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Condensed tannins in birdsfoot trefoil (*Lotus corniculatus*) reduce methane emissions from dairy cows

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ABSTRACT

Ruminant methane production contributes 34% of New Zealand greenhouse gases, but emissions are affected by feed type. A trial conducted at Dexcel measured methane production by 32 Friesian dairy cows grazing either good quality perennial ryegrass pasture or birdsfoot trefoil (lotus, *Lotus corniculatus*), a legume with high nutritive value that contains condensed tannins (CT; 2.62 g CT/100 g DM). Half the cows on each diet were drenched with polyethylene glycol (PEG) to inactivate the CT and allow the effect of CT on methane production to be determined. Total methane production, measured using the SF_6 tracer technique, was similar for the lotus and ryegrass cows (343.2 vs 360.6 g CH_4/cow/d; ns), however, methane production per unit DMI was lower from cows fed lotus (19.9 vs. 24.2 g CH_4/kg DM, $P<0.001$). The CT in lotus reduced methane production by 13% relative to lotus when the CT had been inactivated by PEG, and was responsible for 66% of the difference between lotus and ryegrass. Cows fed lotus produced 32% less methane/kg milksolids compared to good quality ryegrass. The feasability of feeding lotus as a methane mitigation option should now be assessed at a farm systems level to investigate effects on other greenhouse gases, productivity and profitability.

Keywords: condensed tannins; legume; *Lotus corniculatus*; methane emissions; milksolids.

INTRODUCTION

Ruminant methane (CH_4) accounts for 34% of New Zealand greenhouse gas emissions and dairy cattle account for about 23% of ruminant methane. Methane is a sink for hydrogen ions derived from rumen fermentation and the majority is exhaled through the mouth and nostrils. Methane production by ruminants fed forage diets accounts for 6-7% of gross energy intake (GEI) (Ohara *et al*., 2003), equivalent to about 10% of metabolisable energy (ME) intake.

Methane mitigation options for New Zealand's pasture-based dairy industry must be practical and not compromise productivity. A two fold range in methane emissions/dry matter intake (DMI) have been reported in trials when contrasting diets have been fed to sheep, and lowest emissions were reported from the condensed tannin (CT)-containing legume Maku lotus (*Lotus pedunculatus*) (Waghorn *et al*., 2002). Cows fed ensiled birdsfoot trefoil (*Lotus corniculatus*; referred to as "lotus") produced 23% less CH_4/kg DMI than cows fed ryegrass-based pasture silage (Woodward *et al*., 2001), and fresh sulla (*Hedysarum coronarium*) resulted in 19.5 g CH_4/kg DMI compared to 24.6 g CH_4/kg DMI from fresh pasture fed to cows (Woodward *et al*., 2002b).

Both sulla and lotus are highly nutritious and palatable legumes and have resulted in very good performance when fed to lactating cattle as either a sole diet (Harris *et al*., 1998; Woodward *et al*., 2000) or when ensiled and fed with pasture (Woodward *et al*., 2002a). Milk production responses, relative to perennial ryegrass-based pasture, were greater when lotus was fed than white clover (*Trifolium repens*) and part of the response was attributed to the CT in lotus (Harris *et al*., 1998). Lotus yields 10-15 t DM/ha when grown in favourable conditions and CT usually account for 2-4% of lotus DM (Waghorn *et al*., 1998).

CT are found in a number of legumes including *Lotus*es, sulla, sainfoin (*Onobrychis viciifolia*) and in the flowers of white clover (*Trifolium repens*) (Terrill *et al*., 1992). CT bind to plant proteins in the rumen, reducing protein degradation to ammonia and increasing absorption of essential amino acids from the small intestine (Waghorn *et al*., 1987; Wang *et al*., 1996b). The CT in lotus increased milk and milk protein production, and feed conversion efficiency (Wang *et al*., 1996a; Woodward *et al*., 1999). Other benefits include a reduction in the incidence of bloat and amelioration of the effects of parasitism (Hoskin, 1998; Waghorn *et al*., 1999). The CT in Maku lotus reduced methane production by sheep fed at maintenance intakes by about 17% (Waghorn *et al*., 2002).

Other than protein binding, the mechanisms by which CT affect rumen function are not well understood. CT can slow digestion in the rumen (Waghorn *et al*., 1994) and they affect bacterial species to differing extents (Jones *et al*., 1994). The reductions in methanogenesis attributed to CT reported for sheep fed Maku lotus may arise from a reduction in hydrogen production, alternative hydrogen sinks or through direct effects on methanogens. The effects of CT in lotus (cv. Goldie) on milk and methane production from dairy cows, in comparison to conventional ryegrass-based pasture, are reported.

