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_JANIM SCI_ 2009, 87:3342-3350.
doi: 10.2527/jas.2009-1786 originally published online June 19, 2009

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Effect of sward dry matter digestibility on methane production, ruminal fermentation, and microbial populations of zero-grazed beef cattle

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ABSTRACT: Increasing the digestibility of pasture for grazing ruminants has been proposed as a low-cost practical means of reducing ruminant CH4 emissions. At high feed intake levels, the proportion of energy lost as CH4 decreases as the digestibility of the diet increases. Therefore, improving forage digestibility may improve productivity as DM and energy intake are increased. A zero-grazing experiment was conducted to determine the effect of sward DM digestibility (DMD) on DMI, CH4 emissions, and indices of rumen fermentation of beef animals. Twelve Charolais-cross heifers were assigned to 1 of 2 treatments, with 6 heifers per dietary treatment. Additionally, 4 cannulated Aberdeen Angus-cross steers were randomly allocated to each of these 2 treatments in a crossover design. Dietary treatments consisted of swards managed to produce (i) high digestibility pasture (high DMD) or (ii) pasture with less digestibility (low DMD), both offered for ad libitum intake. All animals were zero-grazed and offered freshly cut herbage twice daily. In vitro DMD values for the high and low DMD swards were 816 and 706 g/kg of DM. Heifers offered the high DMD grass had greater \( (P < 0.001) \) daily DMI of 7.66 kg compared with 5.38 kg for those offered the low DMD grass. Heifers offered the high DMD grass had greater \( (P = 0.003) \) daily CH4 production (193 g of CH4/d) than those offered the low DMD grass (138 g of CH4/d). However, when corrected for DMI, digestible DMI, or ingested gross energy, there was no difference \( (P > 0.05) \) in CH4 production between dietary treatments. For cannulated steers, intake tended \( (P = 0.06) \) to be greater for the high DMD grass (5.56 vs. 4.27 kg of DM/d), but rumen protozoa \( (4.95 \times 10^4 \text{ mL}^{-1}; P = 0.62) \), rumen ammonia \( (34 \text{ mg of N/L}; P = 0.24) \), rumen total VFA \( (103 \text{ mM}; P = 0.58) \), and rumen pH \( (6.8; P = 0.43) \) did not differ between treatments. There was no difference in total bacteria numbers, relative expression of the mcrA gene, and numbers of cycles to threshold for fungi when determined using quantitative PCR between dietary treatments with mean values of 73.0 ng/µL, 0.958, and 21.75 CT, respectively. Results of this study demonstrate that there was no difference in CH4 production when corrected for intake or rumen fermentation variables of beef cattle offered a high or low digestibility sward.

Key words: cattle, methane, pasture quality, sulfur hexafluoride, zero-grazed

INTRODUCTION

Irish ruminant production systems are predominantly grass-based with approximately 89% of agricultural land within Ireland dedicated to permanent pasture or rough grazing (CSO, 2007). As a result of its relatively high ruminant population, enteric CH4 emissions contribute to approximately 13% of the total greenhouse gas emissions in Ireland as CO2 equivalents (McGettigan et al., 2008). Globally, animal agriculture is the largest contributor of anthropogenic greenhouse gas production with the largest proportion being CH4 (Steinfeld et al., 2006). The global warming potential of CH4 is approximately 21 times greater than that of CO2 but is relatively short-lived in the atmosphere (IPCC, 1997). Monteny et al. (2006) proposed that the only 2 feasible CH4 mitigation strategies in ruminants were first to increase the ruminal production of propionate through dietary means or second to improve feed intake and feed composition, thus promoting greater animal

1Funding for this research was provided under the National Development Plan, through the Research Stimulus Fund, administered by the Department of Agriculture, Fisheries & Food, Ireland #RSF 06 361.

