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Effects of feeding fresh white clover (*Trifolium repens*) or perennial ryegrass (*Lolium perenne*) on enteric methane emissions from sheep

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ABSTRACT

Enteric CH_4 contributes about one third of New Zealand's greenhouse gas emissions. Measurements from our laboratory using the sulfur hexafluoride (SF_6) technique suggested much lower CH_4 yields (as g CH_4/kg dry matter (DM) intake) from sheep fed fresh white clover (*Trifolium repens*) compared with fresh perennial ryegrass (*Lolium perenne*). Thus, white clover offers an opportunity to reduce CH_4 emissions from pastoral based ruminant livestock systems if its lower CH_4 yield can be confirmed using respiration chambers. This study was comprised of 2 experiments where good nutritional quality freshly harvested white clover and perennial ryegrass were fed to 16 sheep in Experiment 1 (8/diet) and 32 sheep in Experiment 2 (16/diet). Intakes were about 1.6 times metabolizable energy requirements for maintenance ($\times ME_m$) in Experiment 1 and $0.8 \times ME_m$ and $2.0 \times ME_m$ in Experiment 2 (8/diet/feeding level). In both experiments sheep had a 10 d acclimatization to their diets and CH_4 emissions were measured in respiration chambers for 2 consecutive days. Methane yield (g CH_4/kg DM intake) was 12% lower (P=0.04) for white clover (19.8) compared with ryegrass fed sheep (22.5) in Experiment 1. In Experiment 2, there were no effects of forage on CH_4 yield (as g CH_4/kg DM intake) at $0.8 \times ME_m$ (27.1 and 25.5, respectively) but, at $2.0 \times ME_m$, CH_4 yield (as g CH_4/kg DM intake) was 7% higher (P=0.05) for white clover (23.4) compared with ryegrass (21.7). Analyses of combined data from both experiments show that there were no overall dietary effects on CH_4 yield, but increased intakes reduced CH_4 yield (P<0.01). Use of white clover as an alternative fresh forage to ryegrass is not an opportunity to reduce enteric CH_4 emissions from sheep.

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1. Introduction

Methane from enteric fermentation in ruminants is an important contributor to global anthropogenic emissions of greenhouse gases (GHG), with an annual production of about 80 million tonnes (US-EPA, 2007). In addition to effects associated

Abbreviations: ADF, acid detergent fiber; aNDF, neutral detergent fiber; CH_4-E/GE intake, CH_4 energy relative to gross energy intake; CP, crude protein; DM, dry matter; GE, gross energy; GHG, greenhouse gas; LW, live weight; OM, organic matter; RFC, readily fermentable carbohydrates; SF_6, sulfur hexafluoride; $\times ME_m$, multiples of metabolizable energy requirements for maintenance.

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with global climate change, the energy content of CH_4 (55.2 MJ/kg; Brouwer, 1965), represents a loss of 6–7% of gross energy (GE) intake from temperate forages (Ministry for the Environment, 2010); energy which is then unavailable to support animal production.

New Zealand's agricultural system is pastoral based, and animals graze mixed swards consisting predominately of perennial ryegrass (*Lolium perenne*), with white clover (*Trifolium repens*) being 10–20% of the herbage (Waghorn and Clark, 2004). Although perennial ryegrass is the dominant species in New Zealand pastoral systems, white clover and other legumes provide advantages for N fixation and have high feeding value (*i.e.*, nutritive value and intake), which can promote higher levels of ruminant production (Burke et al., 2002; Waghorn et al., 2007).

Environmental concerns over use of nitrogenous fertilizers are re-focusing research towards increased use of legumes in grazing pastures, and previous research (Waghorn et al., 2002) has shown sheep fed white clover have much lower CH_4 yields (*i.e.*, 12–16 g CH_4/kg dry matter (DM) intake) compared to sheep fed ryegrass at 20.9 g CH_4/kg DM intake. These data, as well as those used for the New Zealand Greenhouse Gas Inventory (Ministry for the Environment, 2010) were derived using the sulfur hexafluoride (SF_6) method described by Ulyatt et al. (1999) and Pinares-Patiño and Clark (2008). Low CH_4 yields from sheep fed white clover, lotus (*Lotus pedunculatus*), and other legumes (Waghorn et al., 2002) is an opportunity for GHG mitigation whilst increasing animal productivity, which is difficult in an environment where livestock production is extensive with limited options for dietary manipulation.

