Factors affecting methane production in Friesian x Jersey dairy cattle

L.R. MCNAUGHTON, D.P. BERRY[1], H. CLARK[2], C. PINARES-PATINO[2], S. HARCOURT and R.J. SPELMAN

Livestock Improvement Corporation, Private Bag 3016, Hamilton, New Zealand

ABSTRACT

Methane production was measured in 301, 3-year-old Friesian x Jersey dairy cows, during summer 2004 in stage one of a project to define phenotypes for quantitative trait loci (QTL) mapping and to investigate the relationships between CH_4 and milk production. Animals were allocated into four groups and their methane production was measured during four days. Feed intake was estimated by the alkane technique and by prediction equations. Correlations between the alkane technique and prediction equations were all less than 0.37, but correlations amongst the prediction equations were greater than 0.61. Daily CH_4 output varied from 210 to 498 g and averaged 332 g. Dry matter intake averaged 19.3 g/kg DM. Milk production and live weight gain were negatively related to CH_4 production per kg dry matter intake. Sire was not found to have a significant effect on CH_4 output per kg of dry matter; however, only six sires are represented in the trial. The CH_4 output recorded in the current study was within the ranges observed in other studies and was repeatable between days. High quality data were generated for the QTL study.

Keywords: methane; herbage intake; alkanes; dairy cows.

INTRODUCTION

The Kyoto protocol came into force in early 2005 and commits New Zealand to maintain greenhouse gas emissions at 1990 levels during the 2008-2012 period. New Zealand's agriculture-based economy and low human population has lead to a situation where, compared with other developed countries, an unusually high proportion (49%) of greenhouse gas emisssions come from agriculture. Methane (CH_4) produced by enteric fermentation contributes 31.5% of New Zealand's total greenhouse gas emissions ((Brown & Plume, 2004) www.climatechange.govt.nz)). The methane lost by ruminants has a significant energetic cost, estimated to be 5 to 8 % of gross energy intake in New Zealand dairy cattle (Robertson & Waghorn, 2002; Woodward *et al.*, 2002; Woodward *et al.*, 2004). A reduction in energy lost through CH_4 production could spare energy to be used for production. Therefore reducing CH_4 loss by ruminant animals is likely to have a dual benefit, reducing greenhouse gas emissions and increasing animal productive efficiency.

In order to reduce CH_4 production, the factors that affect CH_4 production need to be better understood. Feed quality, and the presence of condensed tannins have been shown to affect CH_4 production per unit of dry matter intake (Woodward *et al.*, 2001; Woodward *et al.*, 2004). There is also evidence of significant variation in methane production between animals (Ulyatt *et al.*, 2002). Results from a genotype comparison study suggested there could be some genetic component responsible for variation in methane production (Robertson & Waghorn, 2002). The objective of the Friesian Jersey crossbred trial is to discover quantitative trait loci (QTL) relating to production, health and fertility traits. The objective of this paper is to describe the collection of phenotypes relating to CH_4 production for a QTL analysis and to explore the relationship between these phenotypes and milk production variables.

MATERIALS AND METHODS

Experimental Design

CH_4 production was measured in 301, 3-year-old, F2 Friesian x Jersey dairy cattle from the Boviquest quantitative trait loci discovery trial (Spelman *et al.*, 2001). These animals are the progeny of six purpose-bred Friesian x Jersey sires and commercial Friesian x Jersey dams (F1). It was not possible to measure all 301 animals in one group, therefore, animals were allocated into 4 groups, that were balanced for sire, calving date and milk production. The trial was conducted during 4 weeks in January and February 2004 (167 ± 19 days in milk). CH_4 production was measured using the sulphur hexafluoride (SF_6) marker technique (Johnson *et al.*, 1994). Animals were fitted with halters and with evacuated neck yokes for a four-day collection period, with yokes changed daily. Background CH_4 and SF_6 concentrations were measured by placing evacuated yokes (n = 4) around the paddock where cows were grazing, the yokes were changed daily. Yokes were analysed using two pre-calibrated gas chromatographs. Determination of CH_4 concentrations is described by Woodward *et al.* (2004).