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Received January 10, 2009.
Accepted June 12, 2009.
Animals and Experimental Diet

Animals were offered fresh herbage twice daily for ad libitum intake from 1 of the 2 experimental swards, as appropriate. The grass harvested from the high DMD sward was predominately young lush re-growth, whereas that from the low DMD sward was consistently more mature. Refusals were collected daily. Subsamples of grass from each pasture type collected at each harvest were stored at –20°C before subsequent analysis. After a 16-d diet adaptation period, all heifers received a previously calibrated SF6 permeation tube as described by Johnson et al. (1994), in the form of an oral bolus, releasing on average 1.4 mg of SF6/d (SD = 0.12, range 1.3 to 1.7 mg/d). Boluses were blocked by release rate and randomly allocated within treatment. Five days later the 3 heaviest heifers on each treatment and the 4 cannulated steers were moved into individual metabolism stalls for 7 d, and the other 6 heifers remained in group housing. Animals in metabolism stalls were fed individually in the same manner as described earlier. After 7 d in metabolism stalls the heifers were turned outdoors to nonexperimental pasture, whereas the steers returned to individual feeding of zero-grazed pasture indoors and crossed over the opposite treatment to which they had been previously allocated. After a further 14-d period, the remaining 6 heifers and all 4 steers were transferred to the metabolism stalls for 7 d, and after this all animals were turned out to grazing.

Measurements and Analytical Methods

While in the metabolism stalls, intakes were recorded daily and animals were offered 110% of intake of the previous day. Daily CH4 was determined using a calibrated tracer (SF6) technique as described by Johnson et al. (1994). Each heifer was fitted with a head collar that contained a length of calibrated restriction tubing and a filter (Johnson et al., 1994) connected to a length of coiled polytetrafluoroethylene tubing attached to a 2-L evacuated plastic canister suspended above the metabolism stall (Lovett et al., 2003). Five collection apparatus were placed at fixed locations within the shed to determine ambient concentrations of SF6 and CH4. Methane sampling commenced at 0730 h on the second day when the evacuated canister was opened, allowing approximately 0.5 mL of exhaled gas/min to enter. After 24 h each canister was closed off using a tapped valve and replaced with a new evacuated canister. Methane measurements were recorded every 24 h for 5 consecutive days. Subsamples of 24-h fecal outputs were collected from the heifers during the final 3 d. Fecal samples were immediately dried to a constant weight at 55°C. A subsample of solid rumen digesta, taken from 4 locations from the bottom of the fibrous mat, was collected from each steer 4 h after the morning feeding after 5 d in the metabolism stall and stored at –80°C for subsequent quantitative PCR (qPCR) analysis.
Rumens of steers were sampled at 0, 2, 4, 6, and 8 h after the morning feed on d 6 and 7 in the metabolism stalls. Rumen fluid was obtained using a collection tube (#RT, Bar Diamond, Parma, ID) connected to a 60-mL syringe, filtered through 4 layers of cheese cloth, and ruminal pH was measured immediately after sampling using a pH meter. A 1-mL subsample of rumen fluid was added to 9 mL of methyl green solution (0.3 g/L of methyl green, 8 g/L of NaCl made up in 0.1 M formalin) for quantification of protozoa, and 10-mL aliquots of rumen fluid were added to 0.5 mL of 4 mM mercuric chloride and 0.25 mL of 0.5 M sulfuric acid for VFA and ammonia (NH₃-N) analysis, respectively. All rumen samples except those for protozoa were stored at −20°C before subsequent analysis.

