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Effect of *Lotus corniculatus* and condensed tannins on milk yield and milk composition of dairy cows

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ABSTRACT

Experiments at DRC have demonstrated birdsfoot trefoil (*Lotus corniculatus*) increases milk yield and milk protein concentration of grazing dairy cows. An experiment was conducted in March 1998 to determine what proportion of the increased milk yield and milk protein% was due to the condensed tannins (CT) in *Lotus*, and what proportion was due to factors typically associated with legumes (increased herbage intake and improved forage quality). Twenty Friesian cows were housed and fed twice daily on either perennial ryegrass or *Lotus* for 10 days. Five cows on each diet were also drenched with 1.2 l of 50% polyethylene glycol (PEG) three times per day. PEG blocks the action of CT, preventing them binding to plant proteins. Milk yields (l/cow/d) were higher on *Lotus* (16.5) than on *Lotus*+PEG (13.8), ryegrass (10.2) or ryegrass+PEG (9.9) indicating that CT contributed to 42% of the increased milk yield that resulted from feeding *Lotus* rather than ryegrass. CT had no effect on intake, since intakes (kgDM/cow/d) of cows fed *Lotus* (16.8) and *Lotus*+PEG (16.7) were similar and higher than for cows fed either ryegrass (14.7) or ryegrass+PEG (13.7). CT, however, accounted for all of the increase in herbage conversion efficiency as indicated by the higher efficiencies (ml FCM/MJ ME) of cows fed *Lotus* (147) compared with those fed *Lotus*+PEG (126), ryegrass (123) or ryegrass+PEG (127). CT accounted for 57% of the increase in milk protein% as cows fed *Lotus* (3.61) had higher protein% than those fed *Lotus*+PEG (3.44), ryegrass (3.31) or ryegrass+PEG (3.30). Herbage or CT had no effect, however, on the casein or whey protein concentrations. CT had no effect on either milk fat or lactose concentration but cows fed *Lotus* had lower concentrations than cows fed ryegrass ($P<0.05$). Overall the trial showed legumes containing CT have potential as a forage for dairy cows.

Keywords: birdsfoot trefoil; condensed tannins; dairy cows; *Lotus corniculatus*; milk composition; milk yield

INTRODUCTION

Birdsfoot trefoil (*Lotus corniculatus* - referred to as "*Lotus*" in this paper) is a perennial legume which was introduced to New Zealand in the early 1900's for use as a forage legume for sheep in drier hill and high country regions. *Lotus* was shown to increase liveweight gain and wool growth in weaned lambs (Wang *et al*., 1994), liveweight gain in beef cattle (Alison and Hoveland, 1989) and dairy heifers (Marten *et al*., 1987), and milk yield in lactating sheep (Wang *et al*., 1996) compared with yields and growth of livestock fed grass-based diets. Recent experiments demonstrated that Friesian cows grazing *Lotus*-dominant pastures produced more milk and had a higher milk protein concentration than cows grazing either perennial ryegrass or white clover-dominant pastures (Harris *et al*., 1998b). The higher milk yield of cows grazing *Lotus* was due, in part, to improved pasture quality and higher dry matter intake (DMI). The impact of pasture quality and DMI on milk yield was similar to that shown for white clover (Harris *et al*., 1997; Harris *et al*., 1998a,b). The increase in milk production on *Lotus* compared with white clover was probably due to the action of condensed tannins (CT) (Harris *et al*., 1998b) which are present in *Lotus*, but not in ryegrass or white clover (except in white clover flowers).

CT are phenolic compounds which are widespread in the plant kingdom and are found in a number of legumes including *Lotus*, Maku lotus (*Lotus pedunculatus*), sainfoin (*Onobrychis viciifolia*), sericea lespedeza (*Lespedeza cuneata*) and in the flowers of white clover (*Trifolium repens*) (Terrill *et al*., 1992). CT bind to plant protein complexes in the rumen (pH range 3.5 - 7.0) and therefore reduce microbial degradation of soluble protein to ammonia in the rumen. CT-protein complexes dissociate below pH 3.5, increasing non-ammonia nitrogen flux to the abomasum and small intestine, and increasing the apparent absorption of essential amino acids from the small intestine (Waghorn *et al*., 1987). Effectively the CT convert soluble plant proteins into protected or rumen bypass proteins.

The aim of this experiment was to examine the role of CT in the milk production of dairy cows fed *Lotus* and to determine what proportion of any changes in milk yield and milk composition were due specifically to CT in *Lotus*, and what proportion were due to nutritional factors typically associated with legumes (increased DMI and improved forage quality).

