
THE PROCEEDINGS OF THE
NEW ZEALAND SOCIETY
OF
ANIMAL PRODUCTION
VOLUME 62 2002

Condensed tannins in birdsfoot trefoil (*Lotus corniculatus*) affect the detailed composition of milk from dairy cows

S-A. TURNER, G.C. WAGHORN, S.L. WOODWARD and N.A. THOMSON

Dexcel Limited, Private Bag 3221, Hamilton, New Zealand

ABSTRACT

Feeding birdsfoot trefoil (lotus, *Lotus corniculatus*) to dairy cows increases milk solids production and reduces methane production relative to ryegrass pasture. This study has measured the affect of lotus and lotus condensed tannins (CT) on milk protein and lipid composition. The trial involved 32 Friesian dairy cows offered either good quality perennial ryegrass pasture or lotus. Half of the cows given each diet were drenched with polyethylene glycol (PEG) to inactivate the CT. A daily milk sample was collected from all cows prior to and towards the end of the treatment period. Gross milk fat and milk protein concentrations were similar for cows fed lotus or ryegrass. Neither feed, nor CT affected individual proteins in the milk with the exception of lactoferrin (Lf) where the CT in lotus increased concentrations (138 vs 76 mg/l; $P < 0.01$). Forage type affected the composition of fatty acids (FA) in the milk with cows gazing lotus having higher concentrations of *de novo* synthesised ($C_{4:0}$-$C_{15:0}$; 28.3% vs 26.7%; $P < 0.01$) and lower concentrations of pre-formed ($C_{17:0}$ and longer; 34.5% vs 39.0%; $P < 0.05$) FA than those grazing ryegrass. The CT in lotus appeared to affect ruminal biohydrogenation of FA, decreasing the concentration of saturated FA in the milk fat, and increasing in the concentrations of the omega-3 FA (1.5% vs 1.1%; $P < 0.01$). Changes in the saturation and proportions of FA may affect processing characteristics and functionality of milk from cows fed lotus. CT in lotus may also provide potential new avenues for increasing milk value on-farm.

Keywords: detailed milk composition; *Lotus corniculatus*; condensed tannins.

INTRODUCTION

Birdsfoot trefoil (lotus; *Lotus corniculatus*) is a legume with a high nutritive value containing condensed tannins (CT), which, when fed to dairy cows, increases the efficiency of feed utilisation for milk production (Woodward *et al.*, 2004). The CT affect the rumen microflora, reduce feed protein degradation and lower feed energy losses to methane (Waghorn & McNabb, 2003). Previous experiments have shown that grazing cows on lotus increases milk yield and milk protein concentration and decreases fat concentration (Harris *et al.*, 1998; Woodward *et al.*, 1999). However those studies did not measure the effect of lotus or the CT in lotus on protein components, bioactives lactoferrin (Lf) and lactoperoxidase (LP), or the fatty acid profile of milk. Changes in minor components may affect milk processability and quality of manufactured products. Compositional changes that have nutritive or health benefits to human consumers of the milk could be exploited in the future on-farm to increase milk value.

As lotus has nutritive benefits to dairy cows there is a need to determine if feeding lotus to cows affects milk composition and milk processability. This experiment aims to extend our knowledge in this area further by examining the milk composition changes in dairy cattle as a consequence of feeding lotus.

MATERIALS AND METHODS

Experimental design

This project was part of a trial in which the effect of CT in lotus on methane production was being measured in lactating dairy cows (Woodward *et al.*, 2004). The trial conducted at Dexcel No 5 dairy, Hamilton in March 2003 used 32 Friesian cows (211 ± 15 days in milk) grazed on ryegrass dominant pasture for a 7-day uniformity period after which they were assigned to four treatment groups (based on daily milk solids yield and live weight): ryegrass-based pasture + water; ryegrass-based pasture + polyethylene glycol (PEG), lotus + water, lotus + PEG. For the 14-day trial period, 16 cows grazed ryegrass pasture, and 16 grazed lotus with a daily herbage allowance of 40 kg DM/cow for both forages. During this trial period, half (8) of the cows on each diet were drenched twice daily with 800 ml of 50% w/v PEG solution (MW 3350). The remaining cows in each group were drenched with an equivalent volume of water. Although ryegrass does not contain CT (Woodward *et al.*, 2004), inclusion of the ryegrass + PEG treatment enables comparison of both forage and CT effects.

