Effect of divergence in phenotypic residual feed intake on methane emissions, ruminal fermentation, and apparent whole-tract digestibility of beef heifers across three contrasting diets

R. P. McDonnell,*2 K. J. Hart,*3 T. M. Boland,* A. K. Kelly,* M. McGee,† and D. A. Kenny*†4

*School of Agriculture and Food Science, University College Dublin, Lyons Estate Research Farm, Newcastle, Co. Dublin, Ireland; and †Teagasc, Animal and Bioscience Research Department, Animal and Grassland Research and Innovation Centre, Grange, Dunsany, Co. Meath, Ireland

ABSTRACT: This study aimed to examine the effect of divergent phenotypic ranking for residual feed intake (RFI) on ruminal CH4 emissions, diet digestibility, and indices of ruminal fermentation in heifers across 3 commercially relevant diets. Twenty-eight Limousin × Friesian heifers were used and were ranked on the basis of phenotypic RFI: 14 low-RFI and 14 high-RFI animals. Ruminal CH4 emissions were estimated over 5 d using the SF6 tracer gas technique on 3 successive occasions: 1) at the end of a 6-wk period (Period 1) on grass silage (GS), 2) at the end of an 8-wk period (Period 2) at pasture, and 3) at the end of a 5-wk period (Period 3) on a 30:70 corn silage:concentrate total mixed ration (TMR). Animals were allowed ad libitum access to feed and water at all times. Individual DMI was estimated during CH4 measurement and rumen samples were taken at the end of each CH4 measurement period. Diet type affected all feed intake and CH4 traits measured (P < 0.01) but was unavoidably confounded with animal age/size and experimental period. Correlation coefficients between RFI and DMI were significant (P < 0.05) only when animals were fed the TMR. Daily CH4 correlated with DMI (r = 0.42, P < 0.05) only when animals grazed pasture. Daily DMI was lower in low-RFI animals (P = 0.047) but only when expressed as grams per kilogram metabolic BW. Absolute CH4 emissions did not differ between RFI groups (P > 0.05), but CH4 yield was greatest in low-RFI heifers (P = 0.03) as a proportion of both DMI and GE intake. Interactions between the main effects were observed (P < 0.05) for CP digestibility (CPD), DM digestibility (DMD), ruminal propionate, and the acetate:propionate ratio. Low-RFI animals had greater (P < 0.05) CPD and DMD than their high-RFI contemporaries when offered GS but not the other 2 diets. Low-RFI heifers also had greater OM digestibility (P = 0.027). Additionally, low-RFI heifers had a lower concentration of propionate (P < 0.05) compared with high-RFI heifers when fed GS, resulting in a greater (P < 0.05) acetate:propionate ratio. However, these differences were not evident for the other 2 diets. Energetically efficient animals do not have a lower ruminal methanogenic potential compared with their more inefficient counterparts and, indeed, some evidence to the contrary was found, which may reflect the greater nutrient digestive potential observed in low-RFI cattle.

Key words: diet, digestibility, heifers, methane, residual feed intake, ruminal fermentation


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2Present address: Western Dairy Research Development & Extension Hub, PO Box 5066, Bunbury, WA 6230, Australia.

3Present address: Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Penglais, Aberystwyth, SY23 3DD, UK.

4Corresponding author: david.kenny@teagasc.ie

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INTRODUCTION

Residual feed intake (RFI) has increasingly become the measure of choice for estimating energetic efficiency in beef cattle (Herd and Arthur, 2009), given its genetic independence from growth and body size and moderate heritability (Crews, 2005; Crowley et al., 2010). Methane emissions from agriculture are a significant contributor to global greenhouse gas (GHG) emissions, and although estimates vary by country, almost 13% of total Irish GHG emissions (Duffy et al., 2011) are caused by enteric CH₄ production. Waghorn and Hegarty (2011) observed no differences in CH₄ emissions between dairy cows of divergent RFI classification; however, many studies, including our own (Fitzsimons et al., 2013), have shown that cattle ranked as low RFI have lower daily CH₄ emissions than their high-RFI counterparts (Nkrumah et al., 2006; Hegarty et al., 2007; Jones et al., 2011). It is unclear whether these differences in ruminal CH₄ emissions are due to inherent variation in digestive efficiency between cattle of high or low RFI or are merely a function of the reduced DMI associated with the low-RFI phenotype (Kelly et al., 2010a,b; Lawrence et al., 2011). Additionally, there is a dearth of information regarding the consistency of CH₄ emissions across different diet types between cattle divergently ranked on the basis of RFI. The CH₄ mitigation potential of grain as opposed to forage-based diets has been well documented (Grainger and Beauchemin, 2011; Hristov et al., 2013); however, Irish ruminant production systems are predominantly forage based, with animals dependent on grazed and conserved grass for large parts of their life (Drennan and McGee, 2009). Therefore, effective mitigation strategies for pastoral-based beef cattle production systems should account for dietary changes throughout an animal’s life cycle. Our hypothesis was that cattle phenotypically ranked as low RFI will have lower DMI and lower CH₄ emissions than their high-RFI counterparts. We also aimed to investigate whether rumen fermentation indices or apparent whole-tract digestibility differed between RFI phenotypes across 3 commercially relevant diets.

MATERIALS AND METHODS

This study was performed between April and August 2008 at the University College Dublin Lyons Research Farm (Newcastle, Co. Dublin, Ireland; 53°17′36″ N, 6°32′18″ W). All procedures using animals in this study were approved by the University College Dublin Animal Research Ethics Committee and licensed in accordance with the European Community Directive 86-609-EC. During all experimental periods, animals had unrestricted access to clean drinking water.

Animals and Experimental Diets

This experiment was conducted in association with a larger study designed to examine the physiological control of energetic efficiency in growing beef heifers (Kelly et al., 2010a). Briefly, in that study, individual DMI and growth was recorded for 86 yearling Limousin × Friesian heifers offered ad libitum access to a high-energy 70:30 corn silage:concentrate total mixed ration (TMR) diet over a 112-d period. The diet had a DM of 580 g/kg, a CP content of 182 g/kg DM, and an estimated NE concentration of 11.07 MJ/kg DM (NRC, 2001); see Kelly et al. (2010a) for the detailed diet composition. All animals were subsequently ranked, retrospectively, on phenotypic RFI, defined as the deviation of predicted from actual daily DMI (Crews, 2005).

The 14 highest (inefficient; high RFI) and 14 lowest (efficient; low RFI) ranking animals were selected for the current study. Pre-experimental efficiencies, DMI, and BW of the high- and low-RFI animals selected for the current study are presented in Table 1. The subsequent repeatability of these animals within their respective RFI groups was found to be consistent over time ($r = 0.62$) on a diet similar to the one on which the original ranking was based (Kelly et al., 2010b).

Both RFI phenotypes were offered 3 different diets over 3 successive periods of 40, 57, and 41 d duration, respectively. In Period 1, animals were housed in slatted floor pens and were individually offered first cut grass silage (GS) at 110% of their previous day’s intake using Calan Gates (Calan Broadbent Inc., Northwood, NH) at 0930 h daily for a period of 40 d. In Period 2, animals were turned out onto a predominantly perennial ryegrass (Lolium perenne L.) permanent grassland sward (pasture [PAST]) with a pregrazing herbage mass of approximately 2,000 kg DM/ha and rotationally grazed as a single group for 48 d. On d 49, animals were separated into their respective RFI groups and, within the same paddock, grazed adjacent to one another in subplots as 2 separate herds, divided by temporary electric fencing. For the next 8 d, each group was allocated a new area of pasture each day on the basis of 110% of the previous day’s herbage removal. This was estimated as the product of the difference between the pre- and post-grazing herbage mass and area grazed. Herbage mass was predicted using a Filips manual rising plate meter (Jenquip, Feilding, New Zealand). In Period 3, animals were group housed in a pen and bedded on peat mulch with free access to 15 electronic feeding stations (Insectec B.V., Marknesse, The Netherlands) as described by...
Kelly et al. (2010b). They were offered a concentrate and corn silage TMR on a 70:30 DM basis daily at 0930 h. During the first 10 d, the diet was gradually changed from a 0:100 to a 70:30 concentrate:forage ratio. For the subsequent 33 d, each feed station was filled to 110% of the weight of material removed on the previous day to ensure that all animals had constant and unrestricted access to feed. All feed stations were calibrated twice weekly using known weights.

