Hydrogen and methane emissions from beef cattle and their rumen microbial community vary with diet, time after feeding and genotype

John A. Rooke1*, R. John Wallace2, Carol-Anne Duthie1, Nest McKain2, Shirley Motta de Souza2, Jimmy J. Hyslop1, David W. Ross1, Tony Waterhouse1 and Rainer Roehe1

1SRUC, West Mains Road, Edinburgh EH9 3JG, UK
2Rowett Institute of Nutrition and Health, University of Aberdeen, Bucksburn, Aberdeen AB21 9SB, UK

(Submitted 23 May 2013 – Final revision received 12 March 2014 – Accepted 24 March 2014 – First published online 30 April 2014)

Abstract

The aims of the present study were to quantify hydrogen (H2) and methane (CH4) emissions from beef cattle under different dietary conditions and to assess how cattle genotype and rumen microbial community affected these emissions. A total of thirty-six Aberdeen Angus-sired (AAx) and thirty-six Limousin-sired (LIMx) steers were fed two diets with forage:concentrate ratios (DM basis) of either 8:92 (concentrate) or 52:48 (mixed). Each diet was fed to eighteen animals of each genotype. Methane (CH4) and H2 emissions were measured individually in indirect respiration chambers. H2 emissions (mmol/min) varied greatly throughout the day, being highest after feed consumption, and averaged about 0.10 mol H2/mol CH4. Higher H2 emissions (mol/kg DM intake) were recorded in steers fed the mixed diet. Higher CH4 emissions (mol/d and mol/kg DM intake) were recorded in steers fed the mixed diet (P < 0.001); the AAx steers produced more CH4 on a daily basis (mol/d, P < 0.05) but not on a DM intake basis (mol/kg DM intake). Archaea (P = 0.002) and protozoa (P < 0.001) were found to be more abundant and total bacteria (P < 0.001) less abundant (P < 0.001) on feeding the mixed diet. The relative abundance of Clostridium cluster IV was found to be greater (P < 0.001) and that of cluster XIVa (P = 0.025) lower on feeding the mixed diet. The relative abundance of Bacteroides plus Prevotella was greater (P = 0.018) and that of Clostridium cluster IV lower (P = 0.031) in the LIMx steers. There were no significant relationships between H2 emissions and microbial abundance. In conclusion, the rate of H2 production immediately after feeding may lead to transient overloading of methanogenic archaea capacity to use H2, resulting in peaks in H2 emissions from beef cattle.

Key words: Hydrogen: Methane: Rumen microbial community: Beef cattle

Methane (CH4) is a greenhouse gas with a global warming potential that is 25-fold that of CO2 (5). Ruminant livestock production through the enteric fermentation of feed contributes significantly to greenhouse gas production by agriculture, in the UK, CH4 accounted for 37% of all the agricultural emissions in 2005 (2). Enteric production of CH4 also represents a loss of potential that is 25-fold that of CO2 (3), which might otherwise be available for growth or milk production. Understanding the mechanisms of methanogenesis and the micro-organisms involved is important for devising sustainable mitigation strategies to lower the environmental impact of ruminant livestock production.

Molecular hydrogen (H2) plays an important role in intermediary metabolism in the rumen (4). Bacteria, protozoa and fungi produce H2 through the fermentation of carbohydrate. H2 and CO2 are the principal substrates for the production of CH4 by archaea (5,6). H2 is also a vital intermediate or substrate in other reactions. Ruminal inter-species H2 transfer is a process that affects the metabolism of both the microbes that produce H2 and those that utilise it (7). Methanogenic archaea require some accumulation of H2 to grow rapidly enough to avoid being washed out of the rumen (6). On the other hand, the accumulation of H2 exerts a thermodynamic inhibitory effect on H2-producing organisms and causes alterations in the fermentation products of these and other microbial species (7). As fibrolytic Ruminococcus spp. are H2 producers (via acetate formation), their growth and consequently fibre degradation may be inhibited by the accumulation of H2 (4,7). These pure culture studies indicate that decreasing H2 concentrations in the rumen would be doubly beneficial in terms of CH4 emissions and fibre breakdown. Several studies have measured H2 concentrations in ruminal digesta, as reviewed by Janssen (4). The concentrations of H2 increase in vitro after the addition of feed and are diet

Abbreviations: AAx, Aberdeen Angus cross; DMI, DM intake; GEI, gross energy intake; LIMx, Limousin cross; LW, live weight; VFA, volatile fatty acid.