Animal and forage measurements

Daily milk samples (combined from the p.m. + a.m. milkings) were collected from each cow for both gross milk composition and detailed milk composition at the end of the uniformity period and after 12 days of treatment. Milk samples were collected using in-line milk meters (Tru-Test Ltd., Auckland, New Zealand), to obtain a representative sample (2.5%) of the total milk.

Samples of ryegrass and lotus were collected from paddocks immediately prior to grazing, twice daily for the 4-day period prior to the milk sample collection at the end of the trial. Sub-samples were collected across the paddocks (n >10) using hand shears and cutting to an estimated grazing height, then bulked to give a representative sample across the paddock.

Forage samples were frozen immediately following collection and at the end of the experimental period were freeze-dried and ground to pass through a 1mm sieve. Fat from forages were extracted and methylated, and fatty acid analyses were performed by gas chromatography by Michael Agnew (AgResearch, Hamilton, New Zealand).

Milk sample analyses

All milk samples were analysed for fat, crude and true protein, casein, lactose and total solids using an infrared milk analyser (Fourier Transform Infrared Spectroscopy [FT120]; Foss Electric, Hillerød, Denmark). Somatic cell count (SCC) was measured using an automated cell counter (Fossomatic 5000; Foss Electric) and milk pH was measured using a CyberScan 510 pH meter (Eutech Instruments Pte Ltd, Singapore). Urea concentrations in the milk were measured using a kinetic UV assay (Alpha Scientific, Hamilton, New Zealand) and citrate concentrations by UV spectrophometry (Dagley, 1974). Concentrations of magnesium, sodium, potassium and calcium in the milk were measured following wet ashing using inductively coupled plasma optical emission spectroscopy (e-Lab, Hamilton, New Zealand).

Proportions of α-casein (α-CN), β-CN, κ-CN, γ-CN, α-lactalbumin (α-LA) and β-lactoglobulin (β-LG) were measured using SDS-PAGE followed by densitometry (Mackle *et al.*, 1999a; Mackle *et al.*, 1999b).

Bovine serum albumin (BSA) and IgG concentrations were determined using radial immunodiffusion kits, Albumin 'NL' and IgG 'NL' respectively (The Binding Site Ltd, Birmingham, United Kingdom). The following modifications were made to manufacturer's instructions. A total of 5 µl of whole milk was applied per well and the plate incubated at room temperature for 3 days. Ring diameter was determined using the Molecular Dynamics Personal Densitometer SI (Amersham Biosciences, Buckinghamsire, United Kingdom) with ImageQuant for Windows NT version 3.5 (Molecular Dynamics) software. Standard curves were linear. A total of four independent measurements were taken for each well. Samples were analysed twice on separate days and the results averaged (coefficient of variation < 10%).

Lactoferrin concentrations were measured using a bovine Lf ELISA quantification kit (Bethyl Laboratories. Inc, Montgomery, Texas, United States of America) as described by Turner *et al.* (2003).

Lactoperoxidase activity of the milk samples was assayed as follows: 3 ml 0.1 M citrate buffer (pH 5.5), 200 µl 7.5 mM 2,2' azino-di[3-ethylbenzthiazoline-6-sulphonic acid] (ABTS) and 100 µl of milk were mixed together. H_2O_2 solution (10.5 mM; 50 µl) was added, mixed and the absorbance measured at 413 nm at 120 and 240 seconds following the addition of the H_2O_2. One unit of activity was defined as the amount of enzyme resulting in the oxidation of 1 mM ABTS/minute under the standard assay conditions.

Milk fatty acid profiles were analysed as follows: fat was extracted from milk using the Röse-Gottlieb fat extraction procedure (IDF, 1987).

