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Lotus Corniculatus and Condensed Tannins – Effects on Milk Production by Dairy Cows

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ABSTRACT: Birdsfoot trefoil (*Lotus corniculatus*) increases milk yield of dairy cows. An indoor feeding experiment using Friesian dairy cows in mid lactation was conducted to determine what proportions of the milk yield and composition changes were due to the condensed tannins (CT) in *Lotus*, and what proportions were due to factors associated with legumes (higher herbage intake and forage quality). Cows were fed either perennial ryegrass or *Lotus*, and were also drenched with either polyethylene glycol (PEG) to block the action of CT, or water. Milk yields (kg/cow/d) were higher on *Lotus* (21.24) than on *Lotus*+PEG (18.61), ryegrass (15.53) or ryegrass+PEG (15.49) indicating that CT contributed to 46% of the increased milk yield that resulted from feeding *Lotus* rather than ryegrass. CT had no effect on intake, but contributed to increased milk protein concentration, increased efficiency and decreased milk fat concentration.

Key Words : Dairy Cows, Birdsfoot Trefoil, *Lotus corniculatus*, Condensed Tannins, Milk Production

INTRODUCTION

Birdsfoot trefoil (*Lotus corniculatus* - referred to as "*Lotus*" in this paper) is a perennial legume introduced to New Zealand in the early 1900's as a forage legume for sheep in drier hill and high country regions. *Lotus* has been 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 grass-based diets. Recent experiments showed that dairy cows grazing *Lotus*-dominant pastures produced more milk and had a higher milk protein concentration than cows grazing 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). Condensed tannins (CT), in the *Lotus* may also have contributed to the higher milk yield (Harris *et al*., 1998b).

CT are phenolic compounds which are widespread in the plant kingdom and are found in a number of legumes including *Lotus*, lotus major (*Lotus pedunculatus*), sainfoin (*Onobrychis viciifolia*), sulla (*Hedysarum coronarium*) and in the flowers of white clover (*Trifolium repens*) (Terrill *et al.*, 1992). The chemistry of CT is complex. The large variety of chemical structures (Foo *et al*., 1997) together with the wide range in CT concentration results in CT in different plants having different effects on animal metabolism and production. The overall function of CT is, however, uniform. CT bind to plant protein complexes in the rumen (pH range 3.5 - 7.0) and thereby 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, in some cases, increasing the apparent absorption of essential amino acids from the small intestine (Waghorn *et al*., 1987). In this way CT effectively convert soluble plant proteins into protected or rumen bypass proteins.

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

MATERIALS AND METHODS

The trial was conducted over 25 days in January-February 1999 using 20 Friesian cows in mid-late lactation (171±21 days in milk (mean±SE)). For 7 days (uniformity period) before the trial cows grazed perennial ryegrass / white clover pasture. Measurements collected during this period were used for covariate analysis of data. Cows were then individually housed for 9 days (Period A), comprising adaptation (5 days) and measurement periods (4 days), and were fed on either fresh perennial ryegrass (10 cows) or *Lotus corniculatus* cv. Goldie (10 cows) twice daily (0730h, 1600h). Following Period A cows grazed ryegrass / white clover pasture for a second 7 day uniformity period, before beginning a second 9 day period (Period B) housed indoors and again fed either ryegrass or *Lotus*.

Throughout Period A, five cows on each forage were drenched with 1.2 litres 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. During Period B all cows were fed the same forage as in Period A, but those that received the water drench in Period A received PEG in Period B, and vice versa. CT bind to PEG in preference to plant proteins, thereby rendering the CT inactive (Barry and Manley, 1986). Comparing cows on ryegrass or *Lotus* diets with CT-inactivated cows (ryegrass+PEG or *Lotus*+PEG diets) therefore 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 given as percentage values and were calculated for the various parameters using the mean values from the treatments:

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

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

The ryegrass contained no CT so comparison of the ryegrass and ryegrass+PEG treatments was used to confirm that the PEG itself had no effect on the parameters measured.

