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Milk production from pastures and pasture silage with different levels of endophyte infection

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ABSTRACT

Perennial ryegrass (*Lolium perenne L.*) pastures in New Zealand contain the fungal endophyte (*Acremonium lolii*). This association produces a range of toxins; some, such as lolitrem B and ergovaline have been associated with ryegrass staggers, decreased serum prolactin, heat stress and decreased milk yield. Grazing trials with lactating cows have given equivocal results in terms of milksolids yield.

Two experiments examined the effects on autumn milk production of supplementing cows grazing pasture with grass and grass/clover silages containing different endophyte concentrations on autumn milk production. Forty cows were used in a 2 x 2 factorial experiment with low (L) and high (H) levels of endophyte in both pasture and silage. In Experiment 1 (28 March - 6 April 1995) cows were grazing clover free pastures and supplemented with either L or H endophyte grass silage. In Experiment 2 (7-12 April 1995) cows were grazing pastures containing about 17-25% white clover and supplemented with either L or H endophyte grass/clover silage.

At the first milk sampling three days after treatments started in Experiment 1, milk yield from cows given L pastures was 6.9% higher than from those grazing H endophyte pastures (P=0.006), and 4.1% more when L rather than H endophyte silages were given (P=0.10). These effects were additive so the cows fed L endophyte silage and L endophyte pasture produced 11.3% more than those fed H silage and H pasture (P=0.002). The effect of pasture endophyte on milk yield was still evident after seven and ten days of treatment, with 10.3% more milk from L than from H endophyte pastures (P=0.01). Milk protein yields paralleled milk yields after seven and ten days but there was an endophyte x silage interaction (P=0.05) for milkfat yield and protein and fat yields were not effected by treatment. In Experiment 2 when clover was included in the diet there were no significant effects from either pasture or silage endophyte status on any milk yield components. A comparison of milksolids yield from Experiment 1 and Experiment 2 shows that changing to diets containing white clover increased milksolids by 6.4, 13.9 and 21.9% for the L/L, L/H and H/L pasture/silage combinations, respectively, but only increased milksolids by 2.7% for the H/H pasture/silage combination. We conclude that endophyte in both pasture and pasture silage can have a transitory effect on milk and protein yield during the autumn and the nutritional benefits of white clover for increasing milksolids was much reduced by endophyte in both pasture and silage diets.

Keywords: Dairy cows; milksolids; milk protein; milk fat; endophyte; perennial ryegrass; white dover; pasture silage.

INTRODUCTION

The diet of New Zealand dairy cows contains a large proportion of perennial ryegrass (*Lolium perenne L.*). The majority of perennial ryegrass tillers are infected with the fungal endophyte *Acremonium lolii*. The plant-endophyte association produces a range of compounds. The ergopeptines, particularly ergovaline, have been implicated in heat stress, serum prolactin depression and decreased milk production in Holstein cows grazing endophyte-infected tall fescue (Bernard et al., 1993). Sheep grazing ryegrass have shown symptoms of heat stress (Fletcher 1993), and ryegrass staggers is caused by lolitrem B (Gallagher et al., 1981).

Valentine et al., (1993) reported that cows grazing pure swards of irrigated endophyte-infected ryegrass in South Australia gave 4-14% less milk than those grazing endophyte-free ryegrass. However, in the first year of a three year experiment in the Waikato, Thom et al., (1994) could show no direct relationship between pasture endophyte status and milk production in cows grazing either ryegrass or ryegrass-white clover pastures.

To our knowledge the effect of feeding silage made from endophyte-infected ryegrass to lactating dairy cows has not been measured. This paper reports two experiments where pasture silages containing different concentrations of endophyte-infected ryegrass with or without white clover were fed as a supplement to cows grazing pasture in late lactation.

MATERIALS AND METIIODS

Treatments and trial design

The experiments were located at the Dairying Research Corporation, No 5 Dairy, Hamilton, New Zealand. The design was a 2 x 2 factorial with pasture containing low (L) and high (H) concentrations of endophyte and silages made from pasture having low and high concentrations of endophyte. In experiment 1 (28 March - 6 April 1995) cows grazed pastures containing no white clover and were supplemented with either high endophyte silage with no clover (H - C1), or low endophyte silage with no clover (L - C1). In experiment 2 (7 April - 12 April 1995) cows grazed pastures containing white clover and were supplemented with either high endophyte silage containing clover (H + C1), or low endophyte silage containing clover (L + C1).