MATERIALS AND METHODS

Trial design

The trial was conducted in March 2003 at Dexcel's No 5 Dairy, Hamilton, New Zealand using 32 Friesian dairy cows in late lactation (211±15 days in milk). The cows grazed together on perennial ryegrass-based pasture for 7 days (uniformity period) prior to treatment allocation on the basis of daily milksolids yield (milkfat plus milk protein yield; MS) and LW. The 14-day trial period comprised 16 cows grazing ryegrass pasture and 16 cows grazing lotus. During the trial period half (8) of the cows on each diet were drenched twice daily with 800 ml of 50% w/v polyethylene glycol (PEG) solution (MW 3350). The remaining 8 cows on each diet received an equivalent drench of water. PEG supplementation inactivates CT, which bind to PEG in preference to plant proteins (Barry & Manley, 1986). Comparison between control cows fed either ryegrass or lotus diets, with cows given PEG (ryegrass+PEG or lotus+PEG diets) enabled effects of CT in the lotus to be quantified and separated from nutritional attributes of lotus. The CT effects are expressed as a percentage of the treatment effects, calculated using mean values from the treatments:

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

where *x* is the mean of the ryegrass and ryegrass+PEG treatments.

Comparison of the ryegrass and ryegrass+PEG treatments enabled any direct effects of PEG on performance to be determined, although past indications in trials with cows (Woodward *et al*., 1999) suggested this was unlikely.

Forage nutritive characteristics, cow LW, DMI, milk production and composition, and methane emission measurements were made over 2 days at the end of the uniformity period and over the 5-day 'measurement period' at the end of the trial. Cows were maintained on the dietary treatments for 9 days prior to the measurement period to enable rumen adaptation to forages and PEG treatment.

Grazing management and forage measurements

Pre-grazing herbage mass in the ryegrass and lotus paddocks was determined by calibrated visual assessment and measurement of forage DM content. The size of the respective 24-hour grazing breaks was calculated to provide a daily herbage allowance of 40 kg DM/cow. Daily breaks were back-fenced during the measurement period and water was always available.

Pre-grazing ryegrass and lotus samples (cut to grazing height) were collected daily during the measurement period for determination of DM (oven dried 95^{o}C) and chemical composition (oven dried 65^{o}C) using near infrared reflectance spectrophotometry (NIRS systems 6500). Samples of lotus taken during the measurement period were bulked, freeze dried and CT determined using the butanol-HCL colorimetric procedure (Terrill *et al*., 1992). Chemical composition was used to calculate the gross energy (GE) content of the forages.

Cows were given 345 mg C32 alkane marker twice daily throughout the trial and faecal samples were collected from individual cows prior to morning and afternoon milking for 2 days during the uniformity period and over the 5-day measurement period. The faecal samples from each cow were bulked within the uniformity and the measurement period for alkane measurement and estimation of individual cow DMI as described by Harris *et al*. (1998).

Milk measurements

Daily milk yield (pm plus am milking) was measured during the uniformity and measurement periods. Daily milk samples were also collected from each cow and analysed for milkfat and milk protein concentration using an infrared milk analyser (Milkoscan 133B, Foss Electric, HillerØd, Denmark).

Methane measurements

Methane production was measured using the sulphur hexafluoride (SF_6) tracer technique described by Woodward *et al*. (2001). Briefly, the SF_6 intraruminal marker was released at about 3.0 mg/day from permeation tubes given to each cow prior to the uniformity period. Respired air from each cow was sub-sampled continuously over 2x24 hours during the uniformity period and 5x24 hours during the measurement period into an evacuated yoke fitted around the neck of each cow. Background concentrations of atmospheric methane and SF_6 were collected from paddocks adjacent to those grazed by cows on each treatment. Methane and SF_6 concentrations were measured by gas chromatography, and the methane emission rate calculated as:

$$QCH_4 = \frac{QSF_6 \times ([CH_4\ \text{yoke}] - [CH_4\ \text{background}])}{([SF_6\ \text{yoke}] - [SF_6\ \text{background}])}$$

where QSF_6 is the calibrated rate of permeation from the SF_6 capsule.

Statistical analysis

All forage data were analysed using ANOVA. Cow data collected during the measurement period were analysed using ANOVA with cows as replicates and the uniformity period data as a covariate. Forage (ryegrass vs lotus) effects were derived from cows grazing ryegrass (no PEG) and lotus (no PEG) treatments only. CT effects were derived from cows grazing lotus and lotus+PEG treatments only, since the ryegrass contained no CT.