TABLE 1: Yield and composition of milk. Friesian dairy cows were fed either perennial ryegrass pasture or birdsfoot trefoil (lotus, *Lotus corniculatus*) and drenched twice daily (1.6 l/day) with either 50% polyethylene glycol (PEG) or water. Means and SED are given for the treatments (n = 8)

	Ryegrass	Ryegrass + PEG	Lotus	Lotus + PEG	SED	P value: Lotus vs Lotus + PEG	P value: Ryegrass vs Lotus
Milk yield (kg/cow/day)	19.28	19.40	24.55	21.81	1.11	0.020	<0.001
Fat (%)	4.58	4.74	4.29	4.72	0.26	0.112	0.290
Protein (%)	3.57	3.56	3.64	3.54	0.05	0.071	0.194
True Protein (%)	3.32	3.31	3.37	3.26	0.05	0.048	0.323
Casein (%)	2.77	2.75	2.81	2.69	0.06	0.061	0.495
Lactose (%)	4.80	4.77	4.87	4.85	0.04	0.796	0.165
Total solids (%)	13.4	13.4	13.3	13.5	0.30	0.435	0.636
Log_{10} SCC (x 1000 cells/ml)	1.68	1.83	1.77	1.76	0.12	0.937	0.462
pH	6.67	6.60	6.65	6.66	0.04	0.819	0.574
Urea (mM)	8.26	8.66	10.00	10.26	0.34	0.448	<0.001
Citrate (µg/ml)	1.70	1.63	1.51	1.52	0.11	0.884	0.087
Mg (mg/100g)	12.07	12.12	11.82	11.94	0.36	0.732	0.496
Ca (mg/100g)	132.07	132.08	132.26	129.33	2.80	0.304	0.946
Na (mg/100g)	42.60	41.97	40.89	41.64	1.46	0.613	0.253
K (mg/100g)	157.39	157.54	160.83	156.92	3.26	0.241	0.300

Fatty acids were esterified and quantified by gas chromatography by Michael Agnew (AgResearch, Hamilton, New Zealand). Briefly, fatty acids were esterified using potassium hydroxide/methanol and fatty acid methyl esters were quantified using an Agilent 6890 GC (Agilent Technologies, California, United States of America) with a 105 m RTX2330 column (Restek, CA, United States of America). Solid fat content at 10°C (SFC_{10}) of the milk was predicted using the equation reported by Mackle *et al.* (1997).

Statistical analyses

Milk data were analysed using ANOVA with cows as replicates and the uniformity period data as a covariate. Standard errors of the difference (SED) and significance values are given for comparison between all 4 treatments. Forage effects (ryegrass vs lotus) were derived from cows grazing ryegrass (no PEG) and lotus (no PEG) treatments, while CT effects were derived from cows grazing lotus versus cows grazing lotus and receiving the PEG drench (as ryegrass contains no CT). Forage data were compared using a paired *t*-test.

RESULTS

Cows grazing lotus produced significantly more milk than those grazing ryegrass pasture (Table 1) but there were no diet or treatment differences in gross milk composition (fat, protein, casein, lactose and total solids) or SCC. The CT in lotus significantly increased milk yield.

Forage

Total long-chain fatty acids (LCFA; $C_{12:0}$ to $C_{24:0}$; % of dry matter [DM]) of the lotus were higher than that found in the perennial ryegrass pasture (Table 2). The ryegrass had lower concentrations of $C_{18:1}$ and $C_{18:2}$ fatty acids (FA), but higher concentrations of $C_{18:3}$ FA than the lotus. No $C_{15:0}$ FA were detected in pasture. Concentrations of total saturated LCFA were similar for both forages.

Detailed milk composition

No differences in the detailed protein components were apparent in the milk of the cows fed the different forages with the exception of Lf, where cows grazing lotus and receiving the PEG drench had lower ($P < 0.05$) concentrations than the other treatment groups (Table 3). Lf yields were highest in cows grazing lotus. There were no apparent differences in the activity of LP in the milk of the cows receiving the four treatments.