*Corresponding author: Dr J. A. Rooke, email john.rooke@sruc.ac.uk

†Present address: SRUC, Roslin Institute Building, Easter Bush, Midlothian EH25 9RG, UK.
In one study involving two sheep, it was found that the animals were, on average, 2.1% (v/v) of CH4 emissions based on exhaled gas concentrations. There were periods of high H2 study using sheep. Takenaka and colleagues (9) concluded that H2 emissions were, on average, 2.1% (v/v) of CH4 emissions based on exhaled gas concentrations. There were periods of high H2 emission when H2 formation occurred at a faster rate than methanogenesis, particularly when concentrate feeds were included in the diet. To the best of our knowledge, similar studies have reported H2 emissions in vivo. In one study (9), it was found that the animals produced 2-fold different amounts of CH4: the sheep emitting lower amounts of CH4 produced more H2. In another study using sheep, Takenaka et al. (9) concluded that H2 emissions were, on average, 2.1% (v/v) of CH4 emissions based on exhaled gas concentrations. There were periods of high H2 emission when H2 formation occurred at a faster rate than methanogenesis, particularly when concentrate feeds were included in the diet. To the best of our knowledge, similar investigations in cattle have not been published. Therefore, the aim of the present study was to measure both H2 and CH4 emissions from beef steers fed two contrasting finishing diets typical of those produced in the UK: a high-concentrate diet based on barley and a mixed forage–concentrate diet including grass and whole-crop barley silages, barley grain and maize distillers dark grains (similar to maize distillers grains with solubles).

Materials and methods

The present study was conducted at the Beef Research Centre of SRUC (6 miles south of Edinburgh, UK) in 2011. The experiment was approved by the Animal Experiment Committee of SRUC and was conducted in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986.

### Animals, experimental design and diets

The seventy-two cross-bred steers used in the experiment were from a rotational cross between purebred Aberdeen Angus or Limousin sires and cross-bred dams of those genotypes and referred to as AAx and LIMx, respectively. The steers were fed two complete diets using forage wagon, consisting (g/kg DM) of either 480 forage:520 concentrate (mixed) or 75 forage:925 concentrate (concentrate). The composition of the diets and the chemical composition of the feeds are given in Tables 1 and 2, respectively.

Before the start of the present experiment, DM intake (DMI) and live-weight (LW) gain of the steers were measured in a feeding trial for 8 weeks (to be reported elsewhere). The feeding trial was of a 2 x 2 factorial (genotype x diet) design with the steers being stratified by LW on entry. The present experiment was a continuation of the feeding trial and therefore the steers were fed the diet that they were fed during the feeding trial. The steers were allocated to six respiration chambers over a 12-week period, using a randomised block design (six chambers x 4 weeks), which was repeated three times. Within each block, each treatment of the 2 x 2 factorial (genotype x diet) experimental design was replicated once in each respiration chamber. The steers were allocated to blocks to minimise variation in LW (mean LW 674 (SEM 42) kg) on entry into the respiration chambers. Therefore, emissions from each of the seventy-two steers were measured once as described below.

### Respiration chamber design, operation and measurements

In the present experiment, six indirect open-circuit respiration chambers were used (No Pollution Industrial Systems Limited). The total chamber volume (76 m3) was ventilated by recirculating fans set at 450 litres/s. Air was removed from the chambers by exhaust fans set at 50 litres/s, giving approximately 2.5 air changes/h. Temperature and relative humidity were set at 15°C and 60%, respectively. Total air flow was measured using in-line hot wire anemometers that were validated by daily measurements made with an externally calibrated anemometer (Testo 417; Testo Limited). Temperature and humidity were measured using sensor probes in the exhaust air outlet (Johnson...
Controls), and atmospheric pressure, corrected for altitude, was measured using a Vantage Pro2 weather station (Davis Instruments). The chambers were operated under negative pressure (50 N/m²). The concentrations of CH₄ were measured by IR absorption spectrophotometry and those of H₂ using a chemical sensor (MGA3000; Analytical Development Company Limited). The analyser was calibrated with a gas mixture of known composition. The concentrations of gases in each chamber and inflowing air were recorded every 6 min. Before the start of the experiment, gas recoveries were measured by releasing CO₂ at a constant rate into each chamber. The mean recovery was 98 (SEM 3·0)%, which was not different from 100%.

