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In vitro rumen methane output of red clover and perennial ryegrass assayed using the gas production technique (GPT)

A. Navarro-Villa[a,b], M. O'Brien[a], S. López[c], T.M. Boland[b], P. O'Kiely[a,*]

[a] *Animal & Grassland Research & Innovation Centre, Teagasc, Grange, Dunsany, Co. Meath, Ireland*
[b] *School of Agriculture, Food Science & Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland*
[c] *Instituto de Ganadería de Montaña (CSIC-ULE), Departamento de Producción Animal, Universidad de León, E-24071 León, Spain*

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ABSTRACT

The *in vitro* rumen methane output of perennial ryegrass (receiving 0 or 150 kg of inorganic fertiliser N/ha/growth) and two red clover varieties (Merviot and Ruttinova) at three different harvests (early and late primary growths, and an autumn regrowth) was assayed using the gas production technique (GPT). Herbages were produced within a randomised complete block ($n = 4$) design experiment conducted over two consecutive years. The forage samples selected from this field plot experiment were arranged in a 4 (herbages) × 3 (harvests) factorial structure of treatments. Dried milled herbage samples were incubated at 39 °C in a buffered medium inoculated with rumen fluid obtained from fistulated steers. Effects on methane output, feed disappearance, volatile fatty acid (VFA) output and other fermentation variables were evaluated 24 h after inoculation. Red clover (mean of the two varieties) had a lower (P<0.001) methane output per g of feed incubated (CH_4/DMi) than perennial ryegrass (mean of both treatments) but this effect was reversed (P<0.05) when methane outputs were expressed relative to feed disappeared (CH_4/DMd). No differences in methane output were detected between the two red clover varieties (Merviot and Ruttinova) reflecting their similar chemical composition. The application of inorganic N fertiliser to ryegrass reduced (P<0.05) CH_4/DMi resulting in similar output to the red clover. Mature herbage from the primary growth, and the autumn regrowth, had lower (P<0.05) CH_4/DMi than immature herbage from the primary growth. The lowest (P<0.05) CH_4/DMd was associated with the autumn regrowth and was probably due to the presence of non-fermentable soluble compounds in the sward. Overall, a reduction in the *in vitro* rumen methane output was observed with (1) red clover compared to perennial ryegrass, (2) nitrogen-fertilised compared to non-fertilised perennial ryegrass, and (3) mature primary growth or autumn regrowth compared to immature primary growth. The reduction in methane output was associated with a decline in the extent of fermentation of the herbage as indicated by reduced VFA production. In addition, an increase in the nitrate concentration of fertilised ryegrass could have played an important role in the reduction of methanogenesis by decreasing the availability of hydrogen.

Abbreviations: aDMd, apparent DM disappearance; CH_4/DMi, methane output per g DM incubated; CH_4/DMd, methane output per g DM disappeared; CH_4/TGP, methane output relative to total gas production; DM, dry matter; GPT, gas production technique; IVDMD, *in vitro* DM digestibility; Pred.CH_4/DMi, predicted methane output per g DM incubated; TGP/DMi, total gas production per g DM incubated; TGP/DMd, total gas production per g DM disappeared; tVFA/DMi, apparent total VFA output per g DM incubated; VFA, volatile fatty acids; ΔtVFA, change in total VFA concentration in the incubation medium.

* Corresponding author. Tel.: +353 046 9061100.
E-mail address: padraig.okiely@teagasc.ie (P. O'Kiely).

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1. Introduction

Methane (CH_4) represents 14% of total anthropogenic greenhouse gas emissions, of which ruminant livestock are responsible for 28% (IPCC, 2007). Methane, as a by-product of anaerobic fermentation, is produced by autotrophic microbes that use hydrogen (H_2) to reduce carbon dioxide (CO_2) to CH_4 (Mills et al., 2001). This methanogenesis represents the main hydrogen sink in the digestive tract of ruminants, and contributes to maintaining a low partial pressure of hydrogen that is essential for a successful fermentation in the rumen (Argyle and Baldwin, 1988). This enteric methane is not utilised by the host animal and is eventually released to the atmosphere, thereby contributing to global warming. It also represents a dietary energy loss from the ration consumed by ruminants (Johnson and Johnson, 1995).