Dry matter (DM) of ryegrass and *Lotus* was determined by oven drying at 100°C for 24h. Herbage samples were collected daily for analysis of chemical composition (crude protein, available carbohydrate (soluble starch and sugars), lipid, acid detergent fibre (ADF), neutral detergent fibre (NDF), organic matter digestibility (OMD) and metabolisable energy (ME) content) using near infra red spectroscopy (NIRS). Herbage sub-samples were freeze-dried and analysed for extractable (free) and bound (protein + fibre bound) CT concentration using the butanol-HCl colorimetric procedure (Terrill *et al*., 1992).

All cows were fully fed. Individual herbage allowances were calculated, using a quick estimate of herbage DM (sample dried in microwave oven) together with the calculation of Holmes *et al.* (1987), to ensure cows left a refusal at every feeding. Actual DMI was calculated by weighing herbage offered and refused at every feeding together with herbage DMs. 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 was measured throughout Periods A and B, which provided a check that cows had adjusted to their new diets before measurements began, and on 2 days in each uniformity period. Concentrations of fat, protein and lactose were determined daily (pm + am milk sample) during the measurement periods and on 2 days in each uniformity period. Samples were analysed using a fourier transform infra-red spectroscopy milk analyser (MilkoScan FT120, Foss Electric, Hillerød, Denmark).

Milk yield and composition data were analysed for variance (SAS 6.12) using covariate data collected during the uniformity periods. 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 1 and 2. Main effect means, ie. comparison of herbage species, are presented in the text as *Lotus* vs ryegrass means and SEDs.

RESULTS AND DISCUSSION

Although the *Lotus* fed during the trial had a lower DM than the ryegrass, it was, in terms of nutritive value, a higher quality feed with greater crude protein content, OMD and ME, and lower ADF and NDF ($P<0.001$) (Table 1). The ryegrass, however, had a higher ($P<0.001$) available carbohydrate content than the *Lotus*. Previous trials (Woodward *et al*., 1999) have similarly shown *Lotus* to be a high quality feed despite an often low available carbohydrate content.

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

	Ryegrass	*Lotus*	SED
DM (%)	20.4	17.9	0.5
Crude protein (g/100g DM)	16.6	21.7	0.8
Carbohydrate (g/100g DM)	7.2	6.3	0.4
Lipid (g/100g DM)	3.8	3.8	0.1
ADF (g/100g DM)	31.8	26.8	1.1
NDF (g/100g DM)	54.8	40.8	2.0
OMD (g/100g DM)	63.7	70.8	1.8
ME (MJ/kg DM)	10.8	11.1	0.1
CT: free (g/kg DM)	0	13.6	N.A.
bound (g/kg DM)	2.4	11.7	N.A.
Total (g/kg DM)	2.4	25.3	N.A.

CT concentration in the *Lotus* totalled 25.3g/kg DM, with extractable (free) CT comprising just over half (54%) (Table 1). This total CT content was similar to that of *Lotus* used in an earlier experiment in March 1998 (27.3 g total CT/kg DM) (Woodward *et al*., 1999). CT concentrations in *Lotus* do vary however as this CT level was slightly lower than for a *Lotus* pasture used by Wang *et al*. (1996) in a similar experiment with lactating sheep, and only half the concentration reported by Terrill *et al*. (1992). The ryegrass also contained CT although levels were barely detectable. Low CT concentrations have been reported previously in perennial ryegrass (Terrill *et al.*, 1992).

Cows fed *Lotus* readily consumed the herbage, and DMI was higher on *Lotus* (P<0.001) than on ryegrass (17.1 vs 15.2 kg DM/cow/d, SED 0.6). 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 positive effect of feeding *Lotus* on DMI was consistent with previous experiments (Woodward *et al.*, 1999) but was in contrast to the depression in DMI measured when sheep were fed *Lotus pedunculatus* (Barry and Duncan, 1984). These differences in the effects of CT-containing forages on animal performance are due to both CT concentration and type. The lack of a reduction in DMI as a result of feeding *Lotus* to dairy cows in our trial may have been due to the CT concentration in the *Lotus* being below (Table 1) the critical 40g CT/kg DM level reported by Waghorn *et al*. (1990) as having a detrimental effect on DMI. CT concentration in *Lotus pedunculatus* is usually higher. *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).

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.