Grass samples were defrosted at 4°C and dried to a constant weight at 55°C to determine DM. Dried grass and fecal samples were milled to pass through a 1-mm screen (Christy Norris, Ipswich, UK). Sward IVMD and in vitro (IVOMD) disappearance were determined using the method of Tilley and Terry (1963) modified as follows: approximately 0.5 g of dried grass was accurately weighed into a preweighed fiber bag (F57, Ankom Technology, Macedon, NY) and sealed. The bags were placed into a single vessel (Daisy, Ankom) to which 1.6 L of 200 mL/L of buffered rumen fluid was added. Rumen fluid was collected and pooled from 4 ruminally cannulated nonexperimental grazing steers. The vessel was incubated at 39°C for 48 h before being rinsed with 10 L of cold water. Immediately after rinsing, 1.6 L of 0.1 M HCl containing 2 g of pepsin/L (P/1120, Fisher Scientific, Waltham, MA) was added to the vessel before a further 48 h incubation at 39°C. After incubation, the bags were again rinsed with 10 L of water and dried before weighing to determine IVOMD. The dried bags were then placed in a preweighed ceramic crucible and ignited at 550°C for 4 h; after cooling the residue was weighed to determine IVOMD. Crude protein was determined by the Dumas method (990.03; AOAC, 1995) using a FP 528 analyzer (LECO Instruments UK Ltd., Stockport, UK). NDF and ADF were determined sequentially on grass and fecal samples using the method of Van Soest et al. (1991). The NDF was determined without the use of enzymes or sodium sulfite. Lignin was determined on grass samples using the sulfuric acid method (Robertson and Van Soest, 1981) after ADF determination. Acid insoluble ash was determined using 4 M HCl and the method of Van Keulen and Young (1977). Determination of CH₄ and SF₆ was conducted daily as described by Lovett et al. (2003) using a Varian 3800 GLC (Varian Inc., Palo Alto, CA) fitted with a flame ionization detector and electron capture detector. Gross energy was determined on pelletized samples using a bomb calorimeter (Parr Instrument Company, Moline, IL). Ether extract was determined using Soxtek instruments (Tecator, Hoganas, Sweden) and light petroleum ether.

Rumen protozoa were enumerated manually using light microscopy and a Burker counting chamber (Rudolf Brand, Wertheim, Germany). Thawed rumen fluid was analyzed for NH₃-N by the phenol hypochlorite method of Weatherburn (1967). Rumen VFA were determined using a Varian 3800 GLC fitted with a capillary column (CP-WAX 58 FFAP 25 m × 0.53 mm × 1 µm, Varian CP7614) against known standards and corrected using 2-methylvaleric acid (69643, Sigma Aldrich, St. Louis, MO) as an internal standard. All analyses were performed in duplicate.

Genomic DNA from the rumen solid samples and bacterial standard was extracted as described by Yu and Morrison (2004) and quantified using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE). A bacterial DNA standard was extracted from equal volumes, prepared in anaerobic M8 media of overnight cultures of Anaerovibrio lipolytica, Butyryrivibrio fibrisolvens, Clostridium album, Clostridium sticklandii, Lachnospira multipara, Lactobacillus casei, Megasphaera elsdenii, Peptostreptococcus anaerobius, Polyplastron multivesiculatum, Prevotella albensis, Prevotella brevis, Prevotella bryantii, Prevotella ruminicola, Ruminococcus cinnamominum, Ruminococcus albus, Streptococcus bovis, and Veillonella parvula obtained from the Rowett Research Centre culture library (Aberdeen, UK).

The forward primer for detecting anaerobic rumen bacterial 16S rRNA gene sequence was 5′ CGG CAA CGA GCG CAA CCC, and the reverse primer was 5′ CCA TTG TAG CAC GTG TGT AGC C (Denman and McSweeney, 2006). The forward primer for detecting the mcrA gene was 5′ TTC GGT GGA TCD CAR AGR GC, and the reverse primer was 5′ GBA RGT CGW AWC CGT AGA ATC C (Denman et al., 2007). The forward primer for detecting anaerobic rumen fusobacterial 18S rRNA gene sequence was 5′ GAG GAA GTA AAA GTC GTA ACA AGG TTT, and the reverse primer was 5′ CAA TTG CAC AAA GGG TAG GAT GAT T (Denman and McSweeney, 2006). All primers were supplied by MWG Biotech (Ebersberg, Germany) using a scale of 0.05 and were high purity salt free.

