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The effect of endophyte alkaloids on diet selection by sheep

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ABSTRACT

Sheep have a strong preference for clover in summer. One explanation for this may be the avoidance of alkaloids such as lolitrem B, ergovaline and peramine that are produced at that time of the year by endophytic fungi in ryegrass. We tested this hypothesis by exposing groups of three sheep to three food items (ryegrass with wild-type endophyte (E+), ryegrass with no endophyte (E-), and white clover (CL)), in December and in February. Each food was offered singly and in pairs, and time spent grazing on each food, short-term dry matter (DM) intake rate, and preference was measured. In February, sheep given E+ or E- ryegrass alone, grazed for 400 mins/day, compared with 370 mins/day when given a choice between them (P=0.15, SEM=14). The time spent grazing on the E+/E- choice was comprised of 280 mins/day on E- and 90 mins/day on E+ indicating a strong preference for E-. When offered ryegrass and white clover, the total grazing time (335 mins/day), and the grazing time on clover (285 mins/day) and on ryegrass (50 mins/day), were similar for E+/CL and E-/CL. Short-term dry matter intake rate of grass (3.4 g DM/min), and the strong preference for clover in this study, were unaffected by the presence of endophyte. We conclude that while sheep detect endophyte alkaloids, they avoid eating them only when doing so does not reduce daily intake or change the proportion of grass and clover in their diet.

Keywords: diet selection; sheep; perennial ryegrass; endophyte; alkaloids; *Neotyphodium lolii*.

INTRODUCTION

Sheep prefer white clover to perennial ryegrass (Parsons *et al.*, 1994), and white clover has higher nutritive value (Ulyatt *et al.*, 1988). This preference for clover is higher in summer than in autumn (Cosgrove *et al.*, 1996b), and in NZ it varies more from year to year (e.g., 55% - 90% preference for white clover, authors unpubl data), than seen in the UK. One explanation for the variable preference for clover in NZ may be the presence of endophytic fungi (*Neotyphodium lolii*) in perennial ryegrass. This fungus produces several alkaloids. Peramine is a feeding deterrent to insects (Rowan & Gaynor, 1986) and may possibly act in a similar manner for sheep. Lolitrem B, causes ryegrass staggers (Gallagher *et al.*, 1981), and ergovaline causes heat stress and other disorders, and both may be feeding deterrents. These alkaloids may cause the seasonal and year-to-year variation in preference for clover. Alkaloid concentrations tend to be greater in summer and early autumn (Easton, 1999), but vary from year to year, possibly associated with climate (Fletcher *et al.*, 2001).

Sheep discriminate between endophyte-free (E-) and endophyte-infected (E+) ryegrass and reject E+ if alternative grasses are available (Edwards *et al.*, 1993). However, to our knowledge, the effect of endophyte on preference between ryegrass and clover has not been established. The objective of this study was to determine if endophyte in ryegrass affects the preference for white clover and helps explain the seasonal and year-to-year variation in this preference. If sheep avoid alkaloids, we expected they would show a preference for endophyte-free ryegrass, and a greater preference for clover when the grass alternative was endophyte-infected than when it was endophyte-free.

MATERIALS AND METHODS

This study was conducted at the AgResearch, Aorangi research area during December 2000 and February 2001, under the Code of Ethical Conduct for Animal Experimentation of the Palmerston North Crown Research Institutes' Animal Ethics Committee.

Treatments and sward management

Pure swards of perennial ryegrass (cv. Yatsyn) with no endophyte (E-) or with a high level of infection of wild-type endophyte (E+) were sown in October 2000 alongside an established pure sward of white clover (CL). Plots of each of these three food types alone, and each pairwise combination (E+/E-, E+/CL, E-/CL) were created as required by re-configuring the fence layout on these swards. Food choices involved equal, adjacent areas of each food type. Plots were mown frequently to maintain uniform sward height among treatments (9.1 cm and 6.5 cm mean sward surface height in December and February, respectively). Nitrogen fertiliser (urea, 46% N) was applied to ryegrass plots in three applications of 21 kg N/ha each, in November, December and January. Ryegrass swards were treated with Miral 10G (a.i. 100g/kg isazophos) at 10 kg/ha on January 5 to protect the nil-endophyte ryegrass against Argentine stem weevil attack.