Animal Health Management

Before the start of the experiment, animals were treated for internal and external parasites as well as being enrolled in a vaccination program against the respiratory diseases infectious bovine rhinotracheitis (Bovilis IBR Marker Live; MSD Animal Health, Milton Keynes, UK) and bovine respiratory syncytial virus, parainfluenza 3 virus, and Mannheimia (Pasteurella) haemolytica serotype A1 (using Bovipast RSP; MSD Animal Health) as well as bovine viral diarrhea and salmonellosis. Five weeks after turnout to pasture (Period 2), animals were again treated for internal and external parasites using Ivomec Classic pour-on (Merrial Animal Health, Woking, UK). At the start of Period 3, animals were once more treated for internal and external parasites and received a vaccination program similar to that previously described.

Measurements and Analytical Methods

Feed intake for Period 1 was recorded by measuring the individual feed offered and refused daily. Each day, fresh silage was weighed and added to each individual box using an Avery Weigh-Tronix continuous weigh system (Avery Berkel Ireland, Dublin, Ireland). Before this, the residual feed from the previous day was weighed to calculate the daily fresh feed intake for each animal. In Period 2, DMI was estimated using the $n$-alkane technique of Mayes et al. (1986) as modified according to Dillon and Stakelum (1989). Briefly, for 12 d, beginning 7 d before the commencement of CH$_4$ measurement in Period 2, animals were dosed twice daily (at 0700 and 1600 h) with a paper pellet (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) containing 500 mg of dotriacontane (C$_{32}$; Sigma–Aldrich, Gillingham, UK). From d 7 to 12 of dosing, fecal grab samples were collected twice daily before both the morning and evening dosing and stored at $-20^\circ$C pending subsequent analysis. Herbage samples were collected twice daily from each subplot for alkane analysis, beginning 1 d before commencement of CH$_4$ measurement in accordance with the method of Dillon and Stakelum (1989). Cordless hand shears (Accu 60; Gardena International GmbH, Ulm, Germany) were used to collect the herbage samples, which were stored at $-20^\circ$C before subsequent analysis. In Period 3, feed intake was measured automatically for each animal using roughage intake control software (Insentec B.V.). A controlled internal drug release (CIDR) device was administered to each animal on d 31, 47, and 31 of periods 1, 2, and 3, respectively, to inhibit behavioral estrus activity during the subsequent CH$_4$ collection periods. All CIDR devices were removed after 9 d. All animals were weighed on d 5 of each CH$_4$ measurement period, after the collection apparatus had been removed, to determine metabolic BW ($BW^{0.75}$).

Methane Measurement

On d 16 of Period 1, all animals received a previously calibrated permeation tube releasing, on average, 2.9 mg/d of SF$_6$ (SD 0.54; range 1.9–4.1) as an oral bolus. The tubes were prepared 10 wk before the start of the first CH$_4$ measurement period and were

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Mean</th>
<th>SD</th>
<th>Low</th>
<th>SD</th>
<th>High</th>
<th>SD</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RFI, kg DM/d</td>
<td>-0.01</td>
<td>0.67</td>
<td>-0.74$^a$</td>
<td>0.66$^b$</td>
<td>0.09</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCR,$^2$ kg DM/kg ADG</td>
<td>4.48</td>
<td>0.64</td>
<td>3.96$^a$</td>
<td>4.91$^b$</td>
<td>0.15</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>6.82</td>
<td>1.17</td>
<td>6.16$^a$</td>
<td>7.46$^b$</td>
<td>0.26</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid test BW$^{0.75},^3$ kg</td>
<td>63.8</td>
<td>6.91</td>
<td>63.3</td>
<td>63.1</td>
<td>1.75</td>
<td>0.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>1.51</td>
<td>0.13</td>
<td>1.55</td>
<td>1.52</td>
<td>0.03</td>
<td>0.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final BW, kg</td>
<td>316</td>
<td>37.9</td>
<td>315</td>
<td>313</td>
<td>9.7</td>
<td>0.84</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Values within a row with different superscripts differ ($P < 0.05$).

$^1$ High indicates RFI was $>0.5$ SD above the mean; Low indicates RFI was $<0.5$ SD below the mean.

$^2$ FCR = feed conversion ratio.

$^3$ BW$^{0.75}$ = metabolic BW.
stored at 39°C and weighed twice weekly to calculate the SF$_6$ release rate. Permeation tube allocation was balanced between high- and low-RFI groups on the basis of the SF$_6$ release rate. The volume of each gas collection canister was 2 L, and each capillary tube allowed an intake of air at a flow rate of 0.5 ± 0.005 mL/min. During the last 5 d of each experimental period, daily CH$_4$ emissions were determined using the SF$_6$ tracer technique developed by Zimmerman (1993) and modified by Wims et al. (2010). Canisters were evacuated (approximately 90 kPa vacuum) and were replaced on each animal between 0700 and 0830 h daily. The concentrations of CH$_4$ and SF$_6$ were determined against known mixed gas standards (SF$_6$ and CH$_4$; Scott Marrin, Riverside, CA) fitted with a flame ionization detector and an electron capture detector. Daily ambient concentrations of SF$_6$ and CH$_4$ were measured by placing 5 sampling kits (identical to those used on the animals) at representative locations within the building in Periods 1 and 3 and around the periphery of the grazing paddocks in Period 2.

**Rumen Fermentation and Apparent Total-Tract Digestibility**

Fecal samples were collected by rectal palpation from each animal at the time of canister replacement on d 3, 4, and 5 of the CH$_4$ measurement phase of Periods 1 and 3. During Period 2, fecal samples were collected in the morning at the time of canister replacement and at approximately 1600 h. Fecal samples were stored at −20°C before subsequent analysis. On the last day of each gas collection period, a 25-mL sample of rumen fluid was obtained using an esophageal sampling device (FLORA Rumen Scoop; Prosf- products, Guelph, ON, Canada) before feeding and the pH of rumen fluid was immediately determined using a pH meter (Mettler Toledo MP 200; Mettler Toledo Ireland, Dublin, Ireland). The rumen fluid was then divided into 2 aliquots. The first was filtered through 4 layers of cheesecloth and mixed 9:1 with 25% (wt/vol) trichloroacetic acid containing 20 mM 2-methylvaleric acid and stored at 4°C before subsequent analysis. The second aliquot, approximately 15 mL, was frozen on dry ice and stored at −80°C and subsequently used for microbial community analysis as reported in separate publications (Carberry et al., 2012, 2014a,b).

**Feed and Fecal Analyses**

Feed samples were collected daily during all CH$_4$ measurement periods. All feed and fecal samples were defrosted at 4°C and dried at 55°C to a constant weight to determine DM. Feed samples for Periods 1 and 3 were pooled within period before subsequent analysis. Forage samples from Period 2 were pooled within a 24-h period (morning followed by a subsequent evening sample). Daily fecal samples were pooled (10 g of each sample collected) by animal within period for Periods 1 and 3. For Period 2, fecal samples from each individual animal were pooled over five 24-h periods (10 g of each sample collected) by animal (evening and subsequent morning sample) for n-alkane analysis.