MATERIALS AND METHODS

Trial design

The trial was conducted over 10 days (5 day adjustment and 5 day measurement period) in March 1998 using Friesian cows in late lactation (209±17 days in milk). For 7 days before the trial cows grazed together on perennial ryegrass/white clover pasture (uniformity period) and measurements collected during this period were used for covariate analysis of data. During the adjustment and measurement periods cows were individually housed and fed on either perennial ryegrass (10 cows) or *Lotus corniculatus* cv. Goldie (10 cows) which was fed in individual feed boxes

twice daily (0830h, 1600h). The ryegrass and *Lotus* was cut from pure swards which were regularly irrigated, cut and harrowed during the summer-autumn to maintain pasture quality prior to the trial.

Throughout the trial, five cows on each forage were drenched with 1.2kg 50% w/v polyethylene glycol (MW 3350) (PEG) solution three times per day (0630h, 1430h, 2130h), while the remaining cows were drenched with an equivalent volume of water. CT bind to PEG in preference to plant proteins thereby rendering the CT inactive (Barry and Manley, 1986). Therefore, comparing control cows (ryegrass or *Lotus* diets) with CT-inactivated cows (ryegrass+PEG or *Lotus*+PEG diets) enabled any effects of CT in the *Lotus* to be quantified and separated from any nutritional factors of *Lotus* which may have contributed to production differences between *Lotus*- and ryegrass-fed cows . These CT effects are presented as percentage values and were calculated for the various parameters using the mean values from the different treatments:

$$\% \text{ CT effect} = \frac{(Lotus - x^R) - (Lotus\text{+PEG} - x^R)}{(Lotus - x^R)}$$

Note: x^R is the mean of the ryegrass and ryegrass+PEG treatments.

Herbage

Dry matter (DM) of herbage was determined at each feeding by oven drying at 100°C for 24h. Herbage samples were also collected and bulked daily during the measurement period for estimation of chemical composition (crude protein, available carbohydrate, lipid, acid detergent fibre (ADF), neutral detergent fibre (NDF), organic matter digestibility (OMD) and metabolisable energy (ME) content) using near infra red spectroscopy (NIRS). Analysis of chemical composition using wet chemistry techniques in a previous experiment (Harris *et al*., 1998b) confirmed the feasibility of using NIRS for ryegrass and *Lotus* analyses. Subsamples of herbage were also freeze-dried and analysed for extractable (free) and bound CT concentration using the butanol-HCl colorimetric procedure (Terrill *et al*., 1992).

DMI and conversion efficiency

All cows were fully fed, with fresh weight herbage allowances for each cow calculated using a microwave to give a quick estimate of herbage dry matter (DM) and the calculation of Holmes *et al*., (1987) to estimate probable DMI. Actual DMI was calculated by weighing herbage offered and refused at every feeding combined with herbage DM measurements. Conversion efficiencies were based on the calculation of Holmes *et al*., (1987) using the estimates of herbage ME content, and measurements of DMI and fat corrected milk (FCM) yield of individual cows. Results are expressed as ml FCM produced/MJ ME intake excluding maintenance energy requirements.

Milk yield and composition

Milk yield and concentrations of fat, protein and lactose were determined daily (pm + am sample) during the measurement period and on two days in the uniformity period. On the final day of the uniformity and treatment periods, a larger milk sample was collected for the analysis of casein and whey protein concentrations.

Samples were analysed for fat, protein and lactose concentrations using an infra-red milk analyser (Milkoscan 133B, Foss Electric, Hillerød, Denmark). Concentrations of casein and whey protein were determined using macro-Kjeldahl techniques as described by Mackle *et al*., (1998).

Statistical analysis

Milk yield and composition data were analysed for variance (SAS 6.12) using covariate data collected during the uniformity period. DMI and conversion efficiency data were analysed for variance using SAS 6.12, and herbage chemical composition data were analysed for variance using Genstat 5.3, but no covariate data were collected for these parameters. Adjusted means and SEDs for individual treatments are presented in tables. Main effect means ie. comparison of herbage species, are presented in the text as *Lotus* vs ryegrass means and SEDs.

RESULTS AND DISCUSSION

The *Lotus* fed during the trial was of consistently higher nutritive value than the ryegrass, with higher crude protein content, OMD and ME, and lower ADF and NDF (P<0.001) (Table 1). Although the *Lotus* also had a higher (P<0.001) available carbohydrate content than the ryegrass, carbohydrate levels of both the *Lotus* and ryegrass were lower than those accepted as high quality pasture. All other chemical components of both the *Lotus* and ryegrass were consistent with average to high quality herbage.

