"Non-toxic" endophytes in ryegrass and their effect on livestock health and production

L.R. FLETCHER
AgResearch, P.O. Box 60, Lincoln
fletcherl@agresearch.cri.nz

Abstract

Ryegrass/endophyte associations have been developed which produce peramine, but not the endophyte toxins ergovaline or lolitrem B. Sheep grazing these ryegrasses in small paddocks and in a systems trial over three years gained weight as rapidly as those grazing endophyte-free ryegrass, and showed none of the adverse responses associated with grazing ryegrass naturally infected with wild-type endophyte (*Neotyphodium lolii*). Growth rates, rectal temperatures, respiration rates, serum prolactin concentrations, ryegrass staggers scores and scores for dag burdens were equivalent for the selected endophyte and nil endophyte treatments, whereas sheep grazing the wild-type endophyte treatment were adversely affected for all parameters. This was reflected in calculated gross margins for the systems trial of only $605 per hectare for the wild-type endophyte treatment compared with over $700 per hectare for each of the other treatments. To date there have been no conclusive studies of non-toxic endophyte with cattle.

Keywords: AR1, dags, endophyte, ergovaline, liveweight gain, lolitrem B, *Lolium perenne*, *Neotyphodium*, peramine, perennial ryegrass, prolactin, sheep

Introduction

The advantages of wild-type endophyte in perennial ryegrass for insect deterrence and increased persistence, and the disadvantages to the performance and health of grazing animals are well documented (Fletcher & Harvey 1981; Prestidge *et al.* 1982; Fletcher *et al.* 1990; Fletcher 1993; Fletcher & Easton 1997; Fletcher *et al.* 1999).

The potential to capitalise on bio-control elements of this mutualistic relationship while minimising or eliminating animal toxicoses became an obvious objective. Numerous prophylactics and pharmaceuticals have been used in an attempt to prevent or treat ryegrass staggers in New Zealand but to date there are no confirmed successes (Fletcher 1998). Dopamine antagonists, such as Domperidone™, have been effective in alleviating some symptoms associated with the endophyte toxin, ergovaline, but they are relatively expensive and require repeat administrations if the animals continue to graze toxic pastures. Vaccines have been proposed but again there are no reports or knowledge that these have been developed, or that there are any grounds for optimism using this approach to reduce or eliminate endophyte toxicosis. Breeding of resistant animals has been proposed and while research continues in this field, it is likely to be some time before resistant animals are available commercially (Morris *et al.* 1999).

Therefore if the insect bio-control benefits were to be retained, the only option for preventing livestock toxicosis was to eliminate the intake of the toxins, lolitrem B and ergovaline. To achieve this, the potential to produce these toxins had to be eliminated from the ryegrass/endophyte association.

'Endosafe' (187 BB) was the first endophyte strain selected which did not produce the neurotoxin lolitrem B, but was subsequently found to produce higher levels of ergovaline in perennial ryegrass than wild-type associations sold in New Zealand (Davies *et al.* 1993). Sheep grazing perennial ryegrass infected with this strain developed only minor ryegrass staggers compared to the same ryegrass cultivars with their natural endophyte (Fletcher *et al.* 1991), but liveweight gains were no better than on wild-type treatments (Fletcher *et al.* 1993; Fletcher, unpublished data). Most other adverse responses to wild-type infected perennial ryegrass were evident in sheep grazing perennial ryegrass with 'Endosafe', so it was withdrawn from the market. 'Grasslands Pacific' perennial ryegrass, with which it was associated, is available in the market with its wild-type endophyte.

Endophytes which still produce the insect deterrent, peramine, without the mammalian toxins, ergovaline and lolitrem B, have now been isolated and inoculated into perennial ryegrass (Latch *et al.* 1985; Tapper & Latch 1999). Evaluation of these non-toxic associations in sheep, beef and dairy cattle grazing trials has begun and to date there are no indications of major toxicity problems. However further trials, especially with beef and dairy cattle, are needed to confirm these results and test the persistence of the non-toxic associations in different regions throughout New Zealand. Almost all studies to date have been with the non-toxic endophyte AR1 in perennial ryegrass cv ‘Grasslands Nui’. These trials and the current knowledge on animal responses to non-toxic endophytes, including AR1, in perennial ryegrass are discussed.