The qPCR assays were performed using an ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Foster City, CA). Total template rumen DNA was diluted 1:100 with DNase and RNase free water (W4502, Sigma Aldrich) before use. Assay conditions were optimized to determine forward and reverse primer concentrations using Power SYBR Green (Applied Biosystems). An optimal primer concentration of 900 nM was chosen for forward and reverse primers. The qPCR assay conditions were as follows: 1 cycle of 50°C for 2 min followed by 95°C for 10 min for initial denaturation, and 40 cycles of 95°C for 15 s and 60°C for 1 min for primer annealing and product elongation. Fluorescence detection was performed at the end of each denaturation and extension step. Amplicon specificity was performed via dissociation curve analysis of qPCR end products. Standard curves for quantification of rumen bacteria were prepared using a known quantity of DNA, which was serially diluted with DNase and RNase-free water (W4502, Sigma Aldrich).
Calculations

Methane was calculated using the following equation:
\[ \text{CH}_4 \ (g/d) = \text{SF}_6 \ \text{release rate} \ (g/d) \times \frac{[\text{CH}_4 \ (\mu g/m^3)]}{[\text{SF}_6 \ (\mu g/m^3)]} \] (Johnson et al., 1994). Apparent whole-tract digestibility was determined using AIA. The estimated ME was calculated using IVOMD values according to the method of Givens et al. (1990):
\[ \text{Estimated ME (MJ/kg of DM)} = -0.46 + 0.0170[\text{IVOMD}] . \]
Predicted heifer growth rates were calculated according to ARFC (1992). Relative expression of the mcrA gene to total bacteria was calculated using the 2−ΔΔCT method (Livak and Schmittgen, 2001).

Statistical Analyses

The data for heifer feed intake, diet digestibility and CH4 emissions were analyzed using PROC MIXED function (SAS Inst. Inc., Cary, NC) using the model
\[ Y = \mu + P_i + D_j + \varepsilon_{ij}. \]
Steer feed intake and microbial analysis were analyzed using the model
\[ Y = \mu + P_i + D_j + A_k + \varepsilon_{ijk}, \] where \( \mu \) was the overall mean, \( P_i \) was the fixed effect of period (\( i = 1 \) to 2), \( D_j \) the fixed effect of sward DMD (\( j = 1 \) to 2), \( A_k \) the random effect of animal (\( k = 1 \) to 4), and \( \varepsilon_{ij} \) and \( \varepsilon_{ijk} \) were the associated errors for models 1 and 2, respectively. The rumen data were analyzed using PROC MIXED function of SAS using Satterthwaite’s approximation for df, compound symmetry covariance structure. In the following model:
\[ Y = \mu + P_i + D_j + A_k + T_i + (D_j \times T_i) + \varepsilon_{ijkl}, \]
where \( \mu \), \( P_i \), \( D_j \), and \( A_k \) are as described previously, \( T_i \) is the fixed effect of time (\( i = 1 \) to 5), \( D_j \times T_i \) is the interaction between diet and time, and \( \varepsilon_{ijkl} \) is the associated error. Treatments were separated using least squares means, and significances were declared at \( P \leq 0.05 \).

RESULTS

The mean chemical composition of the low and high DMD swards is presented in Table 1. The high DMD sward had greater \( P < 0.001 \) IVOMD than the low DMD sward. There was no difference between the high and low DMD swards for DM and OM with mean values of 189 and 934 g/kg of DM, respectively. The low DMD sward had less \( P < 0.001 \) CP content and a consistently greater NDF \( P < 0.001 \), ADF \( P < 0.001 \), and lignin \( P = 0.04 \) concentration than the high DMD sward. There was no difference in GE concentration of the swards with a mean value of 18.25 MJ/kg of DM. Swards displayed no difference in ether extract content with a mean value of 15.15 g/kg of DM. Estimated ME was greater \( P < 0.001 \) in the high DMD sward compared with the low DMD sward. Predicted growth rates were greater \( P < 0.001 \) in the heifers offered the high DMD sward compared with those offered the low DMD sward with mean values of 1.41 and 0.37 kg/d, respectively (SEM = 0.061).