[1] Moorepark Research Centre, Teagasc, Fermoy, County Cork, Ireland
[2] AgResearch Ltd, Grasslands Research Centre, Private Bag 11008, Palmerston North, New Zealand

TABLE 1: Average methane emissions per cow per day, average dry matter intake (determined using Australian feeding standards) and average methane emissions expressed per kg of dry matter eaten (± SEM) and pasture quality parameters, expressed on a per group basis. Animals were randomly allocated into groups, as it was not possible to measure all animals in one group for logistic reasons.

	Group 1	Group 2	Group 3	Group 4
Dry matter (g/kg)	213	189	241	167
Organic matter (g/kg DM)	888	894	900	889
Crude protein (g/kg DM)	204	205	168	231
Acid detergent fibre (g/kg DM)	275	283	303	291
Neutral detergent fibre (g/kg DM)	501	536	540	584
Soluble carbohydrate (g/kg DM)	79	67	80	67
Digestibility (gOM/kg DM)	687	672	655	640
Metabolisable energy (MJ/kg DM)	11	11	10	10
Methane (g/day)	320.1 ± 4.7	333.0 ± 5.0	338.5 ± 5.3	336.4 ± 4.3
DM intake (kg DM)	17.0 ± 0.2	16.5 ± 0.3	17.4 ± 0.2	18.5 ± 0.2
Methane (g/kg DM)	18.9 ± 0.3	20.5 ± 0.4	19.7 ± 0.4	18.3 ± 0.3

Herbage Intake Estimation

Individual cow herbage intakes were measured using the double alkane technique (Dove & Mayes, 1996). Tablets containing 345 mg of C^{32} alkane markers were dosed twice-daily from 5 days prior to and through the methane collection period. Twice-daily faecal samples were collected during the methane collection period, bulked and analysed to determine alkane concentrations. Intakes were also estimated from measured levels of animal performance using equations based on Australian (CSIRO, 1990), American (NRC, 2001) and French (Jarrige, 1989) feeding standards and equations from the New Zealand Animal Evaluation Unit (Pryce *et al.*, 2005). Where methane output is reported per kilogram of dry matter consumed in the paper (CH_4/kgDMI) the intakes predicted from the Australian equations (CSIRO) have been used for consistency of comparison with previously reported results. Milk yield was measured at each milking and milk component concentrations determined on one day (a.m. and p.m. milkings) during each group's methane measurement period.

Live weight was measured at each milking and prior to and immediately following the methane measurement period. Average live weight was calculated as the mean of live weight measurements during the month surrounding the methane collection period. Linear regressions were fitted to each individual cow's live weights and the slope coefficient was assumed to reflect the change in live weight. These values were then used in the calculations to estimate feed intake.

Paddocks were selected to achieve similar pregrazing dry matter availability between groups, and achieve *ad libitum* pasture intakes. The selected paddocks were located in the same area of the farm. Ryegrass (*Lolium perene*) and white clover (*Trifolium repens*) were the predominat pasture species. In the week preceding CH_4 measurements, each group grazed pasture of a similar quantity and quality to that which would be offered during the trial. Each paddock (4 ha) was divided into four equal daily breaks. Representative pasture samples were collected prior to grazing each break for analysis by near infrared spectroscopy, to determine pasture quality. The daily samples were then bulked, subsampled and frozen to provide a sample for alkane analysis.

Statistical Analysis

Statistical analyses were conducted using SAS version 9.1 (SAS, 2004). Correlations between all variables were determined using the correlation procedure in SAS (Proc CORR). A model was generated using the available variables to explain as much of the phenotypic variation in CH_4 output per kg DMI (CH_4/kgDMI) as possible, using forward selection in the generalised linear model procedure of SAS (Proc GLM). Level of significance for entry into the model was set at $P < 0.10$.

