Performance and Methane Emissions of RFI Selected Cattle in Drylot and Under Open Range Conditions

by

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Abstract

Residual feed intake (RFI) is a moderately heritable trait that can be used to measure feed efficiency in beef cattle, and thereby reduce feed related costs. RFI has been primarily evaluated under drylot conditions where diet, feed intake and activity levels are controlled and foraging behaviour is eliminated. Although previous studies have tried to measure RFI on monoculture pasture, it is difficult to accurately determine individual feed intake. The objective of this study was to determine whether there is a difference in performance (change in weight, change in backfat, and pregnancy status) between cattle with molecular breeding values (MBVs) for high and low RFI while foraging under open range conditions. This research also examined differences in methane (CH_4) emissions and dry matter intake (DMI) of high and low RFI heifers with phenotypic RFI values derived in drylot. This research was conducted at the University of Alberta Mattheis Research Ranch. A total of 450 commercial Hereford/Angus cows, with predicted MBVs for RFI, were separated into groups of high, low and medium efficiency. High RFI cows were bred to high RFI bulls, low RFI cows were bred to low RFI bulls and medium RFI cows were bred to medium RFI bulls, where the bulls had their own phenotypes to produce groups of high, low and medium RFI calves. Production metrics, such as cow weight gain, change in backfat and pregnancy status, along with calf growth were collected for the 2015 grazing season. A subset of 60 replacement heifers, selected based on the MBVs of associated dams (30 high and 30 low RFI) were tested for actual feed intake and CH_4 production using GrowSafe and GreenFeed technologies, respectively. A smaller subset of 18 heifers were tested for individual feed intake and CH_4 production while grazing forage oat pasture using an open-path laser system to monitor emissions and a paired n-alkane methodology to predict feed intake. There were no significant differences in the change in weight, change in backfat or pregnancy status between high and low genomically predicted RFI (gRFI) cows. Additionally, high and low gRFI calves had similar growth over the grazing period. High RFI_{FAT} (RFI corrected for backfat) replacement heifers had significantly greater DMI in drylot when compared to the low RFI_{FAT} heifers. Despite differences in DMI, high and low RFI_{FAT} heifers did not differ in growth while in drylot. Methane production in drylot was not significantly different,

however low RFI_{FAT} heifers had significantly greater CH_4 yields. On pasture, there was no significant difference in DMI or CH_4 production or yield between high and low RFI_{FAT} heifers, although there was a trend towards high RFI_{FAT} heifers having greater DMI and producing more CH_4. Ultimately the results indicate that selection for RFI in cow-calf herds on pasture will not compromise productivity of those cattle and it should not impact their ability to produce offspring. Additionally, selection is likely to reduce feed intake and CH_4 emissions on both pasture and in drylot. Future research is necessary to validate the pasture DMI and CH_4 emission results.

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LIST OF SYMBOLS AND ABBREVIATIONS

AAFC – Agriculture and Agri-Food Canada

ADF – acid detergent fibre

ADG – average daily gain

ARD – Alberta Agriculture and Rural Development

AAF – Alberta Agriculture and Forestry

ANCOVA – analysis of covariance

ANOVA – analysis of variance

BCRC – Beef Cattle Research Council

BFend - backfat at the end of the trial

BW – body weight

C_{31} – alkane with 31 carbons

C_{32} – alkane with 32 carbons

Ca – calcium

CH_4 – methane

CO_2 – carbon dioxide

CP – crude protein

CVAS – Cumberland Valley Analytical Services

DM – dry matter

DMI – dry matter intake

DNA – deoxyribonucleic acid

D_j – dose rate

EFI – expected feed intake

FCR – feed conversion ratio

F_i – fecal C_{31} concentration

F_j – fecal C_{32} concentration

GEMS – GreenFeed Emissions Monitoring System

GHG – greenhouse gas

gRFI – genotypic RFI

H_i – forage C_{31} concentration

H_j – forage C_{32} concentration

HSD – Honest Significant Difference

H+ – Hydrogen ion

IGF-1 – Insulin-like Growth Factor 1

IPCC – Intergovernmental Panel on Climate Change

IS – pellet intake

LSM – least square mean

MAS – marked assisted selection

MBV – molecular breeding value

ME – metabolizable energy

MIDWT – metabolic midweight

NDF – neutral detergent fibre

NRC – National Research Council

OP-FTIR – Open Path Fourier Transform Infrared

P – phosphorus

pRFI – phenotypic RFI

QTL – quantitative trait loci

RFI – residual feed intake

RFID – radio frequency identification

RFI_{FAT} – RFI adjusted for backfat

rRNA – ribosomal ribonucleic acid

SAS – Statistical Analysis Software

SDMI – standardized daily dry matter intake

SF_6 – sulfur hexafluoride

S_i – pellet C_{31} concentration

S_j – pellet C_{32} concentration

SNP – single nucleotide polymorphism

TDN – total digestible nutrients

VFA – volatile fatty acid

50K – fifty thousand

Chapter 1: Introduction and Literature Review

Introduction to Feed Efficiency

Feed efficiency in beef cattle is a concept which came about shortly after World War II when nutrition, management and feeding innovations allowed for more efficient production of beef (Riggs 1958). Riggs (1958), explained that success in the beef industry began with a better understanding of chemical composition and digestibility of feedstuffs. Ultimately, efficiency was achieved when the correct feedstuffs were used in the optimal conformation and ratios. Today, approximately 70-75% of total dietary energy intake is used for maintenance, leaving only about 30% of feed intake for growth and reproduction (Ferrell and Jenkins 1985; Montano-Bermudez et al. 1990; National Research Council (NRC) 1996). Feed is one of the largest variable costs in the beef industry (Alberta Agriculture and Rural Development (ARD) 2005; Ramsey et al. 2005), making it an important determinant of profitability. With intentions of increasing profitability, the beef industry looks to maximize production efficiency while minimizing input costs. Selecting for more efficient cattle is one way that costs may be reduced.

Testing for Feed Efficiency

Historically, cattle efficiency measures were dependent on the feed conversion ratio (FCR), a ratio of feed intake to body weight gain. Animals with a low FCR consume less feed per kilogram of body weight gain, while animals with higher FCR consume more feed for unit of weight gain. Industry has moved away from using FCR however, because it has little value as a trait used to genetically improve efficiency, even with its moderate heritability (Crews 2005). The primary limitation of FCR is that it represents a gross measure of feed intake, meaning it does not distinguish between maintenance and growth requirements (Carstens and Tedeschi 2006). It is difficult to select for low maintenance requirements because the feed to gain ratio is related to growth rate and body size (Arthur et al. 2001). As a result, selecting for increased growth and improved FCR is likely to result in increased maintenance requirements (Van der Werf 2004; Crews 2005) rather than growth. Genetic evaluation for FCR is also unpredictable and can be problematic because usually more emphasis is placed on the trait with greater

genetic variance (Gunsett 1984; Kennedy et al. 1993; Van der Werf, 2004). Koots et al. (1999) and Berry (2012) explained that the genetic correlation between the numerator and the denominator in a FCR relationship is positive, suggesting selection for improved FCR leads to cattle that grow faster, but with a greater mature size and increased maintenance and feed requirements (Bishop et al. 1991; Archer et al. 1999; Herd and Bishop 2000; Crews 2005; Kelly et al. 2010, 2010a). Feed conversion and gross feed efficiency calculations are not phenotypically independent of growth and body size (Meyer et al. 2008), meaning that selection for cattle with superior FCR will inadvertently result in larger cattle with higher maintenance requirements. Selection decisions based on FCR are therefore likely to decrease feed efficiency in the long run.

Residual Feed Intake

Residual feed intake (RFI) is a measure of feed efficiency, calculated as the difference between actual and expected feed intake (Koch et al. 1963; Arthur et al. 1996; Basarab et al. 2003; Nkrumah et al. 2006). RFI is a concept which was initially proposed by Byerly (1941) and first defined by Koch et al. (1963). RFI is now identified as the measure of choice when determining efficiency in beef cattle (Herd and Arthur 2009). Koch et al. (1963) proposed that feed intake should be separated into two components, including (1) feed intake for a given level of production, and (2) the residual portion of feed. Remaining (i.e. residual) feed could be used to identify animals that deviate from the expected level of intake, with animals having greater negative residuals identified as being more efficient (Koch et al. 1963). Calculating RFI requires estimation of an animal's expected feed intake, calculated using performance data from contemporary groups of animals while also considering the animal's weight and their expected growth over a given period of time (Carstens 2006). By definition, RFI is considered independent of production, growth and body size (Koch et al. 1963; Kennedy et al. 1993; Crews 2005). Herd et al. (2014) explained that selection for animals with low RFI resulted in decreased feed intake without compromising body size or growth. Basarab et al. (2003) concluded that low RFI cattle had lower feed costs by $45.80

head^{-1} over a 120-day feeding period ($0.101kg^{-1} as fed); suggesting that selecting for RFI could reduce overall production costs.

RFI can be used as a tool by producers to increase the efficiency of their cattle, especially considering that, along with other feed intake measures, RFI is moderately heritable (h^2 = 0.29-0.46) (Archer et al. 1998; Arthur et al. 2001; Schenkel et al. 2004; Bouquet et al. 2010). Moderate to high heritability in genetic variation suggests that there is an opportunity to select for more efficient cattle with lower maintenance energy requirements (Bishop 1992; Carstens et al. 1989). Arthur et al. (2001a) noted significant improvements in RFI after only two generations of selection by breeding the most efficient dams with the most efficient sires. Selecting for RFI using a multi-trait selection index can result in a 0.75% to 1.0% genetic change per year (Basarab et al. 2013).

RFI is considered to be repeatable across different phases of the beef production cycle, as long as cattle are on similar diets (Kelly et al. 2010a). Durunna et al. (2011) reported lower phenotypic repeatability (rp=0.33 to 0.42) between successive feed intake tests where cattle were on diets differing in energy content (e.g., low vs. high energy). From the grower to the finisher phase, 51% to 58% of cattle were re-ranked (e.g., cattle with a negative RFI value on a grower diet had a positive RFI value on a finisher diet, or vice versa) for RFI due to the change in dietary energy (Durunna et al. 2011). Similar results were noted by Kelly et al. (2010), where 54% re-ranking in RFI occurred. Yet other studies (Crews et al. 2003; Carstens and Tedeschi, 2006; Kelly et al. 2010a) have identified a moderate to high positive repeatability (0.62) between RFI measured in animals on a grower diet and a finisher diet.

RFI in Drylot

Although selecting for RFI is likely to result in more efficient cattle, measuring individual feed intake and body weight gain is an expensive, and sometimes difficult, process (Moore et al. 2009). Phenotypic measures of RFI have been collected in several non-ruminant livestock species including pigs (Gilbert et al. 2007), laying hens (Luiting and Urff, 1991) and fish (Silverstein et al. 2005), where animals are often

fed and housed individually. In beef cattle, phenotypic measures of RFI are commonly collected in a drylot setting where the environment and feed intake are uniform and tightly controlled (Basarab et al. 2003; Wang et al. 2006). GrowSafe® (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) equipment automatically monitors individual animal feed intake and is often used to assess individual feed intake, and RFI, in a group of young growing cattle with a maximum age difference of 60 days (Basarab et al. 2003, 2011). Cattle being tested for RFI in drylot are subject to a 21-28-day dietary adjustment period (Basarab et al. 2003), which is followed by a 76-day test in which individual feed intake and animal growth are measured (Wang et al. 2006). Cattle are weighed on two consecutive days at the start of the 76-day trial, at 14-28-day intervals throughout the trial and then again on two consecutive days at the end of the trial period (Basarab et al. 2003). At least six weights are collected over the duration of the trial; more weights results in increased accuracy of growth rates. RFI values of slaughter cattle, heifers and bulls are adjusted for body fatness, as the latter is independent of RFI (Basarab et al. 2003, 2007, 2011; Schenkel et al. 2004; Crews, 2005). Therefore, ultrasound backfat measurements are also collected at the beginning, and sometimes the end, of the trial (Basarab et al. 2003 and 2011). Due to variation in DMI within and between-animals, as well as variation in growth patterns, tests must be long enough to accurately capture an animal's growth rate while also eliminating within-animal variations of DMI. Although individual feed intake can be accurately measured in 35-42 days (Wang et al. 2006), greater trial durations are necessary to accurately measure an animal's growth rate (Culbertson et al. 2015; Manafiazar et al. 2017) which is why current RFI trials are typically 76 days in length. Although Culbertson et al. (2015) concluded that a 56-day test would provide reliable and stable phenotypic RFI values, current test durations tend to be at least 70 days, allowing for days with data interruptions (i.e. power outages, data interference) to be eliminated, while still collecting sufficient days of data for the calculation of RFI.

RFI on Pasture

Previous RFI studies with cattle on pasture involved grazing of monoculture pastures and they have proven challenging due to the difficulty in calculating individual feed intake (Meyer et al. 2008). Moreover, RFI scores from the same cattle tested in drylot and on pasture will not necessarily be the same because feed type, feed intake, activity levels and the environment can vary widely. Biotic and abiotic factors in the environment can affect forage type and quality across a rangeland (Bailey et al. 1996), meaning that cattle productivity is driven by the forages and habitats which they select. It is important to understand the performance of RFI selected cattle on pasture because it can help determine whether RFI has an effect on the forages which cattle select, and whether foraging on pasture will have an effect on the performance of cattle selected for low RFI. Manafiazar et al. (2015) studied feed intake of 20 (10 high and 10 low RFI) beef heifers on pasture using n-alkanes. High RFI heifers consumed significantly more forage (kg DM day^{-1}) when expressed as a percentage of body weight (Manafiazar et al. 2015). Although not significantly different, Meyer et al. (2008) observed lower grazed forage intake of low RFI cows, when compared to high RFI cows, identified as being high or low RFI as heifers in drylot. Additionally, no differences were noted in body weight change, or body condition score change, between high and low RFI cows, suggesting similar performance on pasture (Meyer et al. 2008). Another pasture-based study, conducted by Herd et al. (2002a), studied divergently-selected backgrounding steers and reported that, although not statistically significant, low RFI steers had lower DMI and superior average daily gain (ADG). These studies prove that selecting for RFI in cattle on pasture should reduce their overall feed intake without hindering their growth and production.

Performance of Cattle with Divergent RFI

Previous studies have shown reduced feed intake by low RFI cattle when compared to their high RFI counterparts (Archer et al. 1998; Basarab et al. 2003; Castro Bulle et al. 2007; Fitzsimons et al. 2013; Manafiazar et al. 2015; McDonnell et al. 2016). Variation in feed intake was most often related directly to

variation in maintenance requirements (Herd et al. 2004). More specifically, Archer et al. (1998) observed that low RFI breeding bulls consumed 2.5 kg day^{-1} less feed than high RFI bulls over a 120 day test period. Similarly, Basarab et al. (2003) found that low RFI steers consumed 0.55 kg day^{-1} and 0.93 kg day^{-1} less feed than medium and high RFI steers, respectively. Castro Bulle et al. (2007) noted that low RFI steers had significantly lower feed intake than high RFI steers (6.61 kg day^{-1} vs. 7.52 kg day^{-1}). Similarly, Fitzsimons et al. (2013) tested heifers with differing RFI and reported that low RFI heifers consumed 9% and 15% less feed than medium and high RFI heifers, respectively. In addition, steers identified as low RFI in drylot consumed 5.3% less forage when fed meadow bromegrass (*Bromus riparius* Rehm) under penned conditions (Manafiazar et al. 2015). Although most studies have identified a lower DMI in low RFI cattle, Lawrence et al. (2012) did not detect any significant differences in DMI between grazing beef heifers of differing RFI classification.

A positive correlation has been identified between DMI and RFI, indicating that low RFI cattle consume less feed than high RFI cattle. RFI is moderately (r=0.47) to highly (r=0.72) correlated with feed intake (Arthur et al. 2001, 2001a; Herd et al. 2002; Basarab et al. 2003, Nkrumah et al. 2007; Kelly et al. 2010a). Nkrumah et al. (2004) found a significant correlation (r=0.77) between RFI and DMI of cattle fed high corn finishing rations. Fitzsimons et al. (2013) also noted a significant positive correlation (r=0.63) between RFI and DMI of beef heifers consuming a grass silage diet. Finally, McDonnell et al. (2016) compared the DMI of high and low RFI cattle across three different diets, including grass silage, perennial rye grass pasture (*Lolium perenne* L.) and a corn silage and concentrate total mixed ration (TMR) and found a significant correlation (r=0.5) between RFI and DMI only when cattle were fed the TMR (McDonnell et al. 2016). These results demonstrate the importance of evaluating RFI using several different feed ingredients, including forages that would be grazed on pasture.

Relationship between RFI and Carcass Characteristics

By definition, RFI is independent of growth and body size (Koch et al. 1963; Kennedy et al. 1993; Crews 2005), implying that there is no expected difference in growth between high and low RFI cattle (Kennedy

et al. 1993), although it should be noted that low growth rates can be seen in both efficient and inefficient cattle. Not only should high and low RFI cattle have similar growth, they also tend to have similar carcass characteristics (Basarab et al. 2003; Hill and Ahola, 2012). Castro Bulle et al. (2007) reported similar dressed carcass yield, backfat thickness, marbling score, and yield grade between high and low RFI cattle; low RFI steers tended to gain less, although not significantly (P= 0.078), compared to high RFI steers. Fitzsimons et al. (2013) also reported no differences in muscle depth, muscularity scores, fat depth or ultrasonic fat measures between high and low RFI heifers. No correlation was found between RFI and the following carcass measures; muscle depth, muscularity score, ultrasonic fat and body condition score (Fitzsimons et al. 2013). In contrast to these investigations, a weak positive correlation has been reported between RFI and ultrasound measures of fat depth (Arthur et al. 2001a; Basarab et al. 2003; Robinson and Oddy, 2004). More specifically, Basarab et al. (2003) and Arthur et al. (2001a), reported slight positive relationships (r=0.14 and r=0.11, respectively) between RFI and ultrasonic measures of fat depth at the 12^{th} and 13^{th} rib. Furthermore, RFI was noted to have a positive correlation (r=0.22) with ultrasound backfat measures (Basarab et al. 2003). Similarly, Richardson et al. (2001) concluded that RFI selection over a single generation was associated with a reduction in overall carcass fat content, indicating low RFI cattle tend to have lower fat measures.

Aside from carcass fat characteristics, Crowley et al. (2010) stated that improvements in feed efficiency should result in cattle with increased muscularity and as explained by Lawrence et al. (2011), increased muscularity appears to be associated with increased concentrations of creatinine, which is produced as a result of muscle breakdown. In sheep, creatinine concentrations were positively and negatively associated with muscle mass and fat depth, respectively (Cameron 1992; Clarke et al. 1996). Similarly, Richardson et al. (2001) reported a relationship between body chemical composition and RFI. More specifically, steer progeny of low RFI cows had less fat and more protein than progeny of high RFI cows (Richardson et al. 2001), and leptin concentrations were greater in high RFI steers (Richardson et al. 2004). Increased leptin concentrations are associated with increased fatness in cattle (Chillard et al. 1998;

Minton et al. 1998), suggesting that high RFI steers are fatter than low RFI steers. Along with leptin concentrations, urea concentrations were reported to have a negative relationship with lean growth in sheep (Cameron 1992; Clarke et al. 1996) and muscle protein content in dairy bulls (Robinson et al. 1992), while having a positive relationship with RFI in growing steers (Richardson et al. 2004). Collectively, these studies suggest that high RFI cattle are fatter than their low RFI counterparts.

Relationship between RFI and Cow Fertility

Past selection for FCR, which resulted in increased mature cow size and increased body weight gain, was also associated with a delay in the onset of puberty (Lesmeister et al. 1973). More recent studies considering the relationship between RFI and cow-calf production efficiency have identified that cows which produced low RFI progeny tended to calve 5 to 6 days later, likely resulting from a delay in the onset of puberty (Basarab, et al. 2007) as a result of lower body fat. Arthur et al. (2005) observed no significant difference in calving date of high and low RFI cows, though low RFI cows tended to calve later; overall cow productivity (i.e. ability to reproduce, maintain body weight and body condition, and ability to raise a healthy calf) remained the same between the two groups (Arthur et al. 2005). This same investigation found that high RFI cows had significantly more backfat, although it did not appear to have an effect on calving date (Arthur et al. 2005). Studies conducted in other multi-parous species have identified a positive correlation between egg number and RFI in poultry (Hagger 1994), as well as a weak positive correlation between RFI and litter size in mice (Hughes and Pitchford 2004), suggesting that selection for RFI over several generations could have a negative effect on fertility. Shaffer et al. (2011) conducted a study on a total of 137 yearling Angus, Angus-cross, and Hereford heifers, and analyzed their performance based on phenotypic RFI values. Results indicated that low RFI heifers reached puberty significantly later, and although not significantly different, tended to have lower pregnancy rates and lower conception rates compared to high RFI heifers. Linear relationships between RFI and age at puberty showed that the latter could be reduced by an average of 7.5 days with a one unit increase in RFI (Shaffer et al. 2011), and overall body fatness could be used to predict the onset of puberty. Greater levels of

backfat tend to increase luteinizing hormone activity (Schillo et al. 1992), resulting in earlier onset of puberty (Shaffer et al. 2011).

Selecting for RFI may also have an effect on fertility of breeding bulls. Wang et al. (2012) found that sperm motility was significantly lower in low RFI bulls as compared to high RFI bulls. More specifically, 10.2% of low RFI bulls, and 4.4% of high RFI bulls, did not meet the minimum sperm motility requirement of 60% (Wang et al. 2012). Although sperm motility was lower in efficient bulls, the average number of progeny per sire was significantly higher ($P < 0.01$) in low RFI bulls (Wang et al. 2012). Wang et al. (2012) speculated that a greater number of progeny per sire may come as a result of low maintenance requirements in low RFI cattle, therefore resulting in greater energy reserves for breeding. Wang et al. (2012) also suggested that feeding behaviors may contribute to the differences in progeny per sire, as low RFI cattle tend to spend less time eating (Durunna et al. 2011), making more time available for breeding. Fox et al. (2004) studied net feed intake of Bonsmara bulls to determine its effects on scrotal circumference and fertility. Net feed intake was not correlated with scrotal circumference and there was no difference in semen concentrations or breeding soundness between bulls with differing net feed efficiencies (Fox et al. 2004). There is a possibility that selecting for RFI can compromise fertility of bulls and breeding females, as a result of reductions in overall body fatness and possible reductions in sperm motility.

Biological Variation in RFI

RFI is a complex concept and differences in RFI come as a result of several different biological processes. Herd et al. (2004a) identified 5 major biological processes that contribute to differences in RFI: 1) feed intake, 2) feed digestion, 3) anabolism and catabolism, 4) activity levels and 5) thermogenesis. Herd and Richardson (2004) reported that 73% of the variation in RFI could be explained by activity, body composition, and metabolic processes, which is in agreement with findings from Nkrumah et al. (2006), Basarab et al. (2003), and Carstens et al. (2002). Further research by Herd and Arthur (2009) revealed that protein turnover, stress susceptibility, and feeding patterns were also potential contributors to differences

in RFI. Maintenance of cell structure and function, along with protein turnover and ion transport, are also possible sources of metabolic expenditure and heat loss (McBride and Kelly 1990; Baldwin and Sainz 1995).