Only the *Lotus* contained CT totalling 27.3g/kg DM, with extractable (free) CT comprising the bulk (69%) (Table 1). This total CT content was similar to that of *Lotus* growing in the same paddocks when used in an experiment in December 1997 (24.9g total CT/kg DM) (Harris *et al*., 1998b), and slightly lower than for a *Lotus* pasture used by Wang *et al*., (1996) in a similar experiment with lactating sheep.

TABLE 1: Chemical composition and condensed tannin (CT) concentration of perennial ryegrass and *Lotus corniculatus*.

	Ryegrass	*Lotus*	SED
Dry matter (%)	22.4	14.1	0.4
Crude protein (g/100g DM)	18.2	25.6	0.7
Available carbohydrate (g/100g DM)	6.7	8.7	0.5
Lipid (g/100g DM)	3.8	4.5	0.1
Acid detergent fibre (g/100g DM)	30.9	22.9	0.6
Neutral detergent fibre (g/100g DM)	52.9	30.4	1.3
Organic matter digestibility (g/100g DM)	65.4	71.4	1.5
Metabolisable energy (MJ/kg DM)	10.6	11.4	0.1
CT: free (g/kg DM)	0	18.9	N.A.
bound (g/kg DM)	0	8.3	N.A.
Total (g/kg DM)	0	27.3	N.A.

Cows fed *Lotus* had higher (P<0.001) milk yields than those fed ryegrass (15.18 vs 10.02 kg/cow/d, SED 0.24) due, in part, to improved pasture quality, particularly higher crude protein and ME levels, and higher DMI. The effect of pasture quality and DMI on milk yield was similar to that previously shown for both *Lotus* and white clover (Harris *et al*., 1997; Harris *et al*., 1998a,b). The higher (P<0.001) milk yield of cows fed *Lotus* compared with

Lotus+PEG (Table 2) indicated that CT also contributed to the increased milk yield and, in this trial, contributed to 42% of the difference in milk yield between cows on *Lotus* and ryegrass. Wang *et al*., (1996) showed CT also had a specific role in increasing the milk yield of lactating ewes grazing *Lotus*.

The higher (P<0.001) conversion efficiency of cows fed *Lotus* compared with those fed either *Lotus*+PEG, ryegrass or ryegrass+PEG (Table 2) suggested that improving efficiency was at least one of the mechanisms by which CT increased milk yield, and that CT were the only factor which affected any change in conversion efficiency. This result was consistent with previous trials in which cows grazing *Lotus*, but not white clover, had higher conversion efficiencies than those grazing ryegrass, thereby suggesting the improved efficiency was associated with the presence CT in *Lotus* rather than pasture quality (Harris *et al*., 1998b).

The action of CT in *Lotus* may also explain the increase in milk protein concentration. Cows fed the *Lotus* and *Lotus*+PEG treatments had higher (P<0.001) milk protein concentrations than those on either ryegrass or ryegrass+PEG (3.53 vs 3.31%, SED 0.03). This difference in protein % may have been associated with the higher pasture protein levels in the *Lotus* compared with the ryegrass (Table 1), along with the increased ME intakes, promoting increased availability and absorption of protein. However, the higher (P<0.05) milk protein % of cows fed *Lotus* compared with *Lotus*+PEG (Table 2) also suggests a specific role for CT in the change, in this case accounting for 57% of the increase above ryegrass-fed cows. Interestingly Wang *et al*., (1996) showed no difference in milk protein concentration between ewes grazing *Lotus* and those grazing *Lotus* supplemented with PEG, showing CT had no effect on milk protein concentration. CT did, however, increase milk protein secretion rate (g/h) in sheep.

TABLE 2: Milk yield, intake, milk composition and herbage conversion efficiency of Friesian dairy cows fed either perennial ryegrass or *Lotus corniculatus* and drenched three times daily (3.6 l/d) with either 50% polyethylene glycol (PEG) or water. The means and SEDs given are for the individual treatments. Main effect (*Lotus* vs ryegrass) means and SEDs are presented in the text.

