), faecal moisture percent (N.S.) and faecal soiling ($P<0.05$) are shown in Table 3.

Ryegrass staggers incidence and severity increased as the removal of leaf by initial grazing treatments forced the consumption of increasing proportions of pseudostem in latter grazing treatments. Faecal moisture was high in all treatments averaging 83% and with a range throughout the experiment from 79-87%. There were no significant differences in faecal moisture due to treatment. Faecal soiling, visually assessed, was significantly greater ($P<0.05$) in animals being offered high pasture allowances; (treatments 1 & 2). Endophyte activity became less obvious as the trial pro-

TABLE 1: Experiment 1 (autumn 1991) pasture mass on offer, residual, disappearance, liveweight gain, staggers incidence, faecal moisture and degree of soiling (0–nil, 5–high) for each treatment.

Treatment	Pasture mass on offer kg DM/ha	Pasture mass residual kg DM/ha	Disappearance kg DM/head/d	Liveweight gain g/d	Staggers % incidence	Average faecal moisture %	Soiling; average dag score (0-5)
1. Nil endophyte	2760	890	0.98	13.0	0	61.9	0.3
2. High endophyte	2810	1030	0.95	13.0	36	70.5	1.0

TABLE 2: Experiment 2 (1992) pasture mass on offer, residual height, disappearance and liveweight gain for each treatment (P< 0.01).

Treatment No.	Pasture mass on offer kg DM/ha	Pasture height, residual cm	Pasture disappearance kg DM/head/d	Liveweight gain g/d
1	5080	11	1.6	164
2	3780	8	1.4	145
3	2750	6	1.0	71
4	2010	4	0.6	28

TABLE 3: Experiment 2 (1992) incidence and severity of ryegrass staggers, faecal moisture and degree of soiling (0=nil, 5=high) for each treatment.

Treatment	% Staggers			Mean faecal	Faecal soiling
	Nil	Slight	Severe	moisture %	(0 - 5 scale)
1	98	2	0	82.9	2.5
2	90	8	2	84.5	3.1
3	42	44	14	83.6	1.9
4	31	29	40	81.2	1.1

gressed with 3.5 hyphal filaments per dissected leaf sheath being observed at the start of the experiment, and 0.6 at the finish. Alkaloid levels from plant components averaged from two samplings in the first and fourth weeks are shown in Table 4.

TABLE 4: Experiment 2 (1992) mean alkaloid levels in plant components from two samplings in March and April. (Adapted from Davies *et al.* 1993).

Ryegrass plant component	Lolitrem B (ppm)	Peramine (ppm)	Ergovaline (ppm)	Paxilline (ppm)
1. Upper leaf (newest)	0.4	25.9	0.3	5.2
2. Second leaf	0.9	22.3	0.4	5.3
3. 3rd and 4th leaves (oldest)	1.9	22.1	0	4.3
4. Upper pseudostem	2.7	23.9	0.6	4.0
5. Lower pseudostem	3.9	25.4	1.5	2.9
6. Dead (sheath)	8.8	7.8	0.3	3.4

Lolitrem B showed higher concentrations toward the base of the plant and had declined by approximately 50% at the time of the second sampling. By contrast peramine was evenly distributed throughout the live plant tissue and remained relatively constant between the first and fourth weeks. Ergovaline was present in the lower pseudostem with correspondingly less in the upper pseudostem and leaf, levels remaining similar at each sampling. Paxilline was evenly distributed throughout the plant initially, but tended to maintain these levels only in leaf fractions at the second sampling.

Rectal temperature averaged 40.3°C ranging from 39.7 - 40.5°C. There were no significant differences in temperature due to treatment.

DISCUSSION

In each of the experiments ingestion of high concentrations of lolitrem B in pseudostems was related to the incidence of ryegrass staggers. The highly significant treatment effect shown in each of these experiments was entirely consistent with other reported work (Fletcher *et al.*, 1990).

Pasture allowance in experiment 1 was restricted by break size to force the consumption of leaf sheath and pseudostem in each treatment. Faecal moisture was considered to be a more accurate and objective parameter than faecal soiling given the low correlation shown by regression of faecal moisture and faecal soiling.

Results obtained in this experiment, supported the hypothesis that staggers and high faecal moisture are caused by different toxins. The results suggested that mycotoxins/toxins other than lolitrems may be present in upper horizons of the pasture canopy. Consequently an attempt was made to progressively remove specific horizons by grazing manipulation the following year in experiment 2.

In experiment 2, visual observations together with height measurements and pasture yield cuts, showed a relatively even and progressive removal of upper leaves, lower leaves and then pseudostem as lambs in each sequential treatment finished grazing. The success of the grazing system used can be partly attributed to the atypical pasture which was a dense high mass sward of pure ryegrass. Initial treatments (treatments 1 & 2), did not differ greatly in pasture consumption or resultant liveweight gain. Lambs in both treatments were offered high proportions of young leaf. Despite the relatively high pasture mass on offer in treatments 3 and 4, animals were forced to consume pasture remaining in lower horizons consisting of necrotic leaf and pseudostem. This resulted in markedly reduced liveweight gains.

In contrast to lolitrem B and ergovaline, peramine and paxilline were evenly distributed throughout the plant. Peramine however, showed higher levels than paxilline. With lambs in initial grazing treatments consuming a high proportion of leaf and the high faecal moisture exhibited by these treatments, it is possible that peramine may be contributing to this problem. Its equal presence in older leaves, and pseudostem, coupled with high faecal moisture in treatment animals grazing these lower horizons throughout the period of the experiment, is consistent with this observation. Paxilline tended to maintain levels only in leaf fractions at the second sampling and while its effects remain unclear, ergovaline does not appear to have contributed to any of the treatment effects in this experiment.

Faecal moisture in experiment 1 was 8.6% higher in animals grazing high endophyte ryegrass compared to endophyte free. Unreported experimental work by the authors has shown 15-20% higher faecal moisture in animals grazing endophyte-infected ryegrass, and while there was no endophyte-free Marsden ryegrass pasture available for use as a control treatment in experiment 2, animals from the same flock grazing a range of adjacent pastures had an average faecal moisture of 68% which was 15% less than in the experiment.

In experiment 2 lambs in all treatments had very high faecal moisture. The significant increase in faecal soiling in treatments 1 and 2 was attributed to the increased faecal output resulting from higher pasture consumption, even though faecal moisture was no higher than in lambs grazing the base of the sward. Other factors contributing to the increased faecal soiling in treatments 1 and 2, included the reduced grazing time and frequent defecation while resting which was observed in these well fed lambs.

The prevention of a confounding effect on faecal softening by gastrointestinal parasitism was achieved by routine anthelmintic administration and the use of "safe pasture". This was confirmed by an absence of parasite eggs in the faeces and third stage larvae on pasture.

CONCLUSION

There is little or no correlation between staggers, diarrhoea and resultant faecal soiling in sheep grazing ryegrass infected with endophyte. Mycotoxins, other than the staggers inducing lolitrem B, which differ in both concentration and distribution within the plant appear to be involved.

Peramine, which offers protection to the plant against stem weevil attack, may be a possible cause of the metabolic disruption in animals which results in diarrhoea. Future research needs to focus on identifying the effects that peramine and other alkaloids in endophyte infected ryegrass may have on animal health.

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