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ARTICLE

Comparative analyses of enteric methane emissions, dry matter intake, and milk somatic cell count in different residual feed intake categories of dairy cows

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Abstract: This study compared the different residual feed intake (RFI) categories of lactating Holsteins with respect to methane (CH_4) emissions, dry matter intake (DMI, kg), milk somatic cell count (SCC, $10^3 \cdot mL^{-1}$), and β-hydroxybutyrate (BHB, $mmol \cdot L^{-1}$). The RFI was calculated in 131 lactating Holstein cows that were then categorized into −RFI (RFI < 0) vs. +RFI (RFI > 0) and low- [RFI < −0.5 standard deviation (SD)] vs. high-RFI (RFI > 0.5 SD) groups. Milk traits were recorded in 131 cows, whereas CH_4 and carbon dioxide were measured in 83. Comparisons of −RFI vs. +RFI and low- vs. high-RFI showed 7.9% (22.3 ± 0.40 vs. 24.2 ± 0.39) and 12.8% (21.1 ± 0.40 vs. 24.2 ± 0.45) decrease ($P < 0.05$) in DMI of −RFI and low-RFI groups, respectively. Similarly, −RFI and low-RFI cows had lower ($P < 0.05$) CH_4 ($g \cdot d^{-1}$) by 9.7% (343.5 ± 11.1 vs. 380.4 ± 10.9) and 15.5% (332.5 ± 12.9 vs. 393.5 ± 12.6), respectively. Milk yield was not different ($P > 0.05$) in −RFI vs. +RFI and low vs. high comparisons. The −RFI and low-RFI cows had lower ($P < 0.05$) SCC in −RFI vs. +RFI and low-RFI vs. high-RFI comparisons. The BHB was lower ($P < 0.05$) in low-RFI compared with the high-RFI group. Low-RFI dairy cows consumed less feed, emitted less CH_4 ($g \cdot d^{-1}$), and had lower milk SCC and BHB without differing in milk yield.

Key words: RFI, methane, SCC, BHB, dairy cows.

Résumé : Cette étude comparait les différentes catégories d'ingestion alimentaire résiduelle (RFI — « residual feed intake ») chez les Holsteins en lactation par rapport aux émissions de méthane (CH_4), à la consommation des matières sèches (DMI — « dry matter intake », kg), à la numération des cellules somatiques (SCC — « somatic cell count », $10^3 \cdot mL^{-1}$) du lait et au β-hydroxybutyrate (BHB, $mmol \cdot L^{-1}$). La RFI a été calculée chez 131 vaches holsteins en lactation qui ont ensuite été catégorisées en groupes : −RFI (RFI < 0) contre +RFI (RFI > 0) et RFI faible [RFI < −0,5 écart-type (SD — « standard deviation »)] contre RFI élevée (RFI > 0,5 SD). Les caractéristiques du lait ont été enregistrées chez les 131 vaches, tandis que les émissions de CH_4 et de dioxyde de carbone ont été mesurées chez 83 d'entre elles. Les comparaisons des groupes −RFI c. +RFI ainsi que les RFI faible c. RFI élevée ont montré une diminution ($P < 0,05$) de 7,9 % (22,3 ± 0,40 c. 24,2 ± 0,39) et 12,8 % (21,1 ± 0,40 c. 24,2 ± 0,45) de DMI des groupes −RFI et RFI faible, respectivement. De façon semblable, les vaches des groupes –RFI et RFI faible avaient des émissions de CH4 ($g \cdot j^{-1}$) plus faibles ($P < 0,05$) à raison de 9,7 % (343,5 ± 11,1 c. 380,4 ± 10,9) et 15,5 % (332,5 ± 12,9 c. 393,5 ± 12,6) respectivement. Le rendement de lait ne différait pas ($P > 0,05$) dans les comparaisons −RFI c. +RFI et RFI faible c. RFI élevée. Les vaches −RFI et RFI faibles avaient des SCC plus faibles ($P < 0,05$) dans les comparaisons −RFI c. +RFI et faible RFI c. RFI élevée. Le taux de BHB était plus faible ($P < 0,05$) chez le groupe RFI faible comparé au groupe

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RFI élevée. Les vaches laitières à RFI faible consommaient moins d'aliments, émettaient moins de CH_4 ($g \cdot j^{-1}$), et avaient de plus faibles SCC et taux de BHB sans différer en rendement de lait. [Traduit par la Rédaction]

Mots-clés : RFI (ingestion alimentaire résiduelle), méthane, SCC, BHB, vaches laitières.

Introduction

Residual feed intake (RFI) can be used as a measure of feed efficiency in dairy cattle (Van Arendonk et al. 1991; Connor et al. 2013; Manafiazar et al. 2013). Differences in total tract digestibility, methane (CH_4) production, heat production, and energy retention are considered to be the main factors affecting variation among dairy cows in RFI (Richardson and Herd 2004; Nkrumah et al. 2006). Herd and Arthur (2009) showed that heat production from metabolic processes, body composition, and physical activity explained 73% of the variation in RFI in Angus steers following divergent selection. The biological mechanisms contributing to these variations included protein turnover, tissue metabolism, stress (37%), digestibility (10%), heat increment and fermentation (9%), physical activity (9%), body composition (5%), and feeding patterns (2%) (Herd and Arthur 2009).

Methane produced from ruminants is a potent greenhouse gas (Ellis et al. 2007) and arises primarily from enteric fermentation (Kebreab et al. 2006). In cattle, enteric CH_4 is produced predominantly in the rumen and to a small extent in the large intestine (Murray et al. 1976). Rumen CH_4 is primarily emitted from the animal by eructation. Enteric CH_4 emission is a major contributor to greenhouse gas emissions and also a loss of feed energy during production (Boadi et al. 2004). Methane represents a significant energy loss to the animal, ranging from 2% to 12% of the gross energy intake (Johnson and Johnson 1995).

Genetic improvement in feed efficiency could reduce feed intake and CH_4 emission leading to economic and environmental benefits (Basarab et al. 2013). Few studies have investigated the relationship between RFI and CH_4 emission in lactating dairy cows. Münger and Kreuzer (2008) reported a weak relationship between RFI and CH_4 emission in Holstein, Simental, and Jersey cows. Recently, Flay et al. (2019) reported that RFI category did not affect $CH_4 \cdot d^{-1}$ or $CH_4 \cdot kg^{-1}$ of body weight (BW) gain, but $CH_4 \cdot kg^{-1}$ of dry matter intake (DMI) was higher in low-RFI Holstein and Jersey heifers. Research in beef cattle showed that CH_4 production ($g \cdot d^{-1}$ or $g \cdot kg^{-1}$ BW) was greater for high- compared with low-RFI heifers (Fitzsimons et al. 2013). However, Jones et al. (2011) found that DMI and CH_4 emission were similar between divergent RFI groups when animals grazed low-quality pastures, but were lower for low-RFI when grazing high-quality pastures. A study in Nellore cattle also showed that there was no evidence that more feed efficient animals release less enteric CH_4 (Mercadante et al. 2015).

The genetic correlation between RFI and energy balance was reported to be 0.85 in Irish dairy cattle (McParland et al. 2014). Cows in negative energy balance mobilize adipose tissue as fatty acids and often have elevated ketone body concentrations (Duffield 2000). Previous studies found that milk and blood β-hydroxybutyrate (BHB) were correlated, and milk BHB ≥ 0.20 $mmol \cdot L^{-1}$ and acetone ≥ 0.08 $mmol \cdot L^{-1}$ are used as threshold levels for hyperketonemia (Denis-Robichaud et al. 2014). Excessive production of ketone bodies can lead to hyperketonemia and can have a negative effect on animal health, production, and profitability (Herdt 2000; McArt et al. 2013). Therefore, it is likely that RFI and hyperketonemia might be correlated.