Dried feed samples were milled to pass through a 1-mm screen (Christy and Norris Process Engineers Ltd., Chelmsford, UK), whereas dried fecal samples were milled using an electric coffee grinder. Crude protein (N × 6.25) was determined by the Du- mas method (method 990.03; AOAC, 1995) using a FP 528 analyzer (LECO Instruments UK Ltd., Stockport, UK), whereas NDF and ADF were determined sequentially on grass and fecal samples according to the method of Van Soest et al. (1991), using an Ankom fiber analyzer (Ankom Technology, Macedon, NY). The NDF was determined without the use of enzymes or sodium sulfite. Gross energy was determined on pelleted samples using a bomb calorimeter (Parr Instrument Company, Moline, IL). The water-soluble carbohydrate content of all feedstuffs was determined by the method of Birch et al. (1974). Ether extract was determined using Soxtec instruments (Tecator, Höganäs, Sweden) and light petroleum ether. In vitro OM degradability was determined by the method of Tilley and Terry (1963) using Ankom fiber bags (F57 Ankom Technology), and ME was estimated according to the Agricultural and Food Research Council (1993). Subsamples of GS and the TMR and respective fecal samples were analyzed for AIA using the 4 N HCl method (Van Keulen and Young, 1977). Alkane concentrations were determined in duplicate on pooled daily samples of forage and feces using the extraction method of Dove and Mayes (2006) with the modification that n-heptane was substituted for n-dodecane as the solvent used to rehydrate the extract before injection onto a GLC (Varian 3800; Varian, Inc.).

The VFA concentration of rumen fluid was determined using a GLC fitted with a capillary column (CP-WAX 58 FFAP 25 m × 0.53 mm × 1 um; Varian CP7614; SGE Analytical Science Pty Ltd., Ringwood, Victoria, Australia) against known standards and corrected using 2-methylvaleric acid (catalog number 69643; Sigma-Aldrich, St. Louis, MO) as an internal standard.
Effect of residual feed intake on methane emissions

Concentrations of CH₄ and SF₆ were calculated from GLC readings, and consequently, CH₄ emissions were calculated using the equation described by Williams et al. (2011), where ambient levels of CH₄ and SF₆ were accounted for in the calculation.

In Period 2, daily herbage intake was estimated for each animal using the ratio between pasture concentrations of C₃₁ and C₃₂ (Mayes et al., 1986). Apparent whole-tract digestibility during Periods 1 and 3 was determined using AIA (Van Keulen and Young, 1977), and in Period 2, it was determined using the n-alkane technique (Mayes et al., 1986).

**Statistical Analysis**

Data were analyzed using the MIXED procedure of Statistical Analysis System (version 9.1; SAS Inst. Inc., Cary, NC) using the following model: $Y = \mu + R_i + D_j + (R_i \times D_j) + \epsilon_{ij}$, in which $Y$ is the measured variable, $\mu$ is the overall mean, $R_i$ is the effect of RFI phenotype ($i = 1$ to 2), $D_j$ is the effect of diet ($j = 1$ to 3), $R_i \times D_j$ is the interaction between RFI phenotype and diet, and $\epsilon_{ij}$ is the associated error. Methane emissions were averaged over the 5-d period and a single mean value for each animal was used in the model. Treatments were separated using least squares means and statistical significance was declared at $P < 0.05$. Pearson correlation coefficients were determined using the CORR procedure of SAS for the association of RFI and CH₄ within diet type, with feed intake and digestibility coefficients.

**RESULTS**

**Diet Composition, Feed Intake, and Methane Emissions**

The mean chemical composition of the diets offered throughout the experiment is presented in Table 2. The diets differed in all measured variables except for OM. The TMR diet had the highest DM and CP contents and GS had the lowest, with the reverse being observed for both NDF and ADF. Pasture had the greatest concentration of water-soluble carbohydrates and GS had the lowest. All diets were similar in GE content with a mean value of 18.0 MJ/kg DM. The mean alkane content of the PAST was 102 ± 8.1 mg/kg for C₂₉, 201 ± 16.3 mg/kg for C₃₁, and 105 ± 6.6 mg/kg for C₃₃.

The effect of phenotypic RFI ranking and diet type on mean feed intake and CH₄ emissions data is presented in Table 3. There were no RFI phenotype × diet interactions ($P > 0.05$) observed for any feed intake- or CH₄ emissions–related variables measured. Daily DMI, BW⁰.⁷⁵, daily CH₄ production, and CH₄ relative to BW⁰.⁷⁵ did not differ ($P > 0.05$) between low- and high-RFI heifers. High-RFI animals had greater ($P = 0.047$) DMI when expressed relative to BW⁰.⁷⁵. Both absolute and relative DMI increased ($P < 0.001$) as animals progressed from GS to the PAST to the TMR. Diet type affected ($P < 0.001$) all measures of DMI and CH₄ production. Total daily CH₄ emissions differed between all periods ($P < 0.05$) and were lowest for the PAST and highest for the TMR. Methane yield (MY), when expressed as grams CH₄ per kilogram DMI, was greater for low-RFI heifers ($P = 0.034$), and MY as a proportion of GE intake (GEI) was also greater for low-RFI heifers ($P = 0.031$). Methane yield (g CH₄/kg DMI) and as a proportion of GEI was greatest ($P < 0.05$) when animals were offered GS and lowest ($P < 0.05$) when animals were offered the PAST whereas, when expressed as grams per kilogram BW⁰.⁷⁵, CH₄ was greatest ($P < 0.05$) in animals offered the TMR and lowest ($P < 0.05$) in animals offered the PAST.

**Rumen Fermentation Variables**

The effect of RFI phenotype and diet on rumen pH, total VFA, and molar proportions of the fermentation acids is presented in Table 4. There were RFI phenotype × diet interactions for the ruminal propionate concentration and for the acetate:propionate ratio whereby, when offered GS, low-RFI heifers had lower (207 vs. 229 mmol/mol; $P = 0.012$) concentrations of propionate and a greater (3.26 vs. 2.87; $P = 0.027$) acetate:propionate ratio. No difference ($P > 0.05$) between the RFI phenotypes in the propionate concentra-

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**Table 2. Mean (SD) chemical composition of the 3 diets offered**

<table>
<thead>
<tr>
<th>Component</th>
<th>GS¹</th>
<th>PAST¹</th>
<th>TMR¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, g/kg</td>
<td>330 (17.7)</td>
<td>218 (5.8)</td>
<td>527 (30.0)</td>
</tr>
<tr>
<td>OM, g/kg DM</td>
<td>938 (1.8)</td>
<td>935 (2.1)</td>
<td>934 (1.0)</td>
</tr>
<tr>
<td>CP, g/kg DM</td>
<td>123 (3.6)</td>
<td>160 (4.3)</td>
<td>171 (3.2)</td>
</tr>
<tr>
<td>EE², g/kg DM</td>
<td>30 (0.3)</td>
<td>16 (0.3)</td>
<td>24 (0.5)</td>
</tr>
<tr>
<td>NDF, g/kg DM</td>
<td>661 (9.9)</td>
<td>552 (6.9)</td>
<td>419 (7.8)</td>
</tr>
<tr>
<td>ADF, g/kg DM</td>
<td>352 (7.6)</td>
<td>206 (2.4)</td>
<td>185 (8.0)</td>
</tr>
<tr>
<td>WSC³, g/kg DM</td>
<td>10 (0.5)</td>
<td>96 (3.2)</td>
<td>33 (2.7)</td>
</tr>
<tr>
<td>pH</td>
<td>3.9 (0.005)</td>
<td>n.d.⁴</td>
<td>n.d.⁴</td>
</tr>
<tr>
<td>GE, MJ/kg DM</td>
<td>17.9 (0.09)</td>
<td>18.3 (0.05)</td>
<td>17.8 (0.07)</td>
</tr>
<tr>
<td>ME, MJ/kg DM</td>
<td>11.5 (0.07)</td>
<td>12.2 (0.05)</td>
<td>11.7 (0.06)</td>
</tr>
</tbody>
</table>

