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Early indications that feeding *Lotus* will reduce methane emissions from ruminants

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ABSTRACT

Ruminant livestock produce at least 75% of New Zealand's total methane emissions and are a major contributor of greenhouse gas emissions. Most ruminant methane arises from microbial activity in the rumen (methanogenesis) and is released through the mouth and nose. Methane production per unit feed intake or per unit production can be reduced by improving diet quality, for example, including legumes in a pasture diet. A preliminary investigation with wether sheep showed lower daily methane outputs per unit dry matter intake (DMI) when fed *Lotus pedunculatus* (a condensed tannin-containing legume) than ryegrass-based pasture or lucerne (14.5 vs. 20.4 vs. 19.0 gCH_4/kg DMI). Friesian dairy cows fed either *Lotus corniculatus* silage or perennial ryegrass silage had similar total methane outputs (376.7 vs. 344.4 g CH_4/cow/day; ns). However, methane emissions were lower from cows fed *Lotus* silage when expressed per unit DMI (26.90 vs. 35.13 g CH_4/kg DMI; $P<0.001$) and per unit production (378 vs. 434 g CH_4/kg milksolids; $P<0.05$) as a result of the higher intake and milksolids yield. The mitigation of methane emissions from animals fed *Lotus* species was due in part to a higher nutritive value relative to pasture but effects of condensed tannins on methanogenesis warrants further investigation.

Keywords: methane; *Lotus*; dairy cows; sheep; ruminants.

INTRODUCTION

Release of greenhouse gases, such as carbon dioxide and methane (CH_4), into the atmosphere is a major cause of global warming. Methane, for example, accounts for about 20% of global warming (Taylor & Smith, 1997). Ruminant animals release significant quantities of methane, as well as carbon dioxide, during digestion, such that farmed livestock are responsible for at least 75% of New Zealand's methane emissions (Lassey *et al*., 1997). Ulyatt *et al*. (1992) reported that sheep, dairy and beef cattle contribute 58, 18 and 21%, respectively, of total ruminant methane production (1.5 Tg CH_4/y) in New Zealand. When methane emissions are expressed per head of human population, values for New Zealand are very high (455 kg CH_4/cap/y) relative to the global average (47 kg CH_4/cap/y), due mainly to our small human population relative to our ruminant population (Taylor & Smith, 1997).

Ruminant methane is produced primarily from microbial activity in the rumen (90%) with a minor contribution from the large intestine (10%). The majority of methane, whether it is produced in the rumen or the large intestine, is released through the mouth and nostrils (Murray *et al*., 1976). Methane production represents an energy loss to ruminants of around 3 to 9% of gross energy (GE) intake (Blaxter & Clapperton, 1965).

New Zealand's agriculturally based economy is vulnerable to imposition of tariffs if international moves to introduce carbon taxes are ratified in an effort to mitigate greenhouse gas emissions. This highlights the importance of defining methane production from forage- fed ruminants, and identifying possible methods for mitigation. Options range from reducing livestock numbers to altering rumen microbial populations. However, modification of diet quality appears to be the most viable option in the short term.

There are several aspects of diet quality (e.g. fibre content) that affect methane production. When forages, such as pasture, are fed, the yield of methane per unit of GE is high relative to concentrate diets, and is further increased at low levels of feed intake (Blaxter & Clapperton, 1965). This means that low yielding animals consuming poor quality diets can produce more methane per unit product than high producing animals consuming higher quality diets at high feed intakes. Hence, methane emission from livestock fed legumes should be lower than emissions from livestock fed grass-based forages.

This paper reports results from two preliminary trials investigating effects of diet on methane emissions from wether sheep and lactating dairy cows fed *Lotus* species vs. pasture. *Lotus corniculatus* (LC; birdsfoot trefoil) and *Lotus pedunculatus* (LP; Lotus major) are high quality legume forages able to support high levels of animal productivity (Harris *et al*., 1998; Waghorn *et al*., 1994). They contain condensed tannins, which affect the site and extent of protein and fibre degradation through alterations in microbial growth (Waghorn *et al*., 1994; 1999).

MATERIALS AND METHODS

Sheep trial

The initial investigation was conducted at AgResearch Grasslands, Palmerston North, New Zealand in February 1995. Three mature (3-4 years) Romney wether sheep (live weight 55-65 kg) were housed indoors and each was fed perennial ryegrass-dominant pasture, followed by lucerne (syn. alfalfa; *Medicago sativa*), and finally LP, each for one week. Freshly cut forage was offered twice daily at maintenance levels of intake (800-900 g DM/sheep/day). Over the final 48h of each week, daily dry matter intakes (DMI) were measured for each sheep (calculated from forage offered and refused, and the DM content of the forage and refusals), in conjunction with faecal output and methane emissions. This enabled methane production to be expressed both as net emissions and in terms of digestible feed intake.