No differences in milk pH, minerals or citrate concentrations were found between cows grazing ryegrass or lotus (Table 1). In contrast, urea concentrations were significantly higher ($P < 0.001$) in the milk of cows grazing lotus than those grazing ryegrass. The binding of the CT in the lotus by the PEG had no effect on milk urea concentrations.

Forage type did not affect milk fat concentration (Table 1), however, detailed milk fat composition did differ (Table 4). Concentrations of the *de novo* synthesised FA ($C_{4:0}$-$C_{15:0}$), were higher and concentration of preformed ($C_{17:0}$ and longer) FA were lower in the milk of cows grazing lotus. Concentrations of *cis*-9, *trans*-11 conjugated linoleic acid (CLA) were higher in the milk of cows fed ryegrass, compared with those grazing lotus, with minor effect of the CT in lotus (Table 4). In contrast, the CT increased the concentrations of *de novo* synthesised FA (28.3% vs 26.7%), and decreased the concentrations of preformed (34.5% vs 37.5%) FA in the cows fed lotus.

TABLE 2: Total lipid and fatty acid profile of perennial ryegrass pasture and birdsfoot trefoil (lotus; *Lotus corniculatus*). Means and SEM are given for both forages (n = 8). Fatty acid profile data are % of total long-chain fatty acids (LCFA; $C_{12:0}$ to $C_{24:0}$) in the pasture.

	Ryegrass	SEM	Lotus	SEM	P value
LCFA[1] (% of DM)	3.17	0.07	3.64	0.09	0.003
Fatty acid (% of LCFA)					
$C_{12:0}$	0.26	0.18	0.19	0.04	0.754
$C_{14:0}$	0.47	0.02	2.17	0.49	0.011
$C_{15:0}$	0.00	-	0.18	0.01	0.000
$C_{16:0}$	16.99	0.30	17.38	0.30	0.437
$C_{16:1}$	0.22	0.01	0.24	0.01	0.288
$C_{18:0}$	1.96	0.04	1.71	0.04	0.010
$C_{18:1\ oleic}$	2.04	0.08	2.37	0.13	0.078
$C_{18:2}$	13.97	0.31	16.17	0.43	0.006
$C_{18:3}$	58.11	0.82	54.51	0.48	0.023
$C_{20:0}$	0.63	0.02	0.97	0.10	0.015
$C_{21:0}$	0.70	0.03	0.55	0.03	0.004
$C_{22:0}$	1.21	0.02	0.82	0.02	0.000
$C_{23:0}$	0.36	0.05	0.56	0.05	0.008
$C_{24:0}$	1.32	0.02	1.11	0.01	0.000

[1]LCFA: Long-chain fatty acids ($C_{12:0}$ to $C_{24:0}$)

The degree of saturation of the milk fat appeared to be affected by the CT in lotus, with decreased concentrations of $C_{18:0}$ and increased concentrations of $C_{18:2}$ and $C_{18:3}$ (Table 4). Concentrations of the omega-3 FA ($C_{18:3}$, $C_{20:5}$ [EPA], $C_{22:6}$ [DHA]) were significantly affected by forage type and were lower in the milk of cows grazing ryegrass than lotus (1.12% vs 1.47%). The CT increased the omega-3 FA in the milk of cows grazing lotus compared with lotus plus PEG (1.47% vs 1.14%). DHA was detected in very low concentrations in only 13 of the 64 milk samples tested and thus the

TABLE 3: Detailed milk protein composition. Friesian dairy cows were fed either perennial ryegrass pasture or birdsfoot trefoil (lotus, *Lotus corniculatus*) and drenched twice daily (1.6 l/day) with either 50% polyethylene glycol (PEG) or water. Means and SED are given for the treatments (n = 8).