To accustom the steers to the chamber environment, 6 d before chamber measurements, groups of steers were moved to the building in which the chambers were located and loose-housed in single pens (4×3 m) identical in design to pens within the chambers. After 6 d, the steers were moved to the chambers and held there for 72 h, with CH₄ and H₂ measurements being recorded in the final 48 h of the experimental period. The steers were fed once daily, and the weight of the feed within the bins was recorded at 10 s intervals using load cells. The front doors of the chambers were briefly opened at about 08.00 hours daily to remove the feed bins and again at approximately 09.00 hours to replace the bins containing fresh feed. The pens were cleaned daily between 08.00 and 09.00 hours. The exact time points at which the doors were opened were recorded.

**Rumen sampling and volatile fatty acid analysis**

Immediately after the steers (within 2 h) left the respiration chambers, samples of ruminal fluid were obtained (one per animal) by inserting a tube (16X2700 mm Equivet Stomach Tube; Jørgen Kruuse A/S) nasally and aspirating manually. Approximately 50 ml of the fluid were strained through two layers of muslin and stored at −20°C until analysis. Samples used for volatile fatty acid (VFA) analysis (1 ml) were deproteinised by adding 0·2 ml of metaphosphoric acid (215 g/l) and 0·1 ml of internal standard (10 ml 2-ethyl n-butyracid/l), and the concentrations of VFA were determined by HPLC(10). For DNA analysis, 5 ml of strained ruminal fluid were mixed with 10 ml of PBS containing glycerol (30%, v/v) and stored at −20°C.

**DNA analysis**

DNA extraction was carried out using a method based on repeated bead beating plus column filtration(11). The concentrations of DNA were determined using a NanoDrop ND 1000 spectrophotometer (NanoDrop Technologies). DNA was diluted to 0·5 ng/μl in 5 μg/ml of herring sperm DNA for amplification of bacterial 16S RNA genes with the universal bacterial primers UniF and UniR and 5 ng/μl in 5 μg/ml of herring sperm DNA for the amplification of other groups(12). Quantitative PCR of 16S RNA genes from different bacterial classes was carried out using a BioRad iQ5 system as described by Ramirez-Farias et al.(13). Calibration curves were constructed using three separate batches in different quantitative PCR runs. Bacterial primer sets, method development and target species have been described in Ramirez-Farias et al.(13). Template DNA from Roseburia hominis A2-183 (DSM 16839T) was used for bacterial calibration. Amplification of archaeal 16S RNA genes was carried out using the primers described by Hook et al.(14) and calibrated using DNA extracted from Methanobrevibacter smithii PS, a gift from M. P. Bryant, University of Illinois. Protozoal 18S rRNA gene amplification was calibrated using DNA amplified from bovine ruminal digesta with the primers 54f and 1747r(15). The coverage of quantitative PCR primers was checked from original references and using the ProbeMatch tool of the Ribosomal Database Project(16).

**Feed analysis**

Feed samples were analysed for DM, ash, crude protein, acid-detergent fibre, neutral-detergent fibre, starch(17) and gross energy contents by adiabatic bomb calorimetry.

**Calculations and statistical analysis**

To minimise bias caused by the entry of air into the chambers on opening the doors for feeding and as steers did not have access to feed during this period (54 min, SD 22·5), the concentrations of gases measured during this period were not used for further analysis. Instead, and to minimise bias, these values were replaced by the mean value of measurements (n 10) made in the last hour before the doors were opened. If a steer had consumed food during this period, mean values recorded during the hour preceding feed consumption were used. All data, including those on gas concentrations, air flow, temperature, humidity, atmospheric pressure and records of feed consumption, were loaded into a database. Dry air flow was calculated and corrected to standard temperature and pressure for each individual record of gas concentration. Daily gas production was then calculated as the average of individual values.

Measurements were not recorded for one steer because of illness and data obtained from three steers were excluded because of an air leak in one chamber; these comprised two LIMx steers fed the concentrate diet, one LIMx steer fed the mixed diet and one AAX steer fed the mixed diet. Data were analysed using Genstat (version 11.1 for Windows; VSN International Limited) using linear mixed models, where the fixed factor was the 2×2 arrangement of genotype and diet with week of the experimental period, VFA data were analysed as a 2×2 factorial arrangement of genotype and diet and the random factors were the block and chamber. As samples for VFA analysis were available for only 7 weeks of the experimental period, VFA data were analysed as a 2×2 factorial arrangement of genotype and diet with week of the experiment and chamber. Data are reported as means with their standard errors of the difference unless indicated otherwise. Multiple linear regression models were fitted to predict CH₄ and H₂ emissions from the whole dataset. Fitted terms included Clostridium clusters IV and XIVa, Bacteroides plus Prevotella, archaea and protozoa (expressed as copy number/ng DNA). To help with variable selection, all the subsets of predictors were examined, with subsets being compared using adjusted R² and Akaike’s information criterion.
Results
Cattle fed the mixed diet consumed less feed (Table 3), whether expressed as total daily DMI ($P<0.001$) or as g/kg LW ($P=0.009$), than those fed the concentrate diet. The DMI of the AAX steers was also greater ($P=0.002$) than that of the LIMx steers.