Animals

Twenty-four Romney ewe hoggets were randomly allocated to eight groups of three, and the required number of groups used for each phase of the experiment. Sheep grazed pure white clover prior to each measurement period to maintain a consistent pre-treatment diet.

Experimental design

There were six treatments (three foods offered alone and three pairwise choices) with four replications (two

spatial replicates and two temporal replicates or runs). The experimental unit was the group of three sheep. Within runs, the six treatments were imposed in two sets, with three foods offered alone (three treatments x two spatial replicates x three sheep) as one set and the three pair-wise combinations as the other set (three treatments x two replicates x three sheep), in random order. Data collected in December was based on two spatial replicates only. Groups of sheep were allocated to treatments in the late afternoon, and measurements made during the following two days. Plots were of sufficient size to ensure that grazing behaviour and preference during the two days were not influenced by sward depletion.

Measurements

Grazing time: Time spent grazing was measured by continuous visual observation of animals from daylight to dark (approximately 16 hours), and the predominant activity (classified as grazing or not grazing) during consecutive one-minute intervals was recorded. Preference was calculated from the relative time spent grazing on each component of a food choice.

Short-term dry matter intake rate: This was measured in February only, by weighing sheep fitted with a collection bag to prevent loss of excreta, before and after a period of grazing of approximately 1 hour in the late afternoon. Gross change in weight during the period of grazing was adjusted for insensible weight loss during a non-grazing period of similar duration preceding grazing, converted to dry matter intake by adjusting for the dry matter % of samples of herbage taken at that time, and finally to intake rate per minute of grazing.

Endophyte alkaloid concentration: In December and again in February, 80 randomly selected tillers of ryegrass were cut at ground level in each replicate plot of endophyte-infected and endophyte-free ryegrass. Leaves from the 80 tillers were removed and bulked together, frozen, and later freeze-dried and ground (1-mm sieve size) and a sub-sample analysed by HPLC (Barker *et al*., 1993) for concentration of peramine, lolitrem B, and ergovaline. The 80 tillers were also used to check for the presence or absence of fungal endophyte using a polyclonal antibody immunoblot procedure (Gwinn *et al*., 1991). In December, 78% of tillers sampled in the E+ treatment were infected and <1% were infected in the E- treatment. In February, the infection levels were 74% and 2% for E+ and E- treatments, respectively.

Herbage chemical composition: Hand-plucked samples of ryegrass and white clover were taken from several sites in each plot during each experimental period. These samples were freeze-dried and ground and then analysed using near infra-red reflectance spectroscopy to estimate protein and fibre concentration and organic matter digestibility (OMD).

RESULTS

Grazing time

There was no difference in total grazing time for each food offered alone, or as pairwise choices, in December or in February (Table 1). When offered a choice between E- and E+ sheep preferred E- (300 vs 150 mins/day grazing on each, respectively, in December, and 280 vs. 90 mins/day grazing on each in February). They also preferred clover to grass. The preference for clover was not affected by the presence of endophyte in the grass, sheep spending similar time grazing on clover and on grass, respectively, for both the E+/CL and E-/CL treatments (Table 1). In February, the mean time spent grazing on E+ (70 mins/day) when it was offered as a choice with E- or with CL, was lower than in December (160 mins/day). The mean time spent grazing clover in these E+/CL and E-/CL choices was similar in December (260 mins/day) and February (285 mins/day).

Intake rate

Short-term rate of intake of dry matter by sheep grazing clover (4.7 g DM/min) was significantly higher ($P<0.01$) than for sheep grazing E+ ryegrass (3.6 g DM/min) or E-ryegrass (3.1 g DM/min). Intake rate on E+ did not differ significantly from E-.

TABLE 1: Time spent grazing (mins/day) by sheep offered each of 3 food items (clover (CL), endophyte-infected ryegrass (E+) and endophyte free ryegrass (E-), alone and as each pairwise choice in December and in February.