Most studies so far have been conducted with sheep, because they are the smallest of the farmed ruminants, and therefore the most efficient models to begin with when seed supplies are limited. Sheep also make a major contribution to New Zealand’s primary production industry. There have been two replicated sheep grazing studies with the lolitrem B and ergovaline-free endophyte, AR1, in Nui ryegrass over the last three years.

Trial 1: Intensive intermittent grazing

Materials and methods

Three replicated paddocks (0.15 ha) of perennial ryegrass (*Lolium perenne* L.) cv Grasslands Nui with its wild-type endophyte, AR1 endophyte or no endophyte were drilled into a well cultivated Wakanui silt loam at Lincoln in 1995, following two cereal crops.

Paddocks were sprayed with 400 g ai/ha of Dicamba each spring to eliminate any volunteer clover and weeds. These ryegrass pastures (no clover) were grazed by a minimum of 10 Coopworth ewe hoggets or lambs per paddock for a minimum period of four weeks in spring (early October–end of November), summer (early January–end of February) and autumn (early March–end of April) from autumn 1996 to autumn 1999.

Herbage dry matter on offer was measured before each grazing from clipped quadrats. Herbage samples, cut to ground level, were taken from each paddock before and midway through each grazing for peramine, lolitrem B and ergovaline analysis (Barker *et al.* 1993).

Animal measurements included all responses known to be affected by wild-type endophyte (Fletcher & Easton 1997). Individually tagged hoggets/lambs were weighed, using Trutest electronic scales, after an 18-hour fast immediately before, at a mid-point and end of each grazing period. All lambs/hoggets were scored for dags, on a 0–5 ascending scale, each time they were weighed (Fletcher 1993). All animals were scored weekly for ryegrass staggers beginning 10 days after the start of each summer/autumn grazing (Keogh 1973). Rectal temperatures were measured using a Beckton Dickonson veterinary digital thermometers, while respiration rates were measured by counting respiratory, thoracic or flank, movements over a 30-second period, indicated by a pre-set audible alarm. Lambs/hoggets were minimally restrained in specially constructed ‘herringbone’ type bales for these measurements.

The effect of endophyte on body temperature and respiration rate only becomes evident when the animal’s thermo-regulatory system is challenged. This of course will be largely dependent on ambient conditions. Such conditions may be rare, short term or fluctuate rapidly in some environments. To eliminate such variability and ensure the animal’s thermo-regulatory system was challenged, lambs or hoggets on these trials were taken from their paddocks and housed for three hours in a room where ambient temperature and humidity were increased to >30°C and 70% relative humidity. Rectal temperatures, respiration rates and prolactin levels where then measured under these conditions.

Respiration rate and rectal temperatures of a healthy resting lamb/hogget, on a cool day (12–15°C) are normally in the range of 20–30 breaths per minute and 39.6–39.9°C respectively.

Results and discussion

Herbage

There was no significant difference in herbage on offer between treatments at the start of any grazing. Mean alkaloid concentrations in herbage are presented in Table 1.

The absence of significant levels of lolitrem B in the endophyte-free and AR1 treatments confirms there was no significant contamination of these treatments by ryegrass with wild-type endophytes. Alkaloid concentrations show a clear seasonal trend with low concentrations in spring and highest concentrations in summer/autumn. Levels were in the ‘normal’ range for these treatments (Tapper & Latch 1999).

Animal responses

There was no significant difference between sheep grazing AR1or endophyte-free treatments in any of the animal parameters measured.

Table 1 The effect of endophytes on mean seasonal alkaloid concentrations (ppm) in Nui ryegrass (Trial 1).

	Spring			Summer			Autumn		
	Per[¶]	Lol B	Ev	Per	Lol B	Ev	Per	Lol B	Ev
Wild –type	9.4	1.7	0.5	25.7	3.8	0.8	19.7	3.6	1.1
Nil	0	0	<0.1	0.4	0	<0.1	0	<0.1	0
AR1	11.3	0	<0.1	26.1	0	<0.1	19.7	<0.1	0

¶ Per = peramine, Lol B =Lolitrem B, Ev = Ergovaline. Limit of detection for all alkaloids 0.1 ppm

Liveweight change: Liveweight gains in lambs from wild-type endophyte treatments were 82% lower than for lambs on other treatments in summer and autumn when endophyte toxins were at their highest, but only 17% lower in hoggets in spring when toxin levels were only beginning to increase (Tables 2 and 3). The smaller reduction in liveweight gains in spring is most likely a reflection of lower ambient temperatures and lower levels of endophyte toxins.