Pasture

Details of pasture establishment are given by Thom et al. (1994). Briefly, the layout consists of paired treatment paddocks randomly arranged within 5 blocks in a 2 x 2 factorial design of high v. low endophyte and clover v. no clover. The ten paddocks (each 0.25 ha) per treatment were considered as a farmlet for grazing purposes. This layout was used in the present experiment, but not all paddocks were used because of limitations on cow numbers. In experiment 1 cows were offered 0.125 ha per day (two days per paddock) and in experiment 2, 0.083 ha per day (three days per paddock).

Silage

All 40 paddocks were cut for silage on 1 November 1994. Silage from each paddock was separately baled, wrapped in plastic, identified by paddock and stored by treatment. At feed-out bales were weighed, a dry matter assessment made, and adjustments made as necessary, to ensure all four treatments received equal silage allocation over the whole experimental period.

Cows

The forty mixed age cows were grazed on nil or low endophyte pastures for a ten day period before experiment 1. Milk production, liveweight and condition score during this period were used as covariates for both experiments 1 and 2. Cows were blocked on the basis of breed, age, liveweight, condition score and milksolids yield and randomly allocated to give 10 cows per treatment. In changing from experiment 1 to experiment 2, treatments were preserved such that the only change was from no clover silage to clover silage. For example, cows on low endophyte pasture plus low endophyte, no clover silage would change to low endophyte pasture plus low endophyte clover silage.

MEASUREMENTS

Pasture

Herbage allowance and pasture intake were extracted from fifty readings by a calibrated rising plate meter (L'Huillier & Thomson 1988) before and after each grazing. Herbage clipped from exclosure cages as part of the main endophyte trial was used to estimate botanical composition. Ryegrass tillers cut to ground level were bulked within treatment, freeze dried and ground before analysis for lolitrem B (Gallagher et al., 1981) and ergovaline (Garner et al., 1991). Ten ryegrass tillers were randomly selected from each paddock in March 1995 for staining and microscopic examination for the presence of endophyte mycellia.

Silage

A sample from each bale fed was taken and analysed for: DM, pH, ammonia-N (g/kg total N), total N, *in vitro* OM digestibility, ergovaline and lolitrem B.

Silage waste was collected and weighed initially, but wastage was so low that subsequent visual estimates were made of wastage and silage intake corrected as required.

Cows

Milk volume and composition (fat, protein and lactose) were measured twice weekly at consecutive milkings. Milksolids are defined as fat plus protein. Cows were weighed and visually scored for condition at the beginning and end of both experiments. Rectal temperatures were measured by hand-held digital thermometers before afternoon milking twice in experiment 1 and once in experiment 2.

Statistical analysis

Analysis of variance and covariance models were used to test treatment differences. Data obtained from individual cow measurements were considered as replicates for analysis of treatment differences. Milk data were analysed separately for days three, seven and ten after treatment initiation. Data from day three are presented separately to highlight the transitory nature of some treatment effects.

RESULTS

In experiment 1, pastures designed to contain no white clover contained trace amounts in all treatments (Table 1), but the ryegrass content in all treatments was decreased by the presence of substantial quantities of summer grass (*Digitaria sanguinalis*) and smooth witchgrass (*Panicum dichotomiflorum*). In experiment 2, pastures contained 17.2-25.4% white clover (Table 1) and much lower proportions of summer grass and smooth witchgrass than those swards in experiment 1. In both experiments dead matter contents were low and similar.

Silage was well-preserved with little evidence of spoilage and moulds. Crude protein concentrations were slightly higher in pasture-white clover silage compared with pasture-silage, 15.9 and 13.8%, respectively (Table 2). However, all silages contained <100 g NH_3-N/kg total N, indicating acceptable levels of true protein degradation. The *in vitro* OM digestibility (Table 2) was slightly lower for the H - C1 silage compared with the other three, but all silages were of medium quality with predicted meta–bolisable energy (ME) contents of >10 MJME/kg DM.

Ergovaline concentrations were low in all silages, 0 and 0.1 µg/g DM in the L + C1 and L - C1 silages and 0.25 and 0.2 mg/g DM in the H + C1 and H - C1 silages respectively (Table 2). Lolitrem B levels were negligible for the L + C1 and L - C1 silages, but were 0.77 and 0.68 µg/g DM for the H + C1 and H - C1 silages, respectively.