		Time spent grazing on				Within treatment
	Treatment	**CL**	**E+**	**E-**	**Total**	comparison
December	CL alone	440			440	
	E+ alone		410		410	
	E- alone			440	440	
	E+ / E-		150	300	450	C4: = P = 0.6 SEM = 122
	E+ / CL	240	170		410	
	E- / CL	280		140	420	
Between treatment comparisons		C1: P = 0.6	C2: P = 0.25		C3: P = 0.85	
		SEM = 12	SEM = 9		SEM = 42	
February	CL alone	370			370	
	E+ alone		410		410	
	E- alone			390	390	
	E+ / E-		90	280	370	C4: P = 0.01 SEM = 24
	E+ / CL	270	50		320	
	E- / CL	300		50	350	
Between treatment comparisons		C1: P = 0.16	C2: P = 0.11		C3: P = 0.15	
		SEM = 10	SEM = 5		SEM = 14	

SEM and P value applies to comparisons of means as follows: Between treatments; C1 = E+/CL vs E-/CL; C2 = E+/CL vs E-/CL; C3 = All treatments. Within treatment; C4 = E+ vs E-.

Alkaloid concentrations

The concentrations of ergovaline, lolitrem B and peramine in the leaves of ryegrass in December and in February are shown in Table 2. Peramine increased in concentration from December to February more than did ergovaline and lolitrem B. The minor concentration of these alkaloids in E- swards in February reflects the small proportion of E+ tillers in these swards (2%).

TABLE 2: Concentrations of three endophyte produced alkaloids in ryegrass leaf lamina in December and in February.

		Ergovaline	Lolitrem B	Peramine
		mg/kg DM		
December	E+	0.2	0.7	18
	E-	N.D.[1]	N.D.	1
February	E+	0.2	0.9	29
	E-	N.D.	N.D.	1

[1] N.D.=Not Detected

Herbage composition

There were no differences between E+ and E- ryegrass in any of the measured parameters of quality in December or in February (Table 3). In December, grass and clover were similar in protein and acid detergent fibre (ADF) concentration, and OMD, but ryegrass had a higher concentration of neutral detergent fibre (NDF), indicating a higher concentration of hemicellulose than in clover (estimated from the difference in concentration between NDF and ADF). In February, ryegrass had declined in OMD ($P<0.05$) and protein ($P<0.05$) compared with clover, and had higher concentrations of both ADF ($P<0.01$) and NDF ($P<0.001$) than did clover, and more hemicellulose than in December.

TABLE 3: Parameters of quality of white clover (CL), endophyte-infected (E+) and endophyte-free (E-) ryegrass during December and February.

		Protein	ADF[1]	NDF[1]	OMD[1]
		g/kg DM			
December	CL	260	210	240	850
	E+	230	240	410	850
	E-	215	230	410	860
	Signif (P)[2]	NS	NS	0.04	NS
	SEM[2]	17	13	16	16
February	CL	240	200	250	820
	E+	200	270	500	680
	E-	200	260	480	710
	Signif (P)	0.03	0.004	<0.001	0.015
	SEM	7	7	8	20

[1]ADF = acid detergent fibre; NDF = neutral detergent fibre; OMD= organic matter digestibility

[2]NS = not significant, [3]SEM = standard error of mean

DISCUSSION

Offering sheep different foods singly and in pairwise choices provided them different opportunities to avoid alkaloid intake, for example, by reducing intake of an endophyte-infected food, or by shifting preference to an endophyte-free food when offered a choice. If endophyte alkaloids were a feeding deterrent, the only option available for sheep to reduce their intake of alkaloids when offered E+ ryegrass alone would be to reduce total dry matter intake. However, estimates of the time spent grazing and short-term intake rate, which together are the components of daily intake, were similar for sheep offered E+ and E- ryegrass. The few previous studies of intake in which sward conditions (e.g., herbage on offer, herbage residuals) and endophyte level have been independently controlled, show no effects of endophyte on daily dry matter intake (Fletcher *et al*., 1999; Cosgrove *et al*., 1996a). However, reduced dry matter intake of E+ ryegrass has been deduced from higher residuals after grazing on E+ swards than on E- swards (R.G. Keogh, *pers comm*., Edwards *et al*., 1993; Watson *et al*., 1999), perhaps caused by a reluctance to graze into the basal pseudostem, or the cumulative effects of alkaloids on sheep in these longer duration studies.