Nutrient Digestibility

About 10% of the variation in RFI can be explained by inherent differences in feed digestibility (Herd and Arthur 2009). Thus far, several studies have reported RFI to be negatively correlated with digestibility (Nkrumah et al. 2006; Krueger et al. 2009; McDonald et al. 2010), indicating that low RFI cattle tend to have greater digestibility (Herd and Arthur 2009). Nkrumah et al. (2006) reported greater crude protein (CP) and dry matter (DM) digestibility in low RFI steers. More specifically, low RFI steers had 75.3% DM digestibility, compared with 73.4% and 70.9% DM digestibility in medium and high RFI steers, respectively (Nkrumah et al. 2006). Low RFI steers had 74.7% CP digestibility, compared with 73.5% and 69.8% CP digestibility in medium and high RFI steers, respectively (Nkrumah et al. 2006). Although Nkrumah et al. (2006) did not report significant differences in DM or CP digestibility (P=0.10, P=0.09, respectively) between RFI groups, these differences were notable. Basarab et al. (2013) found a similar trend, with RFI negatively correlated with DM and CP digestibility (r=-0.55, r=-0.47, respectively). In contrast, research conducted by Gomes et al. (2013) found no significant difference (P=0.18) in apparent DM digestibility between high (72.3%) and low RFI (75.2%) steers on a finishing diet; however, these results were based on the collection of fecal samples, orts, and urine samples. When using lignin and acid-insoluble ash markers, DM digestibility did not appear to differ between high and low RFI cattle (Cruz et al. 2010; Lawrence et al. 2011).

Differences in the efficiency of microbial protein production has also been known to supply varying amounts of amino acids (Kahn et al. 2000), as evidenced by differences in amino acid profiles in the portal vein (Lush et al. 1991), which can affect digestion and overall nutrient availability. Differences in nutrient retention and excretion also help to explain differences in digestibility. When analyzing nitrogen retention and excretion, nitrogen retention was greater, although not significantly (P=0.34),

within low RFI steers (60 g day^{-1}) when compared to high RFI steers (53.2 g day^{-1}) (Gomes et al. 2013). Nitrogen excretion was greater in high RFI steers (97.3 g day^{-1} vs. 108.8 g day^{-1}, P=0.31, Gomes et al. 2013), with no significant differences in nitrogen digestibility between high and low RFI cattle (Cruz et al. 2010; Lawrence et al. 2011).

Blood Metabolites

When studying differences in overall blood composition between high and low RFI cattle, Fitzsimons et al. (2013) noted 9% lower ($P < 0.05$) creatinine concentrations in high RFI cattle, along with significantly higher blood metabolite concentrations of glucose and urea, when compared to low RFI cattle. Creatinine was reported to have a negative correlation ($r = -0.41$) with RFI (Fitzsimons et al. 2013), which supported earlier research by Tatham et al. (2000) where a positive correlation between plasma creatine:urea ratios and RFI was reported. Fitzsimons et al. (2013) also determined the concentrations of the following metabolites in high, medium and low RFI cattle; beta-hydroxybuterate, non-esterified fatty acids, triglycerides, total protein, albumin and globulin, none of which were reported to differ with RFI. High RFI cattle were reported to have greater concentrations of total plasma protein, urea and aspartate amino transferase, providing evidence of greater protein turnover (Richardson and Herd 2004). Higher levels of total plasma protein were also reported in cattle with high RFI (Richardson et al. 2006), reflecting differences in rates of protein degradation and synthesis. In addition, Richardson et al. (2004) detected a genetic association between RFI and white blood cell variables, as well plasma cortisol, suggesting that high RFI cattle are more susceptible to stress due to greater white blood cell counts and cortisol concentrations. Knott et al. (2008) noted similar results in high RFI rams, as they had greater concentrations of plasma cortisol. This suggests that high RFI animals may have greater stress responses, potentially leading to greater energy expenditure, increased protein degradation and lipolysis (Knott et al. 2008). Furthermore, Karisa et al. (2014) examined blood metabolites of 16-high and 16-low RFI steers and successfully validated and accounted for 32% of the phenotypic variation in RFI. In the study, Karisa et al. (2014) found that among other metabolites, creatine, hippurate and carnitine were significantly

associated with RFI in the discovery population when the cattle were 317 days old and in the validation population when the cattle were 360 days old. Although more research is necessary, the findings of Karisa et al. (2014), Knott et al. (2008) and Fitzsimmons et al. (2013) indicate that differences in blood metabolites likely play a significant role in the phenotypic expression of RFI.

Muscle Metabolism

Moore et al. (2005) noted greater concentrations of insulin-like growth factor 1 (IGF-1) in blood of high RFI cattle. IGF-1 concentrations had a genetic correlation of 0.57 with RFI, suggesting that several genes associated with RFI are also associated with IGF-1 concentrations (Moore et al. 2005). Johnston et al. (2002) also noted that beef cattle vary in IGF-1, which they concluded to be associated with muscle metabolism (catabolism and anabolism). Oddy and Owens (1996) explained that increased levels of IGF-1 in calves resulted in significantly greater protein deposition, suggesting that IGF-1 may be associated with muscle metabolism. Furthermore, Oddy et al. (1998) reported a 20% difference in energy expended, calculated on a muscle mass basis, between cattle selected for and against growth rate, suggesting that differences in rates of protein synthesis and degradation do exist. Calpastatin, an inhibitor of the calpain system, reportedly differs between cattle with divergent efficiencies (McDonagh et al. 2001). Calpains are proteases that initiate protein degradation (Huang and Forsberg 1998). Calpastatin activity was significantly less in the *M. Longissimus dorsi* of low efficiency steers (high RFI) than high efficiency steers (low RFI), resulting in greater calpain activity and therefore greater protein degradation (McDonagh et al. 2001). Tatham et al. (2000) also noted greater protein turnover in high RFI bulls as a result of high creatine phosphate turnover, intended to provide short term energy to muscle cells (Bessman and Carpenter 1985). Increased calpastatin activity, which reduces calpain activity, tends to be highly related to reductions in beef tenderness (Morgan et al. 1993; Wulf et al. 1996), suggesting that low RFI cattle produce less tender beef. Although Baker et al. (2006) did not conclude any difference in meat quality and palatability from high and low RFI cattle, it is possible that low RFI steers, with reduced calpain activity (McDonagh et al. 2001), yield less tender meat.

Energy Expenditure and Thermoregulation

Differences in metabolism and tissue turnover are also associated with energy expenditure (Herd et al. 2004). Nearly 90% of cellular energy is produced by mitochondria, which are abundant in the metabolically active cells of the kidney, liver, brain, and muscles (Ojano-Dirain et al. 2007). Kolath et al. (2006) reported no significant difference in mitochondrial function of high and low RFI cattle; however, low RFI steers were reported to have increased rates of mitochondrial respiration, while respiration in high RFI steers appeared to be impaired by continuous electron movement through the electron transport chain. Energy expenditure also appears to be regulated by adrenergic receptors, as manipulation of such receptors by α and β adrenergic agonists results in variations in energy expenditure (Lindsay et al. 1993; Hunter et al. 1993). Blaxter (1962) explained that the primary route of energy expenditure in cattle is through evaporative heat loss due to heat exchange in the nasal turbinates and lungs. High RFI steers reportedly had 9.3% greater heat production, and therefore heat loss, relative to low RFI steers, likely due to a similar difference (10.2%) in metabolizable energy intake (Basarab et al. 2003). Furthermore, a RFI study in chickens revealed that low RFI hens had smaller nude body areas, better feather coverage and less activity, suggesting less energy and heat loss (Luiting et al. 1994). This implies that thermoregulation and energy expenditure may have an effect on RFI in beef cattle. When thinking about thermoregulation it is also important to consider the effects of heat stress. St-Pierre et al. (2003) describes heat stress as a negative balance of net energy flowing from the animal to the environment and the amount of heat produced by the animal. Essentially heat stress occurs when temperatures exceed an animal's thermal neutral zone, resulting in reduced body heat loss. Cattle experiencing heat stress will have a reduced DMI and rate of gain (Lippke 1975). More efficient cattle, with lower DMI, and therefore a lower heat increment of feeding, are likely to be more tolerant of high temperatures, which are common during the summer months in North America.

Activity Levels

Variation in RFI also comes as a result of variation in activity levels (Herd et al. 2004). Activity levels of mice selected for high feed intake with respect to body weight (low efficiency), had activity levels that were three times greater than the activity of mice selected for low feed intake (high efficiency) (Bünger et al. 1998). Similar results were noted in beef cattle (Richardson et al. 2000; Arthur et al. 2001a). More specifically, a phenotypic correlation of 0.32 was reported between RFI and daily pedometer count (Richardson et al. 2000), while high RFI bulls averaged 6% more steps per day than low RFI bulls (Arthur et al. 2001a). Arthur et al. (2001a) explained that the increase in activity and energy expenditure was due to an increase in distance walked, and increased time standing and ruminating, along with variation in energy expended feeding, walking and ruminating (Herd et al. 2004).

Feeding Behavior

Along with energy expenditure and activity levels, Herd and Arthur (2009) suggested that feeding patterns and feeding behavior result in variation in RFI. Past research has confirmed moderate to strong positive correlations (r= 0.08 to 0.62) of RFI to feeding frequency, feeding duration and eating rate (g min^{-1}) (Robinson and Oddy 2004; Basarab et al. 2007; Nkrumah et al. 2007; Golden et al. 2008; Bingham et al. 2009; Montanholi et al. 2010; Kelly et al. 2010; Durunna et al. 2011). High RFI cattle have been shown to require 2 to 5% more energy for feeding activities (Herd et al. 2004; Basarab et al. 2011), in-part due to 14-22% more frequent daily feeding events than low RFI cattle. Basarab et al. (2013) reported that RFI was positively correlated to the number of bunk visits and to feeding event duration. Furthermore, feeding duration and feeding frequency behaviors are moderately heritable (h^2=0.28 to 0.38) and repeatable (r= 0.37 to 0.62) (Nkrumah et al. 2007; Kelly et al. 2010a), suggesting that ideal feeding behaviors can be selected for with relative certainty. Although low RFI cattle have lower DMI, fewer feeding events and lower feeding duration, Basarab et al. (2013) suggested that these animals can still

competitively acquire forage within extensive grazing systems due to lower stress susceptibility and greater adaptability.

RFI and Methane Production

Not only does selecting for low RFI cattle reduce feed costs, in many cases it also reduces methane (CH_4) production. Methane is a greenhouse gas (GHG) produced by ruminants during digestion and fermentation (Beauchemin et al. 2009) and has 25 times the global warming potential of carbon dioxide (CO_2; IPCC, 2014), and therefore making it the focus of much concern amidst concerns of climate change. Beauchemin et al. (2009) explained that nearly 12 to 17% of GHG emissions come from ruminants. In North America, cattle are responsible for approximately 8% of GHG emissions (Beauchemin et al. 2010; Basarab et al. 2012). High levels of CH_4 production tend to be associated with the breeding cow herd because a cow's diet consists largely of forages rather than concentrates (Allen et al. 1992; Verge et al. 2008; Capper 2011), and because cows have a much longer life span than grower and feeder cattle. Reducing cow herd CH_4 production is therefore a key factor in reducing the beef carbon footprint. Previous research shows that feedlot finished beef produced 17 CO2 equivalents kg^{-1} of carcass (Verge et al. 2008; Beauchemin et al. 2010; Basarab et al. 2012), while grass finished beef produced 40 CO2 equivalents kg^{-1} carcass (Cederberg et al. 2011). Selecting for cattle with low RFI may be one way in which beef cattle produce less CH_4, resulting in a smaller carbon footprint and increasing sustainability of the industry. More specifically, low RFI cattle require fewer resources to achieve market weight and they require less feed to maintain their body weight as mature cows, all the while producing fewer GHGs.

Previous studies have indicated that low RFI cattle produce less CH_4 compared to high RFI cattle (Nkrumah et al. 2006; Hegarty et al. 2007; Jones et al. 2011; Fitzsimons et al. 2013). Nkrumah et al. (2006) found that low RFI feedlot steers produce 28% less CH_4 than high RFI cattle on concentrate diets in drylot. The mechanism underlying the difference in CH_4 production is not entirely understood, in-part due to conflicting results from previous studies. Waghorn and Hegarty (2011) observed no differences in CH_4 production between dairy cows with differing RFI classifications. Similar results were reported by

McDonnell et al. (2016); absolute CH_4 production did not differ between heifers with high and low RFI. While CH_4 production is often a function of diet and DMI (Blaxter and Clapperton 1965; Johnson and Johnson 1995; Grainger et al. 2007), it is unclear whether low RFI cattle produce less CH_4 as a result of lower DMI or differences in digestive efficiency (Kelly et al. 2010a; Lawrence et al. 2011).

Hegarty et al. (2007) explained that an increase in feed intake increases fermentation, which also increases the amount of hydrogen available for methanogens to produce CH_4. Lack of consistency in diet between studies may be the cause of contrasting study results. It has been reported that CH_4 production can be reduced by feeding grain rather than forage based diets (Grainger and Beauchemin 2011; Hristov et al. 2013). Moe and Tyrrell (1979) explained that feed sources with low fibre, or high carbohydrate content, can reduce CH_4 production. Similarly, improvements in diet quality can reduce CH_4 production per animal unit (Eckard et al. 2010). Although Jones et al. (2011) observed no difference in CH_4 production between high and low RFI pregnant cows when grazing low quality pasture, they did observe 27% lower CH_4 production within low RFI lactating cows when grazing high quality pastures. Kerley and Lardy (2007) explained that poor quality pastures with low crude protein content (<80-90 g kg^{-1}) fail to meet nitrogen requirements for the rumen microbial population, thereby affecting CH_4 production. Nkrumah et al. (2006) restricted all study animals to a daily feed intake of 2.5 x maintenance in an attempt to determine if factors other than DMI had an effect on CH_4 production. In that study, low RFI animals produced 28% less CH_4 than high RFI animals, suggesting inherently lower CH_4 production occurred in low RFI animals, a response independent of DMI (Nkrumah et al. 2006).

Measures of CH_4 yield, calculated as CH_4 production relative to DMI (g kg^{-1} DMI), more accurately describe inherent differences in CH_4 production. Although CH_4 production (g day^{-1}) is known to differ significantly between high and low RFI cattle, CH_4 yield may not differ. While Fitzsimons et al. (2013) reported CH_4 production to be significantly greater in high RFI heifers, CH_4 yield did not differ between RFI groups. Similarly, although RFI was positively correlated with CH_4 production (g kg^{-1}), RFI was negatively correlated with CH_4 yield (g kg^{-1} DMI) (Fitzsimons et al. 2013). Hegarty et al. (2007) and

Waghorn and Hegarty (2011) noted similar results; CH_4 yield was not significantly different between RFI groups and RFI was unrelated to CH_4 yield. These findings demonstrate that differences in CH_4 production may not be directly associated with RFI, but rather that they may result from differences in DMI due to differences in RFI.

Methanogens and Methanogenesis

Ruminal methanogenesis, the process of CH_4 production, represents an energy loss to ruminants (Hristov et al. 2013). It is estimated that about 3 to 7% of gross energy intake is lost to CH_4 production from cattle fed high grain and forage diets, respectively (Hristov et al. 2013). Methanogenesis is regulated by methanogens, which are CH_4 producing bacteria in the rumen that live in symbiosis with ruminal protozoa (Lange et al. 2005) and bacteria (Wolin et al. 1997). Methanogens work to minimize the partial pressure of hydrogen in the rumen while continuing to produce H+ for fermentation (Wolin et al. 1997; Russell 2002; McAllister and Newbold 2008). Research suggests that diet type often dictates the type of microbes and methanogens present in the rumen (Theodorou and France 2005), and also that RFI has an effect on microbial populations (Guan et al. 2008), both of which can therefore factor into differences in CH_4 production from cattle (Moss et al. 2000).

Zhou et al. (2009, 2010) reported that differences in methanogenic profiles were likely associated with differences in RFI, a conclusion based on studies conducted at the molecular level in which 16S rRNA libraries were constructed from the pooled DNA of the rumen microbial community of high and low RFI steers extracted from their ruminal fluid. Low RFI steers expressed less diversity in the overall methanogenic community compared to high RFI steers, especially when fed low energy diets (Zhou et al. 2009, 2010). Carberry et al. (2012) noted similar results, with the abundance of *Methanobrevibacter smithii* genotypes differing between high and low RFI cattle, independent of their diet. Through the use of metagenomics, differences in microbial enzymes between high and low RFI cattle were identified (Ghoshal et al. 2012). Differences in microbial profiles reportedly have a greater effect on RFI than differences in the total number of microbes (Guan et al. 2008; Zhou et al. 2009, 2010; Hernandez-

Sanabria et al. 2010). These results were evident in steers fed growing (Hernandez-Sanabria et al. 2010) and finishing diets (Guan et al. 2008; Hernandez-Sanabria et al. 2010). Differences in rumen microbial profiles were also noted between high and low RFI heifers on forage diets (Carberry et al. 2012). More specifically, Zhou et al. (2009) reported a greater proportion of *Methanosphaera stadtmanae* and *Methanobrevibacter* sp. AbM4 in high RFI cattle compared to low RFI cattle. Miller and Wolin (1985) explained that *Methanosphaera stadtmanae* utilize methanol, while *Methanobrevibacter* sp. AbM4, which is similar to *Methanobrevibacter smithii*, utilizes acetate as its main substrate for CH_4 production (Zhou et al. 2009). The above results suggest that cattle which favor the use of organic substrates for CH_4 production may be less efficient (Basarab et al. 2013). It is also important to note that differences in dietary energy content affect associations between RFI and overall methanogen profile (Zhou et al. 2010).

Volatile fatty acids (VFA), the final products of ruminal fermentation, are generally determined by diet type (Janssen, 2010). Bacteria within the *Prevotella* genus play a role in the breakdown and use of starch (Cotta, 1992) and proteins (Wallace et al. 1993); nearly 60% of ruminal bacteria can be found within the *Prevotella* genus (Stevenson and Weimer, 2007). Hernandez-Sanabria et al. (2010) identified several *Prevotella* sp. that were dominant in high RFI cattle and appeared to be linked to reduced FCR, lower ratios of straight to branched chain VFAs, and increased butyrate production. Ramin and Huhtanen (2013) linked acetate and butyrate production with hydrogen production, while propionate production was involved with the utilization of hydrogen. Furthermore, when comparing high and low RFI pregnant heifers, low RFI heifers were observed to have greater propionic acid concentrations and lower acetate:propionate ratios while on a grass silage diet (Lawrence et al. 2011). This was confirmed by Fitzsimons et al. (2013), who reported slightly lower acetate:propionate ratios and higher propionic acid concentrations in low RFI heifers. Propionate is the main VFA that contributes to gluconeogenesis, a process that produces glucose from non-carbohydrate precursors (University of California, Davis, 2013). Propionate concentrations depend on the intake of digestible energy, such as starch (Stewart et al. 1997). Starch fermentation encourages propionate production, which provides hydrogen with an alternative

pathway to CH_4 production (Murphy et al. 1982). This may help to explain why high-starch, concentrate-based diets result in reduced CH_4 emissions (McGeough et al. 2010; Grainger and Beauchemin 2011; Hristov et al. 2013).

Methane Production from Cattle on Pasture

Methane production from cattle grazing under open range conditions can be measured using open path Fourier transform infrared spectrometry (OP-FTIR) techniques (Jones et al. 2011; McGinn et al. 2011) or sulfur hexafluoride (SF_6) tracer techniques (Srivastava and Garg, 2002). FTIR techniques are non-invasive, while the SF_6 tracer technique requires administration of a permeation tube into the rumen and attachment of a sampling canister to the animal's head (Srivastava and Garg, 2012). Similar to CH_4 production in drylot, high RFI cattle tend to have greater CH_4 production on pasture (Jones et al. 2011). Jones et al. (2011) noted that low RFI cows had significantly lower (27%) CH_4 emissions than high RFI cows when grazing high quality pastures, although no differences were noted when cattle were on poor quality pasture. McCaughey et al. (1999) also observed differences in CH_4 production from cattle when grazing different forages. Methane production was significantly lower when cattle grazed alfalfa-grass pastures as compared to grazing grass pastures. Methane production, as a percent of gross energy intake, was also lower in cattle grazing alfalfa-grass pastures (McCaughey et al. 1999). Eckard et al. (2010) also reported improved diet qualities to be associated with reduced CH_4 production in ruminants. It is not clear whether cattle on drylot have the same CH_4 production, particularly in comparison to other cattle, as they would on pasture.

Ongoing Research

Although research with cattle on drylot is abundant, it is important that further research be conducted with cattle on pasture. As the majority of Canadian cattle herds spend significant periods of time foraging on native and tame pastures, it is important to understand how selecting cattle for RFI will affect the performance of cattle in such an environment. Cattle in all areas of production are reared from cow herds,

and therefore it is important to consider how selection decisions made in the cow herd will affect subsequent components of the beef production cycle. Further RFI research of cattle on pasture will provide practical implications, in the form of herd selection tools, which producers can use to make herds more efficient. Such tools will allow producers to be more productive and profitable, while practicing more sustainable grassland management.

Thesis Structure

The overall goal of this thesis is to understand whether selecting for RFI in extensive cow calf systems on pasture will have an effect on performance and efficiency of the herd. In this case, performance refers to: weight gain, changes in backfat thickness, CH_4 production and pregnancy status. Analyzing the performance of these cattle will provide an understanding of how to select for the most productive cattle on pasture, ultimately increasing the efficiency of cow-calf herds.

Chapter two focusses on how specific production metrics are affected by selection for RFI. Such production metrics include weight gain, weaning weights, birth weights, changes in backfat thickness, and pregnancy status. Chapter three analyzes DMI and CH_4 emissions of high and low RFI replacement heifers in drylot. While chapter four concentrates on CH_4 production and CH_4 of replacement heifers on pasture. More specifically, it examines CH_4 production and CH_4 yield of individual heifers in drylot as well as CH_4 production and yield of high and low RFI groups of heifers on pasture. The thesis will conclude by setting out the implications of the results for industry and highlighting areas for future research.

Chapter 2: Production metrics of cows and calves on pasture with molecular breeding values for RFI

INTRODUCTION

Profitability in the beef industry depends on a producer's ability to reduce input costs, which are often related to feeding, while optimizing outputs of calf size and gain. Feed costs are one of the largest variable costs (ARD 2005; Ramsey et al. 2005) and account for up to 70% of a producer's total costs (AAF 2014). Approximately 70-75% of dietary energy intake is used to meet maintenance requirements, while less than 30% is used for growth and production (Ferrell and Jenkins, 1985; Montano-Bermudez et al. 1990; NRC 1996). Energy used for production is often allocated towards lean protein and fat accumulation, with the production of adipose tissue being a more energy expensive process than lean muscle production (Ricks et al. 1984). Selecting for feed efficient cattle, with lower maintenance requirements, is one way in which production costs can be mitigated.