RESULTS AND DISCUSSION

Forage quality

The lotus and ryegrass pasture were both good quality forages. The ryegrass had higher DM and neutral detergent fibre (NDF) content than lotus, but values for crude protein (CP), organic matter digestibility (OMD) and metabolisable energy (ME) were reasonably similar for the two forages. The good quality ryegrass was achieved by irrigation and differed from that used in previous summer-autumn trials where ryegrass pasture NDF was higher (52.9-55.0 g/100 g DM (Harris *et al*., 1998; Woodward *et al*., 1999; Woodward *et al*., 2000).

The CT in lotus was 2.62 g/100g DM, most of which was unbound (Table 1) and concentrations were similar to those of fresh and ensiled lotus used in previous trials at this site (Harris *et al*., 1998; Woodward *et al*., 1999; Woodward *et al*., 2000; Woodward *et al*., 2001). Trials with sheep have shown 2-4 g CT/100 g DM in lotus lowered rumen ammonia concentrations, suggesting significant reductions in protein degradation and an increased protein flow to the small intestine (Waghorn *et al,* 1990).

Intake

Cow DMI was higher when fed lotus (P<0.001) compared to ryegrass (Table 2) in line with previous trials (Harris *et al*., 1998; Woodward *et al*., 1999; Woodward *et al*., 2000). There were no effects of CT on intakes of cows fed lotus, and PEG drenching did not affect intakes of cows fed ryegrass. The absence of CT effects on intake is consistent with previous trials, and those with sheep, but differs from the inhibition seen in some instances when Maku lotus was fed (Waghorn *et al*., 1999). Maku lotus contains higher concentrations of a more astringent CT than lotus used here (Waghorn & Shelton, 1997).

Milk production and composition

Cows fed lotus had higher (P<0.001) milk yields than those fed ryegrass (Table 2) due to a combination of higher DMI and forage quality and the action of CT in the lotus. The lotus effect on MS (+0.52kg/day or a 33% increase) was similar to effects of substituting ryegrass pasture with 25-50% white clover (20-30% increase; Harris *et al*., 1998) but less than the 42% increase reported in a comparison between ryegrass pasture and lotus by Woodward *et al*. (1999). The smaller MS response in this trial is probably a consequence of the good quality ryegrass.

The similar milkfat and milk protein concentrations from cows grazing lotus and ryegrass in this trial (Table 2) are further evidence of the good quality ryegrass used here. Previous trials with dairy cows have demonstrated a higher milk protein concentration from lotus than ryegrass (Harris *et al*., 1998; Woodward *et al*., 1999; Woodward *et al*., 2000).

The higher (P<0.001) milk yield of cows fed lotus compared with lotus+PEG (Table 2) showed that CT contributed 41% of the increased milk yield relative to ryegrass. This is similar to the contribution made by CT when cows were fed lotus compared with medium quality ryegrass during two indoor feeding trials in late lactation (42%; Woodward *et al*., 1999) and mid-lactation (46%; Woodward *et al*., 2000). This effect of CT on milk production is not restricted to dairy cows and has also been demonstrated in lactating ewes by Wang *et al*. (1996a) and probably results from an increased flow of protein to the intestine for absorption.

Methane production

Total methane production from cows grazing lotus was similar to cows grazing ryegrass pasture (P=0.367) (Table 2). However, cows ate 2.5 kg more lotus DM than ryegrass DM (+16%) and the methane production per unit DMI was 17% lower from lotus than ryegrass (P<0.001). Measurements showed the CT in lotus accounted for 66% of the reduction in methane/kg DM for lotus compared to ryegrass. The remainder of the difference may have been due to the lower NDF in lotus compared to ryegrass.

When expressed in terms of GEI, cows grazing lotus lost 5.98% to methane compared to 7.50% from cows grazing ryegrass (Table 2; P<0.001). These percentages are similar to reports for lactating dairy cows fed a range of forages (Lassey *et al*., 1997; Woodward *et al*., 2002b). The difference in energy lost to methane from cows fed 15 kg DM/d of ryegrass or lotus can be calculated as 4.24 g CH_4/kg DMI or about 64 g CH_4/d, equivalent to 3.5 MJ or 2% of ME intake. If this energy was absorbed

TABLE 1: Chemical composition and condensed tannin (CT) concentration of the perennial ryegrass pasture and the birdsfoot trefoil (*Lotus corniculatus*). Means and standard errors of the mean (SEM) are given for both forages (n=10). Data are g/100 g DM unless indicated.