						P value	
	Ryegrass	Ryegrass + PEG	Lotus	Lotus + PEG	SED	Lotus vs Lotus + PEG	Ryegrass vs Lotus
bovine serum albumin (mg/l)	183.35	193.56	174.34	183.94	20.96	0.651	0.671
IgG (mg/l)	562.69	600.15	585.07	593.95	38.99	0.821	0.571
α-casein (g/kg)	13.84	13.16	13.41	13.04	0.47	0.438	0.363
β- casein (g/kg)	9.86	10.14	10.39	9.91	0.54	0.392	0.340
κ- casein (g/kg)	3.40	3.43	3.54	3.35	0.15	0.220	0.360
γ- casein (g/kg)	0.48	0.45	0.48	0.58	0.12	0.447	0.981
α-lactalbumin (g/kg)	0.66	0.58	0.66	0.70	0.16	0.807	0.984
β-lactoglobulin (g/kg)	4.25	4.55	4.41	4.31	0.18	0.556	0.368
$\log_{10}$ lactoferrin (mg/l)	2.16	2.06	2.14	1.88	0.09	0.008	0.836
	(144.5)[1]	(114.8)[1]	(138.0)[1]	(75.9)[1]			
$\log_{10}$ lactoferrin yield (g/day)	0.43	0.34	0.51	0.20		0.001	0.373
	(2.7)[2]	(2.2)[2]	(3.2)[2]	(1.6)[2]			
lactoperoxidase activity (U/ml)	7.72	7.61	7.55	8.33	0.58	0.191	0.776

[1]Mean lactoferrin (Lf) concentration (mg/l) following back-transformation
[2]Mean Lf yield (g/day) following back-transformation

results were not included in the statistical analyses, and are not shown in Table 4.

The predicted SFC_{10} of the milk fat was higher from cows grazing the lotus, than those grazing the ryegrass pasture (58.0% vs 52.1%; SED 1.5; P < 0.01).

DISCUSSION

Analysis of milk composition presented here show substantial differences between lotus and pasture, and effects of CT in the lotus on individual FA, the degree of milk fat saturation and changes in some FA of high commercial value (e.g. CLA, omega-3). Payment for milk is based on concentrations of milk fat and protein with a small penalty for volume. As there were no differences in gross fat or protein concentration, the small changes in detailed milk composition would not have affected producer income (apart from the larger quantity of milk from cows fed lotus). However, many of those changes that occurred in detailed milk components, in response to treatments, are of benefit to human consumers. For example, the CT in lotus were responsible for increasing concentrations of CLA, polyunsaturated and omega-3 FA, all of which have been reported as being beneficial to human health (Parodi, 1999; Siddiqui *et al.*, 2004; Wahrburg, 2004). On-farm manipulation of high value components by feeding cows lotus could provide a means with which to increase concentrations of desirable fats and proteins for later extraction.

The lotus forage had a higher LCFA concentration (3.6 vs 3.2 % of DM; Table 2), and cows had a higher intake of this forage than cows grazing ryegrass (Woodward *et al.*, 2004). Further, there was no difference in live weight between treatments, and gains in live weight were similar across all treatments (Woodward *et al.*, 2004). Unexpectedly, however, preformed FAs (those obtained from the diet) were lower in the milk of those cows grazing the lotus despite the higher concentrations of LCFAs in the diet. The lower transfer of dietary FA to milk fat in cows fed lotus suggests FAs were either oxidised or accumulated in body tissue.

Dietary FAs are subjected to substantial alteration during rumen digestion (Harfoot & Hazelwood, 1997). The decreased concentrations of saturated $C_{18:0}$ FA and increased concentrations of the unsaturated FA, $C_{18:2}$ and $C_{18:3}$, suggest that the CT in lotus influenced ruminal biohydrogenation. The reduced hydrogenation of dietary FAs from lotus was unexpected because (Woodward *et al.*, 2004) showed that the CT in lotus also lowered methane production (per feed DM intake) from these cows. Both methane and unsaturated FA are hydrogen sinks for the rumen microflora (Hegarty, 1999) so the 15% reduction in methane emissions attributable to the CT here and elsewhere (Waghorn *et al.*, 2002; Woodward *et al.*, 2004) and the lower saturation of pre-formed FA suggests there was less hydrogen produced during lotus digestion, compared with ryegrass. The CT in lotus is able to affect the activity of specific rumen bacteria (Jones *et al.*, 1994; Min *et al.*, 2002) and may be responsible for the changes in milk composition reported here.