In the past, beef producers selected for cattle with superior feed conversion ratio (FCR), a metric comprised of feed intake to body weight gain. Because the trait is a ratio of characteristics, selecting for cattle with high FCR inadvertently resulted in larger cattle, with increased growth and ultimately greater maintenance requirements that, in turn, required more feed (Van der Werf 2004; Crews 2005; Kelly et al. 2010 and 2010a). Today, residual feed intake (RFI), or net feed intake, is commonly used as a tool for assessing feed efficiency (Herd and Arthur 2009). The concept of RFI was first described by Koch et al. (1963), where the energy necessary for maintenance was quantified separately from the energy necessary for growth and reproduction. RFI is calculated as the difference between an animal's actual intake and their expected intake for a given level of production (Koch et al. 1963; Arthur et al. 1996; Basarab et al. 2003; Nkrumah et al. 2006). Cattle with low RFI values are efficient while cattle with high RFI values are inefficient. RFI is a valuable trait due to its moderate heritability, ranging from 0.29 to 0.46 (Archer et al. 1998; Arthur et al. 2001; Schenkel et al. 2004; Bouquet et al. 2010), and its independence from body weight and size (Koch et al. 1963; Kennedy et al. 1993; Crews 2005; Crowley et al. 2010). This means

that selection for RFI is not likely to affect growth, but it also means that RFI cannot be readily detected from physical appearance alone.

Measuring phenotypic RFI values is an expensive, time consuming, and sometimes difficult, process (Moore et al. 2009). Genomic selection tools have the potential to predict RFI through the use of specific genetic markers which could be used to identify and therefore select more efficient beef cattle for herd retention. Comparison between genotypes and phenotypes of cattle with extreme differences in RFI values (i.e. high vs low) have identified single nucleotide polymorphisms (SNP) that appeared to be associated with RFI (Bardense et al. 2007). Such markers can be used for marker assisted selection (MAS) a method of creating genomic predictions (Lande and Thompson 1990). MAS relies on specific SNPs that appear to be associated with a trait, often times analyzing SNPs that are in linkage disequilibrium with specific quantitative trait loci (QTL) (Lande and Thompson 1990). The accuracy of genomic evaluations depends on the estimated effects of the SNPs as well as the linkage disequilibrium (i.e. correlation of an allele at one locus with a different allele at another locus) between the SNP and its causal variants (Hayes et al 2009). In 2007, Bardense et al. identified 161 SNPs associated with RFI ($P < 0.01$), when using a MegAllele Genotyping Bovine 10K SNP panel (Hardenbol et al. 2005). SNP locations revealed that the RFI trait may be controlled largely by micro-RNA motifs, promoter sequences and mRNA sequences, with several SNPs located in genes that control cellular energy use, cell growth, translation, and transcription (Bardense et al. 2007).

Nkrumah et al. (2007a) found that measures of RFI were associated with eight different QTL (located on chromosomes 5, 6, 7, 11, 14, 16, 17, 18, 19 and 28), which are areas of the genome that are responsible for a portion of the genetic variance in a trait (Soller 1978). Further research conducted by Sherman et al. (2008) mapped the same QTL as Nkrumah et al. (2007a), along with additional QTL, at a greater marker density (14-18 SNPs per QTL region) to identify more SNPs associated with RFI on chromosomes 2, 5, 10, 20 and 28. Although the two experiments were similar, Sherman et al. (2008) likely identified different QTL as a result of mapping at a greater marker density and as a result of

reporting SNPs that were associated with RFI but not associated with dry matter intake (DMI) or FCR. Both studies indicate that genomics can likely be used to predict RFI, which in turn, can reduce the cost and time associated with selecting for RFI. More recently, a different approach, known as genomic selection, has become the preferred method for predicting breeding values. Meuwissen et al. (2001) proposed the idea of constructing breeding values, estimated from genotypic expressions at many marker locations across the entire bovine genome (not just in a small number of QTL regions), in an attempt to increase the rate of genetic improvement. This method sums the effects of each marker to produce a molecular breeding value (MBV) for a specific trait, such as RFI (Meuwissen et al 2001). With the development of the 50,000 SNP panel (Matukumalli et al 2009), it became possible to create genomic selection for RFI. MBV for phenotypic traits (for example RFI) are calculated, whether by MAS or genomic selection, using prediction equations acquired from reference populations of cattle for which both genotypes and phenotypes are available (Boichard et al. 2016). While genotypes are derived from genetic sequences, RFI phenotypes used are normally assessed in drylot under controlled intake conditions (Nkrumah et al. 2007a).

As indicated above, due to its independence from body size and production, selecting for RFI is not likely to compromise cattle performance in other economically important traits, specifically growth (Kennedy et al. 1993), body condition (e.g. backfat thickness) (Castro Bulle et al. 2007; Fitzsimons et al. 2013), or fertility (Arthur et al. 2005). Given that RFI is commonly evaluated in a drylot environment (Basarab et al. 2003; Wang et al. 2006), performance data on these animals must be interpreted in this context (i.e. in a controlled environment where diet is fixed, and many behaviors eliminated). As a result, performance and RFI values of cattle in drylot may not be representative of cattle performance on pasture, where diet (Kelly et al. 2010) and feed intake are likely to differ and complex foraging behaviors are introduced (Bailey et al. 1996). Furthermore, MBV that are constructed using a reference population with phenotypes derived in drylot, may not be the most accurate estimation of their RFI on pasture. The majority of cattle in Alberta and Western Canada are raised in pasture environments. Even feedlot cattle

spend part of their life on pasture, which is why it is important to understand how selecting for low RFI in beef herds may affect their performance under open range conditions.

The objective of this study was to determine if there is a difference in performance, as expressed by body weight gain, change in backfat, pregnancy status and time of conception, between cattle with MBV for high and low RFI while foraging under open range conditions. In addition, the study also evaluated the performance of phenotypically high and low RFI calves on pasture, using phenotypes which were collected in drylot after weaning (Chapter 3). The following three hypotheses were formulated: (1) that there would be no difference in the change in body weight over the grazing season between high and low RFI cows and calves because the trait is independent of body size and growth, (2) that low RFI cows would accumulate more backfat than high RFI cows over the grazing period because low RFI cows have lower maintenance requirements and greater energy available for backfat accumulation (especially considering that growth is independent and thus similar between high and low RFI animals), and (3) that there would be no difference in pregnancy status and time of conception between high and low RFI cows.

MATERIALS AND METHODS

This research was conducted between October 2013 and November 2015 at the University of Alberta Mattheis Research Ranch, in collaboration with Gemstone Cattle Company. All animal procedures were reviewed and approved by the University of Alberta Animal Care and Use Committee (AUP00001284), and all animals were cared for in accordance with the Canadian Council on Animal Care (1993).

Experimental Design

A total of 450 commercial Hereford-Angus cows were genotyped with a BovineSNP50 Beadchip (Illumina Inc., San Diego, CA) 50,000 SNP (50K) panel, to produce molecular breeding values (MBV) for RFI in the winter of 2014; derivation of the MBVs is explained in Appendix A. In addition to producing MBVs, the genotypes were also used to determine breed composition and to calculate retained heterozygosity for each of the cows. Retained heterozygosity explains how much heterosis was retained

over generations of crossbreeding, with crossbred cattle have greater retained heterozygosity than purebred cattle. Breed composition and retained heterozygosity was evaluated using the methods outlined in Appendix A. Cows were split into three groups for breeding in June of 2014 according to their genotypic RFI (gRFI) value, including 80 low gRFI cows (gRFI values ≤ -0.07), 290 medium gRFI cows (gRFI values >-0.07 but <0.04), and 80 high gRFI cows (gRFI values ≥ 0.04). Mean gRFI values for the high, medium and low herds were 0.074 (SD = 0.03), -0.018 (SD = 0.03), -0.108 (SD = 0.04), with accuracies of 0.338 (SD =0.06), 0.390 (SD =0.06), and 0.401 (SD =0.08), respectively. The overall range in RFI was -0.213 to 0.14 (Appendix A). All three groups of cows were kept separate for the entire 2014 breeding season. On July 14, 2014, high RFI cows were exposed to two bulls with known high RFI based on actual drylot tests, low RFI cows were exposed to two bulls of known low RFI, while medium RFI cows were exposed to a battery of 27 bulls having moderate RFI. All bulls were genotyped but selected based on phenotypic RFI values. On September 15, 2014, after approximately three breeding cycles, all bulls were removed. The herds were then combined and thereafter cows were maintained as one herd for the remainder of the study period.

Stringent culling by the herd owner in the fall of 2014 and missing data for some cow-calf pairs reduced the number of cows in the high, medium and low RFI groups the following spring to 74, 219, and 59, respectively. In 2015 at the time of calving, cows ranged in age from three to thirteen years, with the majority (54%) being three years old. High, medium and low RFI cows had an average age of 4.1 years (SD = 2.25), 3.7 years (SD = 1.10), and 3.9 years (SD = 1.25), respectively. After wintering on pasture and receiving supplemental hay/greenfeed as necessary, calving began on April 16, 2015. Production measures were collected on both cows and calves over the duration of the 2015 summer grazing season. The 2015 breeding season began on July 15, 2015 when the bulls were first exposed to the entire herd.

Data Collection

Birth weights were collected on all calves throughout the calving season (April 16 – June 8, 2015), at which time calves were also sexed and tagged for visual identification. Additionally, calf and dam

phenotypic descriptions were recorded and calf DNA samples collected using TypiFix (Gene Check, Inc., Greeley, CO, USA) cattle ear tags. Weights were collected within 24 hours of birth using a duffle bag and a hanging scale (Chatillon Type 140, AMETEK Inc., Largo, FL, USA); calves were placed in the bag and the handles of the bag attached to the hook of the scale, with weights then collected by holding onto the scale and lifting the bag. Actual calf birth weights were collected on just over half of the calves (57%), and excluded those when the environment (cow) was deemed non-safe. Estimated birth weights were recorded for all calves (including those actually weighed, with estimates done prior to weighing), along with the name of the individual making the estimation. All cow-calf pairs were grazed together throughout the summer and fall, and therefore exposed to the same environment at all times. Immediately after weaning, heifers and steers were weighed on two consecutive days, October 27, 2015 and October 28, 2015, respectively. All calves were weighed using a Cattleac (Cattleac Cattle Equipment and Acc. Inc., Weatherford, Oklahoma, USA) squeeze and scale system, while a Gallagher (Gallagher Group Ltd., Hamilton, New Zealand) scale head recorded animal weights to the nearest pound. Weights were subsequently converted to kg.

After the end of the calving period, cow weights and ultrasound backfat measurements were collected on June 10, 2015, just prior to the start of the main grazing season. All cows were temporarily separated from calves and weighed using a Silencer (Silencer Cattle Chutes, Raymond, Alberta, Canada) chute and squeeze system. Ultrasound backfat measurements were taken between the 12th and 13th ribs by a certified ultrasound technician. Cow weights and ultrasound backfat measurements were also collected on October 26, 2015, immediately after weaning, using the same equipment and processes as in June. Both weights in the spring and fall were recorded to the nearest pound using a Gallagher scale head and subsequently converted to kg. All backfat measurements were recorded in cm and later converted to mm of backfat.

All cows were also pregnancy tested on October 26, 2015 via rectal palpation by a certified veterinarian. Palpation indicated the timing of conception, specifically identifying whether the cow

became pregnant in the first breeding cycle, the second breeding cycle, or whether they did not become pregnant at all.

Calculations and Statistical Analysis

General linear models in R (R Core Team 2016) were used to regress estimated calf birth weights to actual birth weights, grouped separately by individual observer (to eliminate observed bias), with the following R^2 values; 0.75 ($P < 0.0001$) (n = 90), 0.51($P < 0.0001$) (n = 123), and 0.53 ($P = 0.101$) (n=3) for estimates taken by three different observers. These relationships were then used to generate predicted actual birth weight values for those 168 calves that only had estimates, which consisted of 46, 104 and 18 high, medium and low gRFI calves, respectively. Only actual or predicted (based on estimated birth weights when actual birth weights were not available) birth weight values were used for further analysis.

Calf weaning weights were individually adjusted to 205-d weaning weights using the following formula; 205-d Weaning Weight = [((Wean Weight (kg) – Birth Weight (kg)) / Age (days)) x 205] + Birth Weight (Gould 2015). This process allowed standardization of weaned weights among calves of different ages which was necessary to directly interpret differences among animals in relation to maternal MBVs for RFI. Calf growth values were then quantified as the difference between adjusted wean weights and birth weights.

Data and residuals were visually examined for normality for all cow and calf parameters using the qqplot and qqnorm functions in R (R Core Team 2016). Five outliers were removed from both cow backfat (3 high and 2 low outliers) and cow weight gain measures (3 low and 2 high outliers) to meet assumptions of normality (different cows were considered outliers in each analysis). This analysis used linear mixed effects models in the nlme package in R (Pinheiro et al. 2016). Five different models were used, each with one of the following parameters as a response variable; birth weight, ADG, weaning weight, growth and 205-d adjusted weaning weight. The fixed effects (independent variables) in the models were gRFI group and the dam's age, while the calves within each group were considered random. Due to the significant effects of the dam's breed composition, it was added as a fixed effect along with the dam's retained heterozygosity as a covariate in the weaning weight, 205-d adjusted weaning weight, and

growth models. Additionally, calf sex was added to the birth weight model. Type III analysis of variance (ANOVA), in the car package (Fox and Weisberg 2011) in R, which accommodates unbalanced designs (i.e., unequal sample sizes among treatment groups), was used to determine differences in the calf parameters among the different gRFI groups. When the models were significant, post-hoc multiple comparisons were done using Tukey's Honest Significant Difference (HSD) Test, in R's agricolae package (de Mendiburu 2016), to determine differences among the groups of predicted high, medium and low RFI calves for all parameters analyzed. Phenotypic, or actual, RFI (pRFI) values were collected for 60 heifers in a subsequent drylot feed intake trial, described in detail in Chapter 3. Simple linear regressions in R were used to compare 205-d adjusted weaning weights (kg) to calf pRFI values, as well as growth (kg) to calf pRFI values. Both of the regression analyses were grouped by age to account for the effect of age on body weight. In both analyses, three different regression lines were produced, one for each of the following age groups, young calves (< 170 days of age), middle-aged calves (173 – 181 days of age) and old calves (> 182 days of age). Regressions were done using the pRFI values to show whether the same growth and weaning weight trends were noted when using gRFI and pRFI values. Significance was determined at p-values lower than 0.05.

The changes in cow backfat and cow weight over the summer grazing period, between genomically predicted high, medium and low RFI cows, was analyzed using linear mixed models in the nlme package in R. Two separate models were used in which the response variables were the change in weight and the change in backfat, while the fixed effects (independent variables) were gRFI group and cow age, and each animal was considered a random effect. Breed composition and retained heterozygosity of the cows was not included in the model as they did not have significant effects. Type III ANOVA was done to identify differences in the changes in cow backfat and weight among the high, low and medium gRFI cows. Post-hoc multiple comparisons were done using Tukey's HSD Test, given that the models were significant. Simple linear regressions in R were used to compare cow weight gain (kg) to cow gRFI value, as well as the change in cow backfat (mm) to gRFI value. In both of the regression analyses the cows were grouped by age to account for effects of cow age on backfat and weight gain.

Each of the analyses had two regression lines, one representing cows younger than five years of age and the other representing cows five years of age and older. Significance was determined at p-values lower than 0.05.

A contingency test was used to evaluate the proportion of predicted high, medium and low gRFI cows that became pregnant in the first and second breeding cycles, as well as the cows that were open. A Chi-square test of independence was conducted in R (R Core Team 2016), to determine if there was a difference in the distribution of pregnancy status among the three groups of cows, including whether gRFI grouping and pregnancy status remained independent of one another.

RESULTS

Calf Responses

All ANOVA values related to the calf production metrics are presented in Table 1. Calf birth weights, weaning weights and 205-day adjusted weaning weights were similar among groups of high, medium and low gRFI calves. Additionally, growth and ADG were similar among all three groups of calves. ANOVA results showed that breed composition had a significant effect on growth, weaning weight and 205-day weaning weight, suggesting that breed differences and heterosis would likely have affected the significance of those results. Least square means (LSM) and standard errors (SE) for the calf production metrics in relation to gRFI of the dams are summarized in Table 2. Although there were no differences, growth and ADG tended to be greatest in high gRFI calves and lowest in low gRFI calves. When using the actual weaning weights as a percentage of mature cow weight, the LSM values remained similar among calves from the high, medium and low gRFI cows, at 33.8%, 33.4% and 33.8% ($P > 0.05$), respectively. When using the 205-day adjusted weaning weights as a percentage of mature cow weight, LSM values were 41.8%, 40.5% and 40.7% ($P > 0.05$) for the high, medium and low gRFI groups, respectively. Regression of phenotypic RFI values and both 205-day adjusted (Fig. 1) and unadjusted

(Fig. 2) calf weaning weights showed little relationship, even when calf age was incorporated ($R^2 \leq 0.027$), with none of the relationships being significant ($P \geq 0.57$).

Cow Responses

Results of the ANOVA analyses of the breeding cow herd are shown in Table 1, with LSM and SE responses in Table 3. High (n=70), medium (n=207) and low (n=58) gRFI cows had similar ages ($P > 0.05$), with an average age of 4.12, 3.70, and 3.95 years, respectively. LSM values for cow weights show that high gRFI cows were significantly heavier than medium and low gRFI cows in both the spring and fall (Table 3). ANOVA results revealed that cow age, as well as RFI, had a significant effect on spring and fall cow weights (Table 1). Weight gain over the summer grazing period tended to be greater in medium gRFI cows, lower in high gRFI cows, with low gRFI cows in the middle, although none of the groups differed significantly. Regressions of cow weight gain against gRFI showed slight negative relationships, in both cases the R^2 values were low (i.e. $R^2 = 0.0018$ and $R^2 = 0.071$) with neither of the relationships being significant (Fig. 3), although it appears that cow age had an effect on the relationships.

LSM and SE values related to the change in cow backfat are summarized in Table 3. The average spring cow backfat measures for high, medium and low gRFI cows did not differ from one another ($P > 0.05$). Although all gRFI cow groupings tended to increase in average backfat thickness over the grazing season, average fall cow backfat measurements were similar among high, medium and low gRFI cows. Changes in backfat over the summer grazing period tended to be greatest in low gRFI cows, lowest in medium gRFI cows, with high gRFI cows in the middle, although once again these remained similar to one another ($P > 0.05$). Regressions of the change in cow backfat over the grazing season against gRFI value showed slight negative relationships, neither were strong (i.e. $R^2 = 0.0007$ and $R^2 = 0.06$) or significant ($P > 0.05$) relationships (Fig. 4). Once again, it was evident that cow age had an effect on the relationships. However the ANOVA revealed that there was no significant interaction between RFI and cow age (Table 1).

Cow pregnancy results, from the chi-square analysis, revealed no significant difference in pregnancy status distribution between high (n=73), medium (n=209) and low (n=59) gRFI cows (Fig. 5). All three groups had the greatest number of cows conceive during the first breeding cycle, while the percentage of open cows was the smallest proportion in all three gRFI groups of cows. More specifically, of the high gRFI cows (n=74) 50% became pregnant in the first breeding cycle, 32% in the second breeding cycle and 18% were open. Of the medium gRFI cows (n=208) the percentage that became pregnant in the first breeding cycle, in the second breeding cycle and the percentage of open cows was 52%, 35% and 13%, respectively. Of the low gRFI cows (n=59) the percentage that became pregnant in the first breeding cycle, the second breeding cycle, and the percentage of open cows was 49%, 41% and 10%, respectively.

DISCUSSION

Cow Production Metrics

It was hypothesized that cow weight gain and growth would not be affected by selection for RFI. Based on the results, this hypothesis was supported as cow weight gain over the summer grazing period was not affected by RFI, and the regression analysis showed no relationship between a cow's gRFI value and change in weight over the summer grazing season. However, these relationships appeared to depend on cow age, with RFI more likely to impact weight gain in older cows than younger cows, with a trend towards low gRFI cows putting on more weight in older cows, likely resulting from a comparatively smaller number of old cows (i.e. greater than five years of age). These results validate other studies of mature lactating cows that also concluded no difference in growth relative to gRFI. Black et al. (2013) separated replacement heifers into groups of high, medium and low RFI, based on phenotypic RFI values. Subsequent performance measures of these same animals as three-year-old lactating cows indicated no difference in growth or ADG over a 70-day trial period, in which the cows were fed forage-based diets (Black et al. 2013). Despite no difference in body weight change or ADG, DMI was different among all

groups, with low RFI cows consuming significantly less than their high RFI counterparts (Black et al. 2013). Results from the current study also confirm earlier results by Arthur et al. (2005) in which 185 Angus cows, with 1.5 generations of divergent selection for RFI, had no significant differences in body weight, or weight gain, when comparing body weights collected 4 times throughout the year.

While it was hypothesized that low gRFI cows would accumulate more backfat over the grazing period as a result of lower maintenance requirements, the current study did not support this notion. Instead, selection for RFI in the cow herd on pasture did not compromise the ability of those cattle to accumulate backfat over the course of the grazing period, as there was no significant difference in the change in backfat of cows among each of the gRFI groups. Despite this, a non-significant negative relationship between gRFI value and changes in backfat was observed, suggesting efficient cows may have had a tendency to put on more backfat. These results are supported by earlier research which failed to find differences in backfat between high and low RFI cows (Black et al. 2013). A previous study conducted by Basarab et al. (2007) examined phenotypic relationships between progeny and maternal productivity of high, medium and low RFI cattle. Basarab et al. (2007) found that cows that produced high RFI calves consistently had less backfat than the cows that produced low RFI calves, somewhat validating the trend identified in the current study in which efficient cattle had a tendency to put on more backfat. Although, body composition is not the same among mature cows, and yearling heifers and steers, previous studies did not identify differences in backfat between high and low RFI steers (Castro Bulle et al. 2007) or heifers (Fitzsimons et al. 2013). Although the current results are not supported by all literature, the results are still positive for the use of RFI as they suggest that selecting for RFI in beef cattle is unlikely have a negative effect on cow body condition.

It was hypothesized that there would be no difference in pregnancy rates among cows in the different gRFI groups. This hypothesis was supported as the results confirmed that cow pregnancy rates did not vary in relation to gRFI. Pregnancy rates also remained very similar across all three gRFI groups with nearly 50% of the cows in each group becoming pregnant in the first breeding cycle. These findings

validate results from Arthur et al. (2005), in which overall pregnancy rates were 90.2% and 90.5% for high and low RFI cows, respectively. Although the low RFI cows in that study had significantly less backfat, which had a tendency to delay their time of calving, it did not affect the overall pregnancy rate (Arthur et al. 2005). A study by Shaffer et al. (2011) found that low RFI beef heifers reached puberty significantly later. Although not significant, the study also found that low RFI heifers tended to have lower pregnancy rates and lower conception rates than their high RFI counterparts (Shaffer et al. 2011). Reproductive performance is known to be related to backfat measures (Drennan and Berry 2006), in which reduced fat can negatively impact conception and pregnancy rates, as well as delay the onset of puberty (Basarab et al. 2007). It is likely that because backfat measures did not differ between cows in the current gRFI groupings examined, neither the timing of conception nor their ability to conceive were compromised.

