



## Effects of feed intake on enteric methane emissions from sheep fed fresh white clover (*Trifolium repens*) and perennial ryegrass (*Lolium perenne*) forages

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### ABSTRACT

Published analyses of enteric methane (CH<sub>4</sub>) emissions from sheep and cattle show an inverse relationship between feed intake and CH<sub>4</sub> yield (g CH<sub>4</sub>/kg dry matter (DM) intake), which suggests opportunities for reducing CH<sub>4</sub> emissions from feed eaten and per unit of animal production. Most relationships between feed intake and CH<sub>4</sub> yield have been based on animals fed conserved feeds, especially silages and grains. Our research is a series of experiments with fresh white clover (*Trifolium repens*) and perennial ryegrass (*Lolium perenne*; ryegrass) forages fed to sheep at a range of feed intake levels. This study was comprised of four experiments where good quality freshly harvested white clover or ryegrass were fed to sheep over a three-fold range in DM intake, and CH<sub>4</sub> emissions were measured in respiration chambers for two consecutive days in each experiment. Measurements were made from 16 sheep in Experiment 1 (fed at 1.6 × metabolizable energy requirements for maintenance; ME<sub>m</sub>), 28 sheep in Experiment 2 (at 0.8 and 2.0 × ME<sub>m</sub>), eight sheep and two measurement periods in Experiment 3 (at 1.6 × ME<sub>m</sub>), and 30 sheep in Experiment 4 (fed at 0.8, 1.2, 1.6, 2.0 and 2.5 × ME<sub>m</sub>). Prior to each experiment, sheep had a 10 d acclimatization period to diets. Apparent digestibility was measured over 7 d from sheep in Experiments 1, 3 and 4, along with collection of rumen digesta for volatile fatty acid (VFA) determination. Although CH<sub>4</sub> yields differed when sheep were fed white clover or ryegrass at similar intakes, the differences were inconsistent and mean values similar across all experiments. This, and a similar structure of all experiments, enabled combined analysis of data from all four experiments using the restricted maximum likelihood (REML) procedure to estimate effects of feed intake level on digestibility, digestible nutrient intake, gas emissions, and VFA concentrations in the rumen. The REML analysis showed that when

**Abbreviations:** ADF, acid detergent fibre; aNDF, neutral detergent fibre; CH<sub>4</sub>/GE intake, methane energy as a proportion of GE intake; CP, crude protein; DDM, digestible DM; DM, dry matter; DOM, digestible organic matter; GE, gross energy; HWSC, hot water soluble carbohydrates; LW, live weight; ME, metabolizable energy; ME<sub>m</sub>, ME requirements for maintenance; MRT, mean retention time; REML, restricted maximum likelihood; SF<sub>6</sub>, sulphur hexafluoride; VFA, volatile fatty acids.

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DM intake increased from 0.40 to 1.60 kg/d, the predicted responses were an increase in CH<sub>4</sub> production (g/d) of 187% (12.4–35.6 g/d;  $P < 0.001$ ), and a decline in CH<sub>4</sub> yield of 21% (25.6–20.2 g/kg DM intake;  $P < 0.001$ ). High feed intake levels were associated with increased molar proportions (mM of total VFA) of propionate from 0.17 to 0.21 ( $P = 0.038$ ). Single and multiple regressions were completed on the data from all experiments, with organic matter (OM) intake predicting 0.87 of the variation in CH<sub>4</sub> production, and molar proportion of propionate predicting 0.60 of the variation in CH<sub>4</sub> yield. Increasing feed intakes by 1 kg/d of DM reduced CH<sub>4</sub> yield by 4.5 g/kg DM intake. Plant chemical composition was weakly related to CH<sub>4</sub> yield. High intakes of fresh forages will lower CH<sub>4</sub> yield from fermentation, but effects of feed composition on CH<sub>4</sub> emissions were minor. The interaction between effects of feed intake and rumen function requires further investigation to understand relationships with CH<sub>4</sub> emissions.

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## 1. Introduction

Ruminants are farmed in New Zealand for food production, mainly milk and meat. Animal production is maximized when high quality feeds *i.e.*, vegetative, with adequate concentrations of nutrients and neutral detergent fibre (NDF) less than about 0.45 of dry matter (DM), is available *ad libitum* and animals have a high genetic merit for production. Under New Zealand pastoral grazing, the prime constraint to production is generally metabolizable energy (ME) intake, with the concentration of crude protein (CP) usually in excess of requirements (Waghorn et al., 2007). Intakes of forages are affected by feed availability and quality and, during lactation, intakes may be three or more times the ME requirements for maintenance (ME<sub>m</sub>) in dry cows (Holmes et al., 2002) and sheep (Kenyon and Webby, 2007).