Hyperthermia: Body temperatures and respiration rates in lambs from wild-type treatments were higher than for the other treatments in summer and autumn, but again the difference was much smaller or non-significant in spring. The similar rectal temperatures and respiration in lambs grazing ryegrass with AR1 endophyte and without endophyte, is further evidence that ergovaline is the main alkaloid associated with hyperthermia in animals grazing ryegrass and tall fescue infected with wild-type endophyte.

Plasma prolactin: Prolactin levels are seasonal, being much higher in late spring than in autumn. Levels in lambs grazing endophyte-free or AR1 treatments were 100% higher than those from wild-type treatments in summer and autumn and over 200% higher in spring (Tables 2 and 3). Prolactin level was used as an indicator of ergovaline ingestion in this study but may also be a mediating factor in other responses (Fletcher *et al.* 1999).

Ryegrass staggers: Ryegrass staggers was confined to the summer/autumn grazings and was moderate to severe in lambs grazing wild-type treatments, with none in lambs grazing endophyte-free ryegrass (Table 3). There were some tremors detected in lambs grazing AR1 which are believed to be associated with low levels of tremorgens other than lolitrem B, but they were of much lower severity than the staggers suffered by sheep on wild-type swards, and are considered to be of little significance to a farming operation. The impact of ryegrass staggers can be substantial in a farming system where it disrupts normal stock management (Fletcher *et al.* 1999).

Dags: The overall incidence of dags has been low in these trials but again they were worse in lambs/hoggets grazing ryegrass with its wild-type endophyte in both summer and autumn (Tables 2 and 3).

Along with individual animal variation there was some year-to-year variation in the magnitude of responses. The significance of these responses to farming systems is discussed later in this paper in relation to the systems grazing trial (Trial 2) and by Fletcher *et al.* (1999).

Trial 2: Systems grazing

Materials and methods

In a second trial, replicated one hectare farmlets (six paddocks in each) were established in perennial ryegrass cv Grasslands Nui without endophyte or with its wild-type endophyte or with one of three ergovaline and lolitrem B-free endophytes (including AR1) in autumn 1996. The trial was drilled into a well cultivated Wakanui silt loam following a cereal and a pea crop. All three treatments were sown at rate of 15 kg/ha for ryegrass and 3 kg/ha of white clover (cv. 'Grasslands Demand'). For the first two years, this trial was irrigated by overhead sprinkler system (approximately 5 × 40 mm) and then was run as a dryland unit for the third year. Monthly herbage measurements included pasture cover and composition, as well as peramine, lolitrem B and ergovaline levels (Barker *et al.* 1993).

Table 2 Lamb responses to grazing Nui ryegrass with AR1 endophyte in summer and autumn. Means of 3 years.

Mean	LWG‡ g/hd/day	Rectal temp °C	Resp rate /min	PRL ng/ml	RGS 0–5 scale	Dags 0–5 scale
Nui/wild-type	23[a¶]	40.5[a]	97[a]	96[a]	3.2[a]	0.6[a]
Nui/nil	120[b]	40.0[b]	73[b]	185[b]	0[b]	0.2[b]
Nui/AR1	131[b]	40.1[b]	79[b]	203[b]	0.3[b]	0.3[b]

¶ Values within a column with a letter subscript in common are not significantly different
‡ LWG = Liveweight gain, Resp = respiration, PRL = prolactin, RGS = ryegrass staggers

Table 3 Hogget responses to grazing Nui ryegrass with AR1 endophyte in spring. Means of 2 years.

Mean	LWG‡ g/hd/day	Rectal temp °C	Resp rate /min	PRL ng/ml	RGS 0–5 scale	Dags 0–5 scale
Nui/wild-type	165[a¶]	40.3[a]	93[a]	101[a]	-	1.4[a]
Nui/nil	191[b]	40.0[a]	83[a]	333[b]	-	0.6[b]
Nui/AR1	212[b]	40.2[a]	88[a]	344[b]	-	0.4[b]

¶ Values within a column with a letter subscript in common are not significantly different
‡ LWG = Liveweight gain, Resp = respiration, PRL = prolactin, RGS = ryegrass staggers

Coopworth breeding ewes (15/ha irrigated, 10/ha dryland) and their lambs (September–April) rotationally grazed the six paddocks in their allotted farmlet throughout the year. Animal measurements were taken at least monthly, and included ewe liveweights, lamb liveweights, dag scores (when applicable), rectal temperatures, respiration rates and plasma prolactin levels. Other measurements taken when appropriate included wool weights, ryegrass staggers scores and flystrike. Measurement techniques were the same as for Trial 1.