Calf Production Metrics

It was hypothesized that there would be no difference in calf size and calf growth among calves of different gRFI groups, this hypothesis was supported. Birth weights, along with weaning weights and 205-day adjusted weaning weights for calves in all three gRFI groups were not statistically different. Additionally, ADG and calf growth over the grazing period was similar among high, low and medium gRFI calves. In addition to similar weaning weights and growth, weaning weights as a percentage of mature cow weight was similar across the three groups, even though the low gRFI cows were significantly lighter than the high gRFI cows but had similar body weights to medium gRFI cows (Table 3). This result may reflect ongoing selection by producers in this commercial beef herd for smaller framed cows. This process was intended as a means of producing more kg of beef per hectare by enabling more cattle to be supported, while also actively selecting for RFI, thereby explaining why low gRFI cows may have been smaller in size. Medium gRFI cows were likely also significantly lighter than high gRFI cows as a result of ongoing selection, which explains why they had significantly lower gRFI values than the high gRFI cows. Regressions of the phenotypic heifer calf RFI values against the adjusted 205-day

weaning weights and actual weaning weights (Fig. 1 and Fig. 2, respectively) showed little to no relationship, which also supports the initial hypothesis that growth would be independent of RFI and is consistent with previous studies (Koch et al. 1963; Kennedy et al. 1993; Crews 2005; Castro Bulle et al. 2007; Fitzsimons et al. 2013).

Previous research conducted by Castro Bulle et al. (2007) examined performance traits of individually penned high and low RFI beef steers, in which their results indicated no differences in initial body weight, final body weight or ADG following a 122-day trial period. Although growth of the steers was not significantly different over the length of the trial, low RFI steers had significantly lower DMI (Castro Bulle et al. 2007). Similar results were noted by Fitzsimons et al. (2013) in which there were no differences in initial weight, final weight and ADG between high and low RFI beef heifers, even though there was a 15% difference in DMI between the two groups of heifers. Results from Castro Bulle et al. (2007) and Fitzsimons et al. (2013) both validated previous studies that identified RFI as being independent of body size and growth (Koch et al. 1963; Kennedy et al. 1993; Crews 2005); also validating the results of the current investigation.

Research Limitations

This research was limited by knowledge of individual cow milk production, a trait which may have provided some insight into the differences in energy supplied to calves in each of the three gRFI groups. Additionally, the cows in this study had a smaller range in RFI when compared to previous studies such as Fitzsimons et al. (2013) and Manafiazar et al. (2015) which had ranges in RFI of -0.50 to 0.54, when compared to the range of -0.21 to 0.14 in the current study, making it difficult to directly compare the results.

CONCLUSION

Based on the current study's experimental design, the observations and the results, selection for RFI in cattle on pasture is not likely to affect cow productivity, in terms of a cow's ability to maintain body

weight, put on backfat and become pregnant. Additionally, selection for RFI is not likely to affect calf growth or fall weaning weights. The results suggest that selecting for more feed efficient cattle will not compromise overall herd productivity. Future research should look into whether differences in grazing behaviour and terrain selection should be considered when trying to increase the efficiency of cattle on pasture. This is important as previous research has identified that activity levels contribute to differences in RFI, which can be readily expressed in a pasture environment.

Table 1. ANOVA table of cow and calf production metrics for high, medium and low gRFI cattle

Calf Production Metrics

	Birth Weight (kg)		Average Daily Gain (kg day^{-1})	
	F-Value	P-Value	F-Value	P-Value
RFI Group	1.27 $_{(2, 206)}$	0.28	6.00 $_{(2, 206)}$	0.003
Calf Sex	18.21 $_{(1, 206)}$	< 0.0001	7.14 $_{(1, 206)}$	0.008
Cow Age	1.45 $_{(5, 206)}$	0.21	2.51 $_{(5, 206)}$	0.03

	Weaning Weight (kg)		205-D Weaning Weight (kg)		Growth (kg)	
	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
RFI Group	0.79 $_{(2, 203)}$	0.46	0.51 $_{(2, 203)}$	0.60	0.76 $_{(2, 203)}$	0.47
Calf Sex	0.01 $_{(1, 203)}$	0.91	0.02 $_{(2, 203)}$	0.90	0.11 $_{(1, 203)}$	0.74
Cow Age	3.71 $_{(5, 203)}$	0.003	3.39 $_{(5, 203)}$	0.006	3.94 $_{(5, 203)}$	0.002
Retained Heterozygosity	0.87 $_{(1, 203)}$	0.35	0.60 $_{(1, 203)}$	0.44	0.40 $_{(1, 203)}$	0.53
Breed Composition	2.89 $_{(2, 203)}$	0.06	3.56 $_{(2, 203)}$	0.03	3.66 $_{(2, 203)}$	0.03

Cow Production Metrics

	Change in Weight (kg)		Spring Weight (kg)		Fall Weight (kg)	
	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
Cow Age	6.92 $_{(1, 286)}$	0.078	29.39 $_{(1, 308)}$	< 0.0001	8.66 $_{(1, 308)}$	0.004
RFI Category	1.17 $_{(2, 286)}$	0.23	5.13 $_{(2, 308)}$	0.006	12.22 $_{(2, 308)}$	< 0.0001
Cow Age: RFI Category	1.96 $_{(2, 286)}$	0.14	1.21 $_{(2, 308)}$	0.301	6.59 $_{(2, 308)}$	0.002

	Change in Backfat (mm)		Spring Backfat (mm)		Fall Backfat (mm)	
	F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
Cow Age	0.611 $_{(1, 286)}$	0.44	1.65 $_{(1, 308)}$	0.199	1.35 $_{(1, 308)}$	0.247
RFI Category	1.22 $_{(2, 286)}$	0.3	0.804 $_{(2, 308)}$	0.449	2.25 $_{(2, 308)}$	0.107
Cow Age: RFI Category	1.22 $_{(2, 286)}$	0.3	0.794 $_{(2, 308)}$	0.453	2.33 $_{(2, 308)}$	0.099

Table 2. Calf production metrics for each of the high, medium and low gRFI calves assessed during the 2015 grazing season.

	Least Square Means	SE
Birth Weights (kg)		
High gRFI	37.40[a]	1.24
Medium gRFI	38.37[a]	1.18
Low gRFI	39.47[a]	1.48
Weaning Weights (kg)		
High gRFI	192.88[a]	6.09
Medium gRFI	187.35[a]	5.88
Low gRFI	184.82[a]	7.55
205-Day Adjusted Weaning Weights (kg)		
High gRFI	229.10[a]	6.24
Medium gRFI	224.02[a]	6.02
Low gRFI	224.85[a]	7.73
Growth (kg)		
High gRFI	197.50[a]	6.23
Medium gRFI	191.41[a]	6.01
Low gRFI	190.63[a]	7.72
Average Daily Gain (kg day^{-1})		
High gRFI	0.96[a]	0.03
Medium gRFI	0.89[a]	0.03
Low gRFI	0.85[a]	0.04

Table 3. Production metrics for each of the high, medium and low gRFI cow groups assessed during the 2015 grazing season.

Spring Weight (kg)	**Least Square Means**	**SE**
High gRFI	518.0[a]	6.31
Medium gRFI	487.0[b]	3.79
Low gRFI	494.0[b]	7.35
Fall Weight (kg)		
High gRFI	566.4[a]	7.23
Medium gRFI	537.6[b]	4.33
Low gRFI	522.8[b]	8.41
Change in Cow Weight (kg)		
High gRFI	45.36[a]	4.22
Medium gRFI	51.78[a]	2.41
Low gRFI	44.67[a]	4.58
Spring Backfat (mm)		
High gRFI	1.21[a]	0.1
Medium gRFI	1.24[a]	0.06
Low gRFI	1.26[a]	0.11
Fall Backfat (mm)		
High gRFI	2.28[a]	0.17
Medium gRFI	2.14[a]	0.1
Low gRFI	2.11[a]	0.2
Change in Cow Backfat (mm)		
High gRFI	0.904[a]	0.1
Medium gRFI	0.833[a]	0.06
Low gRFI	0.914[a]	0.1

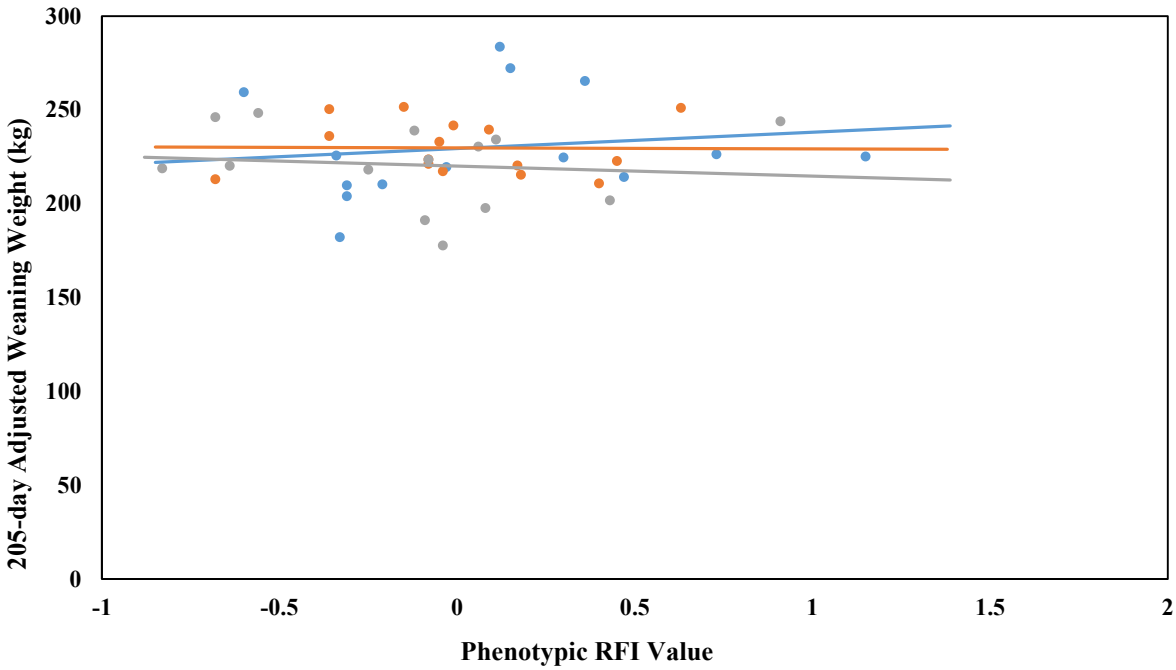

Fig. 1. Regression of phenotypic RFI value with respect to calf 205-day adjusted weaning weights, with calves grouped by age at weaning. Blue points represent the youngest calves (n=14; < 170 days of age), orange points represent the middle-aged calves (n=15; 173-181 days of age) and grey points represent the oldest calves (n=14; > 182 days of age). Corresponding lines represent the linear relationships of the different age groups.
Young calves: $y = 229.42 + 8.65x$, $R^2 = 0.021$, adjusted $R^2 = -0.061$, P-value = 0.62
Middle-aged calves: $y = 229.81 - 0.55x$, $R^2 = 0.0002$, adjusted $R^2 = -0.077$, P-value = 0.96
Old calves: $y = 220.10 - 5.32x$, $R^2 = 0.013$, adjusted $R^2 = -0.069$, P-value = 0.70

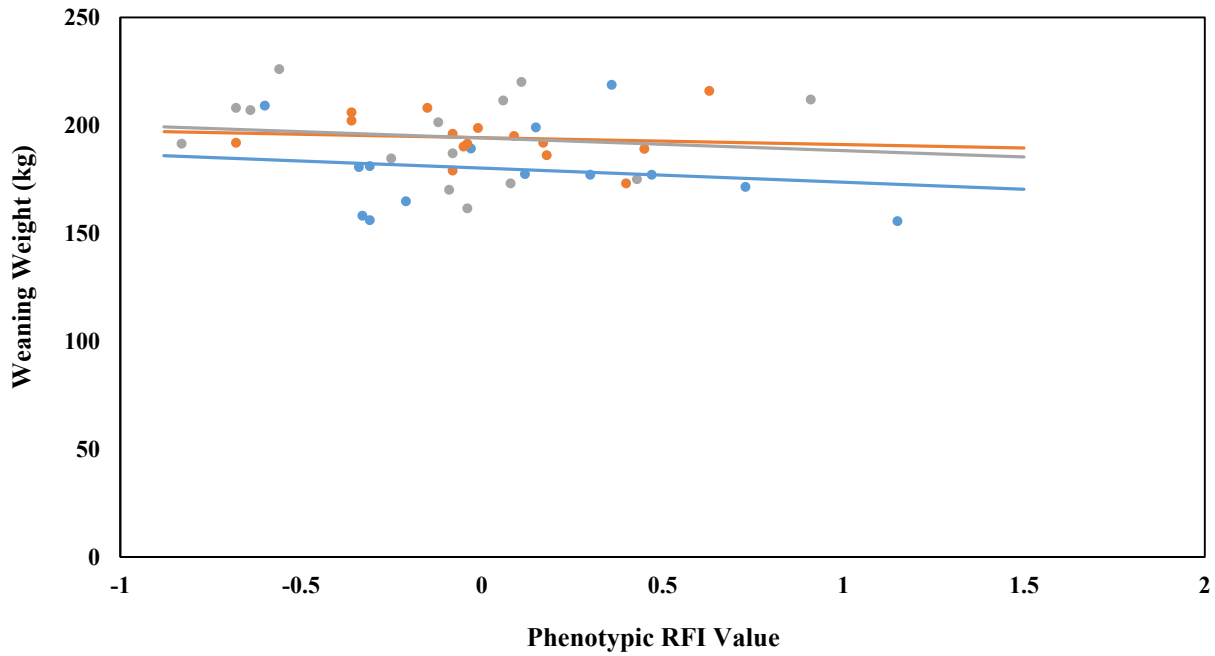

Fig. 2. Regression of phenotypic RFI value with respect to calf weaning weights, with calves grouped by age at weaning. Blue points represent the youngest calves (n=14; < 170 days of age), orange points represent the middle-aged calves (n=15; 173 – 181 days of age) and grey points represent the oldest calves (n=14; > 182 days of age). Corresponding lines represent the linear relationships of the different age groups.
Young calves: $y = 180.12 – 6.54x$, $R^2 = 0.027$, adjusted $R^2 = -0.054$, P-value = 0.57
Middle-aged calves: $y = 184.26 – 3.21x$, $R^2 = 0.010$, adjusted $R^2 = -0.067$, P-value = 0.73
Old calves: $y = 194.14 – 5.87x$, $R^2 = 0.018$, adjusted $R^2 = -0.064$, P-value = 0.65

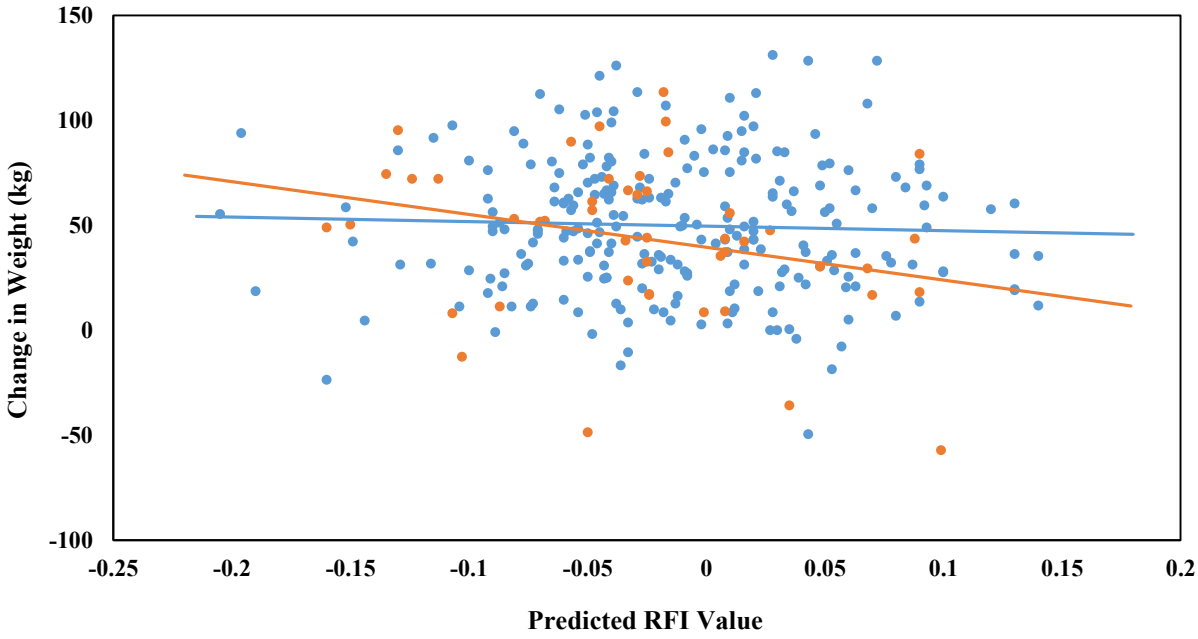

Fig. 3. Regression of the change in cow weight (kg) against predicted RFI value, with cows grouped by age. Blue points represent cows younger than 5 years of age (n=245), and orange points represent cows that were 5 years of age and older (n=47). Corresponding lines represent the linear relationships of the different age groups.
Younger cows: $y = 49.64 – 21.55x$, $R^2 = 0.002$, adjusted $R^2 = -0.0023$, P-value = 0.51
Older cows: $y = 39.56 – 156.49x$, $R^2 = 0.071$, adjusted $R^2 = 0.055$, P-value = 0.061

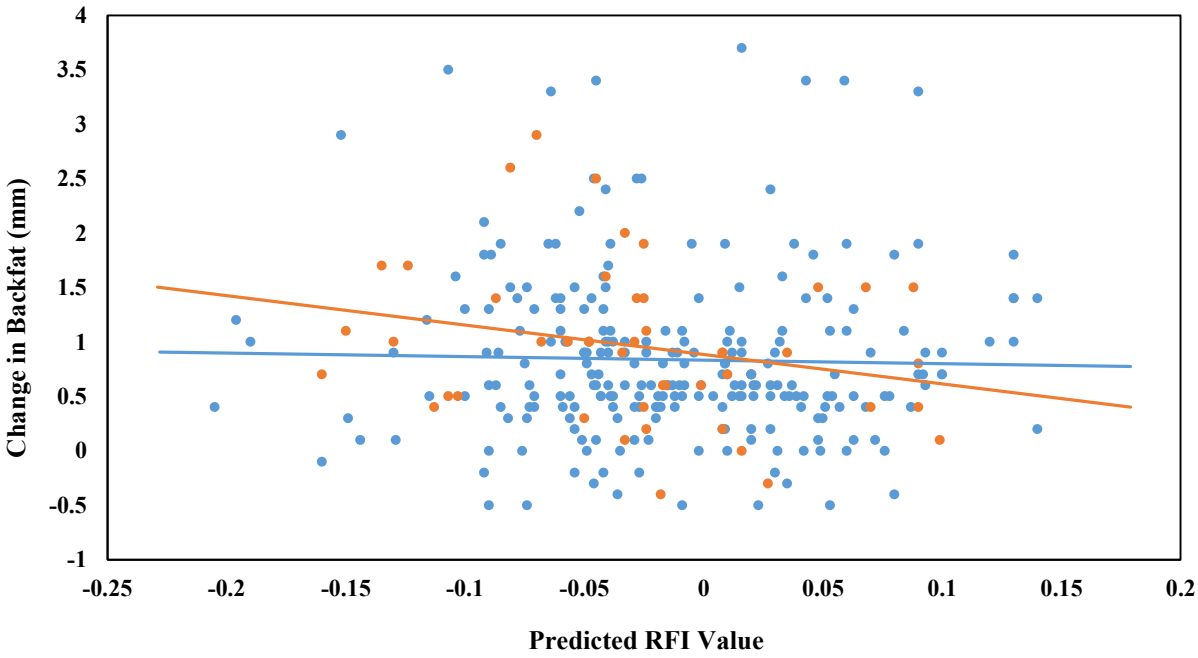

Fig. 4. Regression of the change in cow backfat (mm) against predicted RFI value, with cows grouped by age. Blue points represent cows younger than 5 years of age (n=246), and orange points represent cows that were 5 years of age and older (n=46). Corresponding lines represent the linear relationships of different age groups.
Younger cows: $y = 0.83 – 0.33x$, $R^2 = 0.0007$, adjusted $R^2 = -0.0034$, P-value = 0.67
Older cows: $y = 0.89 – 2.70x$, $R^2 = 0.060$, adjusted $R^2 = 0.039$, P-value = 0.101

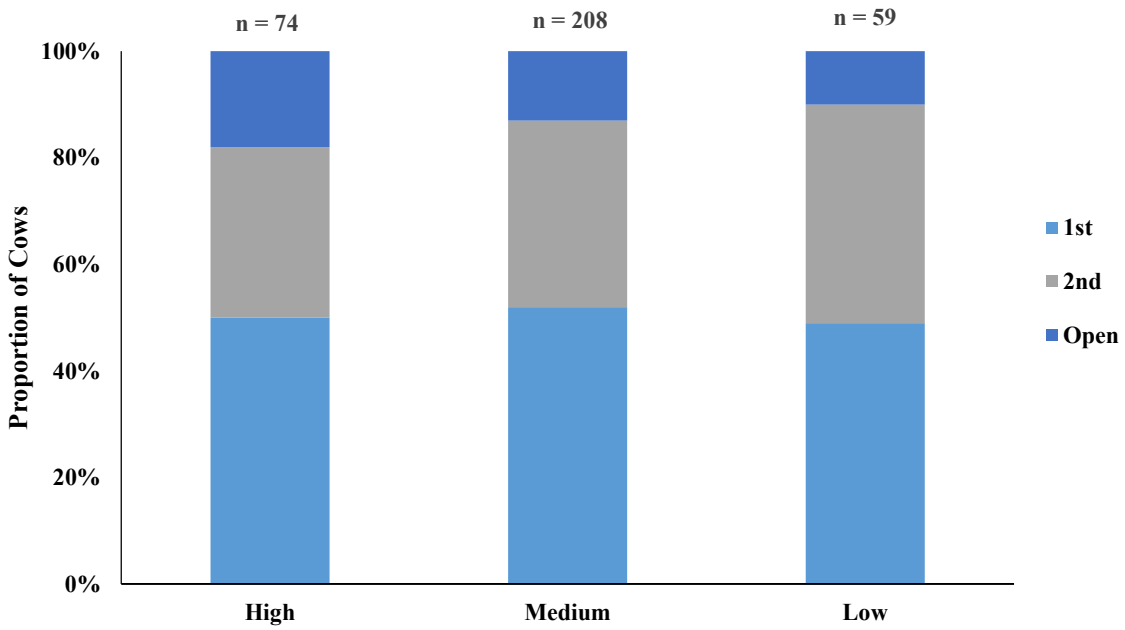

Fig. 5. Proportion of predicted high, medium and low RFI cows that became pregnant during the first and second breeding cycles, as well as the proportion of cows that remained open following the 2015 breeding season.

