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# JOURNAL OF ANIMAL SCIENCE

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# Effect of sward dry matter digestibility on methane production, ruminal fermentation, and microbial populations of zero-grazed beef cattle<sup>1</sup>

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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 CH<sub>4</sub> emissions. At high feed intake levels, the proportion of energy lost as CH<sub>4</sub> 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, CH<sub>4</sub> 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 CH<sub>4</sub> production (193 g of CH<sub>4</sub>/d) than those offered the low DMD grass (138 g of CH<sub>4</sub>/d). However, when corrected for DMI, digestible DMI, or ingested gross energy, there was no difference ( $P > 0.05$ ) in CH<sub>4</sub> 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 ( $4.95 \times 10^4$ /mL;  $P = 0.62$ ); rumen ammonia (34 mg of N/L;  $P = 0.24$ ); rumen total VFA (103 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/ $\mu$ L, 0.958, and 21.75 C<sub>T</sub>, respectively. Results of this study demonstrate that there was no difference in CH<sub>4</sub> 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

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## 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 CH<sub>4</sub> emissions contribute to approximately 13% of the total greenhouse gas emissions in Ireland as CO<sub>2</sub> equivalents (McGettigan et al., 2008). Globally, animal agriculture is the largest contributor of anthropogenic greenhouse gas production with the largest proportion being CH<sub>4</sub> (Steinfeld et al., 2006). The global warming potential of CH<sub>4</sub> is approximately 21 times greater than that of CO<sub>2</sub> but is relatively short-lived in the atmosphere (IPCC, 1997). Monteny et al. (2006) proposed that the only 2 feasible CH<sub>4</sub> 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

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performance. The feed quality of grass for grazing ruminants can be easily manipulated by increasing the leaf proportion through grazing management (Wilkins, 2002). Additionally, DM yields and sward quality can be increased through means of fertilizer application (Chestnutt et al., 2006), resulting in an abundant feed-stuff. The IPCC (1997) has estimated that an average grazing beef animal in Western Europe produces 48 kg of CH<sub>4</sub> annually when fed a predominantly forage-based diet. Improving pasture digestibility leads to a greater estimated ME content (AFRC, 1992) and potentially increased animal performance. The aim of this experiment was to evaluate the enteric CH<sub>4</sub> production and rumen fermentation variables from swards differing in DM digestibility (DMD).

## MATERIALS AND METHODS

All animals used in this study were cared for under license in accordance with the European Community Directive, 86-609-EC (EC, 2002).

### *Grassland Management*

A newly reseeded 4-ha perennial ryegrass (*Lolium perenne*) sward was utilized for this experiment. The entire sward was grazed over winter by sheep until April 3, 2007, to promote plant tillering. The sward was then divided into 2 equal 2-ha sections, and all animals were removed. One section continued to remain un-grazed and un-fertilized for the duration of the experiment, thus promoting the development of a greater stem:leaf ratio and lesser DMD content. After a 21-d rest period, the other section was grazed freely by 20 heifers for a further 21 d and subsequently strip-grazed with only 10 heifers to ensure a greater leaf:stem ratio. This section received 30, 3, and 12 kg/ha of N, P, and K, respectively, on May 24, 2007, and was considered high DMD.

### *Animals and Experimental Diet*

Twelve 14-mo-old Charolais cross heifers, mean BW 470 kg (SD = 22) and 4 ruminally cannulated Aberdeen Angus steers, mean BW 380 kg (SD = 29) were used for this experiment. Before use all animals were treated for liver fluke and gastrointestinal nematodes. All animals were grazed on a nonexperimental sward until housing on June 8, 2007. The heifers were blocked according to age and BW and were randomly allocated within block to the high or low DMD sward. Steers were blocked according to BW and randomly allocated within block to their respective dietary treatments in a crossover design.

All animals were accommodated in slatted floor pens and were fed individually using an electronic feeding system (Calan Broadbent, Northwood, NH). Herbage was harvested twice daily at 0800 and 1430 h from sequential strips within each sward using a Kidd Mk 2 double chop forage harvester (Omitec Ltd., Deviz-

es, Wiltshire, UK) leaving a stubble height of 10 cm. 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 SF<sub>6</sub> permeation tube as described by Johnson et al. (1994), in the form of an oral bolus, releasing on average 1.4 mg of SF<sub>6</sub>/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 CH<sub>4</sub> was determined using a calibrated tracer (SF<sub>6</sub>) 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 SF<sub>6</sub> and CH<sub>4</sub>. 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 (NH<sub>3</sub>-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 IVDMD 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 IVDMD. 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 CH<sub>4</sub> and SF<sub>6</sub> 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 Soxhlet instruments (Tecator, Hoganas, Sweden) and light petroleum ether.