Results and discussion

Herbage

Alkaloid concentrations in the herbage were typical for the treatments where endophyte infection levels were greater than 90% for wild-type and non-toxic endophyte treatments and less than 2% for endophyte-free treatments (Tapper & Latch 1999) (Table 4). There was again a clear seasonal profile with all alkaloid levels peaking in summer or autumn. The absence of significant levels of lolitrem B in non-toxic and endophyte-free treatments confirms the absence of contamination of these treatments by ryegrass with wild-type endophyte.

Table 4 The mean seasonal alkaloid concentrations (ppm) of Nui perennial ryegrass infected with wild-type and selected non-toxic endophytes and the nil-endophyte Nui ryegrass control (Trial 2).

	---------- Alkaloid concentration (ppm)† ----------			
	Winter	Spring	Summer	Autumn
Peramine				
Wild-type	0.8	6.5	13.5	22.0
Nil	0	0.1	0	0.6
Non-toxic¶	1.0	6.0	14.3	10.8
Lolitrem B				
Wild-type	0.5	0.6	2.2	3.8
Nil	<0.1	0	<0.1	0
Non-toxic	0	<0.1	0	<0.1
Ergovaline				
Wild-type	0.2	0.6	1.2	0.7
Nil	0	<0.1	<0.1	0
Non-toxic	0	0	<0.1	0

¶ Endophytes selected for absence of ergovaline and lolitrem B toxins
† Limit of detection for all alkaloids 0.1 ppm

There was always most herbage on the wild-type farmlets especially in late spring and autumn. Non-toxic endophyte farmlets had pasture cover values similar to those on wild-type endophyte farmlets, except for late summer and early autumn, when there was 10% less than on wild-type farmlets in year three. This difference was no longer evident by late autumn. The difference between wild-type and endophyte-free systems increased each year so that in summer and early autumn of the third year there was 50% more herbage on wild-type farmlets. While this difference is large it occurred at a time when overall pasture cover was low in a dryland system and some recovery in late autumn reduced the overall impact on animal performance. The trial was a self-contained systems grazing trial and as such protocol did not allow for adjustment of stock numbers in response to fluctuations in available pasture. Any difference of significance to the farm would be reflected in sheep performance or the need for supplementary feed inputs. The lower herbage on offer on both endophyte-free and non-toxic treatments, in summer and early autumn, was most likely a reflection of higher palatability to sheep, greater insect damage and possibly lower drought tolerance. There was more dead material in wild-type than the other two treatments but endophyte-free treatments had more clover than wild-type and non-toxic endophyte systems, especially from November to January. The greater herbage mass on the wild-type treatments did not translate to increased animal production however.

Animal

There was no difference, measured or observed, in any of the animal responses, between endophyte-free and non-toxic treatments.

Lambing percentage: Mean lambing percentage was 178% with no significant difference between treatments.

Ewe liveweight: There was no difference in ewe liveweight throughout the first year. In years two and three, ewes on the wild-type treatments were 3–6 kg lighter than those from the other two treatments, in summer. However, by mating in mid-March the difference was negligible.

Lamb liveweight: Growth rate of lambs was always slightly lower on wild-type than on the other two treatments, and differences in liveweight gradually increased over the season. The largest difference in growth rates occurred from December to April resulting in 10–15% better overall growth rate for lambs grazing on endophyte-free and non-toxic endophyte systems.

Dags: Lambs and ewes grazing wild-type endophyte treatments had a significantly higher incidence and severity of dags, especially in autumn. Although the increase in dags in sheep is a problem associated with grazing ryegrass with wild-type endophyte there is considerable individual animal, seasonal and year to year variation in the magnitude of the response. This suggests an interaction with other factors within the animal and/or the grazing environment. The endophyte toxin associated with dags has not been identified.

Flystrike: There were three times more ewes and lambs flystruck on wild-type treatments than on the other two treatments (<8% for endophyte-free and non-toxic endophyte systems, >23% for wild-type, averaged over three years). Increased flystrike is one of the most costly problems associated with endophyte apart from reduced liveweight gains. While incidence of flystrike is closely correlated with incidence of dags, the influence of other factors associated with grazing endophyte infected ryegrass cannot be discounted.