Studies on the association of RFI with CH_4 emission and production traits in dairy cattle are scarce, and some of the results in dairy as well as beef are inconsistent. Therefore, this study aimed to investigate the impact of RFI classifications on CH_4 emission, DMI, milk yield, and composition in lactating dairy cows. In addition, we attempted to investigate the association of RFI with milk somatic cell count (SCC) and BHB.

Materials and Methods

Animals and management

The experiment was conducted at the Dairy Research and Technology Center (DRTC) of the University of Alberta. All cows during the study period were housed individually in a ventilated tie-stall barn with free access to water and were fed total mixed ration (TMR) ad libitum. Cows were brought to an exercise area (an open dry lot) for 3 h every second day. Cows were milked twice daily (0300–0500 and 1500–1700) in their stalls. After parturition, cows were gradually switched during the first 7 d to a fresh lactation diet with a higher proportion of grain [up to 50% on a dry matter (DM) basis] to meet the energy demands for high milk production. The animals received either high- or mid-energy dense ration according to their milk production levels. Daily ration was offered as TMR for ad libitum intake to allow approximately 5% feed refusals throughout the experiment. This was achieved by offering cows a measured amount of feed using a CALAN Super Data Ranger. The amount of feed that was offered increased when the cows ate more, and the refusal was less than 5% and decreased when the refusal was more than 5%. This method decreases the amount of feed that can be wasted and reduces the potential for sorting. All cows were fed once daily in the morning at 0800. Individual offered feed weight in the morning and refusal feed weight left on the next morning were recorded daily. The particle size of the TMR ingredients (silage or hay) were chopped to the recommended sizes determined by a Penn State

Table 1. Ingredients and chemical composition of high- and mid-energy density ration for the cows that were in the study.

	High ration	Mid ration
Diet ingredients (% DM basis)		
Alfalfa hay	11.5 ± 0.7	11.02 ± 1.19
Barley silage	33.0 ± 7.79	40.05 ± 5.08
Pea/triticale silage	3.1 ± 3.0	6.47 ± 3.7
Rolled grain[a]	32.8 ± 4.49	27.16 ± 3.25
Protein supplement[b]	19.6 ±1.38	15.3 ± 1.69
Chemical composition		
DM (%)	54.9 ± 4.46	48.68 ± 3.51
Crude protein (% of DM)	17.6 ± 0.61	16.93 ± 1.03
Acid detergent fiber (% of DM)	20.33 ± 1.41	22.24 ± 2.65
Neutral detergent fiber (% of DM)	30.89 ± 1.39	34.29 ± 1.62
NE lactation (Mcal·kg^{-1})	1.83 ± 0.03	1.73 ± 0.03

Note: DM, dry matter; NE, net energy for lactation. The ration changed during the experimental period and the diet ingredients and chemical composition were presented as mean ± standard deviation.

[a]Rolled grain: corn and barley.

[b]Protein supplement: 26.61% amino plus (high bypass soy), 26.25% soy bean meal-47%, 25.75% canola meal, 8.15% F 100 Dairy fat, 4% corn distiller 2010, 2.3% limestone, 2% AFA/canola oil, 1.5% SOD bicarbonate, 1.2% DICAL PHOS-21%, 1% salt, 0.58% MAG OX-56%, 0.4% nutritec-diamond V mills, 0.1% selenium 1000 mg·kg^{-1}, 0.1% ruminant TM Pak, 0.05% ADE VIT PAK-30, and 0.02% biotin 2%-Rovimix H-2.

particle separator, which has an upper sieve (>0.75 inches), middle sieve (0.31–0.75 inches), lower sieve (0.16–0.31 inches), and bottom pan (<0.16 inches), and mixing was done for 5–10 min to minimize sorting. Feed compositions, including DM (%), crude protein (CP, %), neutral detergent fiber (%), and net energy (NE) lactation were determined when the TMR ingredients were changed (Table 1). All experimental procedures were approved by the University of Alberta Animal Care and Use Committee for Livestock, and animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (2009).

A total of 131 mixed parity (63 primiparous and 68 multiparous) lactating cows were enrolled in the study from 1 Apr. 2015 to 30 Dec. 2015, and from 30 May 2017 to 23 Oct. 2018. Two types of data collection were undertaken: (1) daily feed intake, monthly BW, weekly milk samples, and milk yield were collected in all 131 mixed parity lactating cows from 3 to 240 days in milk (DIM); and (2) CH_4 emission was measured on 83 mixed parity (47 primiparous and 36 multiparous) lactating cows (a subset of 131 mixed parity cows) using the GreenFeed system (C-Lock Inc., Rapid City, SD, USA) from 5 May 2015 to 7 July 2015 and 17 Jan. 2018 to 22 June 2018.

Feed intake, milk yield, milk composition, and BW data collection

Daily feed intake was calculated as the difference between the amount of feed offered and the refusal of individual cows on a daily basis. Daily DMI was calculated as the product of daily feed intake, and DM percentage of the feed assuming that the diet and the refusal had similar DM percentages. The DM percentage of the silage was assessed weekly and adjustments were performed when the DM percentage of the silage deviated by 2% from the one used in the latest diet formulation. The nutrient composition and NE density (Mcal·kg^{-1}) of the diets were analyzed when the diet changed.

Cows were milked twice per day, in the morning [AM, milking time (MT): 0300–0500] and afternoon (PM, MT: 1500–1700). Milk samples were collected twice per week every Tuesday (PM) and Wednesday (AM). Both the AM and PM milk samples were analyzed for milk components, separately. Milk sampling bottles were assembled on the semi-automatic milking machines where milk samples were continuously collected automatically from the start to the end of milking in each cow. At the end of milking for each cow, the milk in the bottle was homogenized and the milk sample was collected with a 50 mL bar-coded plastic vial. Samples were stored at 4 °C temporarily and shipped to Lactanet Canada, Edmonton, AB for milk composition analysis. All weekly test-day milk samples were analyzed using the same mid-infrared spectrometer (Foss MilkoScan FT6000; Foss Electric A/S, Hillerød, Denmark) to determine milk composition (milk fat, protein, and lactose), disease indicator traits such as SCC and BHB

concentrations. Milk urea nitrogen (MUN) was analyzed alongside the other milk traits. Weekly test-day records of fat ($n = 7602$), protein ($n = 7650$), lactose ($n = 7650$), SCC ($n = 7608$), MUN ($n = 7650$), and BHB ($n = 7608$) were recorded on 131 unique cows from 3 to 240 DIM. The laboratory analyses of the PM and AM milk samples were performed separately, and the number of records varied in the milk components because of missing records and data cleaning. For example, all the negative values for BHB were removed from the analysis.

Milk yield data corresponding to the milk sampling dates were collected for each cow in the study. Body weight was measured using a Myscale Pro-W810 weighing scale (Gallagher, Canley, UK) at 0600 after morning milking and before feeding.

