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BRIEF COMMUNICATION: Use of novel pasture species to reduce methane emissions from New Zealand’s grazing ruminants.

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INTRODUCTION

In New Zealand, methane (CH$_4$) accounts for approximately 38% of greenhouse gas emissions. The majority of this methane, approximately 98%, is from ruminants (Waghorn & Woodward, 2006). New Zealand’s ruminant production industry relies heavily on a pasture-based system and depends on efficient utilisation of pasture. Variation between plant species in their nutritional and chemical composition provides an opportunity to manipulate rumen fermentation patterns and increase the utilization of pastures, while potentially reducing ruminant livestock CH$_4$ emissions. Preliminary findings are reported from a pilot study which investigated the effects of nutritional composition of a range of forage species on the short-term in vitro production of CH$_4$.

MATERIALS AND METHODS

Methane production from chicory (*Cichorium intybus* cv. Choice), plantain (*Plantago lanceolata* cv. Ceres Tonic), birdsfoot trefoil (*Lotus corniculatus* cv Grasslands Goldie) and ryegrass (*Lolium perenne* cv. Bronsyn), was measured in vitro over a 6 hour incubation. Chicory and plantain samples were harvested in autumn, March 2009, and lotus and ryegrass in winter, July 2009, from the Pasture and Crop Research Unit, Massey University, Palmerston North. All samples were harvested between 8:00 h and 10:00 h to about 4 cm above ground level. Dead plant material and weeds were removed and samples were stored at -20°C until nutritive analysis. Plantain was the only plant species containing reproductive material at the time of harvest. Plant nutritive value was measured from triplicate samples. Frozen samples were pooled, freeze-dried, ground through a 1 mm sieve and analyzed for total nitrogen (N) (Leco 2000; Leco Instruments, Inc., St. Joseph, Michigan, USA), with crude protein reported as N x 6.25. Structural carbohydrate (SC) content (hemicellulose and cellulose) were determined using the methods described by Robertson and Van Soest (1981) using a Tecator Fibertec System. Water soluble carbohydrate (WSC) and in vitro dry matter digestibility (IVDMD) were measured using the methods described by Sadasivam and Manickam (2005) and Roughan and Holland (1977), respectively.

Ground plant material (0.6 g per bottle) from each plant species was transferred to triplicate 125 mL serum bottles. Ryegrass with the addition of 100 mL of 2-bromo-ethylsulfonic acid (BES) as a methanogen inhibitor, was used as a negative standard to simulate the absence of CH$_4$ production. Cumulative CH$_4$ production and rate of CH$_4$ production from batch in vitro fermentation was determined for all plant samples. Rumen fluid was collected from one fistulated cow fed a ryegrass/white clover diet and filtered through a double cheese cloth in a CO$_2$ rich environment. The filtered rumen fluid (20% v/v) was mixed with McDougal buffer (80% v/v) and maintained in a CO$_2$ rich environment at 39°C before dispensing 60 mL into each of the serum bottles containing plant material and incubating for 6 hours at 39°C using a Contherm incubator. A 200 µL sample was taken from the headspace of each serum bottle at 3 hours and 6 hours, and analysed for CH$_4$ in a gas chromatograph (Shimadzu GC-2010, Shimadzu Scientific Instruments, Columbia, Maryland, USA), with a megabore HP-MOLSIV column (30 m × 0.53 mm × 25 µm). A Beta Standard (BOC Gasses, Auckland, New Zealand) containing 5 % hydrogen, 10% CH$_4$, 15% CO$_2$ and 60% nitrogen was used to quantify CH$_4$ production.

Cumulative CH$_4$ production and the rate of CH$_4$ production in the first three and final three hours of in vitro incubation were analysed using a General Liner Model in the statistical software package Minitab® (version 15.1.0.0, Minitab Inc., Cary, North Carolina, USA).

RESULTS AND DISCUSSION

The inclusion of (BES) inhibited the production of CH$_4$ (P <0.05; Table 1). Cumulative short-term CH$_4$ production from chicory, plantain and birdsfoot trefoil did not differ (P >0.05), but was lower (48%, P <0.05) than that of ryegrass and higher (P <0.05) than the negative standard (Table 1). The rate of CH$_4$ production per hour increased (P <0.05) in the final three hours of incubation for all plants species except birdsfoot trefoil. Ryegrass had the highest
TABLE 1: Cumulative methane (CH\textsubscript{4}) production over the 6 hour \textit{in vitro} incubation period and rate of CH\textsubscript{4} production for the first three and final three hours of a six hour incubation, for chicory, plantain, birdsfoot trefoil, ryegrass and the negative standard (Ryegrass + 2-bromo-ethylsulfonic acid). Data shown as mean ± pooled standard error. DM = Dry matter.