Chapter 3: Dry matter intake and methane emissions of heifers with divergent RFI in drylot

INTRODUCTION

Selecting for low RFI cattle reduces feed-related costs as a result of reduced feed intake (Archer et al. 1998; Basarab et al. 2003; Castro Bulle et al. 2007; Fitzsimons et al. 2013; McDonnell et al. 2016). As described by Basarab et al. (2003), efficient (i.e. low RFI) cattle consume less feed and have lower feed costs. Basarab et al. (2003) reported savings of \$45.80 $head^{-1}$ (\$ 0.101 kg^{-1}) over the duration of a 120-day feeding period when compared to high RFI cattle. Not only is RFI associated with dry matter intake (DMI), it is also associated with methane (CH_4) production, which is especially important when considering sustainability of the beef industry. Emissions of CH_4 are often the focus of climate change efforts because CH_4 is a greenhouse gas (GHG) that has 25 times the global warming potential of carbon dioxide (CO_2; IPCC 2014).

Ruminants produce CH_4 as a byproduct of digestion and fermentation (Beauchemin et al. 2009), it is also emitted from manure and manure compost sources (Hao et al. 2001). In North America, ruminant CH_4 production accounts for approximately 8% of GHG emissions (Beauchemin et al. 2010; Basarab et al. 2012), most of which are associated with breeding cow herds as a result of their largely forage-based diets (Allen et al. 1992; Verge et al. 2008; Capper 2011) and longer life span. Increases in feed intake lead to greater CH_4 production due to increased hydrogen available for CH_4 production with more extensive fermentation (Hegarty et al. 2007).

Individual DMI is often evaluated under drylot conditions where cattle diet and feed intake are controlled (Basarab et al. 2003; Wang et al. 2006), and often employ specialized testing technology such as GrowSafe equipment (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). The latter is comprised of automated feed intake monitoring equipment, similar to a feed bunk, but which collects daily DMI information from each animal with each visit to the feed bunk. Wang et al. (2003) explained that feed

intake is monitored over a 76-day period following an initial 21-28-day dietary adjustment phase, with several body weights collected throughout the length of the trial. The duration of the trial is important because it determines accuracy of growth rate and associated daily DMI measures. Longer trials generally increase the accuracy of growth rate (Culbertson et al. 2015; Manafiazar et al. 2017) ultimately enhancing the reliability of DMI measurements. RFI is often adjusted for backfat thickness (RFI_{FAT}), especially in growing heifers as they tend to be at different levels of sexual maturity, which in turn can alter intrinsic productivity (Basarab et al. 2011).

Production of CH_4 is often affected by DMI (Blaxter and Clapperton 1965; Johnson and Johnson 1995; Grainger et al. 2007), as cattle with lower DMI have lower CH_4 production. Measures of CH_4 yield, which standardize the quantification of CH_4 production relative to DMI (g kg^{-1} DMI), provide insight into inherent differences in CH_4 production. High and low RFI cattle often differ in overall CH_4 production; however, that does not mean they differ in CH_4 yield. As described in a study by Fitzsimons et al. (2013), CH_4 production was reduced in low RFI heifers, while CH_4 yield did not differ between the groups (due to correspondingly lower DMI in the low RFI heifers). Similar results were noted by Hegarty et al. (2007) and Waghorn and Hegarty (2011), where CH_4 yield did not differ between RFI groups. These findings indicate that CH_4 production may result from differences in DMI, as a result of differences in RFI. In contrast, Nkrumah et al. (2006) suggested that differences in CH_4 production result from inherent differences in RFI values among cattle. Nkrumah et al. (2006) restricted all study animals to a daily feed intake of 2.5 times maintenance, and found that low RFI cattle produced 28% less ($P= 0.04$) CH_4 than high RFI cattle, ultimately suggesting that CH_4 production is independent of DMI, and there are likely inherent mechanisms which contribute to differences in CH_4 production. Although CH_4 yield values determine which animals produce more CH_4 per kilogram of intake and they reflect inherent differences in efficiency, CH_4 production measurements are more valuable in terms of sustainability as they relate to the overall CH_4 released into the environment.

Similar to CH_4 production, CO_2 production is also affected by DMI. Manafiazar et al. (2016) reported that DMI values, standardized over the length of a test period, were highly correlated with mean daily CO_2 emissions ($R^2 = 0.39$) as well as that over a 14-day period ($R^2 = 0.79$). This suggests that a large proportion of the variation in CO_2 emissions can be explained by DMI. The association between DMI and CO_2 emissions indicates that RFI may be linked to CO_2 production. Differences in CO_2 production can be used to estimate energy expenditure (Stewart et al. 2008) which is associated with activity levels and the muscular work necessary for eating and digestion (Caton and Dhuyvetter 1997), greater CO_2 production results in a greater energy expenditure prediction. Because energy expenditure associated with eating is a result of the amount of time spent eating (Osuji 1974), cattle with greater DMI, that spend more time eating, are more likely to produce more CO_2 as a result of increased respiration. Because selecting for RFI tends to lead to reductions in DMI, it is also likely to reduce CO_2 production, and ultimately decrease GHG emissions, allowing for more sustainable beef production. Along with the negative effects of releasing GHG into the atmosphere, CH_4 and CO_2 emissions also affect the overall efficiency of cattle as it relates to the amount of energy available of growth and production.

Individual CH_4 emissions can be measured using different methods, with one such method using sulfur hexafluoride (SF_6) gas (Johnson et al. 1994). The SF_6 method requires each individual animal to be equipped with a halter-mounted collection canister, connected to a tube to capture the individual animal's exhaled gases, including those expelled during eructation. Permeation tubes containing SF_6 tracer gas are inserted in each animal's rumen, allowing each canister to collect CH_4 and SF_6 gas necessary for gas chromatography analysis (Johnson et al. 1994). Although the SF_6 tracer method is able to assess CH_4 emissions from individual animals, the intrusive nature of the equipment has the potential to interfere with natural animal behaviours. Another method that measures individual CH_4 emissions takes a more technologically advanced approach, wherein animals voluntarily visit an individual feeding stall where non-dispersive lasers within the feeding stall actively measure CH_4 emissions (Manafiazar et al. 2016).

Although the second method does not limit or interfere with individual animal behaviour, it is possible that not all animals will visit the unit.

The objective of this study was to determine if there is a difference in DMI, as well as CH_4 production and yield, within beef cattle fed under drylot conditions. It was hypothesized that (1) high RFI heifers would have higher DMI in drylot, (2) that high RFI heifers would have greater CH_4 and CO_2 production in drylot, (3) that CH_4 yield would be similar between high and low RFI heifers in drylot, and (4) that DMI would be correlated to CH_4 production in drylot.

MATERIALS AND METHODS

This research was conducted between January 2016 and April 2016 at the Agriculture and Agri-Food Canada (AAFC), Lacombe Research and Development Centre (Lacombe, Alberta, Canada) in collaboration with Gemstone Cattle Company. All animal procedures were reviewed and approved by the University of Alberta Animal Care and Use Committee (AUP00001284), together with the AAFC Lacombe Animal Care Committee; all animals were cared for in accordance with the Canadian Council on Animal Care (1993).

A total of 60 replacement heifers from the 2015 calf crop associated with the Gemstone Cattle Company near Gem, AB, were selected based on their dam's predicted RFI values (30 high and 30 low), as well as structural soundness, age and weight. All animals were largely Hereford with some Angus influence. All heifers were tested for individual feed intake using GrowSafe equipment (GrowSafe Systems Ltd, Airdrie, Alberta, Canada) at the AAFC Lacombe Research and Development Centre. Simultaneously, observations of individual animal CH_4 production were collected using a GreenFeed Emissions Monitoring System (GEMS) (C-Lock Inc., Rapid City, South Dakota, USA). The average age of all heifers at the start of the trial was 276 days (SD = 16.275) with a range of 66 days, while the average weight was 267.57 kg (SD = 23.13) with a range of 98 kg. Dams of the high and low RFI heifers had average predicted RFI values of 0.0536 and -0.0774, respectively. Prior to the start of the trial all

heifers were tagged with high performance half-duplex radio frequency identification (RFID) tags (HDX Ultra EID Tag, Allflex USA, Texas, USA) in order to be identified by the GrowSafe and GreenFeed Emissions Monitoring systems. Access to both the GEMS unit and the GrowSafe bunks was limited to a single animal at a time to ensure that only one RFID tag was identified each time the systems were accessed.

Dry Matter Intake

Replacement heifers were subject to 76 days of feed intake observations, from February 19, 2016 to April 26, 2016, following a 21-day acclimatization period, in a drylot pen with eight GrowSafe automatic feed intake bunks. The bunks measured each individual heifer's daily feed intake (kg DM d^{-1}) along with feeding behaviors such as the number of daily feeding events (events day^{-1}), head down time (min day^{-1}) and feeding event duration (min d^{-1}) (Basarab et al. 2007 and 2011). Heifers were weighed before morning feeding on two consecutive days at the beginning of the trial along with two consecutive days at the end of the trial. Two additional weights were collected at approximately 28-d intervals throughout the trial. Ultrasound backfat measurements were collected on the first and last days of the trial. Heifers were on a 100% barley silage diet, and fed twice a day in the morning and afternoon. Bedding (i.e. woodchips and shavings) was added to the pen as needed and water access was *ad libitum.*

Barley silage samples were collected once a week for the entire duration of the trial. Samples from each month were mixed, and then sub-sampled in duplicate. One of the duplicate samples was dried at 80ºC for 72 hours and weighed to determine average dry matter (DM) content, while the other sample was dried at 55ºC for 72 hours, ground to 1mm in a Wiley™ mill and analyzed by Cumberland Valley Analytical Services (CVAS Inc., Maugansville, Maryland, USA) to determine average crude protein (CP), acid-detergent fibre (ADF), neutral-detergent fibre (NDF), calcium (Ca), phosphorus (P) and total digestible nutrient (TDN) content over the length of the trial (Table 1). CVAS analytical procedures are referenced in Appendix B.

Methane Emissions

Emissions of CH_4 were monitored for 28 consecutive days, from February 19, 2016 to March 8, 2016, using a GEMS, at the same time that individual feed intake measurements were collected. Heifers were on a 100% barley silage diet for the duration of the measurement period. Observations were collected as described by Manafiazar et al. (2016). The GEMS attracted heifers by dropping pellets into a feeder. Each heifer received six pellet drops per visit, with a 36 sec interval between each drop; the average drop size was 32.3 g (SD = 0.318). After the sixth drop, heifers had to wait 4 hours to receive another drop, allowing for a maximum of six visits per day. Heifers had to remain at the GEMS unit for 3-5 min in order to gather adequate CH_4 and CO_2 measurements.

During an animal's visit to the GEMS unit, air was drawn past their head and into the collection pipe of the system. The system continuously measured the rate of airflow along with background concentrations of CH_4 and CO_2 to accurately quantify trace gas flux during each visit. A non-dispersive infrared laser, positioned inside the collection pipe, was used to analyze CH_4 and CO_2 concentrations. Measurements were only kept for analysis when the head proximity sensor, positioned within the unit, determined that the animal's head was in the proper position for sampling (Huhtanen et al. 2015). Calibration of the system required the release of propane tracer gas, a semi-pure nitrogen gas and a span-gas mixture of CO_2, CH_4 and pure nitrogen. Propane tracer gas was released inside the unit on a regular basis for a duration of 5 min in order to calculate the dilution air. The span-gas and semi-pure nitrogen were remotely released into the unit on a daily basis to calibrate CH_4 and CO_2 measurements. At the start of every month CO_2 recovery tests were performed by releasing a known amount of CO_2 directly into the GEMS, this was done three times and for three min each time. Calibration measurements were included in the individual CH_4 and CO_2 concentration calculations.

The pellets in the GEMS were wheat-based (pellet 1), and on day 17 of observations the pellets were replaced with a different wheat-based pellet (pellet 2). Pellets used in the GEMS were analyzed to determine DM content and nutritional value. Pellet samples were collected at least once a week, with a

total of 5 samples collected. After collection, samples were split in half in order to assess DM content as well as nutritional value. DM pellet samples were dried at 80^0C for 72 hours, while the nutritional value samples were dried at 55^0C for 72 hours and then ground to 1 mm using a Wiley™ mill. Nutritional samples were analyzed by CVAS (see Appendix B for analytical procedures). The nutritional values of pellet 1 and pellet 2 are described in Table 2.

Calculations and Statistical Analysis

Dry Matter Intake

Out of the 60 heifers selected for the individual feed intake test, three heifers were excluded from the final analysis due to unreasonably low DMI values, potentially resulting from faulty RFID tags. Linear regression of each heifer's observed body weight, against the number of days on test, was used to calculate ADG, on-test body weight, and mid-point weight (Basarab et al. 2003; Wang et al. 2006). Each heifer's average daily feed intake was then converted to total dry matter intake (DMI), which was then used to determine total metabolizable energy (ME) intake based on the known DM and ME content of the diet (Table 4)**.** Total DMI values included any daily pellet intake from heifers visiting the GEMS. Total ME of the diet was determined using the following two formulas (NRC 1996):

$$\text{TDN (\%)} = 96.03 - [1.034 \times \text{ADF (\%)}]$$

$$\text{ME (MJ kg}^{-1}\text{ DM)} = ((\text{\% TDN}/100) \times 4.4 \text{ Mcal kg}^{-1}\text{ TDN}) \times 4.184 \text{ MJ DE Mcal}^{-1} \times 0.82 \text{ MJ ME MJ}^{-1}\text{ DE}$$

Total ME intake for each heifer was divided by 10 to standardize total DMI to an energy density of 10 MJ ME kg^{-1} DM. Standardized DMI for each heifer was divided by 76 (duration on feed) to produce an average standardized daily DMI (SDMI; kg DM d^{-1}). Estimates of SDMI for each heifer were then regressed against ADG and metabolic midweight (MIDWT; BW $kg^{0.75}$). This was done using PROC GLM (SAS Institute, Inc. 2009) and Model 1:

$$\text{Model 1: } Y_{ij} = \beta_0 + \beta_1 \text{ ADG}_i + \beta_2 \text{ metabolic MIDWT}_j + e_{ij}$$

where Y_{ij} is the SDMI for animal *ij,* β_0 is the regression intercept, β_1 is the partial regression coefficient of SDMI on average daily gain, β_2 is the partial regression coefficient of SDMI on metabolic mid-weight and e_{ij} is the random error term. Measured RFI was adjusted for backfat thickness using model 2:

$$\text{Model 2: } Y_{ijk} = \beta_0 + \beta_1 \text{ADG}_i + \beta_2 \text{ metabolic MIDWT}_j + \beta_3 \text{ BFend}_k + e_{ijk}$$

where β_3 is the partial regression coefficient of SDMI on final ultrasound backfat thickness (BFend; mm). The following equations were used to calculate expected feed intake without adjusting for backfat (EFI_I) and expected feed intake adjusted for backfat (EFI_{II}):

$$EFI_I = -1.71 \pm 1.10 + (1.05 \pm 0.60 \text{ ADG}) + (0.10 \pm 0.02 \text{ MIDWT}^{0.75}),\ R^2 = 0.57,\ P < 0.0001$$

$$EFI_{II} = -1.73 \pm 1.24 + (1.05 \pm 0.61 \text{ ADG}) + (0.10 \pm 0.02 \text{ MIDWT}^{0.75}) - (0.002 \pm 0.06 \text{ BF}_{END}),\ R^2 = 0.57,\ P < 0.0001$$

Unadjusted RFI and RFI adjusted for backfat thickness (RFI_{FAT}) were determined for each heifer as the deviation of SDMI from the expected feed intake (EFI; RFI = SDMI - EFI_I) and SDMI from EFI_{II} (RFI_{FAT} = SDMI - EFI_{II}). Three heifers (out of 60) were not assigned RFI values as a result of inadequate feed intake reads likely due to a faulty tag, and as a result of periods of low growth or weight loss during the trial, affecting feed intakes of those animals. RFI_{FAT} was used to rank the heifers from lowest to highest. All subsequent analyses and comparisons were done using RFI_{FAT} values and groupings rather than RFI.

Differences in DMI were analyzed using a mixed effects model in the nlme package in R (Pinheiro et al. 2016) where daily DMI, RFI_{FAT} group and heifer age (days) were fixed effects, with individual animal as a random effect. Type III analysis of variance (ANOVA) in the car package (Fox and Weisberg 2011) in R, was used to determine differences in DMI between the high and low RFI_{FAT} heifers. Post-hoc multiple comparisons were conducted using a Tukey's Honest Significant Difference (HSD) test in the lsmeans package in R (Russell and Lenth 2016). Initial and final body weight and backfat measurements were analyzed using a mixed effects model in the nlme package in R (Pinheiro et al. 2016). The different models had response variables of initial or final body weight or backfat, along with the fixed

effect of RFI_{FAT} group and heifer age as a covariate, with individual animal as random effects. Type III ANOVA was conducted to determine differences in body weight and backfat between high and low RFI_{FAT} heifers. Post-hoc multiple comparisons were done using Tukey's HSD test in the lsmeans package in R (Russell and Lenth 2016). Simple linear regression of average daily DMI and RFI_{FAT} was conducted in R (R Core Team 2016).

Methane Emissions

Heifer CH_4 production was analyzed based on the heifers' phenotypic RFI_{FAT} values, collected in drylot using GrowSafe equipment. Thirteen heifers (out of 60) were excluded from the analysis due to a lack of adequate CH_4 or phenotypic RFI_{FAT} data. Data for the remaining 47 animals were analyzed using mixed linear models in the mixed procedure package in SAS (Statistical Analysis Software Institute Inc. 2013), with the following specifications: CH_4 production was a dependent variable, while RFI_{FAT} group, day of measurement, and their interaction, were fixed effects (independent variables), and the number of visits per day was a covariate. Individual animal was nested within each day, and day was included as a repeated measure. The same model was used to determine CO_2 production, but with CO_2 concentration as the dependent variable. Type III ANOVA, in the car package (Fox and Weisberg 2011) in R was conducted on both resulting datasets. Simple linear regressions of average daily CH_4 production and RFI_{FAT}, as well as average daily CH_4 production and average daily DMI, were analyzed in R (R Core Team 2016).

Daily CH_4 and CO_2 flux observations were analysed to determine the diurnal pattern of CH_4 and CO_2 production throughout the day. Production of CH_4 occurs when cattle are consuming feed, and because high and low RFI cattle have different feeding behaviors (Robinson and Oddy 2004; Basarab et al. 2007; Nkrumah et al. 2007; Golden et al. 2008; Bingham et al. 2009; Herd and Arthur 2009; Montanholi et al. 2010; Kelly et al. 2010; Durunna et al. 2011), it is important to consider that they may also be producing different amounts of CH_4 at different times during the day. Therefore, CH_4 production concentrations were separated into eight different time bins, with each one grouping all CH_4 production

measurements over three hour long time periods starting at 0000 hours. The analysis was conducted using the mixed procedure package in SAS with RFI_{FAT} group, day, interaction between day and RFI_{FAT} group, time bin (i.e. a three-hour time period), and the interaction between time bin and RFI_{FAT} group as fixed effects. The mixed model used a covariate of available time, referring to the time which the heifers spent at the GEMS unit.

CH_4 yield per heifer was calculated by averaging the individual CH_4 production concentrations over time and dividing this average by the average individual DMI calculated over the entire length of the feed intake trial. This analysis was conducted using the GLM procedures package in SAS (Statistical Analysis System Institute Inc., Cary, NC, USA), with the fixed effect of RFI_{FAT} group and the number of measurements collected each day as a covariate. Differences in CH_4 and CO_2 yield were analyzed using a Tukey's HSD test in SAS. Simple linear regressions of average daily CH_4 yield and RFI_{FAT}, as well as average daily CH_4 yield and average daily DMI were analyzed in R (R Core Team 2016).

RESULTS

Dry Matter Intake Observations

Based on the phenotypic values collected from the individual feed intake trial, high RFI heifers (n = 29) had an average RFI value of 0.37 (SD = 0.44) while low RFI heifers (n = 28) had an average RFI value of -0.39 (SD = 0.26). Average RFI values adjusted for backfat were 0.37 (SD = 0.44) and -0.39 (SD = 0.26) for high and low RFI heifers, respectively. The overall range in RFI_{FAT} was -1.05 to 1.95 with high RFI heifers ranging from 0.032 to 1.95 and low RFI heifers ranging from -1.05 to -0.04. Overall RFI and RFI_{FAT} values had means of 0.00 with the same SD of 0.53. Drylot individual feed intake ANOVA results are listed in Table 3 while LSM and SE are summarized in Table 4. Daily dry matter intake was significantly different between high and low RFI_{FAT} heifers, with high RFI_{FAT} heifers consuming 14% more dry matter on a daily basis. Despite a significant difference in DMI between high and low RFI_{FAT} heifers, ADG, feed conversion ratio (FCR), initial trial weight, final trial weight and metabolic mid

weight were similar (Table 4). The number of daily feeding events (events day^{-1}) was significantly higher in high RFI_{FAT} heifers (P = 0.03), however total feeding duration (min day^{-1}) did not differ between the two groups. More specifically, LSM values representing the number of daily feeding events (events day^{-1}) for the high and low RFI_{FAT} heifers was 127 (SE = 5.45) and 101 (SE = 5.56), respectively, while the LSM values for the total daily feeding duration (min day^{-1}) were 132.75 (SE = 4.41) and 135.54 (SE = 4.41) for high and low RFI_{FAT} heifers, respectively. Additionally, linear regression of average daily DMI and phenotypic RFI values show a significant positive relationship (R^2 = 0.41, P-Value < 0.0001) with low RFI heifers consuming significantly less barley silage than high RFI heifers (Fig. 1).

Methane and Carbon Dioxide Emission Observations

Drylot CH_4 and CO_2 production and yield ANOVA results are shown in Table 5. Overall CH_4 production was not significantly different between high and low RFI_{FAT} heifers, as low RFI_{FAT} heifers produced an average of 148.99 g head^{-1} day^{-1} and low RFI_{FAT} heifers produced an average of 148.66 g head^{-1} day^{-1}. Overall average CO_2 production for the high and low RFI_{FAT} heifers was 5413.98 g head^{-1} day^{-1} and 5264.33 g head^{-1} day^{-1}, respectively (Table 6). Although RFI_{FAT} group did not have a significant effect on CH_4 emissions, the day of emission observations, and the time of day that observations were collected, together with the interaction between RFI_{FAT} group and time bin, had a significant effect on CH_4 production, while CO_2 production was also significantly affected by the time in which heifers spent at the GEMS unit (Table 7). Both high and low RFI_{FAT} heifer groups produced the least CH_4 between 2100-2400 hours, with total CH_4 emission values of 128.67 g (SD = 2.49) and 129.59 g (SD = 2.48), respectively (P-Value > 0.05) (Fig. 6). Both groups produced the most CH_4 between 600-900 hours, with high RFI_{FAT} heifers producing a total of 162.92 g (SD = 2.49) and low RFI_{FAT} heifers producing a total of 164.92 g (SD = 2.53) (Fig. 2).

