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The effects of fresh forages and feed intake level on digesta kinetics and enteric methane emissions from sheep

K.J. Hammond[a], D. Pacheco[a], J.L. Burke[b], J.P. Koolaard[a], S. Muetzel[a], G.C. Waghorn[c,*]

[a] AgResearch Ltd, Grasslands Research Centre, Private Bag 11-008, Palmerston North 4442, New Zealand
[b] Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11-222, Palmerston North 4442, New Zealand
[c] DairyNZ Ltd, Private Bag 3221, Hamilton 3240, New Zealand

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ABSTRACT

Published data have shown that in ruminants, methane (CH_4) yields (g/kg dry matter [DM] intake) decline as feed intakes increase and, although the reduction has been attributed to a shorter digesta mean retention time (MRT), there are few supporting data. This study was undertaken to determine the association between digesta kinetics and CH_4 emissions measured from sheep in respiration chambers fed either fresh white clover (*Trifolium repens*; WC) or fresh perennial ryegrass (*Lolium perenne*; RG) (Experiment 1), or RG at several feed intakes (Experiment 2). Measurements included CH_4, whole tract apparent DM digestibility (DDM), total tract and rumen MRT (TMRT and RMRT, respectively) of solid and liquid fractions, as well as passage rates. In Experiment 1, eight sheep each with a rumen fistula were fed hourly either WC or RG forages, repeated over two periods (four sheep/diet/period) at about 1.6 times maintenance requirements for metabolisable energy (ME_m; 1.12 kg DM/d). Diet did not affect apparent DDM (726 g/kg), CH_4 yield (22.3 g/kg DM intake), or TMRT of solid fractions (29.4 h). However, TMRT for the liquid fraction was shorter (P=0.037) for sheep fed RG (17.4 h) compared with WC (23.0 h), and rumen digesta analyses suggested a larger rumen liquid pool size when RG was fed (6.05 L) compared with WC (3.96 L) (P=0.041). Experiment 2 involved 30 sheep offered fresh RG twice daily at about 0.8, 1.2, 1.6, 2.0 and 2.5 × ME_m. The DDM did not differ greatly across RG intakes (625–648 g/kg) but, as RG intake increased (0.49–1.34 kg DM/d), there were corresponding reductions (P<0.001) in CH_4 yield (27.0–23.9 g/kg DM intake), liquid TMRT (31.4–14.2 h), solid TMRT (46.4–24.8 h), liquid RMRT (18.4–7.5 h), and solid RMRT (28.4–15.8 h). When CH_4 yield was plotted against rumen liquid and solid passage rates, the extent of the relationship was best explained (R^2) when RG was fed at different intakes in Experiment 2 (0.71 and 0.66 for liquid and solids, respectively). The 2.7-fold increase in feed intake halved RMRT, but intakes affected passage of the rumen liquid fraction to a greater extent than solids. It can be concluded that reductions in CH_4 yield from fresh forages fed to sheep are associated

Abbreviations: ADF, acid detergent fibre; aNDF, neutral detergent fibre; ANOVA, analysis of variance; CH_4, methane; CO_2, carbon dioxide; Co-EDTA, cobalt ethylene diaminetetraacetic acid; CP, crude protein; Cr-NDF, chromium mordanted NDF; DM, dry matter; DDM, digestible DM; GE, gross energy; H_2, hydrogen; HWSC, hot water soluble carbohydrates; *k*, passage rate; LW, live weight; MRT, mean retention time; REML, residual maximum likelihood; RG, perennial ryegrass; RMRT, rumen MRT; SD, standard deviation; SED, standard error of the difference between means; TMRT, total tract MRT; VFA, volatile fatty acids; WC, white clover; × ME_m, multiples of metabolisable energy requirements for maintenance.

* Corresponding author. Tel.: +64 078583750; fax: +64 078583751.
E-mail address: Garry.Waghorn@dairynz.co.nz (G.C. Waghorn).

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with shorter TMRT and RMRT. Understanding the effects of diet, digestion, feed intake, and feeding frequency on methanogenesis requires more knowledge about rumen digesta kinetics, especially relationships between outflow rates of solid and liquid fractions.

1. Introduction

It is well established that the yield of CH_4 (g/kg DM intake) from digestion of feedstuffs consumed by ruminants decreases as feed intakes increase above ME_m in both sheep (Blaxter and Clapperton, 1965; Hammond et al., 2013) and cattle (Sauvant and Giger-Reverdin, 2009; Yan et al., 2010). Rumen digesta pool size can also increase in response to increasing feed intakes, but to a limited extent. Pinares-Patiño et al. (2003) suggested the decrease in CH_4 yield at high feed intakes may be a consequence of a reduced residence time of digesta in the rumen. However, the extent of the decrease in CH_4 yield is likely to be affected by diet type, due to the time required to chew and reduce the particle size of fibre, thus enabling passage from the rumen (Ulyatt et al., 1986). As a consequence, changes in CH_4 emissions in response to increasing intakes of fresh forages may differ from the relationships that have been previously established for dried forages and concentrate-based feeds (*e.g.* Blaxter and Clapperton (1965) and others).