Derived parameters for RFI calculation

The daily actual energy intake (AEI), monthly metabolic body weight (MBW), weekly milk production energy requirement (MPER), and empty body weight (EBW) for each animal were derived from the recorded raw data. Actual energy intake was derived from the daily DMI recorded 3–240 DIM as a product of the daily DMI and the NE density of the diet. The daily feed intake was multiplied by the DM percentage of the diet to obtain daily DMI. This approach assumed that the ration and refusal DM percentages were equal. Metabolic body weight was calculated as BW powered to 0.75 (NRC 2001). Empty body weight was an adjusted BW for the gut fill (GF), and it is the function of individual daily DMI and the metabolizable energy content of the diet that each animal consumed at the test day (Coffey et al. 2001). It is calculated as EBW (kg) = BW − GF, where GF (kg) = DMI × [11 − (7 × MED/15)], in which MED was the metabolizable energy density (Mcal·kg^{-1}) of the diet (NRC 2001). Milk production energy requirement is the sum of the heat of combustion of milk fat, protein, and lactose and calculated as MPER (Mcal·d^{-1}) = {[0.0929 × fat (%)] + [0.0547 × CP (%)] + [0.0395 × lactose (%)]} × milk yield (NRC 2001).

Predicting daily MBW, EBW, and MPER

Metabolic BW and EBW were derived from BW data recorded once per month. Similarly, MPER was derived from milk yield, fat, protein, and lactose percentages that were recorded weekly. The daily values of MBW and EBW were predicted from monthly values, and daily values of MPER were predicted from weekly values using a random regression model. The Legendre polynomial random regression model was used as described in Manafiazar et al. (2013). Briefly, the Legendre polynomial random regression model was used in this study as follows:

$$y_{it} = F_{it} + \sum_{m=0}^{k1} B_m P_m(t) + \sum_{m=0}^{k2} \lambda_{im} P_m(t) + \varepsilon_{it}$$

where y_{it} is a derived trait (MBW, EBW, and MPER) for animal i on day t, and F_{it} represents fixed effects of the population used to define contemporary groups. The fixed effects were combined month and year of measurement with ration type, the temperature and humidity index at each test month, and the covariate of animal's age at first calving deviation from the population mean (linear and quadratic). Term β_m is the fixed regression coefficient for a particular contemporary group; λ_{im} represents random regression coefficients associated with the animal's additive genetic effects plus its permanent environmental effects; P_m (t) is the mth Legendre polynomial evaluated at time t; the parameters $k1$ and $k2$ are the order of fitted fixed (1–5) and random (1–5) polynomials regression, respectively; and ε_{it} is the residual error associated with an animal i at time t.

Empty body weight change calculation

After predicting daily values of EBW from monthly values, empty body weight change (EBWC) was calculated as a difference in EBW between two consecutive days (days after − day before, e.g., EBW at 4th − 3rd, 5th − 4th DIM) from 3 to 240 DIM. Cows that lose weight after calving had negative EBWC values, whereas cows that gain weight had positive values. Empty body weight change was calculated to account for the body tissue mobilization in the RFI calculation during the study period (3–240 DIM).

Calculation of RFI

Residual feed intake was predicted in 131 lactating Holstein dairy cows according to Manafiazar et al. (2013). In short, RFI values were calculated as the difference between the actual (AEI) and expected NE intake (EEI). Phenotyping for RFI required recording of daily DMI and predicting EEI accounting for multifunctional energy requirements (MBW, EBW, EBWC, and MPER). Mixed parity cows were used in the study, and parity (P) was included in the RFI calculation model. A multiple linear and quadratic regression model was used to predict EEI values from 3 to 240 DIM. The smoothed total AEI was linearly regressed on a total of 237 d predicted traits of MBW, MPER, and EBWC and parity to obtain the individual's 237 d of EEI and RFI as follows.

$$\sum_{i=3}^{240} \mathrm{AEI}_i = P_i + \beta_0 + \beta_1 \sum_{i=3}^{240} \mathrm{MBW}_i + \beta_2 \sum_{i=3}^{240} \mathrm{MPER}_i + \beta_3 \sum_{i=3}^{240} \mathrm{EBWC}_i + \sum_{i=3}^{240} \mathrm{RFI}_i$$

where β_0, β_1, β_2, and β_3 were intercept and regression coefficient of MBW, EBWC, and MPER, respectively.

The 237 d RFI for individual animal i was obtained by subtracting the total energy expenditures from smoothed total 243 d AEI of the ith individual as follows:

$$\sum_{i=3}^{240} \mathrm{RFI}_i = \sum_{i=3}^{240} \mathrm{AEI}_i - \sum_{i=3}^{240} \mathrm{EEI}_i = \sum_{i=3}^{240} \mathrm{AEI}_i - \left(P_i + \beta_0 + \beta_1 \sum_{i=3}^{240} \mathrm{MBW}_i + \beta_2 \sum_{i=3}^{240} \mathrm{MPER}_i + \beta_3 \sum_{i=3}^{240} \mathrm{EBWC}_i \right)$$

The daily average lactation RFI for each individual over 237 d was obtained by dividing the total lactation RFI by the number of days that the animal was in the record.

Methane and carbon dioxide measurements

Methane and carbon dioxide (CO_2) measurement was undertaken at DRTC using the GreenFeed unit (C-Lock Inc.) in 83 mixed parity lactating dairy cows. All cows were managed under the tie-stall system during the entire experimental period, and measurement was conducted in two time periods: first from 5 May 2015 to 7 July 2015 and second from 17 Jan. 2018 to 22 June 2018. During the first measurement, the unit was located in an exercise area where cows from the tie-stall were moved to as a routine farm activity. A total of 39 mixed parity (19 primiparous and 20 multiparous) lactating dairy cows were used in this measurement period. The measurement was conducted in three batches with 11 animals in batch 1 and 14 animals in each of batch 2 and batch 3 (Table 2). The measurement was done twice a day (0900–1200 and 1800–2100) for 14 consecutive test days, and cows had the opportunity to voluntarily visit the unit that monitored their CH_4 and CO_2 emission. In addition to the two-time interval measurements, we measured CH_4 emission on 14 cows (out 39) at eight equally spaced time points during the diurnal cycle as a validation set. We compared the two-time point and eight-time point measurements and observed a strong correlation of DMI ($r = 0.73$; $P < 0.001$), CH_4 g·d^{-1} ($r = 0.74$; $P < 0.001$), and CO_2 g·d^{-1} ($r = 0.72$; $P < 0.001$) production. By plotting the eight-time point measurements with respect to CH_4 production and time of the day, we observed that the time intervals used to measure CH_4 emission (0900–1200 and 1800–2100) included the peak and the lower levels of CH_4 emission during the diurnal cycle.

The GreenFeed system has a radio frequency identification reader that identifies the animal's ear tag when the animal's head is in a correct position within the hood. The GreenFeed system dispenses pellets from the hopper to encourage the animal to maintain a suitable head position for accurate measurements. Once the group of cows (on average 13 cows per group) was released to use the GreenFeed system, any of the animals could visit the unit provided it was not in use by another animal. The animal using the GreenFeed system needed to maintain an appropriate head position in the hood for 3–5 min in order for that visit to result in CH_4 measurement. A modification was done during the second time of measurement. Methane and CO_2 emission

Table 2. Summary statistics (mean ± standard deviation) for parameters used in the CH_4 emission study.