Methane and CO_2 yield of high and low RFI heifers in drylot were significantly different, with the low RFI heifers having greater CH_4 and CO_2 yields. Linear regression of the heifers' phenotypic RFI_{FAT} value with respect to CH_4 production showed no relationship (R^2 = 0.002, P-Value = 0.77)

between CH_4 production and RFI_{FAT} value (Fig. 3), while Fig. 4 shows a positive relationship ($R^2 = 0.27$, P-Value < 0.001) between daily DMI and daily CH_4 production. Linear regressions of CH_4 yield reveal a significant negative relationship ($R^2 = 0.44$, P-Value < 0.0001) between RFI_{FAT} and CH_4 yield (Fig. 5), as well as a significant negative relationship ($R^2 = 0.28$, P-Value < 0.001) between CH_4 yield and dry matter intake (Fig. 6).

DISCUSSION

It was hypothesized that low RFI_{FAT} heifers would have significantly lower DMI compared to the high RFI_{FAT} heifers, and this hypothesis was supported. High RFI_{FAT} heifers consumed 14% more feed throughout the course of the 76-day feed intake trial (Table 4). Previous studies found that low RFI cattle consume significantly less than high RFI cattle without compromising growth (Herd et al. 2014), which is in full support of the findings in this study, as DMI was significantly reduced without any effect on animal size and ADG. Additionally, the results of this study show a significant relationship between RFI_{FAT} and daily DMI ($R^2 = 0.41$, P-Value < 0.0001), representing a correlation of 0.64. Previous studies also identified significant relationships between RFI and DMI (Lancaster et al. 2009; Kelly et al. 2010; Lawrence et al. 2012; Fitzsimons et al. 2013). The current study is similar to that of Fitzsimons et al. (2013) as both involved replacement heifers on forage or grass silage based diets, with the biggest difference being that Fitzsimons et al. (2013) grouped DMI values by RFI rather than RFI_{FAT}. Fitzsimons et al. (2013) reported a significant correlation of 0.63 ($P < 0.01$) while the current study also reported a significant positive correlation ($r = 0.66$, $P < 0.0001$). The relationship in the current study likely presents a stronger and more significant correlation due to a much larger sample size. The current study was conducted with a total of 57 heifers while Fitzsimons et al. (2013) only studied 22 heifers. Lawrence et al. (2012) also identified a significant positive relationship between RFI and DMI ($R^2 = 0.66$, $P < 0.001$) of growing heifers consuming a grass silage diet, once again validating the results of the current study.

Despite significant differences in DMI between the groups of high and low RFI_{FAT} heifers, animal performance was not significantly different. Initial weight, final weight, mid weight and metabolic mid

weight were similar between high and low RFI_{FAT} heifers. Additionally, ADG and end of trial backfat measures were also similar between the two groups. Most of the variation in weights can be attributed to differences in heifer age. As identified in Table 3, age had a moderate or significant effect on initial weight, final weight, midweight, as well as metabolic midweight. Previous research supports the findings of the current study (Basarab et al. 2003; Nkrumah et al. 2004; Kelly et al. 2011; Fitzsimons et al. 2013). High and low RFI bulls that were tested for individual feed intake, on a highly concentrate diet with access to grass hay, had similar initial and final body weights, along with similar ADG and metabolic body weight measures (Kelly et al. 2011). Bulls only differed in rib fat and muscle depth measures in which high RFI bulls had greater muscle depth and greater backfat thickness (Kelly et al. 2011), which does not fully support the findings of similar backfat measures of high and low RFI_{FAT} heifers in the current study. In another study, backfat thickness, initial body weight, final body weight, and ADG of high and low RFI heifers on a grass silage diet was not significantly different (Fitzsimons et al. 2013), fully supporting the results of the current study.

It was hypothesized that CH_4 production in drylot would be significantly greater in high RFI heifers, and this hypothesis was not fully supported. Although high and low RFI_{FAT} heifers had similar overall CH_4 production, the interaction between RFI_{FAT} and day of sampling suggested that CH_4 production differed between RFI_{FAT} groups only on certain days. The differences in drylot CH_4 production were not directly aligned with the findings of previous studies in which high RFI cattle had overall greater CH_4 production than low RFI cattle (Nkrumah et al. 2006; Hegarty et al. 2007; Jones et al. 2011; Fitzsimons et al. 2013). Similar to the current study, Fitzsimons et al. (2013) studied feed intake and CH_4 production of high (n = 7), low (n = 7) and medium (n = 8) RFI heifers in drylot while consuming a grass silage diet. Although the experiments were similar, Fitzsimons et al. (2013) reported a significant difference in DMI as well as CH_4 production between the high and low RFI heifers. The sample size of the current study (n = 42) should not have affected the ability to find a significant difference as Fitzsimons et al. (2013) conducted the study with about half the sample size (n = 22) and

reported significant differences. While the method used to measure CH_4 emissions in the current study has seldom been reported, it should be noted that it was different from the method used by Fitzsimons et al. (2013) (i.e. GEMS rather than SF_6 tracer technique). Manafiazar et al. (2017) concluded that the GEMS unit was a credible and repeatable way in which emissions could be monitored, and as such it was unlikely that the method of measuring CH_4 emissions affected the results of the current study.

Results from the current study are more directly aligned with the results from Waghorn and Hegarty (2011) and McDonnell et al. (2016), in which absolute CH_4 production did not differ between high and low RFI animals. Waghorn and Hegarty (2011) analyzed CH_4 production data of eight high and eight low RFI dairy cows on a forage based diet, while McDonnell et al. (2016) analyzed CH_4 emissions of 14 high and 14 low RFI cattle on various diets including pasture, grass silage and a total mixed ration. More specifically, McDonnell found that CH_4 production was greatest when cattle were consuming the total mixed ration and lowest when cattle were grazing on pasture. Furthermore, CH_4 production was only correlated with daily DMI when cattle were on pasture ($r = 0.42$, P-value < 0.05) and there was no correlation between RFI and CH_4 production (McDonnell et al. 2016), as was also seen in the current study (Fig. 3). McDonnell et al. (2016) reported that errors associated with the SF_6 tracer technique used to measure the enteric CH_4 emissions could have influenced the results. As reported by Waghorn and Hegarty (2011), no differences in CH_4 production existed between high and low RFI cows (P-Value = 0.09), a result which was reportedly associated with the higher CH_4 yields of low RFI cows.

While it was hypothesized that the high and low RFI_{FAT} heifers would have similar CH_4 yields, this hypothesis was rejected. Low RFI_{FAT} heifers had significantly greater CH_4 yields in drylot when compared to high RFI_{FAT} heifers. Previous research related to CH_4 yield is conflicting, as some studies report no differences in CH_4 yield between high and low RFI heifers on grass silage diets (Fitzsimons et al. 2013), nor in steers fed concentrate diets (Hegarty et al. 2007). Whereas both McDonnell et al. (2016) and Waghorn and Hegarty (2011) reported that low RFI cattle had greater CH_4 yields, these differences were only significantly greater in the study conducted by McDonnell et al. (2016). Methane yields

relative to RFI_{FAT} in the current study parallel the findings of McDonnell et al. (2016) and Waghorn and Hegarty (2011). Methane yield in the current study was negatively correlated with RFI_{FAT} ($r = -0.66$, P-Value = < 0.0001) and also negatively correlated with DMI ($r = -0.53$, P-Value < 0.001). This means that low RFI_{FAT} heifers produced more CH_4 per kilogram of DMI.

Although not examined in the current study, it is possible that low RFI_{FAT} heifers had greater CH_4 yield values as a result of greater digestibility and greater rumen retention times, resulting in more CH_4 produced per kilogram of feed consumed. McDonnell et al. (2016) examined apparent total tract digestibility of organic matter and gross energy, and found that low RFI animals had significantly greater organic matter digestibility (P-Value = 0.027), and slightly greater gross energy digestibility when compared to their high RFI counterparts. McDonnell et al. (2016) reported that an increase in dietary digestibility likely provided methanogens with more hydrogen for CH_4 production, hence the reportedly greater CH_4 yields. Along with differences in digestibility, it is possible that differences in rumen retention time could also have contributed to the differences in CH_4 yield, especially considering that increases in digestibility are often associated with greater rumen retention time (Waghorn and Hegarty 2011). The CH_4 yield results in the current study may also arise from greater diet digestibility and greater rumen retention time of low RFI cattle. Greater dietary digestibility could also explain the similar CH_4 production between the two groups. Although low RFI_{FAT} heifers had significantly lower DMI, they produced more CH_4 per kilogram of DMI, resulting in both groups producing similar amounts of overall CH_4.

It was hypothesized that CO_2 production in drylot would be significantly greater in high RFI heifers, and this hypothesis was supported. High RFI heifers produced more CO_2 in drylot than low RFI heifers, suggesting that selecting for low RFI will result in reduced greenhouse gas emissions associated with CO_2, potentially offsetting at least a portion of the increase in CH_4 yield observed therein. As identified in an earlier study, cattle that consume less feed (i.e. low RFI cattle) produce fewer greenhouse gases, with one of those gases being CO_2 (Beauchemin et al. 2010). Although CO_2 has 25 times less the

global warming potential of CH_4 (IPCC 2014), it still influences overall GHG emission levels due to its abundance. According to the results of the current study, CO_2 emissions are of greater importance in terms of selection for RFI because the difference in emissions was much greater between the high and low RFI heifers and the overall amount of CO_2 released into the atmosphere was much more significant than the amount of CH_4. The results from the current study indicate that selection for RFI is more likely to reduce GHG production as a result of less CO_2 emissions.

Research Limitations

There were few research limitations in the current study. This study did not appear to be limited by the number of animals used or by the number of days in which DMI and CH_4 data were collected, particularly in comparison to other studies, which used fewer animals and collected data over a smaller number of days. However, cross-validation of some of the results in the current study was difficult due to the very small number of studies published to date that used the GEMS method to monitor enteric GHG emissions. In hindsight, the addition of apparent total tract digestibility and rumen retention times would have been valuable for interpreting CH_4 emission results.

CONCLUSION

Low RFI_{FAT} heifers have significantly lower daily DMI in drylot compared to high RFI_{FAT} heifers when consuming grass silage diets. Despite differences in feed intake, factors associated with production, such as weight gain and backfat measures, remained similar between high and low RFI_{FAT} heifers. Such results indicate that selecting for RFI will not compromise production traits while still reducing overall feed costs, a substantial economic advantage. Although the low RFI_{FAT} heifers consumed significantly less feed, overall CH_4 production did not differ from that of the high RFI_{FAT} heifers. The similarity in CH_4 production likely stemmed from the significantly greater CH_4 yields reported for the low RFI_{FAT} heifers. Results from the current study suggest that differences in total tract digestibility, as well as rumen

retention time, are likely to influence CH_4 production and may also influence the expression of RFI, something which will need to be examined further in additional studies.

Table 1. Composition of Barley Silage fed to replacement heifers on dry matter basis.	
Dry matter (%)	36.72
Metabolizable energy (MJ kg $^{-1}$)[X]	9.51
Crude protein (%)	10.93
Acid detergent fibre (%)	31.37
Neutral detergent fibre (%)	47.43
Total digestible nutrients (%)	63.03
Calcium (%)	0.39
Phosphorus (%)	0.26

[x]Metabolizable energy (ME), MJ kg^{-1} DM = ((TDN, %/100) x 4.4 Mcal kg^{-1} TDN) x 4.184 MJ DE Mcal^{-1} x 0.82 MJ ME MJ^{-1} DE (NRC 1996).

Table 2. Composition of Diet 1 and Diet 2 pellets used in the GEMS, all percentages on DM basis.

	Diet 1	**Diet 2**
Dry Matter (%)	96.2	97.6
Crude Protein (%)	15.9	17.3
Acid Detergent Fibre (%)	9.55	7.8
Neutral Detergent Fibre (%)	23.2	18.6
Calcium (%)	1.8	1.28
Phosphorus (%)	0.61	0.33
Magnesium (%)	0.28	0.14
Potassium (%)	0.73	0.48
Sodium (%)	0.38	0.33

Table 3. ANOVA table of drylot DMI data.

	Dry Matter Intake		**Feed Conversion Ratio**	
	F-Value	P-Value	F-Value	P-Value
RFI Group	17.57 $_{(1, 54)}$	0.0001	2.32 $_{(1, 54)}$	0.13
Calf Age	0.51 $_{(1, 54)}$	0.48	0.081 $_{(1, 54)}$	0.78
	Initial Calf Weight		**Final Calf Weight**	
	F-Value	P-Value	F-Value	P-Value
RFI Group	0.11 $_{(1, 54)}$	0.74	0.42 $_{(1, 54)}$	0.056
Calf Age	5.78 $_{(1, 54)}$	0.02	3.56 $_{(1, 54)}$	0.065
	Midweight		**Metabolic Midweight**	
	F-Value	P-Value	F-Value	P-Value
RFI Group	0.27 $_{(1, 54)}$	0.60	0.005 $_{(1, 54)}$	0.94
Calf Age	3.81 $_{(1, 54)}$	0.056	3.81 $_{(1, 54)}$	0.056
	Residual Feed Intake		**Residual Feed Intake – Fat Adj.**	
	F-Value	P-Value	F-Value	P-Value
RFI Group	53.97 $_{(1, 54)}$	< 0.0001	53.98 $_{(1, 54)}$	< 0.0001
Calf Age	1.90 $_{(1, 54)}$	0.17	1.91 $_{(1, 54)}$	0.17
	Average Daily Gain		**Backfat**	
	F-Value	P-Value	F-Value	P-Value
RFI Group	0.22 $_{(1, 54)}$	0.64	0.10 $_{(1, 54)}$	0.75
Calf Age	0.039 $_{(1, 54)}$	0.84	0.52 $_{(1, 54)}$	0.47

Table 4. Dry matter intake and performance of heifers fed barley silage for 76 days in drylot at the Lacombe Research Centre.

	High RFI_{FAT}		Low RFI_{FAT}	
	LSM	**SE**	**LSM**	**SE**
Daily Dry Matter Intake (kg DM day^{-1})	6.21[a]	0.13	5.43[b]	0.13
RFI	0.36[a]	0.07	-0.37[b]	0.07
RFI Fat	0.36[a]	0.07	-0.37[b]	0.07
Initial Weight (kg)	269.11[a]	4.27	267.08[a]	4.35
Final Weight (kg)	314.05[a]	5.82	308.61[a]	5.92
Mid Weight (kg)	288.15[a]	5.91	284.35[a]	5.02
Metabolic Mid Weight (kg)	69.88[a]	0.91	69.19[a]	0.93
Backfat (mm)	4.09[a]	0.3	3.96[a]	0.30
Average Daily Gain (kg d^{-1})	0.58[a]	0.03	0.56[a]	0.03
Feed Conversion Ratio	12.22[a]	0.64	10.81[a]	0.65

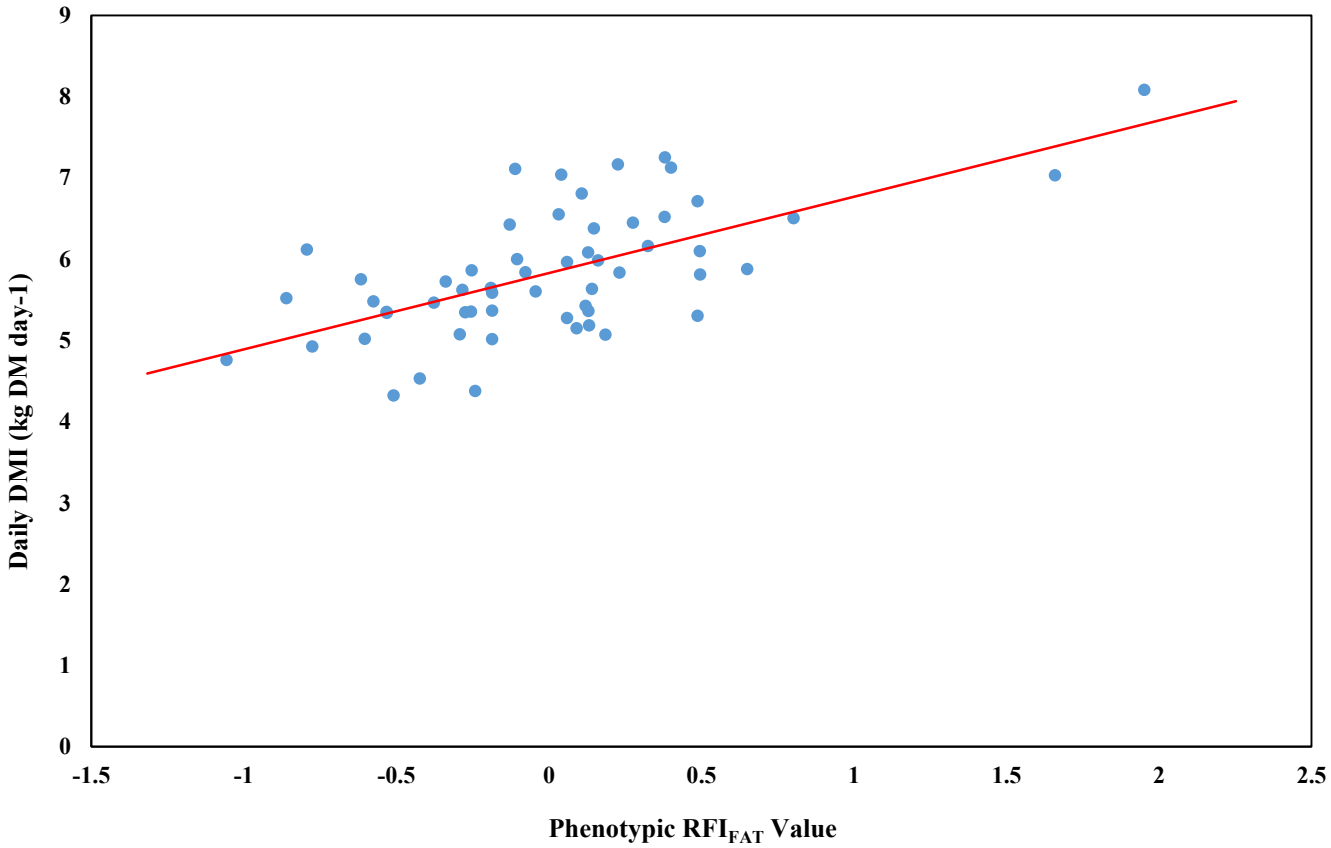

Fig. 1. Linear regression of replacement heifer DMI with respect to phenotypic RFI_{FAT} value.
$y = 5.83 + 0.094x$, $R^2 = 0.41$, Adjusted $R^2 = 0.39$, P-Value < 0.0001.

Table 5. ANOVA table of CH_4 and CO_2 production (g $head^{-1}$ day^{-1}) and yield (g kg^{-1} DMI) of high and low RFI_{FAT} heifers fed barley silage and monitored for emissions using a GreenFeed Emissions monitoring system at the Lacombe Research Centre.

	CH_4 Production		**CO_2 Production**	
	F-Value	P-Value	F-Value	P-Value
RFI_{FAT} Group	0.04 $_{(1, 3644)}$	0.84	30.83 $_{(1, 3644)}$	< 0.0001
Day	6.73 $_{(28, 3644)}$	< 0.0001	7.17 $_{(28, 3644)}$	< 0.0001
RFI_{FAT} Group: Day	1.46 $_{(28, 3644)}$	0.056	1.26 $_{(28, 3644)}$	0.17
Good Time	0.93 $_{(1, 3644)}$	0.33	7.14 $_{(1, 3644)}$	0.0076
Time Bin	44.91 $_{(7, 3644)}$	< 0.0001	23.65 $_{(7, 3644)}$	< 0.0001
RFI_{FAT} Group: Time Bin	2.28 $_{(7, 3644)}$	0.026	4.07 $_{(7, 3644)}$	0.0002
	CH_4 Yield		**CO_2 Yield**	
	F-Value	P-Value	F-Value	P-Value
RFI_{FAT} Group	11.78 $_{(1, 41)}$	0.001	10.10 $_{(1, 41)}$	0.003
Day	0.00 $_{(1, 41)}$	0.98	0.13 $_{(1, 41)}$	0.72

Table 6. Means and standard deviations of CH_4 and CO_2 production and yield from high and low RFI_{FAT} replacement heifers while in drylot.

	High RFI_{FAT}		**Low RFI_{FAT}**	
	Mean	SD	Mean	SD
Methane Production (g $head^{-1}$ day^{-1})	148.99[a]	36.07	148.66[a]	36.11
Methane Yield (g kg^{-1} DMI)	23.63[a]	2.56	26.40[b]	2.59
Carbon Dioxide Production (g $head^{-1}$ day^{-1})	5413.98[a]	734.29	5264.33[b]	696.07
Carbon Dioxide Yield (g kg^{-1} DMI)	870.75[a]	79.26	947.03[b]	73.39

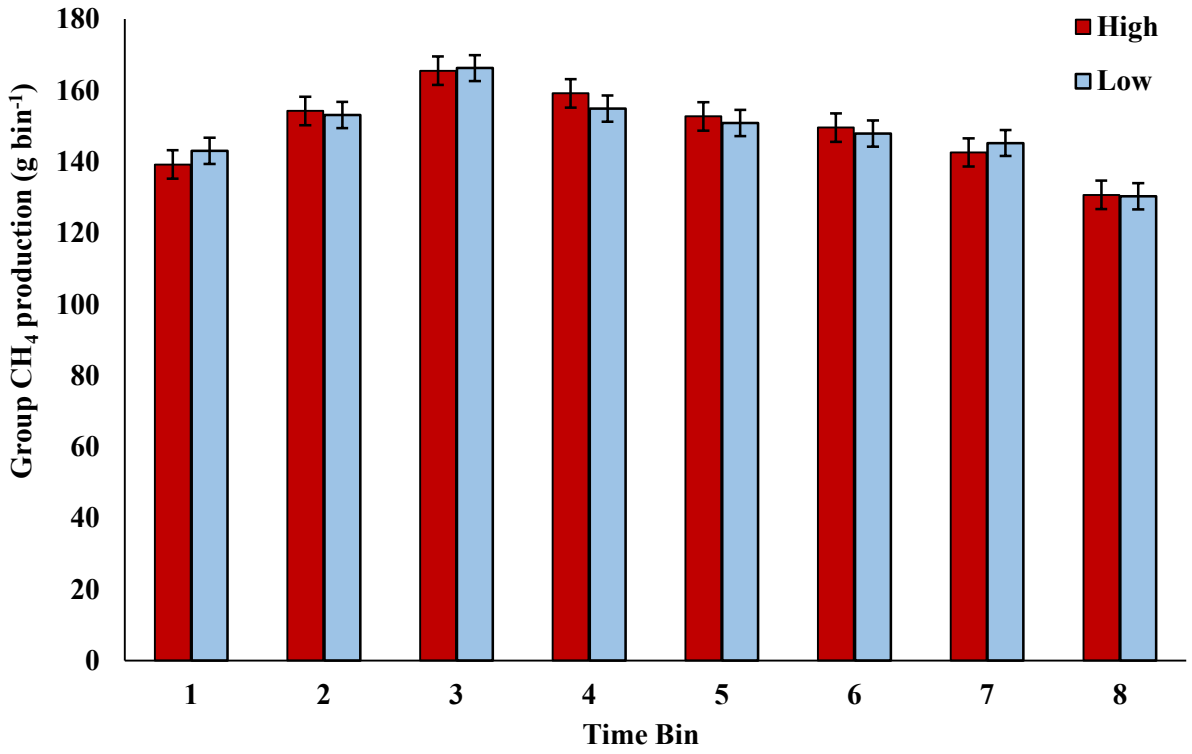

Fig. 2. Bargraph of the total CH_4 production of high and low RFI_{FAT} heifers in drylot (g head^{-1} day^{-1}) in each of the eight successive three-hour time periods, starting at 0000 hours.