Hyperthermia: On warm days in summer and autumn (>23°C in Canterbury), mean rectal temperatures and respiration rates were always higher in ewes grazing wild-type treatments. Plasma prolactin levels measured from samples taken at the same time were significantly lower in ewes grazing wild-type treatments. These measurements and samples were taken under field conditions.

Ryegrass staggers: Ewes and lambs grazing the wild-type treatment had moderate to severe ryegrass staggers in summer and/or autumn in all years, while those grazing non-toxic and endophyte-free treatments showed no symptoms. Lamb deaths due to ryegrass staggers ranged from 5–11% over three years. Moving ewes and lambs, as part of the normal paddock rotation, took up to 3.5 times longer on wild-type treatments when ryegrass staggers was a problem.

Economic analysis

The following economic analysis is based on results, actual costs and returns from the systems grazing trial, using high fertility ewes (178% lambing). Assumptions used included a wool price of $2.50/kg and a lamb value of $1/kg liveweight. Gross returns calculated per hectare were $605 for wild-type treatments, $719 for endophyte-free and $737 for AR1 and non-toxic endophyte treatments. Extra costs associated with grazing perennial ryegrass with wild-type endophyte over and above those for ryegrass without endophyte or with AR1 and other non-toxic endophytes include:

- Extra dagging $18 ha
- Flystrike (surveillance and treatment) $70 ha
- Extra time to move sheep 2.5–3.5 times longer
- Pelt damage (income foregone)
- Withholding period for flystruck lambs before drafting

Dairy cows

There is limited evidence of toxic responses in either dairy or beef cattle to the ryegrass/wild-type endophyte alkaloids, lolitrem B and ergovaline (Easton *et al.* 1996; Clark *et al.* 1996; Blackwell & Keogh 1999; Easton & Couchman 1999). Both of these alkaloids have been eliminated from AR1 and other non-toxic endophytes (Tapper & Latch 1999).

Dairy cow response to non-toxic endophytes including AR1 in ryegrass *per se* has not been studied. However AR1 in Nui ryegrass was part of an ergovaline and lolitrem B-free treatment, which also included endophyte-free ryegrass and endophyte-free tall fescue, in an 'on farm' demonstration trial. Milk production from cows grazing these treatments was higher, especially in summer and autumn, compared to those grazing ryegrass with wild-type endophyte (Blackwell & Keogh 1999). There were no differences in measured body temperatures or respiration rates but on at least one occasion clinical symptoms of heat stress were observed in 19% of the cows grazing ryegrass with wild-type endophyte but in less than 4% of those grazing the ergovaline-free treatments. The ambient temperature at the time was around 26–27°C with a light humid north east breeze. Overnight minimum temperatures however were 18–20°C. Ryegrass staggers was also a significant problem on the wild-type endophyte farmlets only in 1997/98 season.

In recent years there have been many reports, from several regions, of ryegrass staggers in dairy cows. In some cases cows have been dried off prematurely because they were unable to be milked (Fletcher 1998). The elimination of ryegrass staggers, through removal of lolitrem B from non-toxic endophytes such as AR1, has the potential for a significant impact on dairy farms in some regions.

While there is limited evidence of improved health and performance of beef and dairy cows grazing AR1 or other non-toxic ryegrass/endophyte associations, there is an apparent potential for improvement.

Conclusions

Only time will determine how persistent these new non-toxic grass/endophyte associations will be and they have not been available for long enough to make reliable predictions at this stage. Results to date are encouraging (Popay *et al.* 1999).

There have been no serious adverse effects from grazing AR1, in the animals studied, albeit in a limited range of environments and in only one ryegrass cultivar.

The similar growth rates of lambs grazing AR1 and endophyte-free ryegrass during periods of peak endophyte toxicity suggests that any undetected subclinical effects are likely to be minor or inconsequential.

The philosophy that there is often a cost to progress, can apply to biological systems as well, as there is often a "trade off". Two toxins have been removed from the natural ryegrass/endophyte association in New Zealand. What was their purpose, and what has been the cost of their removal?

The development of non-toxic endophytes has been a quantum leap in pastoral agriculture, but it is only one factor in a very complex pasture ecosystem. If new non-toxic endophyte/grass associations, such as AR1, are established and managed well within a grazing system, they are likely to provide a sustainable and environmentally acceptable improvement in animal health and production.

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