Whether expressed as mol/d, mol/kg DMI or kJ/MJ GEI (Table 3), steers fed the concentrate diet produced less CH$_4$ than those fed the mixed diet ($P<0.001$). The AAX steers produced more CH$_4$ (mol/d, $P=0.032$) than the LIMx steers, but this difference disappeared when CH$_4$ production was expressed relative to DMI or GEI.

H$_2$ production by the steers was, on average, 0.10 mol H$_2$/mol CH$_4$ (Table 3). There was a significant diet × genotype interaction such that the concentrate-fed AAX steers produced less total H$_2$ than the LIMx steers, but the opposite trend was observed on feeding the mixed diet. When expressed as mol/kg DMI or kJ/MJ GEI, there was no interaction, and the mixed diet-fed steers produced more H$_2$ than the concentrate diet-fed steers. However, as a proportion of CH$_4$ production between the dietary groups, the frequency of H$_2$ production rates (0.63 $P=0.001$) differed from 0. No microbial predictors were able to explain a significant amount of variability in H$_2$ emissions between individual animals. A relationship ($r^2=0.30$) between CH$_4$ emissions (mol/kg DMI) and copy numbers ($\times 10^3$/ng DNA) of archaea and Clostridium cluster XIVa was observed:

$$\text{CH}_4 \text{ (mol/g DMI)} = 1.07 + 0.00298 \text{ Cluster XIVa (s.e. 0.00083, } P = 0.001) + 0.0094 \text{ Archaea (s.e. 0.0024, } P < 0.001).$$

<table>
<thead>
<tr>
<th>Diets…</th>
<th>Concentrate</th>
<th>Mixed</th>
<th>SED</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AAX</td>
<td>LIMx</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI kg/d</td>
<td>11.4</td>
<td>10.0</td>
<td>0.52</td>
<td>0.002 $&lt;0.001$ NS</td>
</tr>
<tr>
<td>g/kg LW</td>
<td>16.1</td>
<td>15.1</td>
<td>0.76</td>
<td>0.0016 $&lt;0.001$ NS</td>
</tr>
<tr>
<td>H$_2$ mol/d</td>
<td>0.92</td>
<td>1.08</td>
<td>0.106</td>
<td>NS</td>
</tr>
<tr>
<td>mol/kg DMI</td>
<td>0.084</td>
<td>0.112</td>
<td>0.0111</td>
<td>NS</td>
</tr>
<tr>
<td>kJ/MJ GEI</td>
<td>1.27</td>
<td>1.66</td>
<td>0.168</td>
<td>NS</td>
</tr>
<tr>
<td>CH$_4$ mol/d</td>
<td>9.4</td>
<td>8.5</td>
<td>0.72</td>
<td>0.032 $&lt;0.001$ NS</td>
</tr>
<tr>
<td>mol/kg DMI</td>
<td>0.83</td>
<td>0.87</td>
<td>0.077</td>
<td>NS</td>
</tr>
<tr>
<td>kJ/MJ GEI</td>
<td>39.0</td>
<td>39.9</td>
<td>3.31</td>
<td>NS</td>
</tr>
<tr>
<td>H$_2$/CH$_4$ (mol/mol)</td>
<td>0.101</td>
<td>0.126</td>
<td>0.035</td>
<td>NS</td>
</tr>
</tbody>
</table>

Concentrate, high-concentrate diet; mixed, mixed forage–concentrate diet; AAX, Aberdeen Angus cross; LIMx, Limousin cross; G×D, genotype × diet; DMI, DM intake; LW, live weight; GEI, gross energy intake.
Discussion

Enteric fermentation in animals occurs predominantly in the absence of oxygen. Under such conditions, microbial communities adapt differently to the disposal of the reducing equivalents that are generated by glycolysis. Some microorganisms use an internal redox mechanism, such as in the formation of propionate and succinate. However, most of the microbial fermentations result in the formation of molecular H₂.