TABLE 4: Detailed milk fat composition. Friesian dairy cows were fed either perennial ryegrass pasture or birdsfoot trefoil (lotus, *Lotus corniculatus*) and drenched twice daily (1.6 l/day) with either 50% polyethylene glycol (PEG) or water. Means and SED are given for the treatments (n = 8).

						P value	
Fatty acid	**Ryegrass**	**Ryegrass + PEG**	**Lotus**	**Lotus + PEG**	**SED**	**Lotus vs Lotus + PEG**	**Ryegrass vs Lotus**
$C_{4:0}$	3.92	3.96	3.60	3.67	0.10	0.496	0.003
$C_{6:0}$	2.32	2.29	2.30	2.20	0.06	0.116	0.706
$C_{8:0}$	1.36	1.30	1.40	1.30	0.05	0.028	0.400
$C_{10:0}$	2.97	2.78	3.27	2.93	0.13	0.012	0.024
$C_{12:0}$	3.34	3.09	3.80	3.34	0.16	0.007	0.006
$C_{14:0}$	10.71	10.26	11.63	11.16	0.29	0.115	0.004
$C_{14:1}$	0.83	0.72	0.92	0.81	0.04	0.021	0.035
$C_{15:0}$	1.22	1.19	1.31	1.31	0.05	0.887	0.061
$C_{16:0}$	26.27	26.17	29.00	27.70	0.86	0.143	0.004
$C_{16:1}$	1.49	1.42	1.65	1.49	0.09	0.074	0.079
$C_{17:0}$	0.54	0.55	0.49	0.53	0.02	0.033	0.021
$C_{18:0}$	11.43	12.53	9.65	11.19	0.52	0.006	0.002
$C_{18:1,\ trans\text{-}8}$	0.25	0.26	0.26	0.29	0.02	0.203	0.360
$C_{18:1,\ trans\text{-}9}$	0.28	0.28	0.29	0.29	0.02	0.729	0.556
$C_{18:1,\ trans\text{-}11}$	5.39	5.64	3.79	3.60	0.31	0.538	<0.001
$C_{18:1,\ cis/trans}$	1.05	1.13	1.46	1.53	0.07	0.313	<0.001
$C_{18:1\ \mathrm{Oleic}}$	16.69	16.92	14.43	16.00	0.69	0.031	0.003
$C_{18:1,\ \mathrm{cis}\text{-}11}$	0.41	0.41	0.39	0.40	0.02	0.641	0.210
$C_{18:2}$	0.86	0.78	1.13	0.97	0.03	<0.001	<0.001
$C_{18:3}$	1.01	0.93	1.37	1.07	0.05	<0.001	<0.001
CLA, *cis*-9, *trans*-11	2.00	1.94	1.50	1.29	0.10	0.057	<0.001
$C_{20:0}$	0.13	0.14	0.13	0.16	0.01	0.011	0.897
$C_{20:4}$	0.07	0.06	0.07	0.06	0.01	0.019	0.324
$C_{20:5\ \mathrm{(EPA)}}$	0.09	0.08	0.10	0.08	0.01	0.007	0.579
$C_{22:5}$	0.12	0.11	0.14	0.11	0.01	0.004	0.057
short ($C_{4:0}$-$C_{8:0}$)	7.62	7.55	7.30	7.16	0.18	0.411	0.088
medium ($C_{10:0}$-$C_{12:0}$)	6.31	5.86	7.07	6.27	0.28	0.009	0.011
long ($C_{14:0}$-$C_{20:0}$)	50.28	50.86	52.25	51.99	0.83	0.753	0.025
total saturated	64.21	64.25	66.63	65.43	1.06	0.270	0.031
monounsaturated	26.24	26.53	23.21	24.77	0.82	0.067	0.001
polyunsaturated	4.94	4.74	5.18	4.51	0.22	0.005	0.295
total unsaturated	30.33	30.50	27.48	28.52	0.96	0.287	0.006
omega-3[1]	1.12	1.04	1.47	1.14	0.05	<0.001	<0.001
de novo synthesised[2]	26.70	25.53	28.25	26.74	0.63	0.024	0.021
preformed[3]	39.01	40.48	34.51	37.50	1.21	0.020	0.001