	Ryegrass	Ryegrass+PEG	*Lotus*	*Lotus*+PEG	SED
Milk yield (kg/cow/d)	15.53	15.49	21.24	18.61	0.63
Intake (kg DM/cow/d)	15.4	15.0	17.3	17.1	0.6
Fat (%)	4.42	4.36	4.09	4.20	0.06
Protein (%)	3.12	3.08	3.34	3.16	0.05
Lactose (%)	4.93	4.97	4.92	4.92	0.04
Milk solids (kg/cow/d)	1.16	1.15	1.58	1.37	0.05
Efficiency (ml FCM/MJ ME)	150	146	184	148	9

Cows fed *Lotus* had higher (P<0.001) milk yields than those fed ryegrass (19.94 vs 15.51 kg/cow/d, SED 0.46) due, in part, to higher pasture quality, particularly higher crude protein levels, and higher DMI. This positive effect of higher pasture quality and DMI on milk yield was similar to that previously shown for cows fed both *Lotus* and white clover (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, caused 46% 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* while a similar experiment with dairy cows demonstrated CT in *Lotus* contributed to 42% of the increase in milk yield compared with cows fed ryegrass (Woodward *et al*., 1999).

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 improved efficiency was at least one of the mechanisms by which CT increased milk yield. This result was consistent with previous trials in which cows grazing *Lotus* (Harris *et al*., 1998b), and those fed *Lotus* indoors (Woodward *et al.,* 1999), had higher conversion efficiencies than those offered either ryegrass, white clover or *Lotus*+PEG. Although these results all indicate CT are involved in the improved conversion efficiency, the mechanism may be indirect via, for example, a reduction in energy wasted on urea and methane production since CT have been shown to reduce both (Waghorn *et al*., 1998).

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 (3.24 vs 3.10%, SED 0.04). 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<0.001) milk protein % of cows fed *Lotus* compared with *Lotus*+PEG (Table 2) also suggests a specific role for CT in the change, which in this case accounted for 75% of the increase above ryegrass-fed cows. This result was slightly lower than a previous trial with dairy cows where CT in contributed to 57% of the increase in milk protein concentration compared with ryegrass fed cows (Woodward *et al*., 1999). The higher contribution of CT in the current trial may have been associated with the lower crude protein content of the *Lotus*; 21.7 (Table 1) vs 25.6 g/100g DM. Wang *et al*. (1996), however, 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. It is difficult to explain these different responses between lactating cows and sheep. One possibility is that the milk protein concentration response is related to the CT concentration in the *Lotus*, and cows and sheep respond to different threshold concentrations. Support for this type of threshold concentration mechanism regulating the milk protein concentration of dairy cows comes from grazing trials where dairy cows were offered different proportions of *Lotus* in the diet, ranging from 0% (pure ryegrass) to 100% (Woodward unpublished). Cows showed no milk protein response at low levels of *Lotus*, but cows grazing pastures containing 25% or more *Lotus* had the same, elevated protein % regardless of *Lotus* content.

Feeding *Lotus* also resulted in a lower (P<0.001) milk fat concentration compared with cows fed ryegrass (4.14 vs 4.39%, SED 0.10). However, a specific role for CT in this decrease was unclear since the difference in milk fat concentration between the *Lotus* and *Lotus*+PEG treatments was not significant (Table 2) despite a trend toward lower fat % for cows on the *Lotus* treatment. Previous grazing and indoor feeding trials have similarly demonstrated a lower milk fat concentration for cows fed *Lotus* compared with those fed ryegrass (Harris *et al*., 1998b; Woodward *et al*.,. 1999) but also failed to demonstrate a specific role for CT in this decrease. 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.