The fate of H₂ depends on the animal species and its anatomical configuration. In man, with a relatively rapid gut transit time, reductive acetogenesis (H₂ + CO₂ → acetate) and H₂ production tend to predominate as mechanisms for the disposal of H₂. In a study carried out in Europe, about 50% of the human subjects were found to also produce CH₄; CH₄ production competed with other metabolic processes, but H₂ was still produced by these subjects (18). The rates of H₂ emissions from ruminants are known to be proportionally much lower and those of CH₄ emissions much greater (19). Van Zijderveld et al. (20) measured H₂ production by dairy cows hourly for 9 h and reported greater concentrations when nitrate was included in the diet, but, to the authors' knowledge, this is the first study in which total daily H₂ emissions from cattle have been quantified on a large scale using indirect respiration chambers.

Hydrogen emissions

Previous studies have reported lower H₂ concentrations in ruminants fed all-forage diets than in those fed diets containing various proportions of concentrate and forage whether measured as concentrations of H₂ dissolved in the rumen fluid (21) in the rumen gas phase (22) or in the exhaled air (23). There do not appear to be any reports of H₂ emissions from live animals fed high-concentrate diets. In the present study, daily H₂ emissions recorded were similar for both diets and genotypes, but when converted to units per DMI, H₂ production was found to be greater for the mixed diet than for the concentrate diet. Total daily H₂ emissions were about 1 and 10% of CH₄ emissions on a mass basis and a molar basis, respectively. Total H balance was determined from estimates of the amounts of carbohydrate fermented in the rumen and observed mean VFA molar proportions for each diet. Although the amount of H₂ produced per unit carbohydrate fermented on feeding the concentrate diet was less than that produced on feeding the mixed diet (3·6 vs. 4·9 mol H₂/mol of carbohydrate fermented), estimates of total H₂ produced were not dissimilar between the dietary groups (108 vs. 177 mol/d, concentrate vs. mixed) because of both the lower fermentability (due to the presence of fermentation end products in the silages) and the lower daily feed intake values recorded for the mixed diet. Thus, H₂ emissions accounted for less than 2% of the estimated total H₂ production from fermentation. Furthermore, after accounting for H₂ consumed in the synthesis of microbial biomass, the total recovery of H₂ in microbial biomass, H₂ and CH₄ was similar between the dietary groups (108 and 114% of H₂ produced on feeding the concentrate and mixed diets, respectively), indicating that there were no major H₂-consuming processes unaccounted for or that differed between the dietary groups.

Peaks in H₂ emission rates (Fig. 1) were observed after feed consumption, and these peak H₂ emission rates were found to be greater on feeding the mixed diet. Increases in H₂ emission rates after feed consumption were consistent with measurements in sheep of H₂ concentrations in the ruminal fluid (24), rumen head-space gas (25,26) and respiration chambers (27,28). The larger size of the meal-related peaks in H₂ emissions observed on feeding the mixed diet accounted for the differences in daily H₂ emissions (g/kg DMI) observed. One might have expected that there would be correlations between the ruminal microbiota and H₂ emissions, particularly the balance between ciliate protozoa and Clostridium cluster IV as major H₂ producers and archaea as consumers, but no relationships
between H$_2$ emissions and any of the different groups of microorganisms were found. It is possible that the primers used may not have detected all the H$_2$-producing bacteria. Alternatively, the differences between the dietary groups with regard to H$_2$ emissions are more likely to be related to the nature of the diets fed and the consumption patterns of individual cows. First, the peaks in H$_2$ emissions may be caused by the physical displacement of gas from the rumen head space by the feed consumed.$^{27}$ Because the mixed diet contained higher proportions of long forage and had a higher moisture content (443 vs. 853 g DM/kg fresh weight), the bulkier mixed diet may have caused greater displacement of the rumen head-space gas and hence greater H$_2$ emissions. Second, compared with the concentrate diet, the mixed diet contained higher concentrations of more slowly fermented cell wall carbohydrates and less starch and also higher concentrations of soluble feed constituents derived from the silages fed, particularly amino acids and fermentation products. Therefore, there may be an increased production of H$_2$ from the rapid fermentation of soluble feed components immediately after consumption of the mixed diet that exceeds the capacity of methanogens to utilise the H$_2$ produced. The peaks in H$_2$ emissions after feed consumption were also more defined and discrete than the peaks in CH$_4$ emissions (Fig. 1). A possible explanation for this is that while CH$_4$ is an end product of the metabolism of H$_2$ by archaea, the H$_2$ present in the ruminal gas phase can either be emitted by eructation or redissolve in the ruminal fluid and be utilised for CH$_4$ production by the archaea.$^{28}$ This may also explain the poor relationship between CH$_4$ and H$_2$ emissions (Fig. 2), as H$_2$ emissions will depend not only on the rates of production by H$_2$-generating metabolism exceeding the capacity of archaea to consume H$_2$ but also on the rate at which dissolved/gaseous H$_2$ is utilised. Both these will depend on the meal size and the rate of feed consumption of individual animals.

