**Project number:** 5631
**Funding source:** DAFF (RSF 06/R&D/361)
**Date:** November, 2011
**Project dates:** Oct 2007 – Dec 2011

# *In vitro* rumen methane output of feeds consumed by housed cattle

## Key external stakeholders:

Dept. Agriculture, Food and the Marine; rumen function and animal nutrition researchers; policy-makers; livestock industry; livestock farmers

## Practical implications for stakeholders:

Methane is a potent greenhouse gas, and manipulating the diet consumed by ruminant livestock can reduce enteric methane output. The *in vitro* rumen gas production technique (GPT) is a rapid screening tool to quickly estimate the methane output (and related characteristics) for a range of feeds, under standard conditions. It involves incubating a dried, milled sample of feed with rumen micro-organisms and artificial saliva (*i.e.* buffer) at body temperature and air-free conditions for 24 hours.

- Practices such as the use of red clover, the application of inorganic N fertiliser to perennial ryegrass and the ensilage of herbage can reduce enteric methane output.
- Modifications made to the GPT and expressing methane output relative to dry matter (DM) apparently digested provided more meaningful rankings of the methane output of forages *vs.* high starch feeds. The GPT can help identify worthwhile treatments to include in *in vivo* experiments that have the objective of formulating diets to reduce methane output per unit meat or milk produced.

## Main results:

- Red clover produced less methane than perennial ryegrass, but applying inorganic N fertiliser to the ryegrass reduced methane output to the level achieved with red clover.
- Silage produced less methane than the grass from which it was made, and this effect was larger when silage fermentation was more extensive.
- The GPT was modified to allow the pH of the *in vitro* rumen medium to change in response to the digestion of a feed. This altered the digestion characteristics to produce more meaningful methane output values (when expressed relative to the DM apparently digested) for forages *vs.* high starch feeds.

## Opportunity / Benefit:

- These experiments using the GPT have identified factors such as red clover, inorganic N fertilisation of ryegrass, autumn regrowth, herbage nitrate concentration and the ensilage of herbage (especially an extensive silage fermentation) that result in lower outputs of methane. This information can help identify treatments to include in *in vivo* methane output studies, with an objective of formulating diets that produce less methane per unit meat or milk produced.
- The modified GPT will allow more meaningful *in vitro* comparisons of feeds that contrast widely in chemical composition. These comparisons can be undertaken in experiments in Ireland or elsewhere.

## Collaborating Institutions:

University College Dublin (UCD), University of Leon, Spain



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## 1. Project background:

Since enteric methane accounts for about half of the greenhouse gas (GHG) emissions from Irish agriculture and the diet consumed by ruminants has a major impact on enteric methane output, much effort has been invested in measuring the methane output associated with diets consumed by grazing and indoor-fed animals. However, the high cost, large resource requirement, long duration of experiments and considerable variability in methane output estimates (made using sulphur hexafluoride as a marker) associated with *in vivo* studies present a range of considerable challenges. In this context, *in vitro* techniques have been developed to partially simulate rumen conditions and thus provide a standardised methane output index for feeds. They involve incubating a dried, milled sample of feed with rumen micro-organisms and artificial saliva (*i.e.* buffer) at body temperature and air-free conditions for 24 hours. These relatively rapid and low cost methods also permit the use of adequate experimental replication. This single *in vitro* trait of a feed provides useful information about its propensity to produce methane, but this value must be interpreted carefully since methane output by cattle consuming a diet to appetite will also be influenced by the actual intake achieved and by the dynamic activities that occur during rumen digestion. Since at least one-third of the lifetime intake of most Irish cattle occurs when they are accommodated indoors, a ranking of the propensity of conserved feeds to produce enteric methane could help in the formulation of 'low-methane' diets. However, any treatments investigated need to be ultimately submitted to a full life cycle assessment to account for all direct and indirect GHG emissions (and also sequestration).
The *in vitro* rumen gas production technique (GPT) was operated to allow a ranking of the methane output of feeds rather than describing their temporal kinetics of digestion.

## 2. Questions addressed by the project:

- What is the *in vitro* rumen methane output of perennial ryegrass and red clover when harvested at a silage production growth stage? How is this influenced by harvesting the primary growth in late May or mid June, or by harvesting an autumn regrowth? Does the variety of red clover alter the outcome and what is the impact of applying inorganic nitrogen fertiliser to the perennial ryegrass?
- What is the effect of ensiling grass on *in vitro* rumen methane output? How is this influenced by the extent and direction of silage fermentation?
- What is the relative *in vitro* rumen methane output of a range of ingredients commonly included in concentrate feeds and how do these compare to the values expected from some forages?
- Following on from concerns about the methane output of the above concentrate feeds, could the GPT be modified so it would provide results more similar to those expected in *in vivo* experiments?

## 3. The experimental studies:

Herbage samples were obtained from a randomised complete block field plot experiment where, in each of two successive years, two varieties of red clover (Merviot & Ruttinova) were harvested at two stages of the primary growth (late May & mid-June) or in an autumn regrowth. Comparable plots of perennial ryegrass (Greengold – tetraploid, intermediate-heading date) that received 0 or 150 kg fertiliser N/ha/growth were also harvested. Samples were incubated using the GPT and methane output was expressed relative to total gas or volatile fatty acids (VFA) produced, to dry matter (DM) incubated or to DM apparently digested.

Herbage samples from replicated field plots of three grass crops differing in ensilability (likely to undergo (a) extensive but undesirable fermentation, (b) extensive but desirable fermentation, and (c) restricted fermentation) were assessed for methane output using the GPT both pre- and post-ensilage. In each case the direction and/or extent of silage fermentation was further manipulated by chemical and biological additives in order to broaden the range of silage fermentation characteristics being assessed.