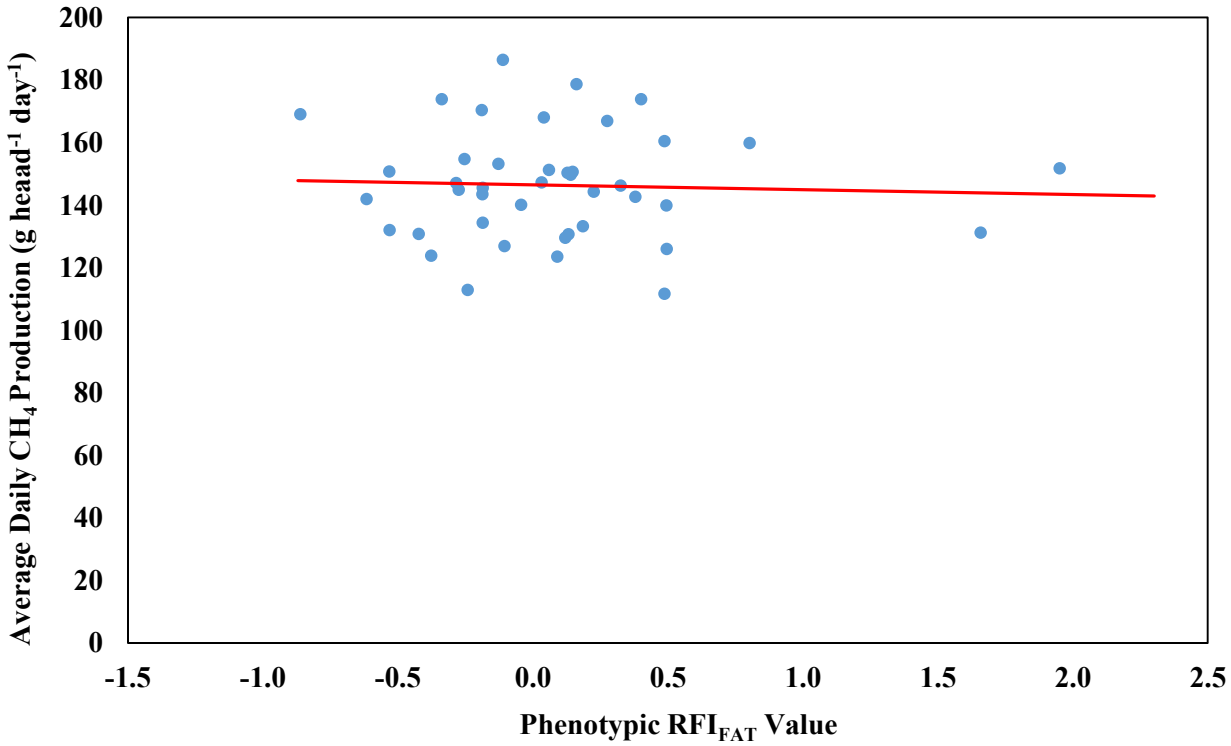

Fig. 3. Linear regression of daily CH_4 production (g $head^{-1}$ day^{-1}) and phenotypic RFI_{FAT} value of heifers in drylot. y = 146.53 – 1.56x, R^2 = 0.002, Adjusted R^2 = - 0.023, P-Value = 0.77.

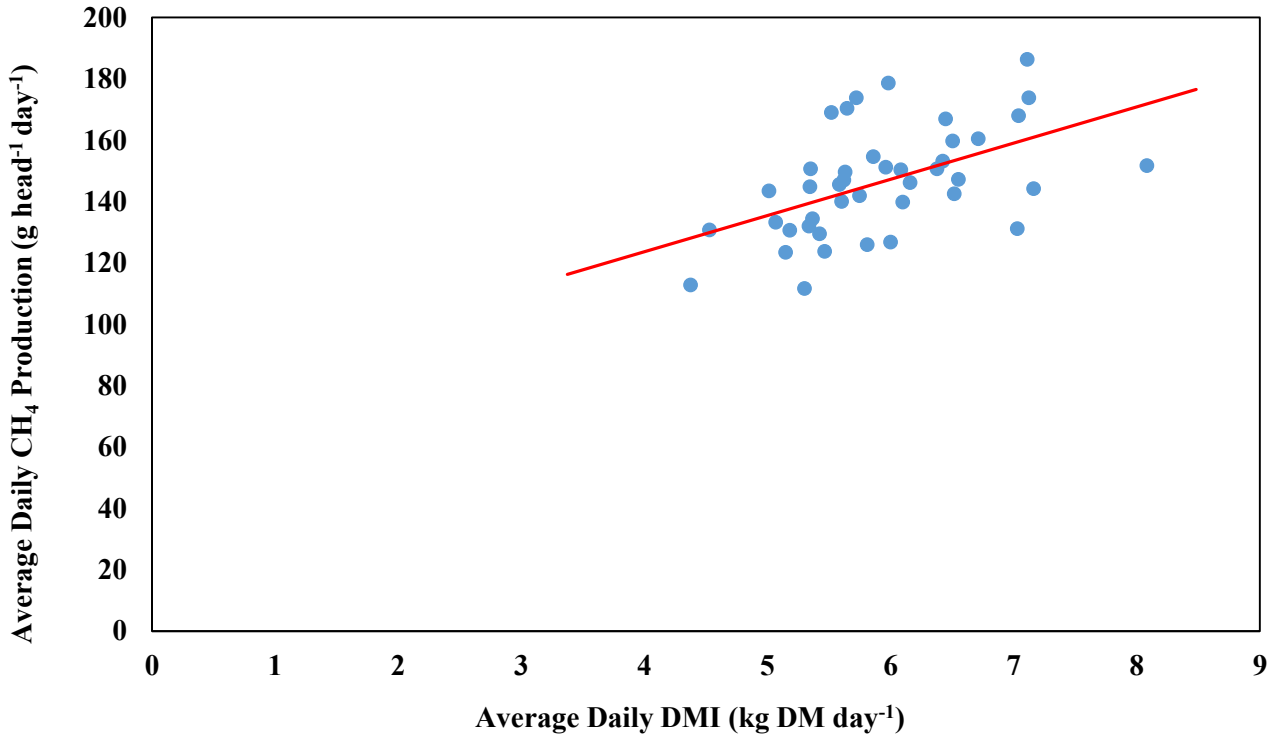

Fig. 4. Linear regression of average daily CH_4 production (g head^{-1} day^{-1}) and average daily DMI (kg DM day^{-1}) of heifers in drylot. y = 76.40 + 11.81x, R2 = 0.27, Adjusted R^2 = 0.25, P-Value = 0.0005.

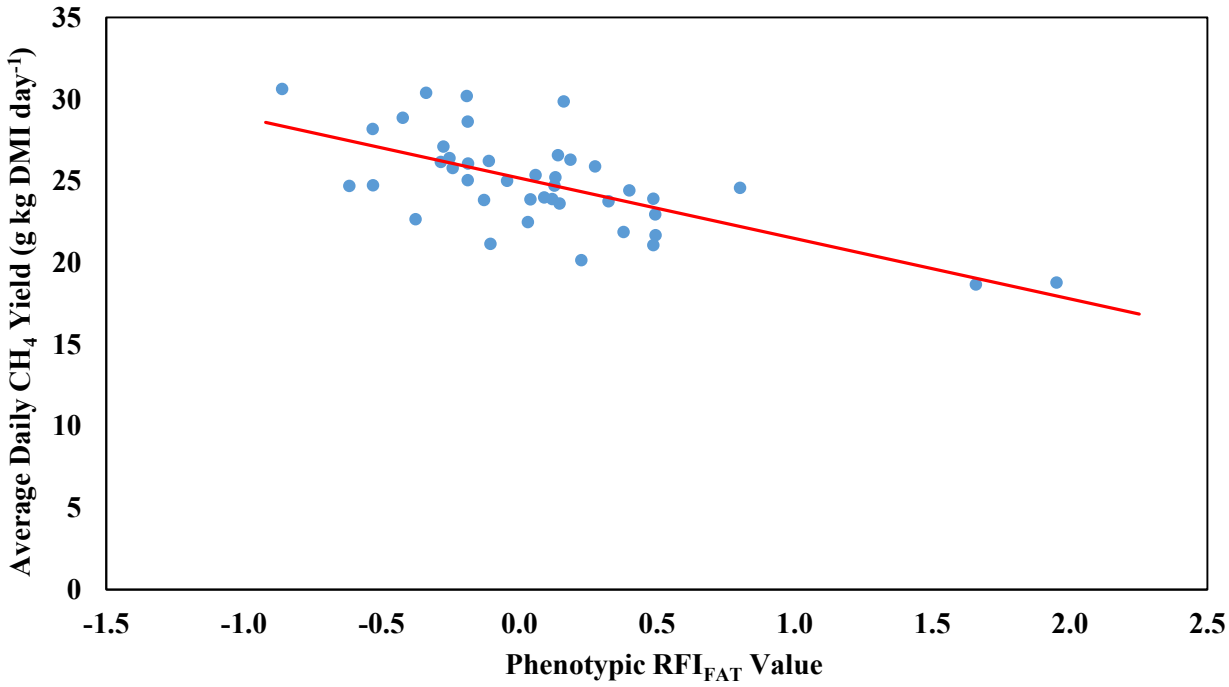

Fig. 5. Linear regression of phenotypic RFI_{FAT} value and average daily CH_4 yield (g kg DMI day^{-1}). y = 25.18 – 3.70x, R2 = 0.44, Adjusted R^2 = 0.43, P-Value = < 0.0001.

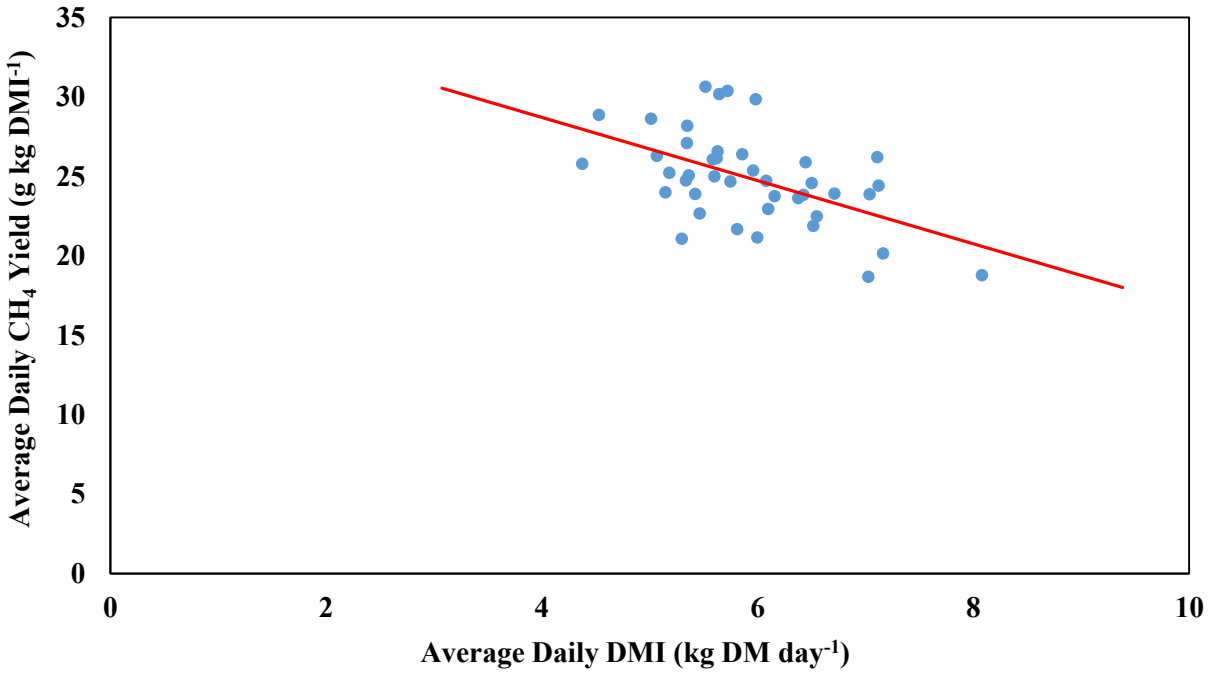

Fig. 6. Linear regression of average daily CH_4 yield (g kg DMI day^{-1}) and average daily dry matter intake (kg DM day^{-1}). y = 36.68 – 1.98x, R2 = 0.28, Adjusted R2 = 0.26, P-Value < 0.001.

Chapter 4: Methane Production and Dry Matter Intake of Replacement Heifers on Pasture

INTRODUCTION

Residual feed intake (RFI) of cattle is most often evaluated in a drylot environment where diet, feed intake and behaviour are controlled, and foraging behaviors are eliminated; all of which are aspects that add to the difficulty of measuring RFI on pasture. Assessing feed intake on pasture is important because it reflects common beef production practices in Alberta and Western Canada. Most cattle, including feedlot cattle, spend a substantial portion of their lives in a pasture environment. Accurately measuring dry matter intake (DMI) of cattle on pasture is challenging because it is difficult to quantify exactly what, and how much, each animal is consuming. Methods involving tracers, such as n-alkanes, can be used to estimate DMI on pasture (Mayes et al. 1986; Charmley et al. 2003; Manafiazar et al. 2015). DMI can be estimated using a paired n-alkane methodology that compares natural alkane concentrations to synthetic dosed alkane concentrations of fecal and feed samples. Information on DMI and growth of cattle grazing on pasture can give insight into RFI, despite the lack of control over feed intake and the environment. Although estimates of DMI on pasture can be determined, it does not mean that RFI values can always be calculated. In order to accurately capture RFI on pasture, feed intake data would need to be collected for at least 35-42 days, alongside approximately 76 days of weight gain data (Wang et al. 2006). Estimating dry matter intake on pasture is a labor-intensive process as it requires individual feeding, fecal sample collection, and feed refusal collection for each animal on trial twice a day (Manafiazar et al. 2015**)**. Manually collecting such data for at least 35 days consecutively is a very physically demanding process, and therefore often only gets done for approximately 14 days (including the dosing and sampling period), which is not long enough to get accurate assessments of RFI on pasture.

Along with the complexity of measuring RFI, comes the difficulty of measuring methane (CH_4) emissions on pasture. CH_4 emissions are important to consider as they affect both sustainability of the industry as well as the efficiency of beef production. Cattle on pasture are subject to forage-based diets, which are typically high in fiber and can be lower in energy content (BCRC 2017), in turn contributing to

increased CH_4 emissions. CH_4 is a potent greenhouse gas, with 25 times the warming potential of CO_2 (IPCC 2014). CH_4 production can be reduced by feeding grain rather than forage-based diets (Grainger and Beauchemin 2011; Hristov et al. 2013) as feed sources with low fibre or high carbohydrate content can reduce CH_4 emissions (Moe and Tyrrell 1979). Furthermore, improvements in diet quality have been shown to reduce CH_4 production per animal (Eckard et al. 2010). Hristov et al. (2013) estimated that about 3 to 7% of gross energy intake was lost to CH_4 production from cattle fed high grain and forage diets, respectively. Cattle on forage-based diets tend to have greater CH_4 emissions as a result of greater ruminal fermentation, while diets with greater starch promote propionate production, creating an alternate hydrogen sink to methanogenesis (Murphy et al. 1982). Because it is not always possible to alter pasture forage quality, selecting for cattle that eat less while maintaining growth may result in lower CH_4 emissions.

CH_4 emissions can be monitored using SF_6 CH_4 collection canisters, which are strapped to each individual animal's head. Although the SF_6 method is a way of measuring individual CH_4 emissions, it has the potential to interfere with normal animal behavior and is limited by wind speeds and wind directions (Harper et al. 1999). Another method that involves CH_4 measurement using infrared lasers, monitors emissions from cattle in a group environment (Jones et al. 2011), the method does not measure individual CH_4 emissions. Using an open-path Fourier Transform Infrared Spectrometry (OP-FTIR) system, CH_4 emissions can be measured directly in a pasture environment while cattle are grazing. An OP-FTIR system utilizes an inverse dispersion technique method (IDM) to estimate CH_4 emissions (Flesch et al. 2004), a method which calculates emissions based on the incremental rise in CH_4 concentration over background levels (Bai et al. 2015).

The objective of this study was to determine if there is a difference in cattle DMI as well as CH_4 production and yield while grazing on pasture. It was hypothesized that (1) high RFI_{FAT} heifers would have greater DMI on pasture, (2) that high RFI_{FAT} heifers would have greater CH_4 production on pasture, and (3) that CH_4 yield would be similar between high and low RFI_{FAT} heifers on pasture.

MATERIALS AND METHODS

This research was conducted between June 2016 and April 2017 at the University of Alberta Mattheis Research Ranch, in collaboration with the Gemstone Cattle Company. All animal procedures were reviewed and approved by the University of Alberta Animal Care and Use Committee (AUP00001284), and all animals were cared for in accordance with the Canadian Council on Animal Care (1993).

Dry Matter Intake and Methane Emissions

Prior to collection of pasture observations, all heifers were maintained as one group in a drylot pen, on a corn silage diet. Heifers were selected based on phenotypic RFI_{FAT} (i.e. RFI corrected for backfat) values in drylot as well as temperament. In total, 18 heifers, nine with relatively low and nine with relatively high RFI_{FAT} values based on a previous drylot test, given that temperaments were suitable for daily handling, were used in the trial. High and low RFI_{FAT} heifers had an average RFI_{FAT} value of 0.61 (SD = 0.280) and -0.56 (SD = 0.178), respectively. On average, the heifers were 515.11 (SD = 16.428) days old, weighed 300.08 kg (SD = 29.933), with a backfat of 3.65 mm (SD = 1.27) at the start of the trial. The trial was conducted from June 13, 2016 to July 5, 2016 at the University of Alberta Mattheis Research Ranch with temporary facilities arranged on an irrigation pivot. Heifers in the trial strip-grazed forage oats (*Avena sativa*; cv. CDC Baler) during the flag-leaf, early boot and boot stages of growth; refer to Appendix D for forage information.

Dry Matter Intake

Dry matter intake on pasture was determined using n-alkanes, in accordance with Manafiazar et al. (2015), which involved a 23-day procedure consisting of an initial warm-up period followed by a dosing period, and then a final sampling period. All 18 heifers spent a total of eight days (day -8 to day -1, inclusive) in the warm-up period, in which heifers were acclimatized to the forage and electric fence (Fig. 1). During the warm-up period, the electric fence was moved on a daily basis to give heifers frequent access to new forage. Twice throughout the warm-up period, heifers were taken to the handling facilities

where they were individually fed small amounts of barley grain to familiarize them with the facilities and the procedure. The dosing period, which followed the warm-up period, took place over nine days (day 0 to day 8, inclusive) where heifers continued to graze together in the warm-up area. On day zero, heifers were weighed, fecal sampled through rectal palpation, and ultrasound backfat measurements taken. Initially the heifers were taken to the handling facilities once a day in the morning (8 a.m.), for feeding of 1000g (± 0.5 g) of alkane pellets in individual 3.05m x 3.05m pens (Fig. 2). On day 5, the procedure was modified to individually feed heifers 500g (± 0.5g) of alkane pellets in the morning (8 a.m.) and in the afternoon (4 p.m.) as heifers would not eat 1000g at one time. Heifers were given a maximum of one hour to eat all of their pellets. Following feeding, all feed refusals were collected and weighed to estimate pellet intakes for each heifer. Orts for each feeding period were composited and stored at -20°C. Samples of alkane pellets were collected on each day throughout the dosing period and stored in a -20°C freezer prior to analysis. Alkane pellet preparation is explained in Appendix C and pellet nutritional information is found in Table 1; pellets were 82.7% digestible, with 17.5% crude protein.

The sampling period, following the dosing period, took place over five days (day 9 to day 14, inclusive). During the sampling period, high and low RFI_{FAT} heifers were strip-grazed separately in two groups and continued to be fed 500g (±0.5g) of alkane pellets in the morning (8 a.m.) and afternoon (4 p.m.), orts continued to be collected and weighed for each heifer. Additionally, all heifers were fecal sampled, through rectal palpation, twice-a-day immediately after alkane pellet feeding; only one fecal sample was collected on day 14. All fecal samples were immediately kept on ice and stored at -20°C following the sampling period. On day 10, two heifers (one high RFI_{FAT}, one low RFI_{FAT}) were removed from the trial due to poor temperament. Additional body weights were collected on day nine, day ten, day 13 and day 14. Final ultrasound backfat measurements were also collected on day 13. Daily forage pluck samples (i.e. emulating selective animal grazing), and daily forage clip samples were collected in all paddocks to determine forage quality (digestibility, crude protein and fiber) and forage dry matter content, respectively. Forage quality during the warm-up period and the sampling period is summarized in Table

2. Pasture had similar digestibility at the start and end of the trial, while protein was initially very high (30%), and by the end remained at 23.5%.

All composited ort samples were oven dried for 72 hours at 80ºC to determine dry matter content. Daily feed samples were oven dried for 72 hours at 55ºC, then ground to 1mm using a Wiley™ mill. Fecal samples were oven dried for 72 hours at 55ºC, then ground to 1mm using a Wiley™ mill. Alkane concentrations of feed and fecal samples were analyzed in the department of Agricultural, Food and Nutritional Sciences (University of Alberta, Edmonton, Alberta) in accordance with the method described by Manafiazar et al. (2015). Samples of 1g each were digested in 15 mL of ethanolic KOH, along with 0.25 mL of tetracontane (C_{34}) internal standard, and saponified at 90ºC overnight. Following digestion, alkanes were extracted with aliquots of heptane, and the solvent layers removed and dried through evaporation. Solutes were re-dissolved in 2 mL of heptane and filtered through a silica column. The eluate was dried through evaporation and re-dissolved in 0.8 mL of n-undecane. Samples were analyzed by gas chromatography (GC) using a Bruker Scion 456 GC (Scion Instruments, Livingston, West Lothian, Scotland) instrument with a flame ionization detector (FID), while using a hydrogen carrier gas at 70 cm sec^{-1}. The instrument used an HP-1 25m x 0.32mm column with an injector and detector temperature of 325ºC. The oven was programmed to an initial temperature of 150ºC, held for 1 min, then increased to 325ºC at 10ºC min^{-1}, and held for 6.5 min. Each sample was run for a total of 25 min.