¹GS = grass silage; PAST = pasture (perennial ryegrass); TMR = total mixed ration (70:30 concentrate:corn silage).
²EE = ether extract.
³WSC = water-soluble carbohydrates.
⁴n.d. = not determined.
⁵pH of corn silage component of TMR was pH 3.6 (SD 0.01).
tion and the acetate:propionate ratio was observed when heifers were offered either the PAST or the TMR.

There was no effect of RFI phenotype on rumen pH; total VFA; the molar proportion of acetate, propionate, isobutyrate, butyrate, and valerate; and the acetate:propionate ratio ($P > 0.05$) with mean values of 6.9; 118 mmol; 665, 198, 8, 107, and 13 mmol/mol; and 3.44:1, respectively. Low-RFI heifers had a greater ($P = 0.017$) concentration of isovalerate than their high-RFI contemporaries. Rumen pH was lower ($P < 0.05$) for the TMR than for the GS or the PAST, which did not differ ($P > 0.05$). Total VFA were lowest ($P < 0.05$) for the PAST and greatest ($P < 0.05$) for the TMR. The molar proportion of acetate was greater ($P < 0.05$) for the PAST than for the TMR, with GS being intermediate ($P > 0.05$). Concentrations of propionate and butyrate differed between all 3 diets ($P < 0.05$); the molar proportion of propionate was greatest for GS and lowest for the PAST, and butyrate was greatest for the PAST and lowest for GS. There were small but statistically significant differences between diets for isobutyrate, isovalerate, and valerate. The acetate:propionate ratio was greatest ($P < 0.05$) for the PAST and lowest for GS, with the TMR being intermediate.

### Apparent Diet Digestibility

The effect of RFI phenotype and diet on mean apparent whole-tract digestibility coefficients is presented in Table 5. There was an RFI phenotype × diet interaction for CP digestibility (CPD; $P = 0.028$) and a tendency ($P = 0.095$) toward a similar interaction for DM digestibility (DMD). The interaction for CPD was manifested as no difference between RFI phenotypes when offered the PAST or the TMR ($P > 0.05$); however, when consuming GS, low-RFI animals had greater CPD than high-RFI animals (594 vs. 558 g/kg DM; $P = 0.028$). Similarly, for DMD, low-RFI heifers had greater ($P = 0.018$) DMD when offered GS compared with their high-RFI counterparts, which was not replicated across the other 2 diets ($P > 0.05$). There was no effect ($P > 0.05$) of RFI on NDF or ADF digestibility coefficients whereas low-RFI animals had greater ($P = 0.027$) OM digestibility and a tendency toward greater GE digestibility ($P = 0.10$) compared with the high-RFI animals. For diet type, digestibility of DM and OM was greatest ($P < 0.05$) on the PAST and lowest ($P < 0.05$) in GS, whereas NDF digestibility was greatest ($P < 0.05$) on the PAST and lowest ($P < 0.05$) on the TMR. Mean ADF digestibility was greatest ($P < 0.05$) on GS and lowest ($P < 0.05$) on the TMR, and GE digestibility was greater ($P < 0.05$) in the TMR and PAST compared with GS.

### Association between Ruminal Methane Emissions and Other Traits

The association of RFI and CH$_4$ with measures of feed intake and diet digestibility is presented in Table 6. There were positive correlations between RFI and both total DMI ($r = 0.497, P < 0.01$) and DMI relative to BW$^{0.75}$ ($r = 0.605, P < 0.001$) when animals were offered the TMR diet. There were tendencies ($P < 0.10$) for negative correlations between RFI classification and DM, OM, and CP digestibilities on the GS diet. There were no other statistically significant

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**Table 3. Effect of residual feed intake (RFI) phenotype and diet on feed intake and CH$_4$ emissions of beef heifers**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>RFI$^2$/ Period and diet$^3$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg/d</td>
<td>Low $n = 14$ High $n = 14$ SEM P1 GS $n = 28$ P2 PAST$^4$ $n = 28$ P3 TMR $n = 28$ SEM RFI Diet RFI × diet</td>
<td></td>
</tr>
<tr>
<td>BW$^{0.75}$ kg</td>
<td>7.18 7.54 0.174 5.52$^a$ 6.53$^b$ 10.02$^c$ 0.210 0.14 &lt;0.001 0.37</td>
<td></td>
</tr>
<tr>
<td>BW$^{0.75}$ kg</td>
<td>87.3 86.4 0.97 80.3$^a$ 86.9$^b$ 93.2$^c$ 1.20 0.52 &lt;0.001 0.99</td>
<td></td>
</tr>
<tr>
<td>DMI, g/kg BW$^{0.75}$</td>
<td>81.4$^a$ 86.3$^b$ 1.74 68.8$^b$ 73.5$^b$ 107.4$^c$ 2.11 0.047 &lt;0.001 0.29</td>
<td></td>
</tr>
<tr>
<td>CH$_4$, g/d</td>
<td>156 146 4.4 136$^b$ 120$^a$ 196$^c$ 5.4 0.11 &lt;0.001 0.85</td>
<td></td>
</tr>
<tr>
<td>CH$_4$, g/kg DMI</td>
<td>22.4$^b$ 20.2$^a$ 0.73 25.4$^b$ 18.6$^a$ 19.9$^b$ 0.09 0.034 &lt;0.001 0.43</td>
<td></td>
</tr>
<tr>
<td>CH$_4$, g/kg BW$^{0.75}$</td>
<td>1.79 1.68 0.053 1.70$^b$ 1.39$^a$ 2.12$^c$ 0.090 0.15 &lt;0.001 0.86</td>
<td></td>
</tr>
<tr>
<td>CH$_4$, % GEI$^6$</td>
<td>6.91$^b$ 6.21$^a$ 0.225 7.87$^c$ 5.62$^a$ 6.18$^b$ 0.276 0.031 &lt;0.001 0.42</td>
<td></td>
</tr>
</tbody>
</table>

$^a$-$c$Values within a row with different superscripts differ ($P < 0.05$).

1 DMI data for each diet are restricted to the 5-d CH$_4$ measurement period.

2 High RFI is inefficient and low RFI is efficient.

3 Period 1; Period 2; Period 3; GS = grass silage; PAST = pasture (perennial ryegrass); TMR = total mixed ration (70:30 concentrate:corn silage).

4 Pasture DMI was estimated using the $n$-alkane technique (Mayes et al., 1986).

5 BW$^{0.75}$ = metabolic BW.

6 GEI = GE intake.
correlations between RFI and measures of feed intake or digestibility. Daily $\text{CH}_4$ emissions were positively correlated with DMI and DMI per kilogram BW$^{0.75}$ ($r = 0.417$ and $r = 0.468$, respectively) when animals were offered the PAST ($P < 0.05$). There were tendencies ($P < 0.10$) for positive correlations between $\text{CH}_4$ and DM, NDF, ADF, and GE digestibilities in GS-fed animals. No further statistically significant correlations between $\text{CH}_4$ and measures of feed intake or diet digestibility were observed.