[1]omega-3 ($C_{18:3}$, $C_{20:5}$ [EPA], $C_{22:6}$ [DHA])
[2]*de novo* synthesised ($C_{4:0}$-$C_{15:0}$)
[3]preformed ($C_{17:0}$ -)

The increase in *de novo* synthesised FA in milk fat of cows consuming lotus may have possibly resulted from reduced ruminal biohydrogenation and the lower levels in milk fat of $C_{18:1}$ FA, which have been reported (Palmquist *et al.*, 1993) as having a negative effect on *de novo* synthesis. During the hydrogenation of $C_{18:2}$ and $C_{18:3}$ to $C_{18:0,}$ vaccenic acid (the precursor FA to the synthesis of c-9, t-11 CLA in the mammary gland) is formed. The concentrations of vaccenic acid (*trans*-11 $C_{18:1}$) and *cis*-9, *trans*-11 CLA were both higher in milk fat from cows grazing the ryegrass, again suggesting reduced ruminal biohydrogenation of FA in the cows fed lotus. The higher concentrations of EPA in the milk fat from those cows fed lotus could be due to the higher concentrations of the precursor FA, $C_{18:3.}$

The SFC_{10} (proportion of fat that is solid at 10^{o}C) of the milk was predicted from the FA profiles. SFC_{10} is used as an indicator of the functional properties of subsequent butter as there is a positive correlation between SFC_{10} and the sectility hardness of butter (an indication of its spreadability; Mackle *et al.*, 1997). The milk from cows fed lotus had approximately a 10% higher SFC_{10} than that from cows grazing pasture suggesting that milk from cows fed ryegrass would produce a more easily spreadable butter.

Dietary crude protein is the dominant nutritional factor influencing milk urea nitrogen (Nousiainen *et al.*, 2004) and thus the higher urea concentration in milk from cows grazing lotus with and without PEG is difficult to explain. The CT in lotus would be expected to reduce net ammonia absorption (Waghorn & McNabb, 2003) despite the higher dietary concentration relative to pasture (27.94 vs 25.06 % of DM; Woodward *et al.*, 2004). If this relationship between CT and rumen ammonia absorption was responsible for milk urea concentrations, a lower milk urea concentrations would be expected in those animals fed lotus without PEG.

Lf is a high-value milk protein that is currently extracted from milk and included in consumer products such as nutritional supplements and infant formulae due to its health-promoting benefits. Drenching cows fed lotus with PEG lowered milk Lf concentrations. The inclusion of PEG in the diet of cows grazing lotus can be equated to a diet of a legume without CT (e.g. white clover) and therefore suggests that a high legume diet may result in decreases in the production of Lf. This maybe important where higher Lf in milk is required, for example for extraction.

In conclusion, lotus can be used to increase milk yields and decrease methane production, without adversely affecting milk protein composition. However, the composition and characteristics of milk fat differed suggesting that lotus could be used on-farm, subject to a suitable agronomic performance, to manipulate milk FA composition for the increased production of high-value milk fat components.

ACKNOWLEDGEMENT

The authors thank the farm and laboratory staff at Dexcel for their technical assistance and Barbara Dow for statistical advice and analyses. We acknowledge the funding support of Dairy InSight.

REFERENCES

Dagley, S. 1974. Citrate: UV spectrophotometric determination. In *Methods of enzymatic analysis 2nd. ed* (ed. H. Bergmeyer), pp. 1562-1569. Weinheim:Verlag Chemie

Harfoot, C. G.; Hazelwood, G. P. 1997. Lipid metabolism in the rumen. In *The rumen microbial ecosystem (2nd Ed).* (ed. P. N. Hobson and C. S. Stewart), pp. 382-426. Blackie Academic & Professional, London

Harris, S. L.; Clark, D. A.; Laboyrie, P. J. 1998. Birdsfoot trefoil - an alternative legume for New Zealand dairy pastures. *Proceedings of the New Zealand Grassland Association 60:* 99-103