Samples of 30 feeds (for which some *in vivo* digestion/metabolism data were available) were assessed for



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methane output using the GPT. These ranged from high starch feeds (*e.g.* barley, wheat, tapioca) to degradable fibre/high sugar feeds (*e.g.* locust beans, beet pulp, citrus pulp), high fibre feeds (*e.g.* grain screenings, rice bran, grass meal, grass silage, lucerne, pollard, malt sprouts, palm kernel) and high protein feeds (*e.g.* maize gluten feed, maize distillers, rolled beans, cottonseed meal, rapeseed meal, sunflower meal, soyabean meal).

A combination of a weaker buffer solution and a higher feed to buffer ratio were used with three contrasting feeds (barley grain, grass silage and barley straw), and the effects on methane output during the GPT were expressed relative to DM incubated and digested, and VFA and total gas produced. A range of associated fermentation characteristics were also monitored.

## 4. Main results:

- Red clover variety had no impact on methane output. Red clover produced less methane per unit feed DM incubated ($CH_4$i) than perennial ryegrass that received no inorganic N fertiliser, but when the ryegrass had 150 kg N applied/ha/growth then $CH_4$i declined to a similar value as red clover (this effect was due to the hydrogen-scavenging by the nitrate in the N fertilised ryegrass). In contrast, methane output per unit feed DM apparently digested ($CH_4$d) during the incubation was similar for these four feeds. Delaying the harvest date of the primary growth reduced $CH_4$i but not $CH_4$d, whereas herbage from the autumn regrowth supported lower $CH_4$i and $CH_4$d than herbage from the early primary growth (due to high nitrate concentrations in the autumn grass).
- Ensilage reduced *in vitro* rumen methane output (whether expressed as $CH_4$i or $CH_4$d), reflecting a reduction in the proportion of acetic acid and an increase in the proportion of propionic acid in the *in vitro* rumen VFA. The magnitude of this decrease in methane output increased as the extent of silage fermentation increased, reflecting the greater change in herbage chemical composition during an extensive silage fermentation (it was related mainly to the higher concentration of lactic acid). However, among silages with a similar extent of fermentation, methane output was positively related to the proportion of lactic acid in fermentation products.
- Mean methane output values ($CH_4$i) were 47.1, 41.1, 28.6 and 33.2 ml/g DM for high starch, degradable fibre/high sugar, high fibre and high protein feed categories, respectively (corresponding $CH_4$d values were 53.9, 56.4, 51.4 and 45.3 ml/g DM). This outcome contrasts with values expected from *in vivo* experiments. Thus, the GPT as originally operated may not be appropriate for ranking methane output of feeds that range from forages to high starch feeds.
- The modified GPT permitted a shift in the pH of the *in vitro* medium to occur in response to the extent of *in vitro* fermentation associated with a feed. This resulted in a more *in vivo*-like ranking of methane output among feed types when expressed as $CH_4$d.

## 5. Opportunity/Benefit:

- These experiments using the GPT have identified factors such as red clover, inorganic N fertilisation of ryegrass, autumn regrowth, herbage nitrate concentration and the ensilage of herbage (especially an extensive silage fermentation) that result in lower outputs of methane. This information can help identify treatments to include in *in vivo* methane output studies, with an objective of formulating diets that produce less methane per unit meat or milk produced.
- The modified GPT will allow more meaningful *in vitro* comparisons of feeds that contrast widely in chemical composition. These comparisons can be undertaken in experiments in Ireland or elsewhere.

## 6. Dissemination:

### Main publications:

Navarro-Villa, A., O'Brien, M., Lopez, S., Boland, T.M. and O'Kiely, P. (2011). 'Modifications of a gas production technique for assessing *in vitro* rumen methane production from feedstuffs.' *Animal Feed Science and Technology*, 166-167: 163-174.

Navarro-Villa, A.. O'Brien, M., Lopez, S., Boland, T.M. and O'Kiely, P. (2011). '*In vitro* rumen methane output of red clover and perennial ryegrass assayed using the gas production technique (GPT).' *Animal Feed Science and Technology*. 168: 152-164.

Navarro-Villa, A.. O'Brien, M., Lopez, S., Boland, T.M. and O'Kiely, P. (2012). '*In vitro* rumen methane output of



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grasses and grass silages differing in fermentation characteristics using the gas production technique (GPT).' *Grass and Forage Science* (under review).

**Popular publications:**

O'Kiely, P., O'Brien, M., McGeough, E., Navarro-Villa, A. and Purcell, P. (2010). 'Reducing methane emissions from cattle.' *TResearch*, 5 (1) Spring :38-39.
http://www.teagasc.ie/publications/2010/6/6_TResearch_201002.pdf

The Investigators (popular science TV documentary series) – Padraig O'Kiely appeared in Programme 2 entitled "Climate Change". http://www.rte.ie/tv/theinvestigators/prog2.html

Public lecture organized by UCD Earth Sciences Institute, TCD TrinityHaus and Dublin City Council, under the TCD-UCD Innovation Alliance and in collaboration with Business in the Community and the main agencies involved involved in delivering policy (Comhar Sustainable Development Council, Enterprise Ireland, EPA, Geological Survey of Ireland, Marine Institute, Met Éireann, Sustainable Energy Authority of Ireland and Teagasc). The lecture ('Future opportunities to reduce bovine greenhouse gas emissions') given by Padraig O'Kiely can be accessed at
http://www.ucd.ie/earth/newsevents/transformingirelandseminarseries2010/seminar1160310/

**7. Compiled by:** Padraig O'Kiely, Alberto Navarro-Villa and Peter Purcell



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