	Ryegrass	SEM	Lotus	SEM
Dry matter (%)	16.10	0.83	12.14	0.57
Crude protein	25.06	0.38	27.94	0.42
Soluble sugars and starch	9.73	0.49	10.09	0.57
Lipid	4.29	0.05	4.81	0.05
Acid detergent fibre	22.98	0.50	20.83	0.61
Neutral detergent fibre	41.10	0.76	28.43	0.84
CT: free	0	N.A.	1.87	0.03
bound	0	N.A.	0.75	0.02
total	0	N.A.	2.62	0.04
Organic matter digestibility	77.28	1.10	81.40	0.68
Metabolisable energy (MJ/kg DM)	10.88	0.15	11.34	0.09

TABLE 2: Cow liveweight, dry matter intake, milk yield, milk composition, milksolids yield, and methane production of Friesian dairy cows fed either perennial ryegrass pasture or birdsfoot trefoil (*Lotus corniculatus*) and drenched twice daily (3.6 l/d) with either 50% polyethylene glycol (PEG) or water. The means and SEDs given are for the individual treatments (n=8).

	Ryegrass	Ryegrass +PEG	Lotus	Lotus +PEG	SED
Liveweight (kg/cow)	538	540	536	537	4.5
Intake (kg DM/cow/d)	14.92	14.93	17.38	17.11	0.46
Milk yield (kg/cow/d)	18.46	18.99	24.37	22.08	0.70
Fat (%)	4.53	4.68	4.63	4.63	0.17
Protein (%)	3.59	3.56	3.63	3.61	0.05
Milksolids (kg/cow/d)	1.49	1.55	2.01	1.81	0.05
Total methane production (g CH_4/cow/d)	360.63	368.30	343.24	391.65	12.40
Methane per unit intake (g CH_4/kg DM)	24.15	24.71	19.91	22.89	0.78
Methane per unit production (g CH_4/kg MS)	249.74	243.66	170.85	216.43	10.59
Methane energy (% of GEI)	7.50	7.66	5.98	6.89	0.27

as volatile fatty acid (VFA) it could contribute about 0.6 kg milk or 48 g MS/d.

The comparisons between ryegrass and lotus incorporate both legume (ie. composition) and CT effects. Use of PEG showed that the CT in lotus lowered methane production by about 3 g/kg DMI, or 13% which is similar to the 17% reported for sheep fed Maku lotus containing 5.8% CT in the DM (Waghorn *et al*., 2002).

The mechanism by which the CT in lotus reduced methane production is not understood but CT can reduce rates and extent of microbial degradation (McAllister *et al*., 1994). Jones *et al*., (1994) demonstrated sensitivity of some bacteria but not others to effects of CT on colonisation of forages, so it is possible that CT could have direct effects on methanogenic archaea in the rumen.

Methane and other greenhouse gases can be expressed on the basis of production, for example MS, but this should only be undertaken with animals of similar age, physiological status and productivity. These similarities are particularly important with dairy cows where feed energy contributes to maintenance and LW change as well as production. In this trial the cows were a similar LW (Table 2) and daily gains ranged from 223-249g/day (treatment group means) and were similar across all treatments. The contribution of feed energy to maintenance would therefore have been similar for all treatments enabling a valid comparison of methane per unit production. Cows grazing lotus had higher MS production ($P<0.001$) than those grazing ryegrass and, as a result, methane production per unit MS was lower ($P<0.001$) for cows grazing lotus than those on ryegrass (Table 2).

CONCLUSION

This trial has clearly demonstrated that grazing dairy cows fed lotus increased milk production and reduced methane emissions per unit DMI or MS. CT played an important role in these benefits. This and previous short-term trials have demanded a more comprehensive analysis of lotus for dairying systems. Future farm systems research will provide a more comprehensive analysis of this forage. Such research should address the use of lotus as a forage supplement rather than as the sole diet within the farm system in order to meet cow feed requirements, and enable evaluation of farm profitability and environmental impacts. The negative implications of cultivation required to maintain lotus will be evaluated in relation to reduced nitrogen application, urinary nitrogen losses and lower methane emissions relative to pasture. These measurements will complement the partial life cycle analysis of Van der Nagel *et al*., (2003) who showed the methane and carbon dioxide emissions associated with grain-based rations substantially exceeded those for pasture when expressed in terms of annual production.

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