**DISCUSSION**

The current study formed part of a larger experimental program examining the biological basis of the RFI trait in beef cattle, which has resulted in the development of a robust animal model, the scientific utility of which has been extensively published to date (Kelly et al., 2010a,b, 2011, 2013; Carberry et al., 2012, 2014a,b). Briefly, the high- and low-RFI cohorts within this population have shown consistency within their respective rankings when offered the same diet over 2 RFI test periods (Kelly et al., 2010b) as well as exhibiting differences when biochemically characterized at both a tissue (Kelly et al., 2011, 2013) and a rumen microbiome level (Carberry et al., 2012, 2014a,b). Therefore, we have confidence in using the current animal model to examine the potential effects of phenotypic RFI status on ruminal methanogenesis and associated indices of digestive efficiency.

### Table 4. Effect of residual feed intake (RFI) phenotype and diet on rumen fermentation variables

<table>
<thead>
<tr>
<th>Measurement</th>
<th>RFI$^1$</th>
<th>Period and diet$^2$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low $n = 14$</td>
<td>High $n = 14$</td>
<td>SEM</td>
</tr>
<tr>
<td>pH</td>
<td>6.9</td>
<td>6.8</td>
<td>0.04</td>
</tr>
<tr>
<td>VFA, mmol/L</td>
<td>116</td>
<td>119</td>
<td>3.5</td>
</tr>
<tr>
<td>Molar proportions, mmol/mol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>667</td>
<td>662</td>
<td>3.9</td>
</tr>
<tr>
<td>Propionate</td>
<td>196</td>
<td>200</td>
<td>3.4</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>8</td>
<td>8</td>
<td>0.3</td>
</tr>
<tr>
<td>Butyrate</td>
<td>105</td>
<td>108</td>
<td>1.9</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>11$^b$</td>
<td>9$^a$</td>
<td>0.4</td>
</tr>
<tr>
<td>Valerate</td>
<td>13</td>
<td>13</td>
<td>0.7</td>
</tr>
<tr>
<td>Acetate:propionate</td>
<td>3.46</td>
<td>3.42</td>
<td>0.068</td>
</tr>
</tbody>
</table>

$^a$–$^c$Values within a row with different superscripts differ ($P < 0.05$).

$^1$High RFI is inefficient and low RFI is efficient.

$^2$P1 = Period 1; P2 = Period 2; P3 = Period 3; GS = grass silage; PAST = pasture (perennial ryegrass); TMR = total mixed ration (70:30 concentrate:corn silage).

### Table 5. Effect of residual feed intake (RFI) phenotype and diet on apparent whole-tract digestibility of heifers

<table>
<thead>
<tr>
<th>Measurement$^1$</th>
<th>RFI$^2$</th>
<th>Period and diet$^3$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low $n = 14$</td>
<td>High $n = 14$</td>
<td>SEM</td>
</tr>
<tr>
<td>DMD, g/kg DM</td>
<td>716</td>
<td>706</td>
<td>4.2</td>
</tr>
<tr>
<td>OMD, g/kg OM</td>
<td>736$^b$</td>
<td>722$^a$</td>
<td>4.2</td>
</tr>
<tr>
<td>CPD, g/kg CP</td>
<td>651</td>
<td>642</td>
<td>5.3</td>
</tr>
<tr>
<td>NDFD, g/kg NDF</td>
<td>669</td>
<td>660</td>
<td>7.6</td>
</tr>
<tr>
<td>ADFD, g/kg ADF</td>
<td>579</td>
<td>579</td>
<td>8.8</td>
</tr>
<tr>
<td>GED, kJ/MJ</td>
<td>698</td>
<td>686</td>
<td>4.8</td>
</tr>
</tbody>
</table>

$^a$–$^c$Values within a row with different superscripts differ ($P < 0.05$).

$^1$DMD = DM digestibility; OMD = OM digestibility; CPD = CP digestibility; NDFD = NDF digestibility; ADFD = ADF digestibility; GED = GE digestibility.

$^2$High RFI is inefficient and low RFI is efficient.

$^3$P1 = Period 1; P2 = Period 2; P3 = Period 3; GS = grass silage; PAST = pasture (perennial ryegrass); TMR = total mixed ration (70:30 concentrate:corn silage).
Although RFI is repeatable, differences in protein turnover, tissue metabolism, indeed, its component traits across diets differing in energy density and chemical composition. A recent study on whether animals rank similarly for RFI, or, ranking of animals across diets varying in chemical and physical digestion and, thus, passage rate through the rumen compared with less-fibrous, readily fermentable feeds, increases the possibility of less restrictive feed intake and nutrient digestibility for each of the 3 diet types used.

### Table 6. Pearson correlation coefficients for the association between residual feed intake (RFI) and CH₄ emissions with measures of feed intake and nutrient digestibility

<table>
<thead>
<tr>
<th>Measurement</th>
<th>RFI²</th>
<th>CH₄</th>
<th>DMI</th>
<th>RFI</th>
<th>BW⁰.⁷⁵</th>
<th>DMI/kg BW⁰.⁷⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS</td>
<td></td>
<td></td>
<td>0.070</td>
<td>−0.004</td>
<td>0.497**</td>
<td>0.134</td>
</tr>
<tr>
<td>PAST</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>−0.048</td>
<td>−0.105</td>
<td>−0.192</td>
</tr>
<tr>
<td>TMR</td>
<td>0.043</td>
<td>0.061</td>
<td>0.026</td>
<td>0.119</td>
<td>−0.082</td>
<td>0.161</td>
</tr>
<tr>
<td>GS</td>
<td></td>
<td></td>
<td>0.067</td>
<td>−0.036</td>
<td>0.605***</td>
<td>0.090</td>
</tr>
<tr>
<td>PAST</td>
<td></td>
<td></td>
<td>0.328†</td>
<td>0.232</td>
<td>0.205</td>
<td></td>
</tr>
<tr>
<td>TMR</td>
<td></td>
<td></td>
<td>0.317</td>
<td>0.274</td>
<td>0.220</td>
<td></td>
</tr>
<tr>
<td>DMD</td>
<td>−0.343†</td>
<td>0.054</td>
<td>−0.272</td>
<td>0.328†</td>
<td>0.232</td>
<td>0.205</td>
</tr>
<tr>
<td>OMD</td>
<td>−0.382†</td>
<td>−0.142</td>
<td>−0.273</td>
<td>0.317</td>
<td>0.274</td>
<td>0.220</td>
</tr>
<tr>
<td>CPD</td>
<td>−0.366†</td>
<td>0.105</td>
<td>−0.328</td>
<td>0.311</td>
<td>0.261</td>
<td>−0.115</td>
</tr>
<tr>
<td>NDFD</td>
<td>−0.085</td>
<td>0.038</td>
<td>−0.291</td>
<td>0.353†</td>
<td>0.239</td>
<td>0.221</td>
</tr>
<tr>
<td>ADFD</td>
<td>−0.091</td>
<td>0.258</td>
<td>−0.178</td>
<td>0.343†</td>
<td>0.145</td>
<td>0.232</td>
</tr>
<tr>
<td>GED</td>
<td>−0.317</td>
<td>0.011</td>
<td>−0.303</td>
<td>0.346†</td>
<td>0.226</td>
<td>0.301</td>
</tr>
</tbody>
</table>

1BW⁰.⁷⁵ = metabolic BW; DMD = DM digestibility; OMD = OM digestibility; CPD = CP digestibility; NDFD = NDF digestibility; ADFD = ADF digestibility; GED = GE digestibility.