The mean intakes, CH4 emissions, and apparent whole-tract digestibility of the heifers offered the high and low DMD swards are presented in Table 2. Heifers offered the high DMD sward had greater \( P = 0.003 \) DM, OM \( P < 0.001 \), and digestible DM \( \text{DDM:} \ P < 0.001 \) compared with those offered the low DMD sward. Daily CH4 production was 55 g/d greater \( P < 0.05 \) for heifers consuming the high DMD sward. However, there was no difference \( P \geq 0.24 \) in CH4 production when corrected for DM and DDM intake with mean values of 25.7 g of CH4/kg of DMI and 30.7 g of CH4/kg of DDM intake, respectively. There was no difference in GE concentration as a proportion of GE intake \( P = 0.92 \) with a mean value of 0.0985 MJ/MJ. Apparent whole-tract digestibilities of OM \( P < 0.001 \), CP \( P < 0.001 \), and ADF \( P < 0.001 \) were greater in the heifers consuming the high DMD sward.

There were no diet × time interactions for any of the rumen variables measured, and thus only the mean treatment values are presented. The mean intakes and ruminal characteristics of the cannulated steers offered the low and high DMD swards are presented in Table 3. There was no difference between dietary treatments in fresh weight intake with a mean value of 25.4 kg/d,
respectively. There was a trend toward a greater DMI ($P = 0.06$) and OM intake ($P = 0.07$) in cattle offered the high DMD sward. There were no treatment differences for fermentation variables, with the exception of isobutyrate; means were rumen pH, 6.8; rumen NH$_3$-N, 34.0 mg of N/L; rumen protozoa, 5.1 × 10$^4$/mL; total rumen VFA, 103 mM; molar proportion of acetate, 0.709 mol/mol; molar proportion of propionate, 0.182 mol/mol; molar proportion of butyrate, 0.135 mol/mol; molar proportion of isovalerate, 0.082 mol/mol; and molar proportion of valerate, 0.008 mol/mol. The difference between treatments in isobutyrate, although significant, was only 0.003 mol/mol and is considered biologically insignificant.

The total ruminal DNA, relative expression of the mcrA gene, and fungal cycles to threshold ($CT$) of the steers offered the low and high DMD swards are presented in Table 4. There was no difference in quantity of total bacterial DNA, the relative expression of the mcrA gene, or the $CT$ for fungi present in the solid ruminal digesta of animals offered pasture divergent in DMD content with mean values of 73.0 ng/µL, 0.958, and 21.75 $CT$, respectively.

**DISCUSSION**

**Forage Quality**

The primary objective of this study was to concurrently generate swards divergent in DMD. This was accomplished with a mean difference of 110 g of IVDMD/kg of DM between swards attributable to the management techniques imposed during the period of normally elevated plant growth. The high DMD sward is of com-

### Table 2. Effect of sward DM digestibility on mean intakes, methane production, and apparent whole-tract digestibility of heifers

<table>
<thead>
<tr>
<th>Item</th>
<th>Sward DM digestibility</th>
<th>SEM</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low, n = 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>High, n = 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed intake, kg/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh weight</td>
<td>31.0</td>
<td>1.1</td>
<td>0.003</td>
</tr>
<tr>
<td>DM</td>
<td>5.38</td>
<td>0.26</td>
<td>0.006</td>
</tr>
<tr>
<td>OM</td>
<td>4.93</td>
<td>0.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Digestible DM</td>
<td>4.30</td>
<td>0.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Methane production</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH$_4$, g/d</td>
<td>138</td>
<td>9.7</td>
<td>0.003</td>
</tr>
<tr>
<td>CH$_4$, g/kg of DMI</td>
<td>25.6</td>
<td>1.5</td>
<td>0.95</td>
</tr>
<tr>
<td>CH$_4$, g/kg of digestible DMI</td>
<td>32.1</td>
<td>1.6</td>
<td>0.24</td>
</tr>
<tr>
<td>CH$_4$, MJ/MJ of GE intake</td>
<td>0.098</td>
<td>0.0056</td>
<td>0.92</td>
</tr>
<tr>
<td>Apparent whole-tract digestibility, g/kg</td>
<td></td>
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</tr>
<tr>
<td>OM</td>
<td>715</td>
<td>12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CP</td>
<td>569</td>
<td>19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NDF</td>
<td>682</td>
<td>15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ADF</td>
<td>667</td>
<td>18</td>
<td>0.001</td>
</tr>
</tbody>
</table>