In experiment 1, pasture intakes would be predicted to be similar because of high allowances on all treatments. The pasture intakes estimated from pre- and post-grazing plate meter readings can only be considered as indicative, because standard calibration equations were used and pasture utilisation was low. Silage utilisation was always above 95% in all treatments and very similar intakes were obtained across all treatments (Table 3).

TABLE 1: Pasture botanical and chemical composition for high (H) and low (L) endophyte combinations of pasture and silage without clover (experiment 1) and with clover (experiment 2).

Treatment Pasture	Silage	Ryegrass	White clover	Other grasses[1] (%)	Weeds	Dead	Ergovaline (µg/g)	Lolitrem B (µg/g)
Experiment 1 - No Clover								
H	H - Cl	50.9	0.7	33.7	0	14.7	0.80	2.62
H	L - Cl	63.0	1.4	19.9	0	15.7		
L	H - Cl	54.0	0.1	29.6	0	16.3	0.40	0.88
L	L - Cl	54.4	0.7	32.9	1.0	11.0		
Experiment 2 - White Clover								
H	H + Cl	49.0	25.4	7.8	3.6	14.2	0.50	1.65
H	L + Cl	61.2	17.2	3.8	5.4	12.4		
L	H + Cl	63.5	18.5	3.6	1.4	13.0	0.30	.48
L	L + Cl	49.9	24.4	9.1	3.9	12.7		

[1] Mainly summer grass (*Digitaria sanguinalis*) and smooth witchgrass (*Panicum dichotomiflorum*)

TABLE 2: Silage composition for high (H) and low (L) endophyte silage without dover (experiment 1) and with clover (experiment 2).

Treatment	DM (%)	pH	NH_3-N (g/kg total N)	Total N (%)	IVOMD (%)	Ergovaline[1] (µg/g)	Lolitrem B[1] (µg/g)
Experiment 1 (No Clover)							
H - Cl	33.5	5.0	98	2.2	70.6	0.2	0.68
L - Cl	50.5	5.2	59	2.2	72.9	0.1	0.03
Experiment 2 (White Clover)							
H + Cl	50.0	4.8	56	2.4	73.2	0.25	0.77
L + Cl	43.7	4.4	84	2.7	72.3	0	0.06

[1] Based on pasture samples from November 1994 silage cut

In experiment 1 there were differences in milk yield between three days after treatments commenced and after seven and ten days of treatment. After three days in Experiment 1 milk yield from cows fed the low endophyte pastures was 6.3% higher than from cows grazing the high endophyte pastures (P=0.006). When low endophyte silages were fed milk yield was 4.1% higher than from high endophyte silage (P=0.09). These effects were additive such that cows given the low endophyte silage supplemented with low endophyte pasture produced 10.6% more milk than those fed high endophyte silage on high endophyte pasture (P=0.002). The effect of pasture endophyte on milk yield persisted in days seven and ten (Table 4), with cows grazing low endophyte pastures producing 10.3% more than those fed high endophyte pastures (P=0.01).

Pasture endophyte had no effect on milk fat yield in Experiment 1. On day 3 of experiment 1 cows grazing low endophyte pasture produced 6.8% more milk protein (P=0.007) than those grazing high endophyte pasture and low endophyte resulted in 4.7% more milk production (P=0.09) than high endophyte silage supplements. These effects did not persist and there were no effects of endophyte on milk protein on days 7 and 10. On day three, milksolids yield from cows fed low endophyte silage was 14.4% (P=0.01) higher than from those fed high endophyte silage, but only when both were grazing high endophyte pasture.

At seven and ten days cows fed high endophyte silage with high endophyte pasture had 6.15% milkfat compared with 5.42% fat from cows fed low endophyte silage on a high endophyte pasture (P=0.03). In the same period there

TABLE 3: Pasture allowance, and pasture and silage intake from high (H) or low (L) endophyte pastures supplemented with high or low endophyte silage (experiment 1).

Treatment Pasture	Silage	Pasture allowance (kg DM/cow/day)	Pasture intake (kg DM/cow/day)	Silage intake (kg DM/cow/day)
H	H - C1	40.8	8.1	5.5
H	L - C1	36.9	9.3	5.5
L	H - C1	31.8	6.1	5.2
L	L - C1	32.2	7.2	5.5

TABLE 4: Milk, fat, protein and milksolids yield from cows grazing high (H) or low (L) endophyte ryegrass pastures supplemented with high or low endophyte silage (experiment 1).