Methane production from ruminants is influenced by the type of feed consumed. Several authors (Johnson and Johnson, 1995; Moss et al., 1995) reported reductions in methane output by increasing the proportion of concentrates in the animal's diet. However, in grassland-based systems ruminants usually receive relatively small amounts of concentrates during their production cycle. In these situations, offering forage of high nutritional quality is essential to enhance animal productivity. It has been suggested that this will simultaneously reduce methane emissions per unit daily weight gain (Hart et al., 2009; Boland et al., 2009) and per unit animal product (Moss et al., 2000).

Several management approaches can be implemented to improve the quality of the pasture offered to cattle such as the inclusion of legumes in the sward, increasing the rate of N fertiliser application or utilising herbage at an immature growth stage. Reductions in methane output when grass-based diets are supplemented with legumes are generally associated with lower fibre and higher protein contents, faster ruminal passage rates and greater intakes (McCaughey et al., 1999; Waghorn and Clark, 2006; Beauchemin et al., 2008). In some legume species the presence of secondary metabolites (condensed tannins, saponins) can also play an important role in inhibiting methane formation (Beauchemin et al., 2007). Non-tanniniferous legumes like red clover have been shown to enhance animal productivity (Vipond et al., 1995; Speijers et al., 2004) concurrent with a decrease in methane emissions compared to grass (Waghorn et al., 2002), although this is not always the case (Van Dorland et al., 2007). Increasing the N fertilisation rate of perennial ryegrass pastures can have relatively similar effects on the chemical composition of grass swards as introducing legumes, with higher CP and lower fibre contents in the herbage. Thus, Lovett et al. (2004) indicated that methane emissions decrease linearly with incrementally higher rates of N fertiliser application. In addition, utilising herbage at a more immature growth stage can increase digestibility through a lower fibre content.

The inclusion of legumes such as white or red clover in pastures providing ruminant feed is common practice in grassland-based livestock production systems in countries with a temperate climate. However, relatively little information is available on enteric methane emissions associated with diets based on red clover compared to ryegrass grown with or without N fertilisation, and on how the seasonal changes in the chemical constituents of the sward effect these emissions.

Due to the cost and the time required to carry out *in vivo* studies, *in vitro* rumen methodologies are often employed. The *in vitro* gas production technique (GPT) is a batch fermentation process that has been used to study the fermentation kinetics and methane output associated with contrasting legumes and grasses (Tavendale et al., 2005; Lovett et al., 2006; Purcell et al., 2011).

The aim of this experiment was to compare the *in vitro* rumen methane output of different forage species (perennial ryegrass vs. red clover), fertilisation managements of ryegrass (0 vs. 150 kg N/ha/growth) and two different varieties of red clover (Merviot vs. Ruttinova), and determine how the methane output associated with these herbages was influenced by early and late stages of maturity in the primary growth and by an autumn regrowth.

2. Materials and methods

2.1. Experimental design

Herbages were produced within a randomised complete block ($n = 4$) design experiment conducted over two successive years (O'Kiely et al., 2006; O'Kiely and Black, 2008). The samples thus selected were arranged in a 4 (herbages) × 3 (harvests) factorial structure of treatments, with year and field replicate effects treated as blocks. The *in vitro* rumen methane output was assayed using the GPT.

2.2. Forage production

The herbage samples were obtained from a field plot experiment conducted at Grange (53°30′N, 6°40′W, 92 m above sea level). Two varieties of red clover (*Trifolium pratense* cv. Merviot and Ruttinova) and two perennial ryegrass (*Lolium perenne* cv. Greengold) treatments (receiving 0 or 150 kg inorganic fertilizer N/ha/growth) were sown (August 2001) in a randomised complete block ($n = 4$) design with two plots (10 m × 2 m each) of each of the above four herbage treatments per replicate block (thus, 8 plots randomly allocated per block). The two plots per herbage treatment were for the early or late primary growth harvest schedules, and every plot was harvested four times in each of two successive years (2002 and 2003). The schedule that commenced with an early primary growth harvest was 2 June, 22 July, 4 September, 10 December in 2002, and 31 May, 16 July, 2 September and 29 October in 2003; the schedule that commenced with a late primary growth was 19 June, 2 August, 13 September and 10 December in 2002, and 13 June, 30 July, 10 September and 29 October in 2003. Three