### Table 3. Effect of sward DM digestibility on mean intakes and selected ruminal characteristics of cannulated steers

<table>
<thead>
<tr>
<th>Item</th>
<th>Sward DM digestibility</th>
<th>SEM</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low, n = 8</td>
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</tr>
<tr>
<td></td>
<td>High, n = 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intake, kg/d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh weight</td>
<td>24.0</td>
<td>1.88</td>
<td>0.22</td>
</tr>
<tr>
<td>DM</td>
<td>4.27</td>
<td>0.40</td>
<td>0.06</td>
</tr>
<tr>
<td>OM</td>
<td>4.01</td>
<td>0.37</td>
<td>0.07</td>
</tr>
<tr>
<td>Rumen variable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>6.9</td>
<td>0.16</td>
<td>0.43</td>
</tr>
<tr>
<td>NH$_3$-N, mg N/L</td>
<td>32</td>
<td>8.0</td>
<td>0.24</td>
</tr>
<tr>
<td>Protozoa, × 10$^4$/mL</td>
<td>4.8</td>
<td>0.67</td>
<td>0.62</td>
</tr>
<tr>
<td>Total VFA, mM</td>
<td>99</td>
<td>7.4</td>
<td>0.58</td>
</tr>
<tr>
<td>VFA, mol/mol total VFA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>0.717</td>
<td>0.009</td>
<td>0.26</td>
</tr>
<tr>
<td>Propionate</td>
<td>0.177</td>
<td>0.006</td>
<td>0.23</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>0.012</td>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>Butyrate</td>
<td>0.080</td>
<td>0.005</td>
<td>0.62</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>0.008</td>
<td>0.002</td>
<td>0.78</td>
</tr>
<tr>
<td>Valerate</td>
<td>0.006</td>
<td>0.001</td>
<td>0.07</td>
</tr>
</tbody>
</table>
parable quality with that typically offered to lactating dairy cows in Ireland (Dunne et al., 1999). The IVOMD were comparable with the IVOMD values obtained. The chemical composition of the newly reseeded swards used here was different than that reported previously (Lovett et al., 2003) with less CP and more fiber. However, this could be attributed to seasonal variation and the absence or low application rate of fertilizer. The concentration of CP in the high DMD sward was approximately 72% greater than that of the more mature low DMD sward. This difference is partly attributable to the difference in N application (Peyraud and Astigarraga, 1998) and the shorter re-growth interval (Wilman, 2004) for the high DMD sward. Herbage GE values in this study were in accordance with published literature for perennial ryegrass swards, which range from 17.7 MJ/kg of DM (Bergero et al., 2002) up to 18.7 MJ/kg of DM (Smit, 2005). Estimated ME, based on laboratory measurements, were greater in the high DMD sward with values equivalent to those reported by Beever et al. (1985) for primary growth of perennial ryegrass. Based on the chemical composition of the diets, the low DMD sward was more protein limiting in terms of microbial protein synthesis (AFRC, 1992) than the high DMD sward, which in turn would reduce animal performance.

**Intake and Predicted Performance**

The intakes reported here were less than initially anticipated, potentially because the animals were housed in metabolism stalls for the duration of CH$_4$ sampling. This is likely to have restricted their level of intake close to maintenance with mean observed intakes of 14 (SD 3.3) and 13 (SD 4.9) g of DM/kg of BW for heifers and steers, respectively. The DMI of the cannulated steers followed a pattern similar to that of the noncannulated heifers, but the difference between treatments for steers was not significant. Therefore, some degree of caution must be employed when extrapolating data from ruminal fermentation of the cannulated steers to explain ruminal CH$_4$ production of the noncannulated heifers.