RESULTS

The Friesian x Jersey cows used in this trial weighed an average of 421 kg (SD = 38.4 kg), at a condition score of 4.85 (SD = 0.3) during the CH_4 measurements. They were producing 12.75 l (SD = 2.4 l) of milk and 1.25 kg (SD = 0.2 kg) of milksolids per day. CH_4 measurements are provided as an average of either two or three (10% of cows) or four (90% of cows) daily CH_4 measurements. Table 1 summarises CH_4 emissions and pasture quality measures, by group. Average CH_4 production was 332.0 g/day (SD = 42.1 g/day). The correlation between CH_4 output (g CH_4) collected on days 1 to 4 ranged from 0.68 to 0.81.

Permeation rate of SF_6 was significantly related to per cow CH_4 production (g/day; R^2 = 0.15, $p < 0.001$), as permeation rate increased, so did CH_4 production. Further investigation revealed a significant effect of group on CH_4 production ($p < 0.001$). Per cow CH_4 output was statistically adjusted by treating group as a block effect and permeation tube flow rate as a linear covariate. This adjusted CH_4 output was used in the remainder of the analysis.

Intakes predicted using the alkane technique and the alternative prediction equations were compared and the correlations between the techniques calculated. Table 2 gives the summary statistics for the alternative approaches to estimating dry matter intake and Table 3 gives the correlation between the approaches. The correlations between dry matter intake predicted by the alkane technique and the prediction equations approaches were low (0.30 to 0.36), whereas those between the alternative prediction equations were moderate to high (0.61 to 0.91). CH_4 output was 19.3 g per kg DMI (SD = 3.0 g per kg DMI), based on an average intake of 17.4 kg DMI (SD = 2.1 kg DMI). This equates to 5.8% of gross energy lost to methane, and 9% of metabolisable energy (ME).

On a gross basis (gCH_4 per day), higher CH_4 output was associated with heavier cows, higher milk production and higher dry matter intake. This changed when CH_4 was expressed per kg DMI. Together, live-weight change, milk yield and fat yield were significantly ($p < 0.05$) associated with CH_4 production (CH_4/ kgDMI; combined $R^2 = 0.46$). The correlation between live weight and gCH_4/kg DMI was effectively zero, but live-weight change was moderately correlated with gCH_4/kg DMI (-0.59). Mean live-weight change was 0.67 kg/day, ranging from –0.56 to 1.67 kg/day. Based on solutions from the generalised linear model, for every kg of live-weight gain methane output decreased by 5.7 g /kg DMI. Correlations between milk yield, fat yield and protein yield and gCH_4/kg DMI were low to moderate and negative (-0.29 to -0.25). For each unit increase in milk yield (litres) and fat yield (kg) CH_4 output decreased by 0.2 and 3.6 g per kg DMI. There was no significant difference in CH_4 production between the daughter groups of the six sires represented in this trial when CH_4 was expressed as g/day, or g/kg DMI.

TABLE 2: Summary statistics for dry matter intake (kg DM/day) estimated using alternative approaches.

	CSIRO	INRA	AEU	NRC	Alkane
Mean	17.4	11.49	11.91	15.53	16.17
SD	2.14	1.21	1.34	1.43	2.55
Min	8.97	5.43	5.20	9.84	7.72
Max	24.80	14.62	16.38	20.21	25.86
CV	12%	11%	11%	9%	16%

CSIRO: Commonwealth Scientific and Industrial Research Organisation
INRA: I'institut National de la Recherché Agronomique
AEU: Animal Evaluation Unit
NRC: National Research Council

DISCUSSION

CH_4 is a by-product of digestion in ruminants and is produced to remove hydrogen that results from microbial digestion (Robertson & Waghorn, 2002). The levels of CH_4 emissions measured in this trial were comparable to those reported in previous New Zealand trials. Methane emissions of 260 to 360 g/cow/day have been reported from cows fed ryegrass in late lactation (Woodward *et al.*, 2001; Woodward *et al.*, 2002; Woodward *et al.*, 2004).