It has been difficult to explain the low CH_4 yields from legumes (McCaughey et al., 1999; Waghorn et al., 2002), because they usually have a higher digestibility than grasses (Ulyatt and Egan, 1979) which provides a better opportunity for H_2 and CH_4 production. However, high digestibility enables high intakes, which contribute to low CH_4 yields (as g CH_4/kg DM intake). A decline of ~3.0 g CH_4/kg DM intake for each multiple increase in metabolizable energy (ME) intake has been shown in sheep fed ryegrass (Hammond et al., 2009) and in cattle fed grain/silage diets (Yan et al., 2010).

Comparisons between the SF_6 marker dilution and respiration chamber techniques to measure CH_4 emissions have shown good agreement (McGinn et al., 2006; Grainger et al., 2007), but there is evidence that CH_4 emissions can be affected by the SF_6 gas permeation rates (Vlaming et al., 2007; Pinares-Patiño and Clark, 2008), which could exacerbate variation in CH_4 yield estimates (Hammond et al., 2009).

This study measured CH_4 yields (as g CH_4/kg DM intake) from sheep fed fresh white clover or perennial ryegrass in respiration chambers, at several intake levels, to validate previous comparisons of these forages using the SF_6 tracer technique. It was hypothesised that sheep fed fresh white clover would have a lower CH_4 yield compared with sheep fed fresh perennial ryegrass.

2. Materials and methods

The study involved 2 experiments, with Experiment 1 in May to June 2009, and Experiment 2 in October to November 2009. Principal measurements were DM intake and CH_4 emissions from sheep fed fresh white clover or ryegrass. In each experiment, sheep had a 10 d acclimatization to dietary treatments and indoor feeding before entering respiration chambers where CH_4 emissions were determined for 2 consecutive days.

Diet and feeding level treatments were applied randomly to sheep and each experiment was a completely randomized design. All procedures were reviewed and approved by the AgResearch Animal Ethics Committee in Palmerston North, New Zealand.

2.1. Forages

The white clover (*cv.* Kopu II) and perennial ryegrass (*cv.* Quartet) used for both experiments were grown near Palmerston North (40°20′S, 175°28′E; 15 m above sea level), and were harvested daily using a sickle bar mower (1995 S.E.P, San Martino in Rio, Italy) and delivered by 14:00 h. Forages were weighed into meal allocations and stored at 4 °C prior to feeding. The white clover and ryegrass fed in both experiments were in a vegetative state when harvested, with no reproductive material, flowers or seed heads. High nutritional quality was achieved by grazing at 4–6 wk intervals for 6 months prior to the experiments, ceasing 4 wk before initial cutting so that an adequate sward height of vegetative forage was achieved for harvesting.

2.2. Animals

Wether sheep aged 1–2 yr were used for both experiments. Sheep were fed white clover and ryegrass at ~1.6 times ME requirements for maintenance ($\times ME_m$) (Australian Agricultural Council, 1990) in Experiment 1 and at $0.8 \times ME_m$ and $2.0 \times ME_m$ in Experiment 2.

Experiment 1 used 16 wethers with an average live weight (LW) of 45.3 ± 1.71 kg. Eight had been previously rumen fistulated and fitted with a 30 mm (o.d.) rumen cannulae (Beruc Equipment Ltd., Benoni, South Africa). Sheep were randomly allocated into 2 groups of 8, with 4 rumen fistulated sheep/group, and fed white clover or ryegrass. Experiment 2 used 32 sheep with 16 (45.7 ± 1.20 kg LW) fed white clover and 16 (48.0 ± 1.20 kg LW) fed ryegrass. Within each diet, 8 sheep were fed at either $0.8 \times ME_m$ or $2.0 \times ME_m$.