2GS = grass silage, PAST = pasture (perennial ryegrass), TMR = total mixed ration (70:30 concentrate:corn silage).

*P < 0.10; **P < 0.05; ***P < 0.01; ****P < 0.001.

**Feed Intake and Residual Feed Intake Classification**

Residual feed intake is gaining widespread acceptance as the most appropriate measure of feed efficiency for beef cattle, although much remains unknown about the underlying biological regulation of the trait. Differences in protein turnover, tissue metabolism, susceptibility to stress, digestibility, activity, heat increment of fermentation, body composition, and feeding patterns have all been proposed as contributing to interanimal variation in RFI (Herd and Arthur, 2009). Although RFI is repeatable across different stages of the production cycle when animals are offered the same diet (Kelly et al., 2010b), there is limited information on whether animals rank similarly for RFI, or, indeed, its component traits across diets differing in energy density and chemical composition. A recent study by Manafiazar et al. (2015) showed that beef heifers ranked as low RFI under drylot conditions, when offered a barley silage and steam-rolled barley TMR, also consumed 5.3% less forage than high-RFI heifers in a follow-up experiment when meadow bromegrass pasture was offered. In contrast, Meyer et al. (2008) observed no difference in grazed forage intake between beef cattle of known divergent RFI classification, although these authors did acknowledge that the methodology used to estimate pasture DMI may have been a limiting factor in that study. Additionally, Lawrence et al. (2012) phenotypically ranked heifers into high-, medium-, and low-RFI groups when consuming GS in winter but subsequently observed a weak, nonstatistically significant correlation between RFI and DMI of these animals when subsequently grazing pasture. The diets used in the current study were representative of those used under Irish pastoral-based beef production systems (Keane et al., 2006; Drennan and McGee, 2009). The quality of the GS used in Period 1 was moderate, with a CP content of 12% and an NDF content of 66%. When the animals were originally selected on the basis of RFI in the 112-d pre-experimental period, mean daily DMI in the high-RFI group was greater (1.30 kg/d) than that in the low-RFI group throughout that period and mean BW and ADG did not differ. The animals were found to remain within their respective RFI groups when they were offered the same TMR diet again subsequent to the current study, with interexperiment repeatability coefficients of 0.61 and 0.62 recorded for DMI and RFI, respectively (Kelly et al., 2010b). However, differences in DMI between the 2 RFI phenotypes were not observed during the DMI measurement periods reported here. This was most likely a result of the short DMI and CH₄ measurement periods used, which were dictated by the necessity to collect CH₄ measurements from all 3 diets during the linear phase of SF₆ release from the intraruminal boluses.

Although not statistically detected within any of the 3 individual DMI recording periods used, when the overall data were expressed on a grams per kilogram BW⁰.⁷⁵ basis, we observed a higher DMI in high-RFI heifers that consumed, on average, 6% more feed across the 3 dietary periods than low-RFI heifers. In addition, we detected a positive correlation (r = 0.50) between DMI and phenotypic RFI although only when the heifers were offered the TMR diet. Previously, Nkrumah et al. (2004) also reported a statistically significant positive correlation (r = 0.77) between RFI and DMI in hybrid beef cattle offered a high corn finishing ration. The lack of an appreciable association between DMI and phenotypic RFI on the exclusively forage-based diets offered here, namely, grazed and ensiled grass, suggests a lack of consistency in RFI ranking of animals across diets varying in chemical composition compared with that on which the animals were originally ranked. Compared with feeding diets based on high levels of concentrate, offering forage-only diets can curb the expression of voluntary DMI of an animal and is dictated by the rumen fill value of the forage (Dulphy et al., 1989). This restriction of feed intake is largely due to the slower rate of mechanical and chemical digestion and, thus, passage rate through the rumen compared with less-fibrous, readily fermentable feeds (Steen et al., 1998; Forbes, 2005). It is also widely accepted that voluntary DMI...
of beef cattle is reduced when offered GS of lower digestibility (Drennan and McGee, 2004). Similarly, we have previously demonstrated that the digestibility of pasture has a significant impact on DMI, with greater intakes achieved on swards of higher DMD offered ad libitum (Hart et al., 2009).

**Nutrient Digestibility**

Herd and Arthur (2009) estimated that variation in digestibility may account for 10% of the interanimal variation between cattle for RFI, although there are little published data to substantiate this. Genetic variation in total-tract digestion of feed, over and above the systemic variation due to the quantity of feed consumed, has been reported in sheep selected for and against weaning weight (Herd et al., 1993; Oddy, 1993). However, the effect of RFI classification on diet digestibility in the published literature is equivocal. A number of studies have found that diet digestibility is negatively correlated with RFI in cattle (Nkrumah et al., 2006; Krueger et al., 2009b; McDonald et al., 2010; Rius et al., 2012). In contrast, Cruz et al. (2010), Lawrence et al. (2011), and Fitzsimons et al. (2014a), using either steers or heifers, all failed to establish an association between these 2 traits. In our study, using AIA as a marker on the GS and TMR diets and n-alkanes on the pasture diet, greater digestibility of DM and CP (only on the GS diets) and OM (across all diets) was observed in the low-RFI heifers. Comparable results were reported by Krueger et al. (2009a) for DM, NDF, ADF, and CPD between low- and high-RFI Brangus heifers consuming a high-roughage diet of nutritional composition similar to the GS used in Period 1. Feeding different levels of forage-only diets has previously been reported to have no effect on total-tract digestibility in beef cattle (Ortigues et al., 1993; Doreau and Diawara, 2003; McGee et al., 2005). Therefore, the higher total-tract DMD in low-RFI heifers consuming GS in the current study may be indicative of an inherent predisposition toward improved DMD, at least on GS-based diets. In addition, Nkrumah et al. (2006) reported that low-RFI animals had a tendency toward higher DMD and CPD in comparison with their high-RFI counterparts when consuming a high-concentrate diet, based on total fecal collection. However, unlike our study, Nkrumah et al. (2006) limited the feed offered to 2.5 × maintenance energy requirements (NRC, 1996). Moreover, when using markers, it is assumed that 100% recovery is achieved, but this is not always the case in practice (Van Keulen and Young, 1977; Dove and Mayes, 2006). Indeed, McGeough et al. (2010b) reported weaker agreement between AIA digestibility and total fecal collection digestibility on a GS-based diet than on a whole crop wheat diet in steers. In summary, the effect of RFI classification on apparent total-tract nutrient digestibility in the current study appeared to indicate an inherent predisposition toward greater digestibility of some dietary nutrient fractions in low-RFI heifers, although this was not consistent across diets. Further studies are warranted to better define this effect across a wider range of diets.