Batch	Cows per GP	PP (n)	MP (n)	DMI (kg·d^{-1})	Milk yield (L)	CH_4 (g·d^{-1})	CO_2 (g·d^{-1})	CH_4 g·kg^{-1} DMI	CH_4 g·L^{-1} of milk	No. of visits	DIM
1	11	6	5	22 ± 2.9	40.2 ± 8.5	385.5 ± 48.0	11 674.7 ± 1037.5	17.6 ± 1.4	9.8 ± 1.6	25 ± 3.6	61.2 ± 9.6
2	14	7	7	21 ± 2.9	41.2 ± 7.9	395 ± 73.2	13 471.5 ± 1852.7	18.1 ± 1.6	9.7 ± 2.0	23 ± 4.0	51.5 ± 12.3
3	14	6	8	20.6 ± 4.2	39 ± 8.0	323.9 ± 80.6	12 358.6 ± 1339.5	15.8 ± 3.2	8.5 ± 1.7	19 ± 5.9	59.8 ± 41.8
4	10	6	4	23.5 ± 2.5	35.8 ± 4.3	411.2 ± 67.8	14 411.7 ± 954.8	17.4 ± 2.1	11.8 ± 2.7	21 ± 1.9	215.4 ± 21.1
5	10	7	3	22 ± 2.7	33.5 ± 3.9	326 ± 74.8	12 545.2 ± 1025.3	14.8 ± 2.7	9.9 ± 2.4	18 ± 3.7	123.5 ± 52.1
6	15	11	4	21.9 ± 2.4	37 ± 4.9	392.9 ± 63.3	11 481.3 ± 1073.8	13.6 ± 3.7	8.1 ± 2.5	18 ± 3.3	96.8 ± 60.4
7	9	4	5	25 ± 3.5	40.3 ± 6.9	319 ± 50.5	11 048.2 ± 1047.5	12.9 ± 2.6	8.2 ± 2.2	19 ± 3.6	177.3 ± 43.0

Note: CH_4, methane; GP, group; PP, primiparous cows; MP, multiparous cows; DMI, dry matter intake; CO_2, carbon dioxide; DIM, days in milk.

measurement was conducted on 44 cows (primiparous and multiparous) in four batches (on average 11 cows per batch). Each batch was measured for 12 d (twice a day, 12 h apart). First day measurement started at 0100 and 1300 then shifted every day by 1 h to cover the 24 h cycle by 12 d of measurements. During CH_4 measurement, the group of cows was arranged in a row in individual tie-stalls, and the GreenFeed system was moved to the cows. It takes 10 min (5 min background sampling and 5 min measurement) to get a measurement from a single cow and about 2 h to finish a group of 12 cows.

During the entire CH_4 emission measurement period, CO_2 recovery tests were performed at the start of each group with four releases of CO_2, each for 5 min into the GreenFeed system. Moreover, gas calibration was performed every week during the experimental period. The details of these procedures were described in Hristov et al. (2015). The GreenFeed system was adjusted so that each cow could receive six drops (40 s apart among each drop) of barley grain from the overhead hopper per visit. The cow visiting the GreenFeed system needed to keep her head in an appropriate head position from 3 to 5 min for a measurement to happen from that specific visit. Methane emission measurement was conducted twice a day, and a cow could receive a maximum of 12 drops. The weight of each drop was on average 38 g·drop^{-1}. Each cow consumed 9.6 drops of barley grain per day resulting in a total of 364.8 g·d^{-1}.

Mass flux of CH_4 and CO_2 was calculated by multiplying the measured increase in concentration from ambient levels related to the animals by the measured air flow, and then applying ideal gas laws. The details of this procedure were described in Manafiazar et al. (2016). We used "time of the day" averaging method that was calculated by aggregating and averaging the visit fluxes by time intervals over the study period. Then, the time interval values were averaged to estimate the daily average emission. The time interval size was specified by 4 h intervals making six time intervals per day, and the six time intervals were defined as 0000–0400, 0400–0800, 0800–1200, 1200–1600, 1600–2000, and 2000–2400. Then, for each averaging period, based on the visit timing, the visits were aggregated into the appropriate time intervals. Subsequently, the mean for the time intervals was calculated as the sum of the visit fluxes in each time interval divided by the number of measurements in the time interval. The time interval averages were averaged to determine the daily individual CH_4 and CO_2 emissions (g·animal^{-1}·d^{-1}). GreenFeed system daily emission data for CH_4 and CO_2 were averaged per cow over the days of measurement, and the averaged data were used in the analyses.

Methane emission measurement was conducted in a total of seven batches. Cows with fewer than eight visits were excluded from the analysis to minimize bias in the CH_4 emission measurement using the GreenFeed system. The summary statistics of CH_4-related parameters including the average number of visits in all the seven batches are presented in Table 2.

Categorizing cows into RFI groups

A total of 131 RFI predicted cows were ranked and grouped using two approaches: (1) cows were categorized into feed efficient (−RFI, RFI < 0) and inefficient (+RFI, RFI > 0); and (2) cows were categorized into most efficient [low-RFI, RFI < 0.5 standard deviation (SD) from the mean] and least efficient (high-RFI, RFI > 0.5 SD from the mean). Fat, protein and lactose percentages, SCC, MUN, and BHB were recorded on 131 cows. Out of the 131 RFI predicted cows, 83 had MBW, DMI, CH_4 production (CH_4·d^{-1}), CH_4 yield (CH_4·kg^{-1} DMI), CH_4 intensity (CH_4 g·kg^{-1} milk), CO_2 emission (CO_2 g·d^{-1}), and milk yield. A similar approach was used to categorize the 83 cows into feed efficient vs. inefficient as well as low-RFI vs. high-RFI groups.

Statistical analyses

A PROC MIXED procedure in the SAS statistical software package (SAS 2016) was used to analyze the MBW, DMI, milk yield, CH_4 production, CH_4 yield, CH_4 intensity, and CO_2 emission average values in the categories of −RFI and +RFI as well as low- and high-RFI. Residual feed intake categories (−RFI, +RFI or low-RFI, high-RFI), CH_4 measurement batches (1–7) and parity (1–3+) were used as fixed effects, whereas DIM was used as a covariate in the model as follows:

$$Y_{ijkl} = \mu + R_i + B_j + P_k + \beta_1 \mathrm{DIM} + e_{ijkl}$$

where Y_{ijkl} is the trait observation for the lth cow tested from the ith RFI group (−RFI and +RFI as well as low- and high-RFI) and kth parity (1–3+); B_j is the effect of the jth CH_4 measurement batches (1–7); β_1 is the regression coefficient of DIM; and e_{ijk} is the deviation due to the $ijkl$th cow or error term.

For the milk traits (fat %, protein %, lactose %, MUN, SCC, and BHB), data were also analyzed using the PROC MIXED model procedure of SAS with fixed effects of RFI (−RFI and +RFI), parity (1, 2, and 3+), MT with two levels (AM and PM), and two-way interactions of RFI × parity, RFI × MT, and random effects of cow nested within week of data collection period and error term. Days in milk was included in the model as a covariate and the model is as follows:

$$Y_{ijk} = \mu + R_i + \mathrm{MT}_j + P_k + \mathrm{RP}_{ij} + \mathrm{MTP}_{jk} + \beta_1 \mathrm{DIM} + e_{ijkl}$$

where Y_{ijkl} is the trait observation for the lth cow tested from the ith RFI group (−RFI and +RFI as well as low- and high-RFI) and kth parity (1–3+); MT_j is the effect of the jth time of day (AM, PM); β_1 is the regression coefficient of DIM; and e_{ijk} is the deviation due to the $ijkl$th cow or error term.

The results were presented as least-square means ± standard error per RFI category. Significance was declared at $P < 0.05$ and tendencies at $0.05 \leq P < 0.10$. The correlation analyses between greenhouse gas emission parameters were also performed using PROC CORR in

Fig. 1. Average daily individual residual feed intake (RFI) predicted from 3 to 140 days in milk expressed in kg of dry matter intake (DMI)·d^{-1}. Each bar indicates daily RFI for each cow ($n = 131$). SD, standard deviation.