Hammond et al. (2009) showed CH_4 yields from sheep fed fresh RG varying widely in quality (neutral detergent fibre [aNDF], 431–626 g/kg DM; crude protein [CP], 103–174 g/kg DM), were only weakly associated with chemical composition. Methane yields (g/kg DM intake) from sheep fed above maintenance requirements were 21.7 *vs*. 23.4 from fresh RG *vs*. WC (Hammond et al., 2011) and 23.8 *vs*. 22.8 from fresh RG *vs*. chicory (*Chicorum intybus*) (Sun et al., 2011), despite very different chemical compositions. However, as feed intakes increased, the rate of decline in CH_4 yield from sheep was greater for the WC diet compared to RG (Hammond et al., 2013). The poor relationship between CH_4 yield and chemical composition or DDM (Johnson and Johnson, 1995) supports the hypothesis of Janssen (2010), who suggests that rumen outflow of liquid (rather than solid) will contribute to the regulation of methanogenesis. Janssen (2010) based his hypothesis on thermodynamic principles associated with hydrogen (H_2), CH_4 and volatile fatty acid (VFA) concentrations, microbial competition, and the growth rate of methanogenic Archaea; all of which are affected by passage rates from the rumen. A high liquid passage rate can reduce Archaeal populations, leading to an accumulation of H_2 and a reduction in CH_4 emission resulting from feedback inhibition on H_2 production. On the other hand, increased H_2 production will drive fermentation toward methanogenesis.

The work presented here measured the effects of fresh feed type (RG and WC; Experiment 1) and feed intake (RG only; Experiment 2) on total tract and rumen digesta kinetics in relation to CH_4 emissions from sheep. It was hypothesised that a shorter MRT of liquid and solid digesta fractions would be associated with decreases in CH_4 yield (g/kg DM intake) from sheep. Most measurements were based on marker kinetics from faecal samples, and *per fistulum* in sheep with rumen fistulae, but exploratory measurements were also undertaken at the trial conclusion to determine rumen MRT based on oral (stomach tube) samples.

2. Materials and methods

This study consisted of two experiments. Experiment 1 used eight sheep, each with a rumen fistula, fed either fresh WC or RG forages (four sheep fed each diet) in hourly meals at about $1.6 \times ME_m$ (1.12 kg DM/d), replicated over two periods. Experiment 2 consisted of 30 sheep offered fresh RG twice daily at about 0.8, 1.2, 1.6, 2.0 or $2.5 \times ME_m$ (0.49–1.51 kg DM/d), with six sheep per intake level. Principal measurements in both experiments were DM intake, apparent DDM, TMRT and RMRT of liquid and solid fractions, as well as passage rates, and total emissions of CH_4, H_2 and carbon dioxide (CO_2) from individual sheep. Details of experimental protocols, animal handling, and gas measurements have been provided by Hammond et al. (2013). Data from both experiments were evaluated in relation to CH_4 emissions from individual sheep measured in respiration chambers at the conclusion of the digestibility period.

Measurements of rumen liquid kinetics were based on digesta sampled *via* the rumen fistula in Experiment 1, but TMRT and RMRT were calculated in both experiments from faecal marker analyses of Co (administered as cobalt [Co] ethylene diamine-tetraacetic acid; Co-EDTA) and Cr (administered as chromium [Cr]-mordanted neutral detergent fibre; Cr-NDF), for liquid and solid fractions, respectively. In addition, after the completion of CH_4 measurements from sheep in Experiment 2, twelve sheep fed RG with intakes of either 0.72 or 1.07 kg DM/d were used in an exploratory trial to determine the efficacy of rumen digesta sampling by stomach tube to estimate liquid digesta kinetics, based on the Co-EDTA marker.

All procedures were reviewed and approved by the AgResearch Palmerston North Animal Ethics Committee and respective Animal Ethic numbers for Experiments 1 and 2 were 11912 and 11918.

2.1. Forages and animals

The WC fed in Experiment 1 was *cv*. Grasslands Kopu II, and the RG used for both experiments was *cv*. Quartet. The forages were grown near Palmerston North (40°20′ S, 175°28′ E; 15 m above sea level), and were harvested daily with a sickle mower