**Methane Emissions**

Ruminal methanogenesis represents an energy loss to the animal, with estimates varying from 3 to 7% of dietary GEI in cattle fed high-grain and high-forage diets, respectively (Hristov et al., 2013). Therefore, a reduction in enteric CH₄ production should, theoretically, result in both economic and environmental benefits to beef cattle production systems. We initially set out with a hypothesis that low-RFI cattle would produce less daily CH₄ than their high-RFI contemporaries. It is not unreasonable to assume this, given that CH₄ production is generally positively correlated with DMI in ruminants (Blaxter and Clapperton, 1965; Johnson and Johnson, 1995; Grainger et al., 2007). To date, however, no clear evidence exists as to whether these differences are simply a function of the well-characterized lower DMI of low-RFI animals (Kelly et al., 2010a,b; Lawrence et al., 2011) or if these energetically efficient animals actually have an inherent disposition toward different rumen methanogen activity (Carberry et al., 2014b) independent of DMI. Furthermore, the majority of published studies, to date, concerning RFI and CH₄ emissions have compared animals on a single diet, and, to our knowledge, this is the first study to compare CH₄ emissions and other indices of ruminal fermentation efficiency in cattle of contrasting RFI phenotypes fed different diets. Although the published literature is sparse, there are conflicting reports regarding the effect of RFI classification on CH₄ emissions. Münger and Kreuzer (2008) observed a very weak relationship between RFI and CH₄ emissions in dairy cows consuming a forage-based ration ad libitum, whereas Freetly and Brown-Brandl (2013) reported no relationship between RFI and CH₄ production in steers fed a high corn ration or heifers consuming a high corn silage ration. However, a number of studies have reported lower CH₄ production from cattle of low RFI classification, across high-concentrate diets (Nkrumah et al., 2006; Hegarty et al., 2007), grazed pasture (Jones et al., 2011), and GS (Fitzsimons et al., 2013). In all but one of these experiments (Nkrumah et al., 2006), this has been a function of a lower DMI in low-RFI animals. Given the experimental design used in the current study, where
diet is confounded by period and age, a more adequate metric for comparison of our results with previously published data is MY. Somewhat surprisingly, our results showed that daily MY was greater in the low-RFI animals. This finding is in contrast to Hegarty et al. (2007), Jones et al. (2011), and Fitzsimons et al. (2013), who all reported no difference in MY between contrasting RFI cohorts in their respective studies. Waghorn and Hegarty (2011), although failing to establish statistical significance, did report numerically higher MY (measured using respiration calorimeters), of a magnitude similar to that observed here, in low-RFI pasture-fed dairy cows when compared with their high-RFI counterparts (22.4 vs. 20.9 g CH₄/kg DMI).

In contrast, Nkrumah et al. (2006) showed CH₄ production was 28 and 24% lower in low-RFI animals compared with high- and medium-RFI animals, respectively. As all animals in their study were restricted to a fixed daily feed allowance of 2.5 × maintenance requirements (NRC, 1996), this resulted in the low-RFI group losing 1.9% less of total GEI as CH₄ than the high-RFI animals for the same level of intake, suggesting that an inherently lower CH₄ production potential existed in low-RFI animals, independent of feed intake. The differences in MY in the current study were reflected in low-RFI animals also losing a greater proportion of GEI as CH₄. As discussed, Nkrumah et al. (2006) reported a lower GE loss as CH₄ in low-RFI animals whereas Hegarty et al. (2007), Jones et al. (2011), and Fitzsimons et al. (2013) all found no differences between RFI phenotypes in the proportion of GEI lost as CH₄. Importantly, however, as alluded to by Waghorn and Hegarty (2011), small differences in MY should be interpreted with caution, particularly in grazing situations where the absolute accuracy of DMI measurements using ći-alkanes is sometimes questionable. In addition, details of some potential limitations to the SF₆ technique, as used in the current study, are discussed in more detail below.

The reasons for the greater MY in the low-RFI animals in our study are unclear. One possible explanation is the higher apparent digestibility of OM and tendency toward greater GE digestibility in low-RFI animals. The increased digestion of some nutrient fractions in the low-RFI group may have resulted in additional availability of H₂ ions in the rumen and, thus, higher methanogenic potential (Keltjens and Vogels, 1996). Another possible explanation for the greater MY of low-RFI animals compared with high-RFI animals may be a potential difference in rumen mean retention time (MRT). Goopy et al. (2014) reported a shorter MRT of both particulate and liquid digesta in sheep selected on the basis of low MY and fed at a fixed level when compared with a corresponding high-MY group. Evidence for a relationship between MRT and RFI phenotypes in the literature is sparse; however, Fitzsimons et al. (2014b) reported that a 1-kg increase in RFI was associated with a 1-kg increase in the empty weight of the reticulorumen of beef bulls. The possibility that low-RFI animals have a longer MRT than high-RFI animals, conceivably leading, in turn, to greater quantities of H₂ generated per kilogram of DMI, may help explain the findings of the current study.

Differences in the abundance of certain rumen methanogenic bacteria between the high- and low-RFI phenotypes in the current study may also provide an explanation for the greater MY observed in the low-RFI group. Carberry et al. (2014b), in a follow-up study to the current experiment and using the same rumen fluid samples used here, showed that although a predominant core group of methanogens existed across diet and phenotype, the abundance of Methanobrevibacter smithii genotypes (an important archaeon involved in the recycling of H₂ into CH₄) was different between the high- and low-RFI animals irrespective of diet type. These authors concluded that it is possible that different genotypes of the same methanogen species may associate with different strains of H₂-producing organisms. However, in a recent study by Freetly et al. (2015), it was found the bacterial concentrations of methanogen 16S rRNA as a proportion of total bacterial 16S rRNA did not differ in rumen, cecum, and rectal contents of steers differing in residual gain (animals that gained more or less BW on a similar amount of feed). These authors concluded that it is likely that postdigestive differences in metabolic efficiency are responsible for variation in residual gain and RFI, and as CH₄ is a byproduct of ruminal fermentation efficiency, it is likely to be less affected by inherent variation in RFI or residual gain.

Although absolute CH₄ emissions were greatest for the TMR diet and lowest for the pasture diet, interpretation of the direct effect of diet is confounded in the current study by the independent contribution of increased body size and, thus, DMI potential of the animals as the experiment progressed. Despite this limitation, the greater MY on the GS diet compared with that on the TMR diet is in accordance with the work of McGeough et al. (2010a), who found that high-starch forages or concentrate-based diets reduced enteric CH₄ emissions of finishing beef cattle compared with more fiber-based forages. Starch fermentation promotes propionate production in the rumen, creating an alternative hydrogen sink to methanogenesis (Murphy et al., 1982). In contrast, the constitutively high cellulose content of GS stimulates extensive digestion by cellulolytic microbes during fermentation, leading to a growth in acetate-dominated fermentation, which
increases ruminal H₂ available for methanogenesis (Moe and Tyrrell, 1979). The average MY as a proportion of GEI on the grazed pasture diet was lower than expected at 5.62%. A recent meta-analysis of experiments over a 10-yr period in Australia, using open circuit respiration chambers, indicated an average MY of 6.2% of GEI for beef heifers consuming diets with forage levels in excess of 70% (Charmley et al., 2016). The pasture allowance to all animals in Period 2 was high, as we wanted to ensure maximal pasture DMI. Consequently, selective grazing by heifers may have resulted in a greater proportion of leafy herbage being consumed relative to the overall pasture composition. It has been previously demonstrated that consumption of higher digestibility, lush pasture results in a lower MY as a proportion of GEI (Wims et al., 2010; Boland et al., 2013). Indeed, the mean MY as a proportion of GEI in the current study on the PAST, although low, is comparable with the study of Boland et al. (2013), on grazed pasture, who used contemporaries of the heifers used in the current study. Furthermore, recent studies by Williams et al. (2011) and Lassey (2013) highlighted that in earlier work using the SF₆ technique, insufficient attention was paid to placement of canisters to measure ambient gases. Williams et al. (2011) suggested this can cause inaccuracies in CH₄ measurements ranging from –6.2 to +0.8%, although this is more important in indoor experiments where air flow may be limited (Lassey, 2013). Background levels of both gases were quantified and corrected for in all 3 measurement periods in our study. However, this potential source of error is likely to have had the least impact during Period 2 when animals were grazing. It was also surprising in the current study that molar proportions of propionate were greatest on GS and lowest on the TMR; however, total VFA production was greater on the TMR diet, meaning that absolute propionate production was also greater on the TMR diet.