Hegarty, R. S. 1999. Mechanisms for competitively reducing ruminal methanogenesis. *Australian journal of agricultural research 50:* 1299-1305

IDF. 1987. Milk: Determination of fat content - Rose Gottlieb gravimetric method (reference method) IFIL-IDF standard no 1C. International Dairy Federation, Brussels

Jones, G. A.; McAllister, T. A.; Muir, A.; Cheng, K. J. 1994. Effects of Sainfoin (Onobrychus viciifolia Scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. *Applied environmental microbiology 60:* 1374-1378

Mackle, T. R.; Bryant, A. M.; Petch, S. F.; Hill, J. P.; Auldist, M. J. 1999a. Nutritional influences on the composition of milk from cows of different protein phenotypes in New Zealand. *Journal of dairy science 82:* 172-180

Mackle, T. R.; Bryant, A. M.; Petch, S. F.; Hooper, R. J.; Auldist, M. J. 1999b. Variation in the composition of milk protein from pasture-fed dairy cows in late lactation and the effect of grain and silage supplementation. *New Zealand journal of agricultural research 42:* 147-154

Mackle, T. R.; Petch, S. F.; Bryant, A. M.; Auldist, M. J. 1997. Variation in the characteristics of milkfat from pasture-fed dairy cows during late spring and the effects of grain supplementation. *New Zealand Journal of agricultural research 40:* 349-359

Min, B. R.; Attwood, G. T.; Reilly, K.; Sun, W.; Peters, J. S.; Barry, T. N.; McNabb, W. C. 2002. Lotus corniculatus condensed tannins decrease in vivo populations of proteolytic bacteria and affect nitrogen metabolism in the rumen of sheep. *Canadian journal of microbiology 48:* 911-921

Nousiainen, J.; Shingfield, K. J.; Huhtanen, P. 2004. Evaluation of milk urea nitrogen as a diagnostic of protein feeding. *Journal of dairy science 87:* 386-398

Palmquist, D. L.; Beaulieu, A. D.; Barbano, D. M. 1993. Feed and animal factors influencing milk fat composition. *Journal of dairy science 76:* 1753-1771

Parodi, P. W. 1999. Conjugated linoleic acid and other anticarcinogenic agents of bovine milk fat. *Journal of dairy science 82:* 1339-1349

Siddiqui, R. A.; Shaikh, S. R.; Sech, L. A.; Yount, H. R.; Stillwell, W.; Zaloga, G. P. 2004. Omega 3-fatty acids: health benefits and cellular mechanisms of action. *Mini-reviews in medicinal chemistry 4:* 859-871

Turner, S.-A.; Williamson, J. H.; Thomson, N. A.; Roche, J. R.; Kolver, E. S. 2003. Diet and genotype affect milk lactoferrin concentrations in late lactation. *Proceedings of the New Zealand Society of Animal Production 63:* 87-90

Waghorn, G. C.; McNabb, W. C. 2003. Consequences of plant phenolic compounds for productivity and health of ruminants. *Proceedings of the Nutrition Society 62:* 383-392

Waghorn, G. C.; Tavendale, M. H.; Woodfield, D. R. 2002.

Methanogenesis from forages fed to sheep. *Proceedings of the New Zealand Grassland Association 64:* 167-171

Wahrburg, U. 2004. What are the health effects of fat? *European journal of nutrition 43 Suppl 1:* I/6-11

Woodward, S. L.; Auldist, M. J.; Laboyrie, P. J.; Jansen, E. B. L. 1999. Effect of Lotus corniculatus and condensed tannins on milk yield and milk composition of dairy cows. *Proceedings of the New Zealand Society of Animal Production 59:* 152-155

Woodward, S. L.; Waghorn, G. C.; Laboyrie, P. J. 2004. Condensed tannins in birdsfoot trefoil (Lotus corniculatus) reduce methane emissions from dairy cows. *Proceedings of the New Zealand Society of Animal Production 64:* 160-164