Dairy cow trial

The second trial was conducted in April 2000 at the Dairying Research Corporation (Dexcel Limited), Hamilton, New Zealand, using 12 Friesian cows in late lactation (240 ± 13 days in milk). Six cows, balanced for

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daily milksolids yield (milkfat plus milk protein yield; MS) and live weight, were allocated to either perennial ryegrass (*Lolium perenne*) or LC dietary treatments. The trial comprised a 7-day adjustment period when cows grazed either ryegrass- or LC-dominant pasture, followed by a 5-day measurement period during which cows were individually housed in metabolism stalls. It was intended that fresh ryegrass and LC pasture would be cut and fed indoors, but an aversion to both cut forages (possibly a consequence of faecal contamination of irrigation water) resulted in a change to pure ryegrass silage or pure LC silage diets. Cows were offered either ryegrass silage or LC silage *ad libitum* for 4 days (from day 2 until day 5 of the measurement period). Results presented are for data collected on days 4 and 5 of the indoor feeding period, by which time intakes, milk yield and methane output had stabilised.

Silages were fed twice daily (0800h and 1630h) with daily DMI calculated from forage offered and refused. The cows were milked twice daily (0730h and 1600h), individual milk yields determined, and milk sampled for composition analysis.

Measurements

In both trials, forage samples were taken to determine chemical composition by NIRS (NIRS systems 6500), and concentration of condensed tannins in *Lotus* by the butanol/HCl method (Terrill *et al*., 1992). Milk fat and milk protein concentrations were measured using an infrared milk analyser (Milkoscan 133B, Foss Electric, Hillerød, Denmark).

Methane production was measured in subsamples of respired air using the sulphur hexafluoride (SF_6) tracer technique (Johnson *et al*., 1994). This procedure involved collecting a continuous aliquot of respired gas over 24-h periods and measuring methane and SF_6 concentrations in the accumulated sample. The SF_6 intraruminal marker was released at a known rate (approx. 0.7 mg/day) from brass permeation tubes (approx. 35mm long x 10mm external diameter) inserted into the rumen of each animal 2 weeks prior to each trial (1 tube per sheep, 2 tubes per cow).

Once housed indoors, each animal was fitted with a halter that supported the sampling probe adjacent to the nose, and connected via a fine-bore capillary tube and Teflon tubing to the evacuated collection yoke (Lassey *et al*., 1997) suspended above the animals. The yokes were changed every 24 hours. Background concentrations of atmospheric SF_6 and methane were collected from the respective animal houses over the duration of both trials. Methane and SF_6 concentrations were measured by gas chromatography, and the methane emission rate was then calculated as:

$Q_{CH4} = Q_{SF6} \times ([CH_4\,\text{yoke}] - [CH_4\,\text{background}]) / ([SF_6\,\text{yoke}] - [SF_6\,\text{background}])$ the SF_6 capsule.

Statistical analysis

The sequential design of the sheep trial and small number of sheep meant that only a restricted statistical analysis of data was possible. Despite its preliminary nature, the trial did, however, provide quantitative data for methane emissions from sheep having a known feed intake and did indicate substantial differences between pasture and LP diets.

Milk yield and milk composition data collected during the dairy cow trial were analysed for variance using Genstat 5.3 with the covariate data collected during the uniformity period. Methane emission and DMI data, together with herbage chemical composition data, were also analysed for variance using Genstat 5.3, but no covariate data were collected for these parameters.

RESULTS AND DISCUSSION

Sheep trial

The pasture, lucerne and LP fed to sheep were of good quality (Table 1) with low DM and high protein contents, but with higher concentrations of fibre in the pasture than the legumes. The LP contained 8.0% condensed tannin, and there was no CT in ryegrass or lucerne. DMI and digestible DM intake (DDMI) were at maintenance level and similar for all three diets (Table 2). However, methane production was much greater from sheep fed pasture than LP, with lucerne intermediate (16.0, 10.6 and 13.5 g CH_4/day respectively). These values equate to 29.3 g CH_4/kg DDMI for sheep consuming pasture compared with 19.6 g CH_4/kg DDMI for the LP diet. When expressed in terms of gross energy intake the energy loss to methane can be calculated as 6.2% (pasture), 5.7% (lucerne) and 3.9% (LP). Values for pasture are comparable with published estimates for sheep of (4.5 – 6.5%GEI) (Lassey et al., 1997; Ulyatt *et al*., 2001). The low values for *Lotus* prompted the measurements on lactating cows to be made.