DeRamus et al. (2003) demonstrated that cattle rotationally grazed on cool season annuals and perennial ryegrass had greater growth rate than intensively stocked cattle. This was attributed to maximized animal selection at less stocking density. The cattle offered the high DMD sward were offered a diet that contained substantially more primary re-growth and CP than the low DMD sward, and as a result they had a greater predicted growth rate. The CP intake of the animals offered the low DMD diet failed to meet the requirements for sufficient microbial protein synthesis (AFRC, 1992) and hence would have contributed to less growth rate. A further factor for the differences in predicted growth rates is the ME content of the forages. Waldo (1986) reported that ruminants offered forages of greater energy density had greater growth rates. The growth rates predicted here are within the range recorded by Wright and Whyte (1989) for beef cattle grazing perennial ryegrass forages. The predicted growth rate for the heifers consuming the high DMD sward was comparable with the mean value of 1.68 kg/d reported by Kenny et al. (2001) for heifers grazing good quality swards.

**Methane Production**

With respect to CH$_4$ production, this study is unique in that it compares the same forage at 2 different DMD levels simultaneously. We hypothesized that increasing the DMD of the forage would result in less CH$_4$ emissions per unit of DDM consumed, but this was not the case. In a meta-analysis, Pelchen and Peters (1998) reported that there was no difference in daily CH$_4$ production of sheep offered diets ranging from 600 to 800 g of DMD/kg of DM, but when DMD was less than 600 g/kg of DM significant differences were observed. This also appears to be the case with cattle based on very limited evidence. Pinares-Patiño et al. (2003) reported that there was no difference in daily CH$_4$ production from grazing cows, corrected for digestible OMI, over a grazing season where the OMD of timothy varied from 596 to 701 g/kg of OM. In contrast, Kurihara et al. (1999) reported a significantly greater CH$_4$ production corrected for digestible OMI when cattle consumed Angelon grass with a DMD of 413 g/kg of DM compared with those offered Rhodes grass with a DMD of 603 g/kg of DM.

Results from our experiment are in agreement with work by Kirkpatrick et al. (1997), McCaughey et al. (1997, 1999), and Pinares-Patiño et al. (2003) who have shown that the greatest driver of total daily CH$_4$ production in cattle is DMI. Daily CH$_4$ production was greater in cattle offered the high DMD sward; however, based on the predicted growth rates these cattle would
finish substantially quicker than those on the low DMD diet, thus resulting in less overall lifetime $\text{CH}_4$ emissions.

As expected, $\text{CH}_4$ emissions expressed on a per unit of GE intake basis were at the upper end of the range (0.002 to 0.120 MJ/MJ of GEI) reported by Johnson et al. (1993) due to the diets being solely forage-based. It has also been demonstrated by Johnson et al. (1993) that level of feed intake above maintenance energy requirements is a driving factor that affects the proportion of energy lost as $\text{CH}_4$ with animals on fewer multiples of maintenance having a greater energy loss as $\text{CH}_4$.

We initially hypothesized that $\text{CH}_4$ corrected for DMI would have resulted in a difference between treatments due to an increased outflow rate in the animals consuming the high DMD diet, but this was not the case. Low digestibility forages are digested slowly and remain in the rumen for longer periods, limiting rumen capacity (Allen, 1996). Studies have shown that intake and performance from forage is greater when the digestibility of the forage is greater (Steen and McIlmoyle, 1982; Rook and Gill, 1990; Steen et al., 1998), which is in agreement with this study where the intakes and predicted growth rates of the cattle were greater for the high DMD sward.