Treatment Pasture	Silage	Milk (kg/cow/day)	Fat (kg/cow/day)	Protein (kg/cow/day)	Milksolids (kg/cow/day)
Period 1					
H	H - C1	9.4	0.52	0.36	0.88
H	L - C1	9.8	0.62	0.38	1.00
L	H - C1	10.0	0.55	0.39	0.94
L	L - C1	10.4	0.55	0.40	0.95
Endophyte		**			
Silage		†[1]	†	†	†
Endophyte x silage			†		†
Period 2					
H	H - C1	7.5	0.45	0.29	0.74
H	L - C1	7.0	0.38	0.27	0.65
L	H - C1	8.1	0.44	0.29	0.74
L	L - C1	7.9	0.42	0.29	0.71
Endophyte		**			

[1] Significance level (P<0. 10)

was an endophyte effect on protein content. Cows fed high endophyte silage on high endophyte pasture had 3.93% protein compared with 3.67% for cows for high endophyte silage on low endophyte pasture (P=0.04).

In experiment 2 pasture allowances (Table 5) were similar across all treatments. Silage utilisation was always above 95% in all treatments and similar intakes were obtained for all treatments (Table 5). The only major difference in milk composition occurred in period 1, when cows on high endophyte pasture had a higher fat content (5.96%) than those on low endophyte pasture (5.42%) (P=0.02), but with an endophyte x silage interaction (P=0.04).

There were no significant effects for either pasture or silage endophyte status on any milk yield components in Experiment 2 (Table 6). A comparison of milksolids yield from experiment 1 (Table 4) and experiment 2 (Table 6) shows that changing to diets containing white clover increased milksolids by 12.7, 12.2 and 21.5% for the L/L, L/H and H/L pasture/silage combinations, respectively, but only increased milksolids by 2.7% for the H/H pasture/silage combination.

Milk protein content was 4.10% for cows consuming low endophyte silage containing white clover compared with 3.92% for cows consuming high endophyte silage containing white clover.

There were pasture (P<0.05) and silage (P<0.01) effects and a pasture x silage interaction (P<0.05) on rectal temperatures measured at the end of experiment 1 (day 10). Cows on the H/H - C1 treatment had higher temperatures (P<0.001) than the mean of the other three treatments which were not significantly different, 39.7°C v. 39.1°C.

Cows on the H/H + C1 treatment had higher temperatures than the mean of the other three treatments which were not significantly different, 39.3°C v. 39.1°C and there was a pasture x silage interaction on day 10 of Experiment 2 (P<0.05).

TABLE 5: Pasture allowance, and pasture and silage intake from high (H) or low (L) endophyte pastures containing white clover supplemented with high or low endophyte ryegrass-white clover silage (experiment 2).

Treatment		Pasture allowance	Pasture intake	Silage intake
Pasture	Silage	(kg DM/cow/day)		
H	H + C1	34.0	13.9	6.7
H	L + C1	30.3	10.6	6.2
L	H+ C1	30.4	11.4	6.6
L	L + C1	31.4	13.2	6.4

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TABLE 6: Milk, fat, protein and milksolids yield from cows grazing high (H) or low (L) endophyte ryegrass-white clover pastures supplemented with high or low endophyte silage containing white clover (experiment 2).

Treatment		Milk	Fat	Protein	Milksolids
Pasture	Silage	(kg /cow/day)			
H	H + C1	8.2	0.45	0.31	0.76
H	L + C1	7.9	0.47	0.32	0.79
L	H + C1	8.8	0.49	0.34	0.83
L	L + C1	8.0	0.47	0.33	0.80

Note: No significant (P<0.05) treatment or interaction effects for any component.

DISCUSSION

TThe effect of high endophyte pasture in reducing milk yield in experiment 1 supports the finding of Valentine et al., (1993), but not that of Thom et al., (1994). On irrigated pure ryegrass pastures in autumn, Valentine et al., (1993) measured a 14% advantage in milk yield when cows grazed high endophyte compared to low endophyte Ellett perennial ryegrass; there was also a 10% advantage in milksolids yield with both protein and fat yield increased. Lolitrem B levels for the low and high endophyte ryegrass were zero and 0.21 mg/g DM, respectively. Neither levels were sufficiently high to cause ryegrass staggers. Unfortunately, ergovaline levels were not reported.