It is important to determine effects of feed intake level on CH<sub>4</sub> emissions to improve the accuracy of the emissions inventory and to define benefits of increasing intake levels on reducing CH<sub>4</sub> yield (g/kg DM intake) in productive animals. For example, Ulyatt (1981), and more recently Burke et al. (2002), have ranked feeds for their capacity to achieve high lamb liveweight (LW) gain, which ranged from the national average of about 120 g/d (Waghorn et al., 2007) to about 300 g/d with legumes such as sulla (*Hedysarum coronarium*) or white clover (*Trifolium repens*; Burke et al., 2002).

Most studies designed to determine relationships between feed intakes and CH<sub>4</sub> emissions have been based on conserved forages, and there is a good agreement that increasing feed intake reduces CH<sub>4</sub> per unit feed eaten (Blaxter and Clapperton, 1965; Johnson et al., 1993; Pinares-Patiño et al., 2003a,b; Hart et al., 2009; Yan et al., 2010). Many of these analyses, however, have been undertaken with cattle and there are few published data relating feed intake level to CH<sub>4</sub> emissions from sheep fed fresh forages. Further, some measurements of CH<sub>4</sub> emissions have been made using the sulphur hexafluoride (SF<sub>6</sub>) tracer technique, which have resulted in more variation and weaker correlations between CH<sub>4</sub> yield, feed intake and diet composition than chamber measurements (Hammond et al., 2009). Also, estimates of CH<sub>4</sub> emissions from sheep and cattle grazing pasture rely on estimates of feed intake (*e.g.*, Lassey et al., 1997; Ulyatt et al., 1997, 2002a,b; Pinares-Patiño et al., 2003a; Ulyatt et al., 2005; Molano et al., 2006; Cavanagh et al., 2008), and therefore the estimates of CH<sub>4</sub> yield were less accurate than those from indoor experiments where feed intake can be measured. Accurate determination of CH<sub>4</sub> yield requires direct measurement of both feed intake and emission of CH<sub>4</sub>.

The causes of lower CH<sub>4</sub> yield as feed intake increases are usually attributed to a shorter time of digesta retention in the rumen, and consequently less extensive fermentation (Ulyatt et al., 1984; Pinares-Patiño et al., 2003b). Less time available for microbial fermentation at high feed intake levels may drive end products of fermentation towards more propionate production (Janssen, 2010) and less H<sub>2</sub>, and consequently reduced CH<sub>4</sub> per unit feed eaten. However, variations in feed intake do not always affect apparent whole tract digestibility (*e.g.*, Molano and Clark, 2008) and, although fibre in forages affects intake, it does not necessarily affect CH<sub>4</sub> yield (Hammond et al., 2011).

The inverse relationship between feed intake level and CH<sub>4</sub> yield offers an opportunity to lower emissions whilst increasing animal productivity, relative to the unit of animal product produced. Thus the main objectives of this study were to measure CH<sub>4</sub> yield (g/kg DM intake) from sheep fed freshly cut white clover and ryegrass forages, and to define relationships between feed intake level and CH<sub>4</sub> yield. The secondary objective was to gain a better understanding of the effects of feed intake level, digestibility and rumen volatile fatty acid (VFA) concentrations on CH<sub>4</sub> emissions from sheep. It was hypothesized that increasing intakes of white clover and ryegrass in sheep would decrease CH<sub>4</sub> yield (g/kg DM intake) and that differences in total tract digestibility and fermentation end products would account for the variation in CH<sub>4</sub> emissions.

## 2. Materials and methods

Experiments 1, 2, 3 and 4 were conducted between May 2009 and May 2010. Experiment 1 occurred between May and June 2009, Experiment 2 from October to November 2009, Experiment 3 from November to December 2009, and Experiment 4 from April to May 2010. Measurements in each experiment were DM intake and emissions of CH<sub>4</sub> and H<sub>2</sub> from sheep fed either white clover or ryegrass forages over a range of feed intake levels. An additional measurement in Experiments 1, 3 and 4 was apparent digestibility, while rumen digesta samples were collected from both rumen-fistulated and intact sheep for

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