When calculated on an annual basis, its is estimated that cattle grazing the low and high DMD swards would produce 50.4 and 70.4 kg of $\text{CH}_4$/yr, respectively, assuming that intakes and sward quality remained unchanged over the grazing season. The IPCC (1997) estimate of 48 kg $\text{CH}_4$/yr is comparable with that of the cattle grazing the low DMD sward. In terms of total greenhouse gas production, application of chemical fertilizers is detrimental due to the amount of energy used in its production and subsequent transport (Steinfeld et al., 2006). To finish cattle quicker, the option remains to supplement grazing cattle with a high energy concentrate, which should in turn reduce $\text{CH}_4$ production. However, Boadi et al. (2004) reported that cattle receiving a high grain:forage (89.5:11.5 DM basis) when corrected for DMI, but when corrected for daily BW gain, cattle on the high grain:forage diet produced significantly less $\text{CH}_4$.

**Ruminal Fermentation and Microbial Populations**

Park et al. (1994) reported that ruminal VFA concentrations of beef steers increased with an increasing forage digestibility. Additionally, Rinne et al. (1997, 2002), examining the effects of silage digestibility on rumen variables of beef and dairy cattle, demonstrated a positive linear relationship between increasing silage digestibility and increasing ruminal concentrations of total VFA. However, this was not the case observed here because there was no difference in rumen VFA concentration between animals offered the 2 swards.

Bacterial populations in the rumen are affected by protozoa numbers, with a decrease in protozoa being linked to an increase in bacteria, due to a reduction in predation (MacMuller et al., 2003). Protozoa numbers are greatly influenced by the type of basal diet with more protozoa being observed with a greater proportion of forage in the diet (Christiansen et al., 1964). Rinne et al. (1997, 2002) reported that protozoa numbers decreased when beef cattle and dairy cows were offered silages produced from increasingly more mature grass. However, in the current study no difference in protozoa numbers was observed with increased pasture IVMD. This further supports the lack of difference in $\text{CH}_4$ production corrected for intake in the heifers offered the low and high DMD swards. Many studies have reported close associations of methanogens with ciliate protozoa due to roles in interspecies hydrogen transfer (Vogels et al., 1980; Krumholz et al., 1983; Finlay et al., 1994) and that ruminal defaunation reduces $\text{CH}_4$ production (Demeyer and Van Nevel, 1979; Jouany et al., 1981; Whitelaw et al., 1984). However, a recent study by Hegarty et al. (2008) reported no difference in enteric methane production between faunated and defaunated lambs.

Using molecular approaches to ascertain ruminal methanogenic populations via amplification of the mcrA gene is still relatively novel and has been limited to in vitro experiments. Studies by Hart et al. (2006), Goel et al. (2008), and Zhang et al. (2008) have demonstrated that dietary additives that significantly reduced in vitro $\text{CH}_4$ production have also resulted in a significant decrease in expression of the mcrA gene. The mcrA gene encodes for methyl coenzyme-M reductase (Ermiller et al., 1997). This enzyme is essential in the final step of $\text{CH}_4$ production within anaerobic environments and is ubiquitous in methanogens (Reeve et al., 1997). Denman et al. (2007) reported that the numbers of copies of the mcrA gene were reduced when a potent $\text{CH}_4$ inhibitor was assessed in vivo. This study resulted in no difference being observed between intake corrected $\text{CH}_4$ production and relative mcrA gene expression in vivo. Additionally, there was no difference observed in this current study in C$_7$ values for rumen fungi further supporting the lack of difference in $\text{CH}_4$ production. An increase in rumen fungi has been reported to increase $\text{CH}_4$ production in vitro by direct fermentation (Nakashimada et al., 2000) or by increasing fiber degradability (Windham and Akin, 1984).

**Conclusions**

Enhancing the DMD of perennial ryegrass swards through improved pasture management offers a cost-effective, short- to long-term opportunity for the reduction of $\text{CH}_4$ emission from grazing beef cattle. Despite the lack of a difference in intake corrected daily $\text{CH}_4$
emissions, rumen fermentation variables, and rumen populations of protozoa, total bacterial DNA, relative expression of methanogens, or fungal C₄, improved animal performance would reduce overall lifetime greenhouse gas emissions per unit of animal product. At a constant annual stocking density, this could reduce the environmental impact of grazing beef cattle, thus improving the sustainability of the grazing enterprise.

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