When sheep have the opportunity to choose freely between E+ and E- ryegrass, rather than reduce total intake, they can satisfy their daily intake requirements by expressing a preference. Given this choice, sheep preferred E- ryegrass, and this preference was stronger in February when the concentrations of alkaloids were higher, than in December. This stronger preference in February resulted from a reduction in the time spent grazing on E+, not an increase in time grazing on E-. Edwards *et al*. (1993) also found that sheep preferred E- ryegrass, but in their experiment, sheep reduced apparent intake rate as they avoided grazing into the pseudostem strata of the sward, and as they searched for E- tillers, rather than reduce the time spent grazing on E+ ryegrass. In that experiment, only 40% of tillers in the E+ sward were infected, providing sheep an opportunity to select within the E+ swards for E- ryegrass. In this experiment, E+ and E- ryegrass were sown in large discrete patches, and sheep "selected" at the patch scale, rather than at the finer scale of individual plants or tillers.

When sheep, as in the present study, are offered the choice between grass and clover, they again have the opportunity to sustain daily nutrient intake, even in the presence of E+ grass, by expressing a preference. We expected they would show a greater preference for clover when the alternative grass was infected with endophyte. In December and in February, preference for clover was not affected by the presence of endophyte in the ryegrass, in contrast to the results of Fribourg *et al*., (1991) using the tall fescue-endophyte association.

The higher preference for clover in February (85%) than in December (63%), based on a reduction in the time spent grazing on ryegrass, indicates an avoidance of grass. This reduction cannot be directly attributed to endophyte alkaloids because it was similar for E- and E+ ryegrass. Sheep were offered fresh leafy ryegrass that was high in organic matter digestibility (similar to clover) in December, with high concentrations of protein and low concentrations of fibre. In February, despite the swards being maintained in a leafy condition, the ryegrass had declined in organic matter digestibility by 160g/kg DM, and increased in fibre concentration, particularly in the refractory hemicellulose component. It appears that changes in these constituents have caused the avoidance of ryegrass and the apparent increase in the preference for clover. With a diet rich in clover, the presence of

alkaloids in the ryegrass that formed only a small proportion of intake did not further affect preference.

The concentrations of lolitrem B and ergovaline in ryegrass leaf in December and in February were at the lower end of the range measured previously at this site at similar times of year in swards of Nui ryegrass containing wild-type endophyte (Cosgrove *et al*., 1996a), but still high enough to affect sheep grazing endophyte-infected swards. The concentration of lolitrem B in intact tillers (leaf and pseudostem above ground level, recalculated from the proportion of the components analysed separately) was 3.1 mg/kg DM in February (c.f. 2.0 in December), above the threshold of 2-2.5 mg/kg DM for clinical effects suggested by Di Menna *et al*., (1992). Ryegrass staggers (caused by lolitrem B) had been observed in sheep grazing similar swards on the research area during January, indicating that concentrations were high enough to cause clinical symptoms given sufficient intake and prolonged exposure. Peramine concentrations were at the upper end of the range of previous measurements, and this compound may affect preference of sheep between E+ and E- ryegrass.

While the presence of endophyte and the concentrations of alkaloids produced under the environmental and sward management conditions of this experiment did not affect preference for clover, continued efforts to select novel, less toxic endophytes would still have important and direct benefits for animal health. Ensuring a higher proportion of clover in mixed pastures, or alternatively, offering animals grass and clover growing separately also increases nutrient intake and decreases alkaloid intake.

CONCLUSIONS

Sheep respond to the alkaloids produced in ryegrass by the fungal endophyte. However, whether they avoid them depends on the opportunity for alternatives. When avoidance would reduce daily intake, as when offered only a single food item, they did not change eating behaviour. When they have opportunity to avoid alkaloids by eating endophyte-free ryegrass, they exercise a partial preference, thus reducing alkaloid intake to below reported clinical thresholds. When the alternative food is clover, preference is determined by many differences between ryegrass and clover, and, in this study was not influenced by alkaloids in the ryegrass, perhaps due to the strong preference for clover and the low intake of grass. The presence of endophyte is unlikely to intensify the selection for clover when clover in the sward and in the diet is abundant, as under the experimental conditions used, or to account for the seasonal and year-to-year variation in preference. Whether this also applies under conditions of higher concentrations of alkaloids (different environments, shorter, less leafy swards), or when the proportion of clover is low, remains to be tested.

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