As previously stated, CH₄ emissions are generally highly correlated with DMI in ruminants. However, we detected only weak, insignificant correlations between CH₄ and DMI on the GS or TMR diets. The statistically significant correlation (r = 0.42) observed between estimated grazed pasture DMI (determined using n-alkanes) and daily CH₄ production is very much in agreement with that reported for grazing sheep (Lassey et al., 1997). It should also be acknowledged, however, that estimation of feed intake using n-alkanes is more prone to error than direct measurement of feed intake. Potential reasons for inaccurate estimations of feed intake using n-alkanes are the use of a single marker, inaccuracies in dosed marker preparation, nonrepresentative grassland sampling, and incomplete fecal recovery of the markers (Dove and Mayes, 2006). This may be of particular importance if data are being used for GHG inventories where both CH₄ and DMI are indirect measurements. de Haas et al. (2011) observed strong positive correlations between predictions of CH₄ emissions and RFI in lactating dairy cows, which implies that reductions in emissions should result from selection for low RFI. In contrast, no statistically significant correlations between RFI and CH₄ production were observed in the current study, which differs from the work of Nkumah et al. (2006), who reported a positive correlation (r = 0.44) between these 2 variables in beef cattle offered a high-concentrate diet.

Another source of variation that should be considered when comparing our results with other published studies is the detail of the methodology used to measure CH₄ emissions. The SF₆ technique, in the form it was used when the experiment was conducted, had been widely validated by several authors to provide an accurate estimate of daily CH₄ emissions (McGinn et al., 2006; Grainger et al., 2007). More recently, however, Williams et al. (2011) and Deighton et al. (2013, 2014) have published a series of papers that identify, in detail, a number of aspects of the technique that can increase the level of error encountered. In relation to the current study, one area of potential variability is the linearity of SF₆ release rate from intraruminal boluses. We assumed that the SF₆ release rate was linear throughout the study, but in fact, Deighton et al. (2013) have demonstrated that the recalibrated SF₆ release rate of boluses declines slightly over time, meaning that CH₄ emissions in Period 3 will have been confounded to a greater degree than Period 1. The decline is apparently due to the decrease in SF₆ permeability of polytetrafluoroethylene membranes due to their exposure to pressurized SF₆ within a permeation tube (Deighton et al., 2013). However, as SF₆ boluses were blocked across both RFI groups according to release rate, in the current study, this should not have influenced interpretation of the effect of RFI ranking on ruminal CH₄ emissions.

A further aspect of the SF₆ technique worth discussing in the context of this study is the varying diurnal patterns of feed intake in each period, due to the feed presentation methods used (i.e., grazing in Period 2 vs. bin feeding in Periods 1 and 3). This results in a large diurnal fluctuation in CH₄ emissions on a daily basis in response to feed intake patterns throughout the day (Grainger et al., 2007; Deighton et al., 2014). Therefore, due to the assumption that gas inflow rates through capillary tubes remain constant throughout the 24-h measurement period used in the current study, it is important that a constant, representative sample of each animal’s breath was collected throughout the day. However, Deighton et al. (2013) showed that the
gas inflow rate through capillary tubes into evacuated sample collection canisters is not constant but actually declines slightly over time, which may have introduced an extra element of variability into our estimates of daily CH$_4$ emissions.

Although we acknowledge these potential sources of error in the SF$_6$ technique methodology as it was used in the current study, it is important to point out we used best practice procedures available at the time and estimated CH$_4$ from a relatively large sample size over a 5-d period and also that the experimental design ensured that all sources of potential error, where possible, were equalized across both RFI groups.

**Repeatability of Methane Emissions**

Our results suggest that the repeatability of CH$_4$ emissions between the 2 RFI phenotypes were weak when successively measured across different diet types. Goopy and Hegarty (2004) showed that steers selected as having either high or low CH$_4$ emissions on a concentrate diet maintained these emissions when measured for a second period on the same diet; however, no difference between these groups was observed when the diet was subsequently changed to sorghum hay. Similar work performed by Pinares-Patiño et al. (2003) also resulted in poor repeatability of CH$_4$ emissions over 4 successive time periods from sheep grazing perennial ryegrass/white clover pastures.

**Rumen Fermentation Indices**

There is some evidence to suggest that interanimal variation in ruminal VFA concentrations may contribute to the observed variation in RFI. Krueger et al. (2009b) found no inherent differences in ruminal pH or VFA concentrations among cattle divergent for RFI when consuming a high corn finishing diet, whereas Krueger et al. (2009a) found that heifers of low RFI status, consuming a high-roughage diet, had a higher ruminal acetate:propionate ratio and lower propionate concentrations than their high-RFI contemporaries, which is in agreement with our findings from Period 1. However, this effect was not observed when the animals were fed pasture or the TMR. In agreement, both Rius et al. (2012) and Fitzsimons et al. (2014a) also reported no differences in ruminal VFA concentrations or molar ratios between feed-efficient and -inefficient lactating dairy or beef cows, respectively.

Guan et al. (2008), using samples collected during the study of Nkrumah et al. (2006), reported that low-RFI cattle tended to have an increased total VFA concentration and a greater concentration of butyrate and valerate compared with high-RFI animals. In our study, there was no difference in the total VFA concentration or butyrate production; however, rumen fluid from the low-RFI animals had an increased concentration of iso-valerate. Ruminal VFA concentrations are an indicator of the metabolic activity of the rumen microbial ecosystem but do not directly reflect how the animal utilizes these products (Hristov et al., 2013). Further studies are required to examine the effect of phenotypic RFI status on the rumen microbial ecosystem to establish whether, and to what extent, the host actually has a consistent influence over the composition and activity of the milieu of microorganisms inhabiting its rumen.

The effect of diet on VFA production was more pronounced. As expected, we observed that feeding the high-concentrate TMR diet reduced rumen pH compared with either of the 100% forage diets, consistent with previous work from our laboratory (Drennan et al., 2006; Owens et al., 2008). A potential limitation to the rumen digesta sampling technique used in this study is the possibility of saliva contamination and the representativeness of the sampled digesta (Duffield et al., 2004). Furthermore, the silage fermentation pattern can have a considerable effect on the rumen fluid VFA concentration (Van Vuuren et al., 1995). Nonetheless, the rumen fermentation characteristics observed in Period 1 are similar to results obtained by Owens et al. (2008) where ruminal cannulated steers were fed a GS diet comparable with that used here.

**Conclusions**

This study has provided both contrasting and supporting evidence to that already published on the relationship between RFI status and ruminal CH$_4$ emissions of cattle. In contrast to our original hypothesis, MY (per kg DMI and as a proportion of GEI) was marginally higher for low-RFI cattle. There was some evidence for improved digestibility of some nutrients in low-RFI animals, which may have been a contributory factor to their greater MY. Few relationships between measures of feed intake, RFI classification, and CH$_4$ production were observed, although the relatively short duration of measurement periods used most likely influenced this. Notwithstanding this, however, the widely established correlation between negative RFI classification and reduced feed intake, allied to the commonly observed relationship between feed intake and CH$_4$ production, suggest that benefits in terms of increased life cycle efficiency of performance on cumulative lifetime CH$_4$ emissions could still be achieved. Selection for improved RFI as a CH$_4$ mitigation strategy is also appealing due to the fact that the associated productivity benefits should make it attractive to producers, an incentive that many alter-
native mitigation strategies do not have. However, as for any trait, ongoing care needs to be maintained in the selection of animals for low RFI so as to avoid any potentially unfavorable relationships with other economically important production traits.

**LITERATURE CITED**