Thom et al., (1994) reported interactions between endophyte and proportion of clover in pastures for milk and milksolids yield of cows grazing autumn pastures. Milk and milksolids yields were 45 and 39% higher from cows fed grass/clover pastures with low endophyte compared to high endophyte but there were no significant differences for the low endophyte-clover compared with high endophyte-clover. In the latter case, cows grazing high endophyte ryegrass showed serious ryegrass staggers with lolitrem B concentration of 3.4 mg/g DM, whereas those on low endophyte pastures showed no signs of staggers with lolitrem B concentration of 0.6 mg/g DM. The authors concluded that lolitrem B was not responsible for differences in milk production on the pastures containing clover. They noted that treatment differences in milksolids production were closely correlated with estimated green DM intake. Ergovaline levels of 0.5 - 1.0 mg/g DM were reported, but effects were considered to be minimal because no significant treatment differences in rectal temperature were recorded.

In the present experiment, minor symptoms of ryegrass staggers occurred on all treatments in a few cows, and lolitrem B concentration(Table 2) were much lower than in the March experiment the previous year (Thom et al., 1994).

Ergovaline intakes were calculated for experiment 1 from estimated feed intakes, ryegrass content, and ergovaline content of silage (Table 2) and pasture (Table 1). Estimated ergovaline intakes for high and low endophyte pastures assessed across silage treatments were 5.6 and 3.0 mg/cow/day (14.0 and 7.5 mg/kg LW/day). Debassai et al., (1993) established ergovaline dose response curves for lambs fed tall fescue seed containing ergovaline. Levels of 6.8 and 13.2 mg/kg LW decreased intake by 4.2 and 6.4%, respectively, with serum prolactin levels reduced from 44.6 mg/ml in the controls to 2.9 and 2.3 mg/ml, respectively. Similar dose response curves are not available for lactating cows. However, the lamb research shows that ergovaline is capable of influencing intake and hormone concentration at amounts being ingested in experiment 1.

Estimated ergovaline intakes for cows given high and low endophyte silages averaged across pasture treatments were 4.6 and 4.0 mg/cow/day (11.5 and 10.0 mg/kg LW/day). The combination of ergovaline from both pasture and silage sources led to an intake of 14.6 mg/kg LW/day for the H pasture + H silage treatment compared to 6.7 mg/kg LW/day for the L pasture + silage treatment with a 10.6% advantage in milk yield to the latter.

Pasture chemical analyses (not presented here) and silage analyses (Table 2) show that these differences in milk yield are unlikely to arise from differences in nutritional components of the diet.

Rectal temperatures taken at the end of experiment 1 (5 April 1995) showed that cows grazing H pasture supplemented with H silage had a mean rectal temperature of 39.7°C vs 39.1°C for the average of the other three treatments. This substantially higher temperature coincides with the highest level of estimated ergovaline intake (14.6 mg/kg LW/day). Strahan et al., (1987) compared lactating cow performance on Kentucky 31 endophyte-free (0% endophyte) and Kentucky 31 endophyte-infected (63% endophyte) tall fescue. Rectal temperatures were 38.9°C and 39.4°C respectively ($P<0.05$). Ergovaline levels were not reported, but if we assume a concentration of 1 mg/g DM then estimated ergovaline intake would have been 11.1 mg/kg LW/day.

There is much circumstantial evidence that associates ergovaline with decreased intake and milk yield. However, it is known that grass-endophyte associations produce a wide range of chemicals, and it is possible that compounds, other than ergovaline, are of primary importance, or act synergistically with ergovaline. There is a need to improve estimates of grazing intake and the concentration of key endophyte compounds in grazed forage so that dose response relationships can be developed under realistic systems.

In experiment 2 there were no treatment effects on milk or milksolids yield (Table 6). Similar calculations were performed as in experiment 1 to estimate ergovaline intake. Values ranged from 2.4 mg/kg LW/day for cows on L pasture + L silage to 9.1 mg/kg LW/day for cows on H pasture + H silage. The change of diet from ryegrass only (Experiment 1) to ryegrass-white clover (Experiment 2) increased milksolids substantially for the L/L, L/H and H/L pasture/silage combinations, but not for the H/H combination. The estimated ergovaline intake of cows in this group decreased from 14.6 to 9.1 mg/kg LW/day; whereas all the others decreased by <7 mg/kg LW/day. We suggest that values above approximately 10 mg ergovaline/kg LW/day may be sufficient to decrease the expected response in milk yield when feeds of higher nutritive value are offered.

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