Rumen protozoa were enumerated manually using light microscopy and a Burker counting chamber (Ru-

dolf Brand, Wertheim, Germany). Thawed rumen fluid was analyzed for NH<sub>3</sub>-N by the phenol hypochlorite method of Weatherburn (1967). Ruminal 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*, *Butyrivibrio fibrisolvens*, *Clostridium aminophilum*, *Clostridium sticklandii*, *Lachnospira multipara*, *Lactobacillus casei*, *Megasphaera elsdenii*, *Peptostreptococcus anaerobius*, *Polyplastron multivesiculatum*, *Prevotella albensis*, *Prevotella brevis*, *Prevotella bryantii*, *Prevotella ruminicola*, *Ruminococcus ruminantium*, *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 3' CGG CAA CGA GCG CAA CCC, and the reverse primer was 3' 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 fungal 18S rRNA gene sequence was 3' GAG GAA GTA AAA GTC GTA ACA AGG TTT, and the reverse primer was 5' CAA ATT 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).

**Table 1.** Mean chemical composition of the low and high DM digestibility swards

Item	Sward DM digestibility		SEM	P-value
	Low, n = 10	High, n = 10		
DM, g/kg	175	203	9.9	0.06
OM, g/kg of DM	940	928	5.8	0.15
CP, g/kg of DM	59	102	3.5	<0.001
NDF, g/kg of DM	671	616	7.9	<0.001
ADF, g/kg of DM	392	340	5.9	<0.001
Lignin, g/kg of DM	60	50	3.3	0.04
IVDMD, g/kg of DM	706	816	11.6	<0.001
IVOMD, <sup>1</sup> g/kg of DM	724	829	10.8	<0.001
GE, MJ/kg of DM	18.3	18.2	0.08	0.48
Ether extract, g/kg of DM	13.8	16.5	1.3	0.16
Estimated ME, <sup>2</sup> MJ/kg of DM	11.8	13.6	0.18	<0.001

<sup>1</sup>In vitro OM disappearance.

<sup>2</sup>Estimated according to Givens et al. (1990).

### Calculations

Methane was calculated using the following equation:  $\text{CH}_4$  (g/d) =  $\text{SF}_6$  release rate (g/d)  $\times$   $[\text{CH}_4$  ( $\mu\text{g}/\text{m}^3$ )] /  $[\text{SF}_6$  ( $\mu\text{g}/\text{m}^3$ )] (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): 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  $2^{-\Delta\Delta\text{CT}}$  method (Livak and Schmittgen, 2001).

### Statistical Analyses

The data for heifer feed intake, diet digestibility and  $\text{CH}_4$  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, and the following model:  $Y = \mu + P_i + D_j + A_k + T_l + (D_j \times T_l) + \varepsilon_{ijkl}$ , where  $\mu$ ,  $P_i$ ,  $D_j$ , and  $A_k$  are as described previously,  $T_l$  is the fixed effect of time ( $l = 1$  to  $5$ ),  $D_j \times T_l$  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,  $\text{CH}_4$  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 intakes of fresh weight ( $P = 0.003$ ), DM ( $P = 0.006$ ), OM ( $P < 0.001$ ), and digestible DM (DDM:  $P < 0.001$ ) compared with those offered the low DMD sward. Daily  $\text{CH}_4$  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  $\text{CH}_4$  production when corrected for DM and DDM intake with mean values of 25.7 g of  $\text{CH}_4$ /kg of DMI and 30.7 g of  $\text{CH}_4$ /kg of DDM intake, respectively. There was no difference between the 2 diets in  $\text{CH}_4$  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 ( $P < 0.001$ ), NDF ( $P < 0.001$ ), and ADF ( $P = 0.001$ ) were greater in the heifers consuming the high DMD sward.