TABLE 3: Pearson correlations among dry matter intake predicted using the various methods.

	CSIRO	INRA	AEU	NRC	Alkane
CSIRO	1.00				
INRA	0.61	1.00			
AEU	0.89	0.90	1.00		
NRC	0.67	0.91	0.84	1.00	
Alkane	0.31	0.36	0.36	0.33	1.00

See Table 2 for explanation of abbreviations.

The low correlations between feed intakes predicted by the alkane method and other prediction equations is a concern. This seems to indicate that the alkane method did a poor job of measuring feed intake under the prevailing conditions. Waghorn *et al.* (2004) discussed some of the problems associated with this technique. In particular, that the recovery of different alkanes in the faeces is not equal, and it is difficult to select a pasture sample that accurately represents the actual herbage selection of the grazing animal.

Comparison of gCH_4 per kg DMI across studies is difficult due to the problems in measuring herbage intake in grazing ruminants. In the current trial the CSIRO equations were used to estimate herbage intake, to allow comparison with data for national CH_4 emisssion estimates, which also use the CSIRO equations to predict herbage intakes. If the Animal Evaluation Unit equations (which have been developed specifically for New Zealand) had been used, the resulting phenotypes would be expected to give very similar results in a QTL analysis, due to the high rank correlation (0.89) between these two prediction methods. Phenotypes derived from both prediction methods will be used in the QTL analysis. The estimated gCH_4 per kg DMI value would have been substantially greater had the AEU method been used to estimate intake (27.9 vs 19.3 gCH_4/kg DMI). The gCH_4/kg DMI value in the current trial (19.3 gCH_4/kg DMI) is comparable to the value of 21.3 gCH_4/kg DMI that has been adopted by MAF policy and the Climate Change Office to estimate CH_4 emissions of dairy cattle (Brown & Plume, 2004). Similarly, the proportion of gross energy lost through CH_4 is similar to that reported in the literature (Woodward *et al.*, 2001; Woodward *et al.*, 2002).

Group had a significant effect on CH_4/cow/day. Pasture quality, which can change rapidly during January, is a possible explanation as feed quality affects CH_4 production (Woodward *et al.*, 2001). The metabolisable energy content of the pasture declined from 11 during the first 2 weeks of the trial (Groups 1 and 2) to 10 during the last 2 weeks (Groups 3 and 4).

The negative relationship between milk production, live-weight gain and methane output per kg of dry matter intake is encouraging. This relationship suggests that high milk producers use more energy from each kg of DMI for productive purposes, rather than to produce CH_4. Potentially, selecting cows that produce less CH_4 could have a small positive impact on feed efficiency. If selection for decreased CH_4 per unit of dry matter intake was able to decrease energy loss to 4.5% of ME this could potentially spare around 8 MJ ME per day (based on an average intake of 17.4 kg DMI x 10.5 MJ ME/kg DM x 4.5%). Ideally, future experimental work should explore the effects of variations in CH_4 production on individual cow production.

Average CH_4 production was not different between the daughters of the six sires used to generate the animals in the current study, however this does not mean that there are no genetic differences between animals. Heritabilities have not been calculated from this data set, as estimates of genetic variation from F2 populations are not comparable with within-breed estimates. This is due to the admixture of the two breeds, which is desirable from a QTL detection point of view (Kayis & Spelman, 2003). The phenotypes collected in this study will be combined with phenotypes from another 400 animals that have had CH_4 measured in early 2005, to search for QTL that affect CH_4 production. This population of animals is part of a breeding experiment specifically designed to identify QTL and has been genotyped at over 230 markers across the 30 bovine chromosomes. In addition to potential QTL discoveries, an experiment of this size provides a wealth of information on factors affecting CH_4 production, particularly the inter-relationships between liveweight, milk production and CH_4 emissions.