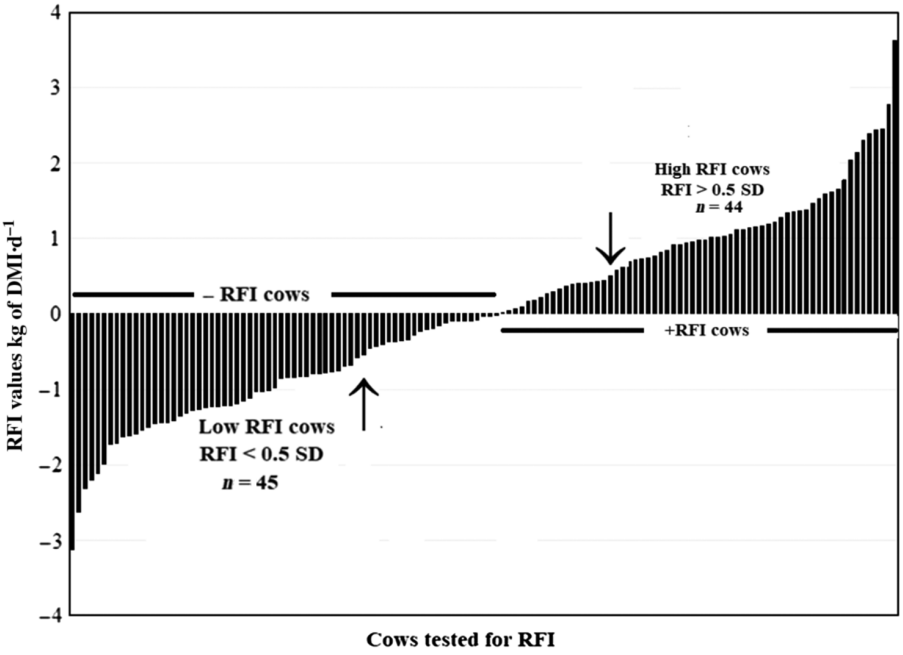
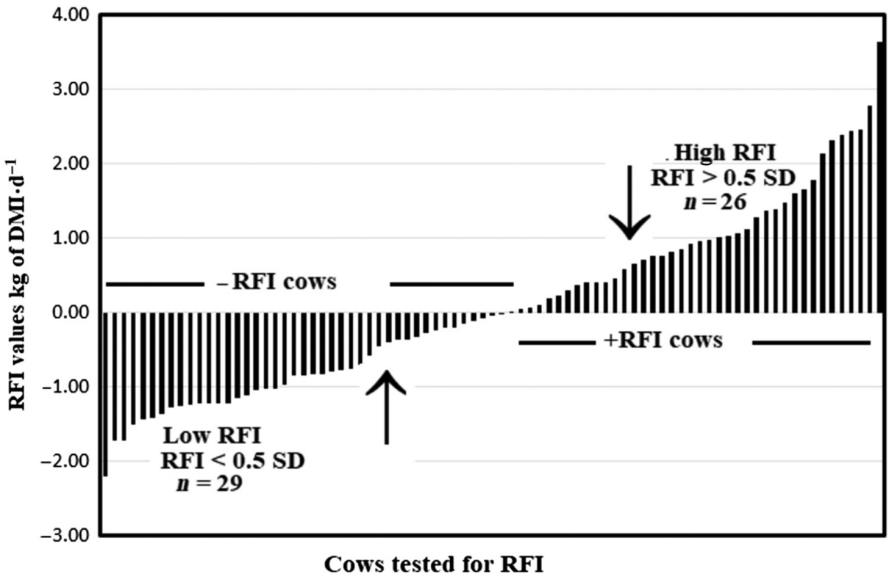


Fig. 2. Average daily individual residual feed intake [RFI; kg of dry matter intake (DMI)·d^{-1}] predicted from 3 to 240 days in milk. Each bar indicates daily RFI for 83 cows (subset of 131) that had methane and carbon dioxide emission data. SD, standard deviation.



SAS, and results were presented with Pearson's correlation coefficients and *P* values. Post hoc mean comparisons were applied to compare differences among means with adjusted *P* value when applicable.

Results

Prediction of RFI for lactating dairy cows

The adjusted R^2 for the RFI prediction model was 0.86. The average daily RFI values for the 131 cows ranged from −3.13 to 3.63 kg of DMI·d^{-1} with a mean value of zero (0.00 ± 1.23). Out of the 131 cows, 68 were −RFI (feed efficient), and the remaining 63 were +RFI (feed inefficient). The individual RFI values for the 131 lactating dairy cows are shown in Fig. 1. The RFI values for the 83 cows (a subset of the 131 cows) ranged from −2.20 to 3.63 kg of DMI·d^{-1} with the mean value close to zero (0.09 ± 1.20). The individual RFI values for the 83 cows are shown in Fig. 2.

Table 3. Comparisons (LSM ± SEM and corresponding *P* values) of metabolic body weight, DMI, milk yield, methane production, methane yield, methane intensity, and carbon dioxide emission in −RFI and +RFI groups.

	RFI groups (LSM ± SEM)			Significance level for main effects		
Parameters	−RFI	+RFI	*P* values	Batch	Parity	DIM
Number of cows	43	40	—	—	—	—
DIM[a]	106 ± 69	102 ± 67	—	—	—	—
Metabolic body weight ($BW^{0.75}$, kg)	127.0 ± 1.3	127.1 ± 1.3	0.958	***	***	NS
DMI (kg·d^{-1})	22.3 ± 0.40	24.2 ± 0.40	<0.001	NS	***	NS
Milk yield (kg·d^{-1})	40.1 ± 0.95	40.9 ± 0.93	0.555	NS	***	NS
Methane production (g·d^{-1})	343.5 ± 11.1	380.4 ± 10.9	0.014	***	*	NS
Methane yield (g·kg^{-1} DMI)	15.5 ± 0.48	15.9 ± 0.48	0.600	***	NS	NS
Methane intensity (g·kg^{-1} milk)	8.9 ± 0.39	9.7 ± 0.38	0.099	**	NS	NS
Carbon dioxide emission (g·d^{-1})	12 614 ± 174.7	12 949 ± 172.0	0.153	***	***	NS

Note: LSM, least square means; SEM, standard error of mean; DMI, dry matter intake; RFI, residual feed intake; DIM, days in milk; BW, body weight; NS, not significant. Significance levels of main effects: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; NS, $P > 0.05$.

[a]DIM was expressed as mean and standard deviation.

Table 4. Comparisons (LSM ± SEM and corresponding *P* values) of metabolic body weight, DMI, milk yield, methane production, methane yield, methane intensity, and carbon dioxide emission in low- and high-RFI groups.

	RFI groups (LSM ± SEM)			Significance level for main effects		
Parameters	Low-RFI	High-RFI	*P* values	Batch	Parity	DIM
Number of cows	29	26	—	—	—	—
DIM[a]	123 ± 70	94 ± 66	—	—	—	—
Metabolic body weight ($BW^{0.75}$, kg)	126.4 ± 1.6	125.8 ± 1.5	0.218	***	***	NS
DMI (kg·d^{-1})	21.1 ± 0.46	24.2 ± 0.45	<0.001	NS	***	*
Milk yield (kg·d^{-1})	39.3 ± 1.1	40.7 ± 1.1	0.355	NS	***	NS
Methane production (g·d^{-1})	332.5 ± 12.9	393.5 ± 12.6	0.004	***	*	NS
Methane yield (g·kg^{-1} DMI)	15.4 ± 0.58	16.0 ± 0.41	0.304	***	NS	NS
Methane intensity (g·kg^{-1} milk)	8.7 ± 0.46	10.1 ± 0.45	0.074	**	NS	NS
Carbon dioxide emission (g·d^{-1})	12 500 ± 205	13 168 ± 200	0.048	***	***	NS

Note: LSM, least square means; SEM, standard error of mean; DMI, dry matter intake; RFI, residual feed intake; DIM, days in milk; BW, body weight; NS, not significant. Significance levels of main effects: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; NS, $P > 0.05$.