### Methane production

As has been found in other studies,$^{2,29}$ CH$_4$ production (mol/d) was found to be substantially lower on feeding the diet containing more than 900 g concentrate/kg DM than on feeding the mixed forage–concentrate diet, thus confirming

---

**Table 4. Volatile fatty acid molar proportions (mmol/mol) in ruminal fluid samples obtained from steers fed either a high-concentrate diet or a mixed forage–concentrate diet**

<table>
<thead>
<tr>
<th>Diets...</th>
<th>Concentrate</th>
<th>Mixed</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes...</td>
<td>AAX</td>
<td>LIMx</td>
<td>AAX</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>557</td>
<td>562</td>
<td>670</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>290</td>
<td>306</td>
<td>172</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>105</td>
<td>92</td>
<td>114</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>16</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Branched-chain acids*</td>
<td>32</td>
<td>24</td>
<td>30</td>
</tr>
</tbody>
</table>

*Concentrate, high-concentrate diet; mixed, mixed forage–concentrate diet; AAX, Aberdeen Angus cross; LIMx, Limousin cross; GxD, genotype x diet.

**Table 5. Microbial numbers in the samples of ruminal digesta**

<table>
<thead>
<tr>
<th>Diets...</th>
<th>Concentrate</th>
<th>Mixed</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes...</td>
<td>AAX</td>
<td>LIMx</td>
<td>AAX</td>
</tr>
<tr>
<td>Archaea*</td>
<td>30.4</td>
<td>25.7</td>
<td>46.4</td>
</tr>
<tr>
<td>Protozoa†</td>
<td>37.2</td>
<td>40.0</td>
<td>102.1</td>
</tr>
<tr>
<td>Total bacteria*</td>
<td>669</td>
<td>761</td>
<td>492</td>
</tr>
<tr>
<td>Clostridium Cluster IV*</td>
<td>138</td>
<td>122</td>
<td>179</td>
</tr>
<tr>
<td>Cluster XIVa*</td>
<td>127</td>
<td>122</td>
<td>75</td>
</tr>
<tr>
<td>Bacteroides plus Prevotella*</td>
<td>218</td>
<td>302</td>
<td>157</td>
</tr>
<tr>
<td>Relative abundance‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium Cluster IV*</td>
<td>0.21</td>
<td>0.17</td>
<td>0.35</td>
</tr>
<tr>
<td>Cluster XIVa*</td>
<td>0.19</td>
<td>0.16</td>
<td>0.15</td>
</tr>
<tr>
<td>Bacteroides plus Prevotella*</td>
<td>0.33</td>
<td>0.40</td>
<td>0.32</td>
</tr>
<tr>
<td>Sum§</td>
<td>0.74</td>
<td>0.73</td>
<td>0.82</td>
</tr>
</tbody>
</table>

* Results are expressed as copy numbers ($\times 10^{3}$/mg DNA) as determined by quantitative PCR of 16S rRNA.
† Results are expressed as copy numbers ($\times 10^{3}$/mg DNA) as determined by quantitative PCR of 18S rRNA.
‡ Relative abundance as a proportion of total bacteria.
§ Sum is the abundance of Clostridium cluster IV plus that of cluster XIVa plus Bacteroides plus Prevotella.