	Ryegrass	Ryegrass +PEG	*Lotus*	*Lotus* +PEG	SED
Milk yield (kg/cow/d)	10.2	9.9	16.5	13.8	0.5
Intake (kg DM/cow/d)	14.7	13.7	16.8	16.7	0.7
Fat (%)	5.05	5.04	4.86	4.72	0.17
Protein (%)	3.31	3.30	3.61	3.44	0.05
Lactose (%)	4.74	4.72	4.65	4.69	0.05
Casein (%)	2.66	2.72	2.66	2.62	0.07
Whey protein (%)	0.84	0.84	0.84	0.83	0.03
Casein: whey protein	3.17	3.22	3.20	3.19	0.11
Milksolids (kg/cow/d)	0.85	0.81	1.40	1.13	0.05
Efficiency (ml FCM/MJ ME)	123	127	147	126	6.4

Casein is generally considered to be the most valuable protein in milk for manufacturing purposes. Thus it would be advantageous if the increased in milk protein concentration due to feeding *Lotus* (Table 2) was achieved through an increase in the casein fraction, as opposed to the whey fraction. Although milk protein % was increased by feeding *Lotus* in the current study, the concentrations of casein and whey protein were not affected by either forage type or PEG supplementation (Table 2). This indicates that while feeding *Lotus* did not increase the casien:whey ratio there was also no detrimental effect on the manufacturing potential of the milk. Further research is required to confirm this observation, and to determine whether feeding *Lotus* has any effects on the individual proteins that make up the casein and whey fractions.

Unlike Wang *et al*., (1996), we found CT had no significant effect on either milk fat or lactose concentration. However, as with previous grazing experiments (Harris *et al*., 1998b), cows fed *Lotus* had lower (P<0.05) milk fat % than those fed ryegrass (4.78 vs 5.04, SED 0.08). Wang *et al*., (1996) suggested the effect on milk fat concentration was due to simple dilution caused by the action of CT increasing the secretion rates of lactose and protein and increasing milk volume, rather than a direct effect of CT on milk fat levels.

Cows fed *Lotus* readily consumed the herbage, and DMI was higher on *Lotus* (P<0.001) than on ryegrass (16.74 vs 14.18 kg DM/cow/d, SED 0.34). This positive effect of feeding *Lotus* on DMI was consistent with previous grazing experiments (Harris *et al*., 1998b). CT in the *Lotus* did not, however, have any effect, either positive or negative, on DMI since DMI was similar for cows fed *Lotus* or *Lotus*+PEG (Table 2). This may have been because the level of CT in the *Lotus* cultivar (Goldie) used in our trial was lower (Table 1) than the 40g/kg DM level reported by Waghorn *et al*., (1990) as having a detrimental effect on DMI. High CT concentrations, for example, may reduce the availability of protein N for microbial use, so that microbial growth is sufficiently reduced to lower the rate of plant fibre degradation in the rumen (Waghorn *et al*., 1990). Also, Barry and Duncan (1984) reported Maku lotus containing high concentrations of CT (60 - 110g/kg DM) reduced DMI when fed to sheep. *Lotus corniculatus* cultivars also have proportionately lower levels of free CT, which have been linked to the detrimental effects of CT, than other *Lotus* species (Barry and Manley, 1986).

SUMMARY

Lotus corniculatus clearly shows potential as a forage for dairy cows due to its positive effects on milk yield and milk protein concentration. *Lotus* also increases DMI and herbage conversion efficiency compared with a perennial ryegrass diet, and slightly decreases milk fat concentration. Results from this trial indicated the effect on conversion efficiency was due only to the action of CT in *Lotus*. CT also contributed, in part, to the increased milk yield and milk protein concentration, which were also due to the higher DMI and nutritive value of the herbage - features typical of legume diets. The positive effects that CT have on milk yield and composition indicate the potential benefits which could be gained if plant breeding and genetic technologies could be used to successfully introduce CT into perennial ryegrass or white clover, the forages commonly grazed by New Zealand dairy cows.

Overall, the effects of *Lotus* on milk protein and milk fat concentrations comply with goals for milk composition set by the New Zealand Dairy Board. The increased protein % combined with the increased milk yield result in a large increase in the milksolids (daily milk fat plus milk protein yield) yield of cows fed *Lotus* compared with those fed ryegrass. From the New Zealand dairy farmers' perspective the large increase in milksolids yield measured on *Lotus*-fed cows (Table 2) could significantly increase farm income since payout is based upon milksolids production. But although *Lotus* is tolerant of dry summers and appears well adapted to rotational grazing, reported difficulties with establishment, low competitive ability and poor winter growth (Chapman *et al*., 1990; Scott and Charlton, 1983) may limit its use on dairy farms as a substitute for white clover in ryegrass-based pastures and could result in higher farm costs. Research is underway at DRC to test whether *Lotus* could instead be used as a perennial green-feed crop, largely during summer-autumn, and the economic viability of this in a farm system.

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