[a]DIM was expressed as mean and standard deviation.

Enteric CH_4-related traits and RFI in lactating Holsteins

The results in −RFI vs. +RFI comparison groups indicated that −RFI cows had ($P < 0.05$) lower DMI (22.3 ± 0.40 vs. 24.2 ± 0.39) compared with their +RFI counterparts. The result showed a 7.9% relative decrease in DMI (kg·d^{-1}) in −RFI cows considering +RFI group as a reference. Dry matter intake was calculated as the average DMI over the CH_4 emission measurement period (14 d), and CH_4 emission measurement was conducted in seven batches. Batch, parity, and DIM were included in the model (DMI, dependent variable) as fixed effects, and only parity was significant ($P < 0.001$). Similar to DMI, CH_4 production (CH_4 g·d^{-1}) was significantly lower in −RFI compared with +RFI with 343.5 ± 11.1 and 380.4 ± 10.9 (g·d^{-1}) average daily emission, respectively. Among the fixed effects considered in the model (batch, parity, and DIM), batch ($P < 0.001$), and parity ($P = 0.02$) were significant. The comparison between −RFI and +RFI (343.5 ± 11.1 vs. 380.4 ± 10.9) showed 9.7% relative decrease in the daily CH_4 production for feed efficient (−RFI) cows. Methane intensity (CH_4 g·kg^{-1} milk) tended ($P = 0.097$) to be lower in the −RFI group. However, CH_4 yield (CH_4 g·kg^{-1} DMI), CO_2 emission (g·d^{-1}), MBW (kg), and milk production (kg) did not differ ($P > 0.05$) between −RFI and +RFI groups (Table 3).

In the low- vs. high-RFI comparison, DMI, CH_4 production, CH_4 yield, CH_4 intensity, MBW, and milk production showed similar significance levels as in the comparison between −RFI and +RFI cows (Table 4). Carbon dioxide emission was significantly ($P < 0.05$)

Table 5. Correlations between methane emissions-related traits in dairy cows (Pearson's correlation coefficients, *r* and *P* values).

	DMI (kg·d^{-1})		CH_4 g·d^{-1}		CH_4 g·kg^{-1} milk		CO_2 emission (g·d^{-1})	
Parameters	*r*	*P* values	*r*	*P* values	*r*	*P* values	*r*	*P* values
RFI (kg DMI·d^{-1})	0.34	0.001	0.32	0.003	0.13	0.239	0.15	0.187
Metabolic body weight (kg)	0.66	<0.001	0.18	0.089	−0.06	0.582	0.32	0.004
Dry matter intake (kg·d^{-1})	1.00	—	0.43	<0.001	0.05	0.671	0.38	0.004
Methane yield (g·kg^{-1} DMI)	−0.21	0.052	0.78	<0.001	0.75	<0.001	0.43	<0.001
Methane production (g·d^{-1})	0.43	<0.001	1.00	—	0.74	<0.001	0.66	<0.001
CO_2 emission (g·d^{-1})	0.38	0.004	0.66	<0.001	0.42	<0.001	—	—
Methane intensity (g·kg^{-1} milk)	0.05	0.671	0.74	<0.001	1.00	—	0.42	<0.001
Milk production (kg·d^{-1})	0.53	<0.001	0.20	0.065	−0.48	<0.001	0.26	0.018

Note: Data from 83 cows were used for the correlation analyses.

lower in the low-RFI group. The low-RFI group showed a 5.1% relative decrease in CO_2 emission with daily average values of 12 500 ± 205.5 and 13 168 ± 200 g·d^{-1} in low- and high-RFI groups, respectively. Dry matter intake was significantly ($P < 0.001$) lower in low-RFI groups (21.1 ± 0.40 vs. 24.2 ± 0.45), and the most efficient cows consumed less by 12.8%. The low-RFI groups also showed significantly ($P < 0.05$) decreased CH_4 production (332.5 ± 12.9 vs. 393.5 ± 12.6) by 15.5%. A tendency ($P < 0.1$) of reduction in CH_4 intensity was observed in the high- vs. low-RFI group (8.7 ± 0.46 vs. 10.1 ± 0.45). The significance levels of fixed effects (batch, parity, and DIM) were similar in both −RFI vs. +RFI and low- vs. high-RFI comparisons, except for DIM (Table 4).

Correlations between CH_4 emission-related traits

With the intention to scrutinize the association between CH_4 emission-related traits used in the study, we performed a correlation analysis between these traits, shown in Table 5. DMI was positively correlated with RFI ($r = 0.34$; $P < 0.05$), MBW ($r = 0.66$; $P < 0.001$), CH_4 production ($r = 0.43$; $P < 0.001$), CO_2 emission ($r = 0.38$; $P < 0.001$), and milk production ($r = 0.53$; $P < 0.001$). Methane production (CH_4, g·d^{-1}) was also positively correlated with RFI ($r = 0.32$; $P < 0.05$), CH_4 yield ($r = 0.78$; $P < 0.001$), CO_2 production ($r = 0.66$; $P < 0.001$), and CH_4 intensity ($r = 0.74$; $P < 0.001$). Methane intensity was positively correlated with CH_4 yield ($r = 0.75$; $P < 0.001$), CH_4 production ($r = 0.74$; $P < 0.001$), and negatively correlated with milk production ($r = -0.48$; $P < 0.001$).

Milk composition and RFI

Protein content was lower ($P < 0.05$) for −RFI cows with least-square means of 3.11 ± 0.009 and 3.14 ± 0.009 in −RFI and +RFI groups, respectively. Milk fat and lactose contents did not differ between groups. All fixed effects considered in the analysis for fat content were not significant in the model, except MT ($P < 0.001$). For protein content parity, MT and DIM were significant, and a tendency ($P < 0.1$) was observed for RFI by parity interaction. However, RFI × MT was not significant. For lactose, all main effects were significant ($P < 0.001$), except for RFI by MT interaction (Table 6).

In the high- vs. low-RFI comparison, protein content was lower ($P < 0.05$) in the low-RFI group (3.11 ± 0.01 vs. 3.15 ± 0.01) and lactose content was higher ($P < 0.001$) low-RFI cows (4.57 ± 0.006 vs. 4.56 ± 0.006), but no difference in fat content was observed. Among the main effects considered, fat content was only affected by MT. Milk protein content was affected by parity, MT, DIM, and RFI × MT. Interaction of RFI by parity had no effect on protein content. All the main and interaction effects had a significant effect on lactose content, except for the RFI by MT interaction (Table 6).

Comparison of SCC, BHB, and MUN in RFI categories

The analyses showed that SCC was significantly ($P < 0.001$) lower in −RFI than the +RFI group (Table 6). The PROC MIXED model analysis for SCC (10^3·L^{-1}) showed least-square means of 168.9 ± 21.6 and 302.9 ± 22.0 in −RFI and +RFI cows, respectively. Parity and RFI × parity were significant ($P < 0.05$) in the model. Days in milk and MT showed tendencies ($P < 0.01$). Milk BHB was not different between −RFI and +RFI groups. Interestingly, all the main effects were significant in the model.

In the low-RFI vs. high-RFI comparison, both SCC and BHB were lower in low-RFI groups. Milk somatic cell count had least-square mean of 152.3 ± 26.1 and 326.8 ± 25.5 in low- and high-RFI groups, respectively (Table 6). Parity and the interaction of RFI by parity were significant in the model. The BHB was affected by all the main effects and interactions (parity, MT, DIM, RFI × MT, and RFI × parity).