In conclusion, this trial has demonstrated CH_4 production can be successfully measured in large groups of cattle, although estimating feed intake remains problematic. Phenotypes for CH_4 production have been successfully defined. No sire effects were detected, however this does not preclude the discovery of QTL. The effect of permeation tube release rate on CH_4 production should be investigated in future trials.

ACKNOWLEDGEMENTS

The authors acknowledge the farm and technical staff at the Fonterra Whareroa farm and also the advice of Sharon Woodward, Dave Clark and Garry Waghorn, of Dexcel. Thanks also to Adrienne Cavanagh and the AgResearch team for analyzing the CH_4 samples and to the Pastoral Greenhouse Gas Research Consortium for funding this trial.

REFERENCES

Brown, L.; Plume, H. (2004): National Inventory Report: 1999 - 2002. N. Z. C. C. Office. Wellington, Ministry of the Environment*:* 155

CSIRO 1990: Feeding standards for Australian livestock ruminants. Melbourne, CSIRO Publishing

Dove, H.; Mayes, R. W. 1996: Plant wax components: A new approach to estimating intake and diet composition in herbivores. *Journal of nutrition 126:* 13-26

Jarrige, R. 1989: Ruminant nutrition: recommended allowances and feed tables. Paris, INRA publications

Johnson, K. A.; Huyler, M. T.; Westburg, H. H.; Lamb, B. K.; Zimmerman, P. 1994: Measurement of methane emissions from ruminant livestock using a SF_6 tracer technique. *Environmental science and technology 28:* 359-362

Kayis, S. A.; Spelman, R. J. 2003: Heritability analysis of natural nematode challenge. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics 15:* 123-126

NRC 2001: National Research Council. Nutritional requirements of dairy cattle: seventh revised edition. Washington, DC., National Academy Press

Pryce, J.E.; Harris, B. L.; Montgomerie, W.A.; Jackson, R.; MacDonald, K. A.; Glassey, C.B.; Thorrold, B.S.; Holmes, C. W. 2005: Comparing feed allowances to inferred energy intake using data from grazing farm trial studies. *Proceedings of the New Zealand Society of Animal Production 65:* 225-230

Robertson, L. J.; Waghorn, G. C. 2002: Dairy industry perspectives on methane emissions and production from cattle fed pasture or total mixed rations in New Zealand. *Proceedings of the New Zealand Society of Animal Production 62:* 213-218

SAS 2004: SAS Version 9.1. Cary, NC

Spelman, R. J.; Miller, F. M.; Hooper, J. D.; Thielen, M.; Garrick, D. J. 2001: Experimental design for QTL trial involving New Zealand Friesian and Jersey breeds. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics 14:* 393-396

Ulyatt, M. J.; Clark, H.; Lassey, D. K. R. 2002: Methane and climate change. *Proceedings of the New Zealand Grassland Association 64:* 153-157

Waghorn, G. C.; Woodward, S. L.; Clark, D. A. 2004: Accuracy of intake measurements for cows grazing grass/legume pastures using the alkane marker technique. *Journal of dairy science 87:* 292

Woodward, S. L.; Waghorn, G. C.; Laboyrie, P. G. 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

Woodward, S. L.; Waghorn, G. C.; Lassey, K. R.; Laboyrie, P. G. 2002: Does feeding sulla (*Hedysarum coronarium*) reduce methane emissions from dairy cows? *Proceedings of the New Zealand Society of Animal Production 62:* 227-230

Woodward, S. L.; Waghorn, G. C.; Ulyatt, M. J.; Lassey, K. R. 2001: Early indications that feeding *Lotus* will reduce methane emissions from ruminants. *Proceedings of the New Zealand Society of Animal Production 61:* 23-26