There were no diet  $\times$  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,

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

Item	Sward DM digestibility		SEM	P-value
	Low, n = 6	High, n = 6		
Feed intake, kg/d				
Fresh weight	31.0	37.6	1.1	0.003
DM	5.38	7.66	0.26	0.006
OM	4.93	7.14	0.27	<0.001
Digestible DM	4.30	6.73	0.23	<0.001
Methane production				
CH <sub>4</sub> , g/d	138	193	9.7	0.003
CH <sub>4</sub> , g/kg of DMI	25.6	25.7	1.5	0.95
CH <sub>4</sub> , g/kg of digestible DMI	32.1	29.3	1.6	0.24
CH <sub>4</sub> , MJ/MJ of GE intake	0.098	0.099	0.0056	0.92
Apparent whole-tract digestibility, g/kg				
OM	715	830	12	<0.001
CP	569	748	19	<0.001
NDF	682	810	15	<0.001
ADF	667	781	18	0.001

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<sub>3</sub>-N, 34.0 mg of N/L; rumen protozoa,  $5.1 \times 10^4$ /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 ( $C_T$ ) of the steers offered the low and high DMD swards are pre-

sented in Table 4. There was no difference in quantity of total bacterial DNA, the relative expression of the *mcrA* gene, or the  $C_T$  for fungi present in the solid ruminal digesta of animals offered pasture divergent in DMD content with mean values of 73.0 ng/ $\mu$ L, 0.958, and 21.75  $C_T$ , 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 3.** Effect of sward DM digestibility on mean intakes and selected ruminal characteristics of cannulated steers

Item	Sward DM digestibility		SEM	P-value
	Low, n = 8	High, n = 8		
Intake, kg/d				
Fresh weight	24.0	26.8	1.88	0.22
DM	4.27	5.56	0.40	0.06
OM	4.01	5.14	0.37	0.07
Rumen variable				
pH	6.9	6.7	0.16	0.43
NH <sub>3</sub> -N, mg N/L	32	36	8.0	0.24
Protozoa, $\times 10^4$ /mL	4.8	5.3	0.67	0.62
Total VFA, mM	99	106	7.4	0.58
VFA, mol/mol total VFA				
Acetate	0.717	0.701	0.009	0.26
Propionate	0.177	0.186	0.006	0.23
Isobutyrate	0.012	0.015	0.001	0.01
Butyrate	0.080	0.083	0.005	0.62
Isovalerate	0.008	0.007	0.002	0.78
Valerate	0.006	0.008	0.001	0.07

**Table 4.** Effect of sward DM digestibility on total ruminal bacterial DNA, relative *mcrA* gene expression, and fungal cycles to threshold ( $C_T$ ) of cannulated steers

Item	Sward DM digestibility		SEM	P-value
	Low, n = 8	High, n = 8		
Total bacteria, ng/ $\mu$ L	65.0	81.1	6.5	0.22
<i>mcrA</i> , $\Delta\Delta CT$	0.9494	0.9668	0.0057	0.15
Fungi, $C_T$	22.3	21.2	0.48	0.19

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 Angelton 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 CH<sub>4</sub> emissions.

As expected, CH<sub>4</sub> 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 CH<sub>4</sub> with animals on fewer multiples of maintenance having a greater energy loss as CH<sub>4</sub>.

We initially hypothesized that CH<sub>4</sub> 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, it is estimated that cattle grazing the low and high DMD swards would produce 50.4 and 70.4 kg of CH<sub>4</sub>/yr, respectively, assuming that intakes and sward quality remained unchanged over the grazing season. The IPCC (1997) estimate of 48 kg CH<sub>4</sub>/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 CH<sub>4</sub> production. However, Boadi et al. (2004) reported that cattle receiving a high grain:forage (89.5:11.5 DM basis) diet had similar CH<sub>4</sub> emissions to cattle consuming less grain:forage (58.2:41.8 DM basis) when corrected for DMI, but when corrected for daily BW gain, cattle on the high grain:forage diet produced significantly less CH<sub>4</sub>.

### ***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 (MacMüller 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 IVDMD. This further supports the lack of difference in CH<sub>4</sub> 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 CH<sub>4</sub> 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 CH<sub>4</sub> production have also resulted in a significant decrease in expression of the *mcrA* gene. The *mcrA* gene encodes for methyl coenzyme-M reductase (Ermler et al., 1997). This enzyme is essential in the final step of CH<sub>4</sub> 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 CH<sub>4</sub> inhibitor was assessed in vivo. This study resulted in no difference being observed between intake corrected CH<sub>4</sub> production and relative *mcrA* gene expression in vivo. Additionally, there was no difference observed in this current study in C<sub>T</sub> values for rumen fungi further supporting the lack of difference in CH<sub>4</sub> production. An increase in rumen fungi has been reported to increase CH<sub>4</sub> 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 CH<sub>4</sub> emission from grazing beef cattle. Despite the lack of a difference in intake corrected daily CH<sub>4</sub>

emissions, rumen fermentation variables, and rumen populations of protozoa, total bacterial DNA, relative expression of methanogens, or fungal C<sub>T</sub>, 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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