Milk urea nitrogen was higher in −RFI cows with least-square means of 13.6 ± 0.11 and 12.7 ± 0.12 in −RFI and +RFI cows, respectively. Parity, MT, DIM, and RFI × parity had a significant effect on MUN. Similar results were observed when MUN was compared between high- and low-RFI groups with least-square means of 13.6 ± 0.14 and 12.3 ± 0.13 for low- and high-RFI cows, respectively (Table 6).

Table 6. LSM and SEM of fat, protein, lactose, SCC, MUN, and BHB in −RFI vs. +RFI and high- vs. low-RFI comparisons.

	RFI groups (LSM ± SEM)			Significance levels for main effects and interactions				
Parameters	−RFI	+RFI	*P* value	Parity	MT	DIM	RFI × MT	RFI × parity
Number of cows	68	63	—	—	—	—	—	—
Fat (%)	3.6 ± 0.10	3.8 ± 0.11	0.122	NS	***	NS	NS	NS
Protein (%)	3.11 ± 0.009	3.14 ± 0.0009	0.007	***	***	***	NS	NS
Lactose (%)	4.6 ± 0.005	4.6 ± 0.005	0.436	***	***	***	NS	***
SCC (10^3·mL^{-1})	168.9 ± 21.6	302.9 ± 22.0	<0.001	***	NS	NS	NS	***
MUN (mg·dL^{-1})	13.6 ± 0.11	12.7 ± 0.12	<0.001	***	***	***	NS	***
BHB (mmol·L^{-1})	0.089 ± 0.002	0.091 ± 0.002	0.637	***	***	*	**	*
Comparison between low-RFI (RFI < 0.5 SD) and high-RFI (RFI > 0.5 SD) categories								
	Low-RFI	High-RFI						
Number of cows	45	44	—	—	—	—	—	—
Fat (%)	3.5 ± 0.13	3.9 ± 0.12	0.105	NS	***	NS	NS	NS
Protein (%)	3.11 ± 0.01	3.15 ± 0.01	0.002	***	***	***	**	NS
Lactose (%)	4.57 ± 0.006	4.56 ± 0.006	0.001	***	***	***	NS	***
SCC (10^3·mL^{-1})	152.3 ± 26.1	326.2 ± 25.5	<0.001	***	NS	NS	NS	***
MUN (mg·dL^{-1})	13.6 ± 0.14	12.3 ± 0.13	<0.001	***	***	***	NS	**
BHB (mmol·L^{-1})	0.088 ± 0.002	0.093 ± 0.002	0.048	***	***	*	NS	***

Note: LSM, least square means; SEM, standard error of means; SCC, somatic cell count; MUN, milk urea nitrogen; BHB, β-hydroxybutyrate; RFI, residual feed intake; MT, milking time; DIM, days in milk; SD, standard deviation; NS, not significant. The *P* values were given for the comparisons between the RFI groups and significance levels were indicated for main effects. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; NS, $P > 0.05$.

Discussion

Enteric CH_4 emission and RFI

Feed efficient (−RFI) and inefficient (+RFI) lactating cows differed in CH_4 production (CH_4 g·d^{-1}); where −RFI cows showed lower CH_4 production by 9.7%. The decrease in CH_4 production was 15.5% when high- and low-RFI groups were compared. In addition, the correlation analysis revealed a positive correlation between RFI and daily CH_4 emission (CH_4 g·d^{-1}). This agrees with the findings that among animals, differences in CH_4 production are mainly driven by the amount of feed consumed (Brask et al. 2015). Therefore, the reduction in CH_4 production (g·d^{-1}) in the −RFI or low-RFI groups suggests that cows in these groups had less CH_4 production because they consumed less feed. In addition, the decreased CH_4 production in the −RFI and low-RFI group observed in our study could be influenced by differences in genetic and rumen microbiome composition (Difford et al. 2018). The ruminal bacterial community is dynamic in terms of membership and diversity in dairy cows, and specific members are associated with high and low milk production efficiency over two lactation cycles (Jewell et al. 2015). In addition, the decreased CH_4 production in feed efficient or most efficient cows could be partly due to the energy loss associated with CH_4 production. Energy loss due to CH_4 ranges from 2% to 12% of gross energy intake (Johnson and Johnson 1995), indicating that the −RFI and low-RFI cows lose less energy because they emit less CH_4 compared with +RFI and high-RFI cows. Nkrumah et al. (2006) reported 28% and 24% less CH_4 production (g·kg^{-1} BW$^{0.75}$) in low-RFI animals compared with high- and mid-RFI animals, respectively. Similarly, Fitzsimons et al. (2013) reported that CH_4 production (g·kg^{-1} BW$^{0.75}$) was highest in high-RFI, intermediate in mid-RFI, and lowest in low-RFI beef heifers. In another study, 25% lower CH_4 production (g·d^{-1}) between high- and low-RFI RFI steers was reported (Hegarty et al. 2007). However, this result contradicts with the recent report that showed no difference between high- and low-RFI categories of Holstein and Jersey heifers in CH_4 production (Flay et al. 2019). The reason for the inconsistency between our result and Flay et al. (2019) could be due to differences in diet, experimental design, and physiological status of the experimental animals. Flay et al. (2019) used growing Holstein and Jersey heifers ($n = 28$) and measured CH_4 emission 2 mo after RFI prediction. This approach is prone to re-ranking of cows and may have affected detection of a difference between the comparison groups. In our study, we used mixed parity lactating dairy cows (−RFI vs. + RFI, $n = 83$; low- vs. high-RFI, $n = 55$), and we measured CH_4 emission and RFI at the same time. Previous reports on the relation between RFI and CH_4 emission are more available in beef than dairy, and our result can provide the missing experimental evidence for the dairy cattle.

The −RFI vs. +RFI and low- vs. high-RFI comparisons did not show a difference ($P > 0.05$) in CH_4 yield (CH_4 g·kg^{-1} DMI), and the result is consistent with a previous study in beef cattle (Fitzsimons et al. 2013) where no difference in CH_4 yield was reported between high- and low-RFI groups. Methane intensity tended to be lower in both −RFI vs. +RFI and low- vs. high-RFI comparisons. Velazco et al. (2016) reported that beef cattle divergent for RFI groups did not necessarily differ in CH_4 emission intensity. Methane emission intensity is increasingly proposed as a mechanism to value livestock emissions as it relates the emissions with the level of saleable products from the animal (Hristov et al. 2013). Methane intensity (CH_4·kg^{-1} milk) was positively correlated with RFI but negatively correlated with milk production, indicating that selection for cows with higher milk yield could lead to lower CH_4 intensity.

Carbon dioxide emission was lower for the low-RFI group compared with the high-RFI by 5.1% and positively correlated with CH_4 production ($r = 0.66$, $P < 0.001$; Table 4). Given CO_2 is a by-product of rumen fermentation and reduced to CH_4 in the process of methanogenesis, the observed positive correlation is not a surprise. Methanogenesis often uses the hydrogen and CO_2 produced by carbohydrate fermentation, as volatile fatty acids are formed (Hungate et al. 1970). The proportions of volatile fatty acids affect the amount of CH_4 produced because propionate formation consumes reducing equivalents, whereas acetate and butyrate formation generate H_2 for methanogenesis (reviewed in Knapp et al. 2014). The amount of feed fermented is one of the factors determining variations in CH_4 production between animals (Brask et al. 2015), and the decreased CO_2 emission observed in low-RFI categories may be explained by lower DMI (Table 4) of cows in this group.