CONCLUSIONS

Unlike other CT-containing legumes, the type and concentration of CT present in *Lotus corniculatus* result in increased milk yield and milk protein concentration in dairy cows. While CT may interfere with amino acid absorption from the small intestine, *Lotus* appears to be unique in that interference with intestinal function is minimal (Waghorn *et al*., 1998). Results from this trial quantified the contribution CT in *Lotus* made to these changes in milk production and highlighted the potential of *Lotus* as a forage for dairy cows. The positive effects of CT on milk yield and composition also indicate possible benefits 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 fat concentrations comply with goals for milk composition set by the New Zealand Dairy Board. The elevated protein % together with the increased milk yield result in a large increase in the milk solids (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 increase in milk solids yield measured on *Lotus*-fed cows (Table 2) could significantly improve farm income since payout is based upon milk solids production. But although *Lotus* is tolerant of dry summers and appears well adapted to rotational grazing, difficulties with establishment, low competitive ability and poor winter growth (Chapman *et al*., 1990) may limit its use on dairy farms as a substitute for clover in ryegrass-based pasture. Research is underway at DRC to test alternative ways of economically incorporating *Lotus* into the farm system.

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REFERENCES

Alison, M. W. and C. S. Hoveland. 1989. Root and herbage growth response of birdsfoot trefoil entries to subsoil activity. Agron. J. 81:677.

Barry, T. N. and S. J. Duncan. 1984. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 1. Voluntary intake. Brit. J. Nutrition 51:485.

Barry, T. N. and T. R. Manley. 1986. Interrelationships between the concentrations of total condensed tannin, free condensed tannin and lignin in Lotus sp. And their possible consequences in ruminant nutrition. J. Sci. Food and Agri. 37:248.

Chapman, H. M., W. L. Lowther and K. D.Trainor. 1990. Some factors limiting the success of *Lotus corniculatus* in hill and high country. Proc. NZGA 51:147.

Foo, L. Y., Y. Lu, W. C. McNabb, G. C. Waghorn and M. J. Ulyatt. 1997. Proanthocyanidins from *Lotus pedunculatus*. Phytochem. 45:1689.

Harris, S. L., M. J. Auldist, D. A. Clark, E. B. L. Jansen. 1998a. Effects of white clover content in the diet on herbage intake, milk production and milk composition of New Zealand dairy cows housed indoors. J. Dairy Res. 65:389.

Harris, S. L., D. A. Clark, M. J. Auldist, C. D. Waugh and P. G. Laboyrie. 1997. Optimum white clover content for dairy pastures. Proc. NZGA 59:29.

Harris, S. L., D. A. Clark and P. G. Laboyrie. 1998b. Birdsfoot trefoil - an alternative legume for New Zealand dairy pastures. Proc. NZGA 60:99.

Holmes, C. W., G. F. Wilson, D. D. S. MacKenzie, D. S. Flux, I. M. Brookes and A. W. F. Davey. 1987. Milk Production from Pasture. Butterworths Agricultural Books Ltd, Wellington, New Zealand.

Marten, G. C., F. R. Ehle and E. A. Ristau. 1987. Performance and photosensitisation of cattle related to forage quality of four legumes. Crop Sci. 34:1074.

Terrill, T. H., A. M. Rowan, G. B. Douglas and T. N. Barry. 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. J. Sci. Food and Agri. 58:321.

Waghorn, G. C., W. T. Jones, I. D. Shelton and W. C. McNabb. 1990. Condensed tannins and the nutritive value of herbage. Proc. NZGA 51:171.

Waghorn, G. C., G. B. Douglas, J. H. Niezen, W. C. McNabb and A. G. Foote. 1998. Forages with condensed tannins – their management and nutritive value for ruminants. Proc. NZGA 60:89.

Waghorn, G. C., M. J. Ulyatt, A. John and M. T. Fisher. 1987. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus* L. Brit. J. Nutrition 57:115.

Wang, Y., G. B. Douglas, G. C. Waghorn, T. N. Barry and A. G. Foote. 1996. Effect of condensed tannin in *Lotus corniculatus* upon lactation performance in ewes. J. Agri. Sci., Camb. 126:353.

Wang, Y., G. C. Waghorn, G. B. Douglas, T. N. Barry and G. F. Wilson. 1994. The effects of condensed tannin in *Lotus corniculatus* upon nutrient metabolism and upon body and wool growth in grazing sheep. Proc. NZSAP 54:219.

Woodward, S. L., M. J. Auldist, P. J. Laboyrie and E. B. L. Jansen. 1999. Effect of *Lotus corniculatus* and condensed tannins on milk yield and milk composition of dairy cows. Proc. NZSAP 59:152.

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