<table>
<thead>
<tr>
<th>Material</th>
<th>Cumulative CH\textsubscript{4} production (mL CH\textsubscript{4}/g DM)</th>
<th>Rate of CH\textsubscript{4} production (mL CH\textsubscript{4}/g DM/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incubation hours 1 to 3</td>
<td>Incubation hours 3 to 6</td>
</tr>
<tr>
<td>Chicory</td>
<td>7.9 ± 0.6\textsuperscript{b}</td>
<td>0.5 ± 0.2\textsuperscript{a}</td>
</tr>
<tr>
<td>Plantain</td>
<td>6.9 ± 0.6\textsuperscript{b}</td>
<td>0.6 ± 0.2\textsuperscript{a}</td>
</tr>
<tr>
<td>Birdsfoot trefoil</td>
<td>7.3 ± 0.6\textsuperscript{b}</td>
<td>1.2 ± 0.2\textsuperscript{b}</td>
</tr>
<tr>
<td>Ryegrass</td>
<td>13.3 ± 0.6\textsuperscript{c}</td>
<td>1.8 ± 0.2\textsuperscript{a}</td>
</tr>
<tr>
<td>Negative standard</td>
<td>1.3 ± 0.9\textsuperscript{a}</td>
<td>0.1 ± 0.3\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\textsuperscript{abcd} Different superscripts within columns indicate values that significantly differ (P <0.05)

TABLE 2: Mean nutritive values for chicory, plantain, birdsfoot trefoil and ryegrass, including measures of dry matter (DM), \textit{in vitro} dry matter digestibility, crude protein, water soluble carbohydrate, hemicellulose, cellulose and lignin contents and the ratio of water soluble carbohydrate (WSC) to structural carbohydrates (SC).

<table>
<thead>
<tr>
<th>Component</th>
<th>Plant species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chicory</td>
</tr>
<tr>
<td>Dry matter (%)</td>
<td>17.7</td>
</tr>
<tr>
<td>\textit{In vitro} dry matter digestibility (%)</td>
<td>89.2</td>
</tr>
<tr>
<td>Crude protein (g/kg DM)</td>
<td>129.6</td>
</tr>
<tr>
<td>Water soluble carbohydrate (g/kg DM)</td>
<td>180.3</td>
</tr>
<tr>
<td>Hemicellulose (g/kg DM)</td>
<td>37.8</td>
</tr>
<tr>
<td>Cellulose (g/kg DM)</td>
<td>54.6</td>
</tr>
<tr>
<td>WSC:SC</td>
<td>2.0</td>
</tr>
</tbody>
</table>

rate (P <0.05) of CH\textsubscript{4} production irrespective of time. The rate of CH\textsubscript{4} production from chicory and plantain in the first three hours of incubation was similar to the negative standard (P >0.05). The rate of CH\textsubscript{4} production from birdsfoot trefoil did not change (P >0.05) during the six hour incubation period and resembled (P >0.05) values observed for plantain during the last three hours of incubation. The rate of CH\textsubscript{4} production from chicory increased rapidly (P <0.05) in the final three hours of incubation to a level similar (P >0.05) to that of ryegrass.

The lower CH\textsubscript{4} production from chicory and plantain may be due in part to their high digestibility and WSC:SC ratio (Table 2). High WSC:SC ratios are associated with increased particle breakdown, decreased rumen retention time, increased passage rate, increased propionate production and decreased CH\textsubscript{4} production (Moss \textit{et al.} 2000; Barry 1998). The high WSC:SC ratio of chicory and plantain would have resulted in an initial fermentation that promoted propionate production, leading to the rate of CH\textsubscript{4} production during the first three hours of incubation being similar to that of the negative control. Additionally, Mould \textit{et al.} (2005) demonstrated that the high energy supply post-incubation provided by WSC decreases \textit{in vitro} gas release kinetics, which may also explain the short-term reductions in CH\textsubscript{4} release observed in the chicory and plantain incubations. However, as the fermentation progressed, the readily fermentable material would become limiting and hemicellulose and cellulose fermentation would begin to predominate resulting in a reduction of the propionate to acetate and butyrate ratio, resulting in an increase in CH\textsubscript{4} production.

Although the fermentation of birdsfoot trefoil produced less CH\textsubscript{4} than ryegrass, its rate of CH\textsubscript{4} production in the first three hours of incubation was significantly higher than that of plantain and chicory. Birdsfoot trefoil had a similar WSC:SC ratio to that of plantain but lower digestibility than both chicory and plantain (Table 2). Birdsfoot trefoil was harvested at a later stage of maturity compared to the other plant species. This potentially resulted in a fermentation pattern that did not promote increased production of propionate and therefore no initial reduction in CH\textsubscript{4} was observed. Interestingly, unlike chicory, plantain or ryegrass, the rate of CH\textsubscript{4} production from birdsfoot trefoil did not increase in the final three hours of incubation. Ramirez-Restrepo and Barry (2003) suggested that in addition to a high WSC:SC, the nutritional characteristics responsible for decreased production of CH\textsubscript{4} from birdsfoot trefoil are CT content and possibly other secondary compounds.

In conclusion, this study suggests that forage herbs and legumes, such as chicory, plantain and birdsfoot trefoil with high WSC:SC ratios, result in lower short-term \textit{in vitro} production of CH\textsubscript{4} compared to ryegrass and may be of use to New Zealand’s ruminant livestock industry to reduce methane emissions. Further research is needed to understand the role of CT and other secondary compounds in these plant species on the rate of CH\textsubscript{4} production.
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REFERENCES