Dry matter intake and RFI

Dry matter intake was decreased by 7.9% and 12.8% in −RFI vs. +RFI and high- vs. low-RFI categories, respectively. Flay et al. (2019) reported comparably lower DMI (9.3%) in low-RFI Holstein and Jersey heifers. Our result is slightly lower than the 15% DMI reduction (Connor et al. 2013) in low-RFI mixed parity Holstein cows when compared with high-RFI cows. Williams et al. (2011) also showed a reduction in DMI in Holstein-Friesian heifers where the bottom 10% consumed 15% to 20% less feed relative to heifers in the top 10% for RFI. The observed differences in DMI in −RFI vs. +RFI and high- vs. low-RFI cows may be explained by feeding behavior (Fitzsimons et al. 2017), which includes frequency and duration of individual feeding events. High-RFI cattle spent more time eating and had a faster eating rate than their low-RFI counterparts (Kenny et al. 2018). Low-RFI cows had higher digestive ability (Bonilha et al. 2017) most likely due to lower DMI (Cantalapiedra-Hijar et al. 2018).

The positive correlation of DMI with RFI that we observed in this study agrees with the report in Potts et al. (2015), where least efficient cows consumed less under high- and low-starch diet conditions (DMI, kg·d^{-1}: 25.4 vs. 29.6, $P < 0.01$ and 23.0 vs. 28.8, $P < 0.01$, respectively). Moreover, stronger phenotypic and genetic correlations of DMI and RFI were reported in the Netherlands and US dairy populations (Manzanilla-Pech et al. 2016). The heritability of RFI and DMI in primiparous Holstein cows was reported as 0.13 and 0.23, respectively (Hardie et al. 2017). Therefore, the lowered DMI that we observed in −RFI and low-RFI compared with their respective +RFI and high-RFI counterparts reaffirms the potential of reducing feed cost by selecting for feed efficient (−RFI) or the most efficient (low-RFI) lactating dairy cows from the herd.

Milk composition in RFI groups

The comparison between efficient (−RFI) vs. inefficient (+RFI) as well as high- vs. low-RFI cows showed no difference in milk fat percentage. This is expected given that RFI is adjusted for milk fat content. The result is in agreement with the study by Montanholi et al. (2013) in beef cattle, where no relationship was observed between RFI vs. milk fat ($r = 0.17$, $P > 0.05$). Olijhoek et al. (2018) reported that milk fat content was higher for low-RFI cows compared with high-RFI cows. Milk protein content was lower ($P < 0.05$) in the low-RFI group and lactose content was higher ($P < 0.05$) in the low-RFI group. Both milk protein and lactose content were adjusted in the RFI calculation for the NE, and the differences observed in low- vs. high-RFI cows in protein and lactose might be due to differences in protein and lactose metabolism and absorption between RFI groups.

Somatic cell count, BHB, MUN, and RFI in dairy cows

Our results showed that −RFI and low-RFI cows had lower SCC and BHB compared with +RFI and high-RFI counterparts. The present study is in agreement with the study by Potter et al. (2018) that reported the association of increased SCC and reduced feed efficiency in lactating dairy cows. Olson et al. (2011) reported that an incidence of mastitis reduced feed efficiency in Holstein, Jersey, and reciprocal F1 crossbred cows. It is important to note that the health status and activity of the immune function of the animal will exert an effect on feed efficiency (Bach et al. 2020), and that the energy cost of activating the immune system has been reported to be 0.64 g of glucose·kg^{-1} of metabolic BW per hour in dairy cows (Kvidera et al. 2017). Therefore, cows with increased SCC could spend more energy on immune function than cows with lower SCC, and this could be the likely link between lower SCC and increased feed efficiency in dairy cattle. Conversely, it is not possible to rule out a favorable genetic correlation between SCC and feed efficiency. Therefore, devising a mechanism to account for the energy cost of the immune response in the RFI calculation could help to fine tune the accuracy of the RFI estimates.

It is well established that SCC in milk samples is used as a diagnostic test for subclinical mastitis (Hillerton 1999,

Viguier et al. 2009, Sharma et al. 2011), and a threshold is established above which cows are categorized as subclinical mastitis positive at 200 000 cells·mL^{-1} of raw milk. Somatic cells are mostly cells of the immune system and include lymphocytes, macrophages, polymorphonuclear cells, and some epithelial cells (Pillai et al. 2001). Somatic cells are a reflection of the inflammatory response to an intramammary infection or another trigger of the immune system (Schukken et al. 2003); therefore, SCC could be used as a measure of udder health.

The most efficient cows had decreased milk BHB ($P < 0.05$) when compared with the least efficient cows. Milk BHB is a diagnostic marker for ketosis and its elevated concentration in milk or blood implicated in development of hyperketonemia (Denis-Robichaud et al. 2014) and other metabolic disorders (McArt et al. 2013). Rathbun et al. (2017) reported absence of relation between RFI and hyperketonemia in dairy cows where RFI was calculated 50–200 d relative to calving. The result agrees with our study for the comparison of milk BHB in −RFI vs. +RFI that showed lack of relationship between RFI and milk BHB; however, disagrees with the comparison between low- and high-RFI group that showed an increased ($P < 0.05$) BHB concentration in high-RFI group compared with the low-RFI. The discrepancy between our results and those of Rathbun et al. (2017) could be due to sample size and method of comparison, where in our case, we compared the two extreme groups (low vs. high) to increase between-group variation, which could result in a significant difference. Even though the high-RFI cows had significantly increased milk BHB compared with the low-RFI cows, the mean BHB (mmol·L^{-1}) was lower than the threshold level for hyperketonemia. This implies that the observed difference between low- and high-RFI groups in milk BHB falls short to claim association between milk BHB concentration and hyperketonemia. Even though subclinical ketosis primarily occurs during early lactation, decreased levels of prevalence has been reported in mid and late lactation stages. The prevalence of subclinical ketosis for cows in early (<65 DIM), mid (65–149 DIM), and late (>149 DIM) lactation stages were 14.1%, 5.3%, and 3.2%, respectively (Duffield et al. 1997). The current study included milk BHB records between 3–240 DIM and intended to see a general perspective of the association between milk BHB concentration and RFI during the lactation period. Breaking down the association analyses into the different lactation stages could show more specific results.

Powell et al. (2014) reported that MUN and urine urea nitrogen are strongly correlated with dietary CP, and monitoring of MUN may be used to enhance dietary CP use and to reduce urine urea nitrogen excretions and nitrogen emissions from dairy farms. The conversion efficiencies of dietary nitrogen into milk nitrogen in dairy cows range from 25% to 35% (Gourley et al. 2012), and in our result, higher MUN was observed in both −RFI and low-RFI groups compared with the +RFI and high-RFI counterparts, suggesting feed efficient or most efficient cows had higher conversion efficiency of dietary nitrogen to MUN. Given that urea synthesis is an energy consuming process, the observation that −RFI and low-RFI cows had elevated MUN appears contradicting. However, this difference might be due to increased protein catabolism or muscle efflux of amino acids that could promote ureagenesis and gluconeogenesis in −RFI and low-RFI cows. Most of the amino groups of the excess amino acids are converted into urea through the urea cycle, whereas their carbon skeletons are transformed into other intermediates, mostly glucose (Schutz 2011). Therefore, even if the urea cycle is energy consuming process, it contributes to the de novo glucoses synthesis, which is the most important source of energy.

Conclusions

This study found that −RFI and low-RFI cows had decreased DMI and CH_4 production (g·d^{-1}) when compared with +RFI and high-RFI cows, respectively. Moreover, our results showed that the low-RFI cows had lower milk SCC and BHB than the high-RFI counterparts. This study was conducted in a single herd, and results need to be confirmed with a larger sample size.

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