TABLE 1: Composition of the pasture, lucerne and *Lotus pedunculatus* fed to sheep during methane measurements. All units are g/100g dry matter unless otherwise stated.

	Pasture	Lucerne	*Lotus*
Dry matter content (%)	21.1	15.2	12.1
Crude protein	30.0	26.8	30.0
Lipid	3.6	4.2	4.5
Neutral detergent fibre (NDF)	38.6	31.6	30.4

TABLE 2: Intakes and methane production (mean ± standard deviation from all observations (SD)) from three sheep fed fresh pasture, lucerne or *Lotus pedunculatus.*

	Pasture		Lucerne		*Lotus*	
	mean	SD	mean	SD	mean	SD
Intake (DMI) (g DM/sheep)	797	34	725	74	733	15
Digestible DMI (g DM/sheep)	549	6.5	540	48.3	543	12.2
Methane production ($g\ CH_4$/sheep/d)	16.0	1.00	13.5	0.89	10.6	0.56
Methane ($g\ CH_4$) / digestible DMI	20.3	2.13	19.0	1.53	14.5	1.04
Methane energy / gross energy intake (%)	6.2		5.7		3.9	

Cow trial

The compositions of the ryegrass and LC silages are given in Table 3. Both the ryegrass and *Lotus* silages were of average quality as indicated by the predicted metabolisable energy (ME) content, although fibre concentrations and the low crude protein for ryegrass suggested that ryegrass harvest had probably been later than optimum. As in previous trials, in which fresh LC was fed to dairy cows (Harris *et al*., 1998; Woodward *et al*., 2000), the LC silage had a much higher crude protein and soluble carbohydrate concentration than the ryegrass silage, in part due to reduced protein degradation in the presence of condensed tannin (Niezen *et al*., 1998).

TABLE 3: Chemical composition (g/100g dry matter, unless otherwise stated) of the perennial ryegrass and *Lotus corniculatus* silages fed to lactating dairy cows. Values are the mean of four samples per treatment, and standard error of the difference (SED).

	Ryegrass	***Lotus***	**SED**
Dry matter (%)	41.5	31.0	1.72
Crude protein	14.7	21.4	0.35
Lipid	5.0	4.6	0.12
Acid detergent fibre (ADF)	37.1	32.1	0.70
Neutral detergent fibre (NDF)	55.1	39.3	1.23
Soluble carbohydrates	1.8	4.5	0.29
In vitro digestibility	67.1	68.6	0.78
Metabolisable energy (MJ/kg DM)	10.7	11.0	0.11
pH	4.10	4.83	4.05
Lactic acid (g/kg DM)	7.6	7.3	0.67
Ammonia-N (mg/100g DM)	198	311	9.4
Ammonia-N as fraction total N (% of total N)	8.4	9.1	0.25
Condensed tannin	0	2.59	N.A

Cows offered LC silage had a higher DMI ($P<0.001$) and milk production ($P<0.01$) than those fed ryegrass silage (Table 4). The methane production was similar for cows fed LC and pasture silage (344 vs. 377 g CH_4/d; ns), but the higher DMI of cows fed LC silage produced significantly lower emissions per kg DMI ($P<0.001$), and a lower methane yield per kg milksolids produced ($P<0.05$; Table 4).

TABLE 4: Milk production, milk composition, intake and methane emissions of Friesian dairy cows fed either perennial ryegrass or *Lotus corniculatus* silage. Values are the mean of six cows on each treatment, and standard error of the difference (SED).

	Ryegrass	***Lotus***	**SED**
Intake (DMI) (kg DM/cow)	10.22	14.07	0.64
Milk yield (kg/cow/d)	9.48	11.94	0.64
Milkfat concentration (%)	4.94	4.57	0.16
Milk protein concentration (%)	3.50	3.86	0.09
Milksolids (MS) yield (kg MS/cow/d)	0.80	1.01	0.04
Methane production (g CH_4/cow/d)	344.4	376.7	19.07
Methane per unit intake (gCH_4/kg DMI)	35.13	26.90	1.58
Methane per unit production (g/kg MS)	434.0	378.0	22.3

Methane emissions ranged from 322-374 g CH_4/cow/d for cows fed ryegrass silage, and from 332-429 g CH_4/cow/d with the LC diet. When methane emissions were expressed per unit feed intake, between-cow variation in methane production was 33.4 – 37.9 g CH_4/kg DMI with pasture silage and 22.3 – 31.8 g CH_4/kg DMI with LC silage. The extent of between cow variation was similar to that previously shown across a range of diets by Blaxter & Clapperton (1965). Variation between cows in gross methane production may result from differences in rumen retention time and rumen volume (Pinares-Patino *et al*., 2000), and an understanding of factors affecting this variation may provide opportunities for future mitigation of methane emissions.