---
the well-established strategy of reducing CH$_4$ emissions by increasing the concentrate proportion of the diet. Mean CH$_4$ yields of 0.039 and 0.062 MJ/MJ GEI were recorded on feeding the concentrate and mixed diets, respectively. These compare with values of 0.030 MJ/MJ GEI (for the diet containing more than 900 kg concentrate/kg DM) and 0.065 MJ/MJ GEI (for all other diets) adopted by the IPCC$^{(1)}$ for estimating CH$_4$ emissions. Thus, the values predicted by the IPCC$^{(1)}$ for CH$_4$ production for the mixed diet slightly differed from those recorded (predicted v. observed; 298 v. 287 litres/d). However, the IPCC$^{(1)}$ underestimated CH$_4$ production values for the concentrate diet (predicted v. observed; 155 v. 200 litres/d). The reason for the higher CH$_4$ production values recorded on feeding the concentrate diet in the present study was probably that the cereal fed was barley rather than maize. When high-concentrate diets based on maize and barley were fed to feedlot cattle$^{(28)}$, CH$_4$ production values of 0.028 and 0.040 MJ/MJ GEI were recorded, respectively. Similarly, CH$_4$ production values of 0.033 and 0.046 MJ/MJ GEI were recorded for maize- and barley-based concentrates (800 g concentrate/kg DM), albeit in different years$^{(30)}$. Finally, a CH$_4$ production value of 0.04 MJ/MJ GEI for a barley-based diet (900 g/kg diet DM)$^{(31)}$ and recently a value of 0.03 MJ/MJ GEI for a maize-based concentrate$^{(32)}$ have been reported. Thus, the value of 0.030 MJ/MJ GEI suggested by the IPCC$^{(1)}$ for high-concentrate diets is probably inappropriate for diets based on barley and a value of 0.04 MJ/MJ GEI might be more appropriate. The reasons for the difference between barley and maize have been discussed$^{(29,32)}$ and are most probably due to the more rapid and complete fermentation of barley grain in the rumen and the higher fibre concentration in barley. The simple approach used by the IPCC$^{(1)}$ does not account for variations in diet digestibility or differences in the efficiency of utilisation of absorbed nutrients for productive purposes. CH$_4$ emissions recorded in the present study were estimated first relative to metabolisable energy (estimated from the feed analysis) intake as a proxy for digestibility and second with respect to steer LW gain during the feeding trial that preceded the present experiment. Estimates recorded on feeding the concentrate diet were 0.058 MJ CH$_4$/MJ metabolisable energy intake and 6.5 mol CH$_4$/kg LW gain compared with 0.101 and 11.7 recorded on feeding the mixed diet. Relative to the concentrate diet, the mixed diet resulted in 1.74-fold (metabolisable energy basis) and 1.80-fold (LW gain basis) higher CH$_4$ emissions in comparison with 1.58-fold expressed on a gross energy basis. Thus, the difference in CH$_4$ emissions between the dietary groups is amplified when expressed on a metabolisable energy or a LW gain basis.

Although total daily CH$_4$ emissions were greater in the AAx steers, this difference was accounted for by differences in DMI. Thus, CH$_4$ emissions (mol/kg DMI) did not differ between similar genotypes, although there were effects of individual sires$^{(33)}$.

**Diet and microbial numbers**

The analysis of the rumen microbial community provided information on how diet affected the main groups of bacteria, total ciliate protozoa and archaea. The three groups of bacteria were chosen to represent the main groups of bacteria (Firmicutes and Bacteroidetes) that are known to colonise the rumen$^{(34–36)}$, but it should be noted that the primers used would not account for all the species of Firmicutes or Bacteroidetes. The three groups of bacteria accounted for more than 0.70 of total bacteria copy numbers and this was true for the AAx steers but not for the LIMx steers in the present study. Part of the variation in the relative abundance (proportion of total bacteria) of the two *Clostridium* clusters was due to diet. The abundance of *Clostridium* cluster IV, encompassing the highly cellulolytic *Ruminococcus* and several *Eubacterium* spp.$^{(13)}$, was found...
to be greater on feeding the mixed diet. Clostridium cluster XIVa, the abundance of which was found to be lower on feeding the mixed diet, contains Butyrivibrio and related species\(^{(13)}\), none of which are known to possess the ability to break down crystalline cellulose\(^{(37)}\). Ciliate protozoa were found to be more abundant on feeding the mixed diet, a result which seems to be at odds with the general observation that adding a concentrate to a forage diet usually increases the number of protozoa\(^{(19,38)}\). There are a limited number of reports on the effects of diets containing high proportions of concentrate on rumen microbial community. The abundance of archaea increased when the concentrate proportion was increased from 100 to 500 g/kg diet\(^{(29)}\) and decreased when it was increased from 500 to 900 g/kg\(^{(40)}\) (similar to that done in the present experiment). However, when Popova et al.\(^{(41)}\) compared starch- and fibre-rich concentrates in a diet containing 870 g concentrate/kg, no differences in the abundance of methanogens were observed between the dietary groups. When the dietary concentrate proportion was increased\(^{(42)}\) from 0 to 700 g/kg, higher proportions were found to reduce the numbers of Fibrobacter succinogenes and increase the numbers of members of the genus Prevotella, but no differences in the abundance of Ruminococcus albus or R. flavefaciens were observed between the diets. This is in contrast to the decrease in the abundance of Clostridium cluster IV and no change in that of Bacteroides plus Prevotella observed when the concentrate proportion was increased in the present study. Similarly, increases in the number of protozoa were reported\(^{(41,42)}\) when the proportion of concentrate or dietary starch was increased, again in contrast to the decrease in numbers reported herein and elsewhere\(^{(31)}\). These differences are probably explained by the different dietary protocols and approaches to community analysis used in the experiments. For example, Carberry et al.\(^{(42)}\) compared 0 and 700 g concentrate/kg, while we compared 500 and 920 g concentrate/kg in the present study.

In terms of our focus on H\(_2\) emissions, it was perhaps surprising that the \(\text{H}_2\)-producing Ruminococcus spp. of cluster IV and total protozoa, which produce abundant \(\text{H}_2\)\(^{(43)}\), were not more correlated with \(\text{CH}_4\), as \(\text{H}_2\) is the main substrate for methanogenesis in the rumen\(^{(28,44)}\). There is no obvious explanation, except perhaps that any effect of the abundance of \(\text{H}_2\) producers was swamped by the effects of long-term adaptation to the diets fed. Alternatively, a more detailed taxonomic description within the groups, best derived from metagenomic information, might help identify key genera and species that dictate \(\text{H}_2\) production and thereby influence methanogenesis.

Many researchers believe, and some studies are beginning to show, that the host animal exerts a controlling effect on its own gut microbiota\(^{(45–47)}\). The findings reported herein that the relative abundance of Bacteroides plus Prevotella was lower and that of cluster IV greater in the AAX steers than in the LMx steers on feeding the corresponding diets would support such a hypothesis and may provide a mechanism for the greater feed intakes observed in the AAX steers.

### Implications

Recently, considering interactions between \(\text{H}_2\) and other gases in the atmosphere\(^{(48)}\), it has been proposed that \(\text{H}_2\) is an indirect greenhouse gas with a global warming potential of 5-8 compared with 25 of \(\text{CH}_4\) on a CO\(_2\) mass equivalent basis. On a daily basis, total (\(\text{CH}_4\) plus \(\text{H}_2\)) mean emissions from enteric fermentation were 3·6 and 5·1 kg CO\(_2\) for the concentrate and mixed diets, of which \(\text{H}_2\) contributed 12 and 13 g CO\(_2\) daily. Thus, although inefficiency in capturing \(\text{H}_2\) during inter-species \(\text{H}_2\) transfer is a loss of energy from the system, in terms of overall greenhouse gas production by ruminants, its contribution will be negligible with the exception of circumstances where methanogenesis is severely disrupted, e.g. when halogenated compounds are used to inhibit methanogenesis\(^{(25)}\).

In conclusion, this large-scale study of the effect of diet, feeding pattern and cattle genotype on \(\text{H}_2\) emissions from cattle has revealed that \(\text{H}_2\) emissions can be up to 10\% on a molar basis of \(\text{CH}_4\) emissions from beef cattle on feeding commonly used diets. Most of the \(\text{H}_2\) was produced shortly after feeding, and the concentration followed that of \(\text{CH}_4\). However, the feeding-related increases in \(\text{H}_2\) emissions were not related to the microbial populations and therefore are more likely to occur due to between-diet differences in feeding patterns and the nutrients rapidly fermented upon feed ingestion. Cattle genotype affected \(\text{H}_2\) emissions via differences in feed intake and this may be related to differences in microbial community structure. The observations are consistent with the review by Janssen\(^{(4)}\) that the capacity for archaeal methanogenesis is in balance with the rates of \(\text{H}_2\) production, such that some accumulation of \(\text{H}_2\) is required for methanogenesis to occur. The quantities of \(\text{H}_2\) emitted and the lower radiative forcing potential of \(\text{H}_2\) suggest that \(\text{H}_2\) emissions present a minor environmental problem in comparison with \(\text{CH}_4\) emissions.

### Acknowledgements

The authors are grateful to Lesley Deans and all support staff at the Beef Research Centre, Easter Howgate, for their technical assistance and also to Graham Horgan of BioSS, Aberdeen, for conducting multivariate analysis.

The present study was funded by the Scottish Government and by DEFRA and the devolved administrations through the UK Agricultural Greenhouse Gas Inventory Research Platform (http://www.ghgplatform.org.uk). The funders had no role in the design and analysis of the study or in the writing of this article.

The authors’ contributions are as follows: T. W., R. J. W. and R. R. initiated the research; C.-A. D., J. J. H. and D. W. R. participated in the planning and facilitation of the animal work; N. Mck. and S. M. de S. carried out DNA extraction and quantitative PCR; J. A. R supervised the respiration chamber studies and wrote the manuscript with inputs from R. J. W. All authors provided feedback on the manuscript.

None of the authors has any conflicts of interest to declare.
References