It is interesting to note that methane emissions from cows fed ryegrass silage in this study were substantially greater than in previous reports for lactating dairy cows grazing fresh ryegrass-white clover pasture. For example, Lassey *et al*. (1997) measured an average methane output of 263 g CH_4/cow/d from Friesian cows producing 14.1 l milk/cow/d, and Ulyatt *et al*. (2001) measured 182 g CH_4/day from cows producing 12.1 l milk/cow/d. This suggests relatively greater methane yields from ensiled compared with fresh pasture. Furthermore, in the trials reported here, the methane outputs per unit DMI of the cows fed silage were higher than the methane outputs of the sheep fed fresh forage for both the pasture (35.1 vs. 20.3 g CH_4/kg DMI) and the *Lotus* (26.9 vs. 14.5 g CH_4/kg DMI). Since published data have shown similar yields of methane from sheep and cattle given diets of similar type and composition (Johnson & Johnson, 1995; Lassey *et al*., 1997), the comparatively high emissions from silages in the dairy cow study may have been due to feed quality or to changes in feed composition during ensiling, or the short adaptation period, rather than differences between animal species.

Differences in methane production attributable to forage species and conservation processes may have important implications for potential greenhouse gas mitigation strategies. The actual amount of potentially useful energy lost to methane is also worthy of consideration. Data presented here suggest 3.9 – 6.6% of the gross energy (GE) of forages given to sheep was lost as methane. This equates to approximately 6.1 – 9.8% of ME and represents a substantial diversion of feed energy away from production. The extent of loss from silage fed to dairy cows was even greater. Ryegrass silage resulted in a loss of 10.8% of GE and 17.0% of ME to methane. Equivalent values for LC silage were 8.6% of GE and 13.5% of ME. These values are at the upper limits of published data and suggest silages, especially pasture silage of low or average quality, may make a significant contribution to greenhouse gas emissions when fed to cattle, and this should be investigated in animals properly adapted to diets containing silage.

The reasons for the lower methane production from both sheep and cattle fed *Lotus* (fresh and ensiled) are not known, but may be explained by the effects of condensed tannin in the *Lotus* on digestion. Tannins have wide-ranging effects on microbial, ruminal and intestinal function, and productivity (Waghorn *et al*., 1999). For example, Woodward *et al*. (2000) showed that 40 to 50% of the increase in milksolids production when cows are fed LC compared with ryegrass was due to the action of condensed tannins. The increase in milk protein and decrease in milkfat percentage from cows fed LC silage relative to pasture silage in the current study, mirror previous trials in which fresh LC and pasture diets were fed to cows (Harris *et al*., 1998; Woodward *et al*., 2000).

Condensed tannins bind to the plant proteins in the rumen and reduce breakdown of plant protein to ammonia by rumen microflora, effectively converting degradable protein to ‘rumen-protected protein’. Waghorn *et al*. (1994) showed that tannins reduced rates of DM degradation, turnover and proteolysis in the rumen of sheep fed LP, while *in vitro* studies have also shown that condensed tannins in LC reduced protein solubility and degradation rate (Waghorn & Shelton, 1997). Tannins also reduced the ability of some bacterial species to colonise plant particles (Jones *et al*., 1994), suggesting substantial direct or indirect effects on microbial activity.

Although we are not aware of any information concerning effects of condensed tannins on methanogenic bacterial populations or their activity, we suggest that the populations will be affected by dietary tannins. The data presented in this paper suggests further investigation of the effects of condensed tannins on rumen methanogens may offer excellent opportunities for methane mitigation in high producing ruminants.

Conclusion

The results of sheep and dairy cow trials confirm the beneficial effects of *Lotus*: improved feeding value for both growing sheep and lactating cows, together with reduced methane emission per unit of feed intake. These observations support the need to fully explore the ability of dietary mixtures to enhance dairy cow productivity and welfare, and to reduce methane emissions in sustainable farming systems.

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