Methane Emissions

Enteric CH_4 emissions were measured on pasture from the same high and low RFI_{FAT} heifer groups using the OP-FTIR. Emissions were measured for 6 days, starting on day 8 (i.e. the dosing period) of the pasture DMI observations. High and low RFI_{FAT} heifers were kept in separate groups and grazed in long narrow strips (Fig. 1). The paddock design outlined in Hu et al. (2016) was modified to allow concurrent emission monitoring from two groups; details of the paddock layout are given in Appendix D. Emissions were calculated using an IDM, where CH_4 concentrations were measured upwind and

downwind of each RFI group. The downwind enhancement of CH_4 (i.e. the rise above the upwind concentration level) together with wind information, defined the group emission rate (Harper et al. 2013).

CH_4 concentrations were measured with an OP-FTIR system, its set up is shown in Fig. 3. The OP-FTIR spectrometer (Matrix-M IR Cube, Bruker Optik, Ettlingen, Germany) sent an infrared beam of light to a distant retroreflector, from which the beam was returned to a detector. The average gas concentration along the path was determined from an analysis of the infrared spectrum of the returned beam (Griffith et al. 2012). Trace gas concentrations were measured along four different OP-FTIR paths running outside and parallel to the long axis of the two paddock strips (Fig. 3). Depending on the wind direction, one set of paths measured upwind concentrations, and the other set measured the enhanced downwind concentrations. The four paths were measured with the single OP-FTIR instrument through the use of an automated aiming motor and mirrors. The aiming motor automatically cycled through the sequence of four paths every 7.5 min, and this was repeated continuously except for periods of adjustment or shut down (i.e. due to high wind and lightning storms). Due to the importance of the height of the concentration path above the ground for emission measurements in this type of configuration (Hu et al. 2016), the heights of the mirrors and reflectors were adjusted to create nearly constant path heights (as measured every 10 to 30 m along each path). A tower-mounted three-dimensional sonic anemometer (CST3, Campbell Scientific Inc., Logan, UT, USA), located next to the grazing strips, provided the necessary wind information for the emission calculations: friction velocity (u*), Obukhov length (L), roughness length (z_0), wind direction (β) and turbulent velocity fluctuations ($\sigma_{u,v,w}$). This information is described in detail by Flesch et al. (2004).

Emissions were calculated concurrently from the two RFI_{FAT} groups in a time series of 15 min average emission rates. The software, WindTrax (www.thunderbeachscientific.com), was used for this calculation, which combines the IDM model described by Flesch et al. (2004) with an interface allowing gas sources and sensors to be mapped (Fig. 4). The paddock boundaries and OP-FTIR paths were GPS located and mapped in WindTrax, with emissions calculated from the time series of OP-FTIR

concentrations and the wind information. Each group of heifers was represented in WindTrax as point sources located in the centre of the strip, with an emission source set to approximately mouth height, 0.5 m above the ground. Not all 15-min periods were suitable for emission measurements. Observations were filtered based on wind and concentration sensor criteria (Flesch et al. 2004 and 2014; Hu et al. 2016). Observation periods were eliminated under the following four circumstances:

1. Very low wind periods with friction velocities (u^*) ≤ 0.1 m s^{-1}.
2. Roughness length (z_0) ≥ 0.2 m. Lengths which exceeded this threshold were unrealistic for the surface cover and they indicated wind flows that did not meet model assumptions.
3. Wind directions (β) within $\pm 30^0$ from the long axis of the paddocks. This was done to ensure that all animal emissions passed through the downwind concentration measurement path, and not out the end of the strips. WindTrax plume maps (Fig. 4) were also created for each 15 min period and compared to photographs of the animal's positions during the period to ensure the plume passed through the proper measurement path.
4. Concentration observations that did not meet quality assurance parameters such as signal strengths, as measured by Specmax (reported by the OP-FTIR), of < 0.20; often times due to dew and heavy rain, which reduced signal strength to unreliable levels.

Methane measurements were collected 24 hours day^{-1}, with heifers given access to a new strip of grass every morning (9 a.m.). Each time heifers were moved to a new strip of forage, the RFI_{FAT} groups were alternated between paddock blocks (i.e. moved to the other side of the OP-FTIR) to minimize potential differences in environmental conditions (including feed) between the two areas of the field (Fig. 5). Animal moves to new pastures required repositioning of the OP-FTIR, mirrors and reflectors every two days. Stationary field cameras, located on either side of the FTIR system, took photos every 5 min to verify the location of heifers within the paddock.

Calculations and Statistical Analysis

Dry Matter Intake

Daily fecal, feed and pellet samples were used to determine alkane marker concentration in feces over the entire 15 day test period (i.e. dosing and subsequent sampling period). Fecal concentrations of endogenous alkane was determined for all seven days in which fecal samples were collected. This was done using knowledge of pellets consumed and the concentration of endogenous alkanes in feed and feces. Estimates of forage intake were calculated for days 9 to 15. Feed intake estimates were calculated in accordance with Manafiazar et al. (2015), in which both naturally occurring alkanes and synthetic alkanes were analyzed in a paired *n*-alkane methodology. The following formula was used to estimate DMI for each individual heifer,

$$\text{DMI (kg DM d}^{-1}) = [(F_i/F_j) \times (D_j) + ((IS \times S_j) - (IS \times S_i))] / [H_i - (F_i/F_j) \times H_j]$$

where H_i, S_i, and F_i, are forage, pellet and fecal concentrations of C_{31} (mg kg^{-1} DM), respectively. H_j, S_j, and F_j, are forage, pellet and fecal concentrations of C_{32} (mg kg-1 DM), respectively. IS represents pellet intake (kg DM day^{-1}) while D_j represents the dose rate of C_{32} (mg day^{-1}).

Forage DMI was averaged for all animals and any DMI beyond three standard deviations of the mean were removed, in total one DMI measurement for each animal was removed, this estimate being from the very first fecal sample collected (i.e. 1 of 7 values)**.** DMI values were not included for the high and low RFI_{FAT} heifers that were removed from the trial. Final DMI values for each heifer were averaged over the six day period. Analysis of covariance (ANCOVA) was conducted in R using the Car package (Fox and Weisberg 2011), in which the model included DMI as a response variable, with fixed effects of heifer RFI_{FAT} group and sampling day (i.e. during sampling period, days 9-15) as a covariate. Differences in initial and final body weight and backfat were analyzed with an lme model in the nlme package in R (Pinheiro et al. 2016), in which RFI_{FAT} group and heifer age were fixed effects, and each individual heifer was considered a random effect. Values smaller than 0.05 were considered to be significant. Multiple comparisons were done using Tukey's Honest Significant Difference (HSD) test in the lsmeans program

in R (Lenth 2016). Linear regression of individual pasture DMI and drylot RFI_{FAT} value was conducted in R (R Core Team 2016) and grouped by each individual sampling day.

Methane Emissions

CH_4 concentrations were grouped into three hour time periods, dividing all of the CH_4 concentration data into eight different time bins throughout the day. Concentrations were grouped into time bins in order to accurately account for differences in high and low RFI_{FAT} heifer feeding behavior. Subsequent analysis was conducted using linear mixed models in the lme package in R (Pinheiro et al. 2016) in which RFI_{FAT} group and time bin were fixed effects, CH_4 concentration was a response variable, and day (n=6) was a random effect. ANOVA was conducted to determine the differences in total CH_4 production.

Methane production was also analyzed on a standardized kg of body weight basis. Because individual animal CH_4 measurements were not collected on pasture, CH_4 production was compared solely between the two RFI_{FAT} groups. Body weights of heifers in each of the RFI_{FAT} groups were averaged and divided by the overall average CH_4 production for that group. Differences in average CH_4 production per average body weight was analyzed using an lme model in R (Pinheiro et al. 2016), with CH_4 production per unit body weight as a response variable, RFI_{FAT} group and time bin (i.e. one of the three hour times periods in the day) as fixed effects, and day as a random effect. Methane yield values were calculated by dividing each RFI_{FAT} group's CH_4 production by average DMI. Differences in CH_4 yield were analyzed using a linear model in R in which methane yield was the dependent variable with fixed effects of methane yield, RFI_{FAT} group and sampling day. Multiple comparisons were conducted using Tukey's HSD test in the lsmeans package in R (Lenth 2016).

RESULTS

High RFI_{FAT} heifers had an average RFI_{FAT} value of 0.52 (SD = 0.49) while low RFI_{FAT} heifers had an RFI_{FAT} value of -0.59 (SD = 0.30) ($P < 0.0001$), both values based on feed intake in drylot (i.e. see Chapter 3). ANOVA results for differences in DMI between high and low RFI_{FAT} heifers are summarized

in Table 3. Day of sampling had a significant effect on DMI. While age of the heifer had a significant effect on final body weight, as well as initial and final backfat, it did not have a significant effect on initial body weight. On average high and low RFI_{FAT} heifers were 427 (SD = 20.9) and 442 (SD = 6.7) days of age, respectively (P = 0.053) at the start of the pasture feed intake trial. High RFI_{FAT} heifers consumed an estimated average of 8.13 kg DM day^{-1} (SD = 1.71) while low RFI_{FAT} heifers consumed an estimated average of 7.88 kg DM day^{-1} (SD = 1.30). Least square means (LSM) of DMI, initial and final body weights, as well as start and end-of-trial backfat measurements are summarized in Table 4. High and low RFI_{FAT} fat heifers had similar body weight measurements, along with similar final backfat measurements, the only significant difference being in final backfat, with high RFI_{FAT} heifers having more backfat. Although there were no significant differences, both high and low RFI_{FAT} heifers gained weight and accumulated backfat while on the grazing trial. Multiple linear regression analysis of drylot phenotypic RFI_{FAT} values, with individual pasture DMI and day of sampling, resulted in a significant relationship (R^2 = 0.21, P-Value < 0.0001). In contrast, simple linear regression analysis of drylot phenotypic RFI_{FAT} values and individual total DMI revealed no relationship (R^2 = 0.0001, P-Value = 0.92). Regression of DMI and RFI_{FAT} plotted for each day of the pasture intake trial showed differences among heifers as well as differences within each animal from day to day (Fig. 6).

ANOVA results evaluating differences in CH_4 production and CH_4 yield between high and low RFI_{FAT} heifers are summarized in Table 5. Time of day had a significant effect on CH_4 production on a g day^{-1} basis, as well as CH_4 production on a g kg^{-1} of body weight basis. Time of day also had a significant effect on CH_4 yield (g kg^{-1} DMI). LSM values for high and low RFI_{FAT} heifer CH_4 production and yield are summarized in Table 5. Although high RFI_{FAT} heifers produced more CH_4 (203.3 ± 27.5SD g $head^{-1}$ day^{-1}), it was not significantly greater than the low RFI_{FAT} heifers (195.6 ± 27.5SD g $head^{-1}$ day^{-1}). Standardized CH_4 production and CH_4 yield also did not differ significantly between high and low RFI_{FAT} heifers. Due to the difficulty of measuring feed intake on pasture, CH_4 emissions were standardized on an animal weight basis. The diurnal pattern of CH_4 production, as shown in Fig. 7, revealed that CH_4

production was greatest for both high and low RFI_{FAT} heifers between the hours of 0900 and 1200, right after heifers were given access to a new strip of forage. Methane production between high and low RFI_{FAT} heifers was only significantly different between the hours of 0000 and 0300. Methane production for both high and low RFI_{FAT} heifers was lowest between the hours of 0600 and 0900. Methane production was steady from approximately 1800 to approximately 0600, and the greatest fluctuations occurred between approximately 0900 and 1800.

DISCUSSION

It was hypothesized that DMI on pasture would be significantly different between high and low RFI_{FAT} heifers, as seen in drylot (see Chapter 3); however, that hypothesis was rejected. High and low RFI_{FAT} heifers had similar DMI on pasture. Previous research conducted in drylot has found that low RFI cattle had lower DMI than their high RFI counterparts (Lancaster et al. 2009; Kelly et al. 2010; Lawrence et al. 2012; Fitzsimons et al. 2013). However, research regarding the measurement of DMI on pasture is limited. Manafiazar et al. (2015) found a significant difference in DMI on pasture between high and low RFI_{FAT} heifers using the alkane method, which was also used in the current study. More specifically, Manafiazer et al. (2015) found that high RFI_{FAT} heifers had a daily DMI of 8.66 kg DM day^{-1}, while low RFI_{FAT} heifers had a daily DMI of 8.20 kg DM day^{-1}, a difference of 0.46 kg DM day^{-1}, as opposed to a difference of 0.25 kg DM day^{-1} in the current study. It is possible that the results in the current study could have been more significant if a larger number of heifers were tested. In the current study, DMI was only measured on nine high and nine low RFI_{FAT} heifers while Manafiazar et al. (2015) analyzed DMI on 24 high RFI_{FAT} heifers and 24 low RFI_{FAT} heifers from pasture intake trials in 2012 and 2013. The greater sample size in the latter study may have increased the ability to find significant differences. Using our variance and effect size (0.25), a power analysis suggests that a total of 214 heifers would have been needed to detect a significant difference in DMI between low and high RFI groups at an alpha of 5%. Although DMI was not significantly different between high and low RFI_{FAT} heifers, it is notable that the daily DMI values we report are similar to those reported by Manafiazar et al. (2015).

Differences in daily DMI may also have been more detectable between high and low RFI_{FAT} heifers had observations been collected over a longer period of time. Because measurements were only collected on pasture for six days, daily fluctuations in DMI could have affected the accuracy of the overall DMI estimates. Drylot DMI trials run for at least 35 days in order to collect representative (and therefore accurate) measurements (Wang et al. 2006). The length of a 35-day trial eliminates variation within and between animals, including variation in environmental conditions during the trial. The short length of the pasture feed intake trial coupled with high daily fluctuations in DMI suggests the data collect may have been insufficient to capture stable long-term DMI values of heifers. Due to the labor intensive process of strip-grazing animals and the facilitation of CH_4 measures, DMI values were not evaluated over a longer period of time, something which could have increased the accuracy of the DMI estimates.

The use of n-alkanes to predict DMI could have affected the accuracy of the heifers' DMI values. A review by Dove and Mayes (1991), along with studies by Mayes et al. (1986) and Moshtaghi-Nia and Wittenberg (2002), reported that comparing dosed and natural alkanes provided accurate DMI estimations of beef cattle on pasture; however, estimations were only accurate when cattle were consuming the same forage, and the material consumed were also the forage sampled for quality and analyzed for alkane concentrations. Consequently, the accuracy of DMI values observed here could have been affected by the consumption of diverse plants or forages with differing alkane profiles, such as weeds (Dove and Mayes 1991). Although efforts were made to have the high and low RFI_{FAT} heifers consume a monoculture of forage (i.e. the pasture had been sprayed to eliminate broadleaf weeds), some weeds were still found within the forage sward, and spraying would not remove volunteer grasses growing with the forage oats. Thus, it is possible that the heifers consumed small amounts of weeds such as dandelion, Canada thistle, buckwheat or lambs-quarter's, as well as other grassy vegetation, which assuming they had different alkane concentrations, may ultimately have affected DMI estimations. In doing so, it is possible that the consumption of small amounts of weeds affected the overall difference in DMI detected between high and low RFI_{FAT} heifers.

While it was hypothesized that CH_4 production on pasture would be significantly different between high and low RFI_{FAT} heifers, this hypothesis was rejected. CH_4 production did not differ between the two RFI_{FAT} groups. Very few studies have used the OP-FTIR method to monitor CH_4 emissions of cattle with differing RFI values; instead, many studies have used the SF_6 tracer method on pasture. The study conducted by Jones et al. (2011) was one of the first to utilize OP-FTIR technology to measure emissions of cattle on pasture. The latter study used the method to monitor CH_4 emissions of high and low RFI beef cows foraging on high quality and low quality annual Mediterranean pastures in Western Australia. Jones et al. (2011) reported no significant difference in CH_4 emissions between high and low RFI cows when consuming low quality forages, a result that was likely driven by low crude protein concentrations, which were too low to meet the minimum nitrogen requirements of rumen microbial populations (Kerley and Lardy 2007). This result is in agreement with the results of the current study, as CH_4 emissions did not differ between high and low RFI_{FAT} heifers. However, Jones et al. (2011) also found that when cows were grazing high quality pastures there was a significant difference in CH_4 emissions between high and low RFI groups, a finding inconsistent with the results of the current study. Although heifers in the current study were also consuming high quality, high protein forages, CH_4 emissions did not differ between the two RFI_{FAT} groups. The lack of a difference in CH_4 production closely followed heifer DMI, which also failed to differ. This response was expected given that CH_4 production tends to mirror DMI (Blaxter and Clapperton 1965; Johnson and Johnson 1995; Grainger et al. 2007). As a result, CH_4 production was unlikely to differ between high and low RFI_{FAT} heifers as a result of the similarity in DMI.

It is also possible that the results of the current study are not in full agreement with the results of Jones et al. (2011) due to a relatively low number of overall CH_4 observations. In the current study, a total of 101, 15-min observation periods, collected over the six-day sampling period, were considered to be quality observations (i.e. not affected by weather, turbulence or winds moving parallel to the grazing strips). In personal communication with Dr. Flesch (November 2016), a minimum of 100 quality

observations were necessary in order to conduct an accurate analysis. Due to wind and lightning storms affecting most of the six days of the observation period, the OP-FTIR unit was shut down several times throughout the trial. System shut-down reduced the number of 15-min observations, limiting the overall number of quality observations collected. Although additional grazing strips were fenced off, CH_4 observations could not be extended due to time constraints on equipment use. Had CH_4 observations been collected over a longer period of time, there could have been an increase in the number of quality emission observations, possibly increasing the likelihood for more significant results. Jones et al. (2011) also collected CH_4 observations for six consecutive days, 24 hours each day, with the data from one of those days being omitted due to inclement weather. Although Jones et al. (2011) reduced the number of quality observations collected, it did not appear to have an effect on observations of overall CH_4 production; this is likely because Jones et al. (2011) tested 48 animals as opposed to 16. According to a power analysis, using an effect size of 0.1, at a significance level of 0.05, it would take 25 days to detect a significant difference in methane emissions between the high and low RFI heifers on pasture.

Although poor diet quality had an effect on CH_4 production in the study conducted by Jones et al. (2011), it is unlikely that diet quality had an effect on the CH_4 production data presented in the current investigation. Kerley and Lardy (2007) reported that dietary crude protein concentrations lower than about 80 g kg^{-1} do not meet the nitrogen requirements of several rumen microbes potentially affecting overall CH_4 emissions. Therefore, poor quality diet in that study likely affected feed intake and digestion rates (Waldo 1986). In contrast however, crude protein content of heifer diets in the current study were nearly 30% on a dry matter basis during the warm-up period and dropped to just below 25% on a dry matter basis during the sampling period, both values of which were well above the crude protein content of common forages found in Alberta and Western Canada (BCRC 2017). This suggests that low protein content probably did not affect the methane emissions observed here.

When considering the diurnal pattern of CH_4 production, Jones et al. (2011) showed that emissions throughout the day were highly variable for high and low RFI_{FAT} cattle on both high and low

quality forages. Peak standardized CH_4 production (g kg BW^{-1} day^{-1}) occurred just before 12 p.m. on most days, on both the poor quality and high quality pastures (Jones et al. 2011), which is in agreement with the findings of the current study. CH_4 production (g $head^{-1}$ $hour^{-1}$) for both high and low RFI_{FAT} heifers here was greatest between the hours of 0900 and 1200, shortly after entry to fresh pasture. CH_4 production was only significantly different between the first three hours of the day (i.e. 0000 to 0300 hours) and well after satiation on new pasture, with high RFI_{FAT} heifers briefly producing significantly more CH_4 than their low RFI_{FAT} counterparts. This pattern may reflect an important difference in post-ingestive fermentation of very high quality fresh forage consumed in the middle of the previous day right after pasture entry. For example, this may reflect differences in the efficiency of more complex feed breakdown by microbes in the rumen of animals with differing RFI as long as 15-18 hr after consumption, and warrants further investigation.

McGinn et al. (2006) used the SF_6 tracer method to collect enteric methane emissions from grazing cattle and compared them to enteric emissions from cattle on the same diets in CH_4 collection chambers and found that the technique was most appropriately used with grazing cattle. McGinn et al. (2006) concluded that using the SF_6 tracer technique would result in greater uncertainty when cattle were fed high concentrate diets, suggesting that the technique is ideal for monitoring CH_4 emissions of high and low RFI cattle on pasture. Although the SF_6 tracer technique is well suited for monitoring emissions on pasture, most previous studies that used the technique are limited to cattle in a drylot environment (Hegarty et al. 2007; Fitzsimons et al. 2013), and are therefore not representative of the current study. Fitzsimons et al. (2013) monitored CH_4 emissions of heifers selected for divergent RFI while on a grass silage diet, which may be more representative of the current study than results from Hegarty et al. (2007) as they were collected from steers on concentrate-based diets. Fitzsimons et al. (2013) reported different results than those of the current study as there was a significant difference in both CH_4 production and DMI, results of which were not significantly different in the current study. It is likely that the

environment, and method of CH_4 measurement, resulted in inconsistent results, signifying the need for further research of DMI and associated CH_4 emissions by beef cattle grazing on pasture.

Research Limitations

This research was limited by the number of animals in the study and the number of days in which intake and methane data could be collected. The results might have been more conclusive had a larger number of high and low RFI_{FAT} heifers been used, resulting in greater statistical power. Additionally, more days of data collection, specifically CH_4 observation days, may have resulted in a greater opportunity to detect significant results. Due to unfavorable weather conditions the OP-FTIR unit did not capture as many CH_4 observations as initially expected, resulting in a relatively low number of overall emissions, barely achieving the minimum number of observations necessary to conduct a statistical analysis. As a result of time constraints and limitations in the availability of the OP-FTIR unit, additional experimental days could not be added to increase the overall number of CH_4 observations. The labor-intensive process of animal handling also limited the number of heifers that could be worked with in the trial and poor animal temperament posed a safety risk, resulting in the removal of additional animals from the trial, potentially reducing the overall statistical power of the study.

CONCLUSION

Measuring DMI and CH_4 production of high and low RFI_{FAT} heifers on pasture resulted in similar results between the two groups of heifers. Although neither of the results were significantly different, both metrics showed a (non-significant) trend towards low RFI_{FAT} heifers consuming less forage and producing less overall CH_4, a response that may have been constrained by sample sizes. Furthermore, the OP-FTIR method has only been used to measure CH_4 emissions of high and low RFI cattle grazing cattle in one previously published study (Jones et al. 2011), meaning that the results of the current study need to be further tested in future investigations. The results of this study indicate that cattle selected for low RFI or RFI_{FAT} will, at a minimum, have similar overall CH_4 emissions on pasture, and therefore should not reduce the sustainability of the beef industry, especially considering that a large percentage of cattle in

North America are exposed to pasture environments. Future studies should be done on larger groups of animals that are more representative of common production practices, including open-range grazing, ultimately providing the beef industry with more rigorous information on the role of RFI in altering cattle DMI and CH_4 emissions.

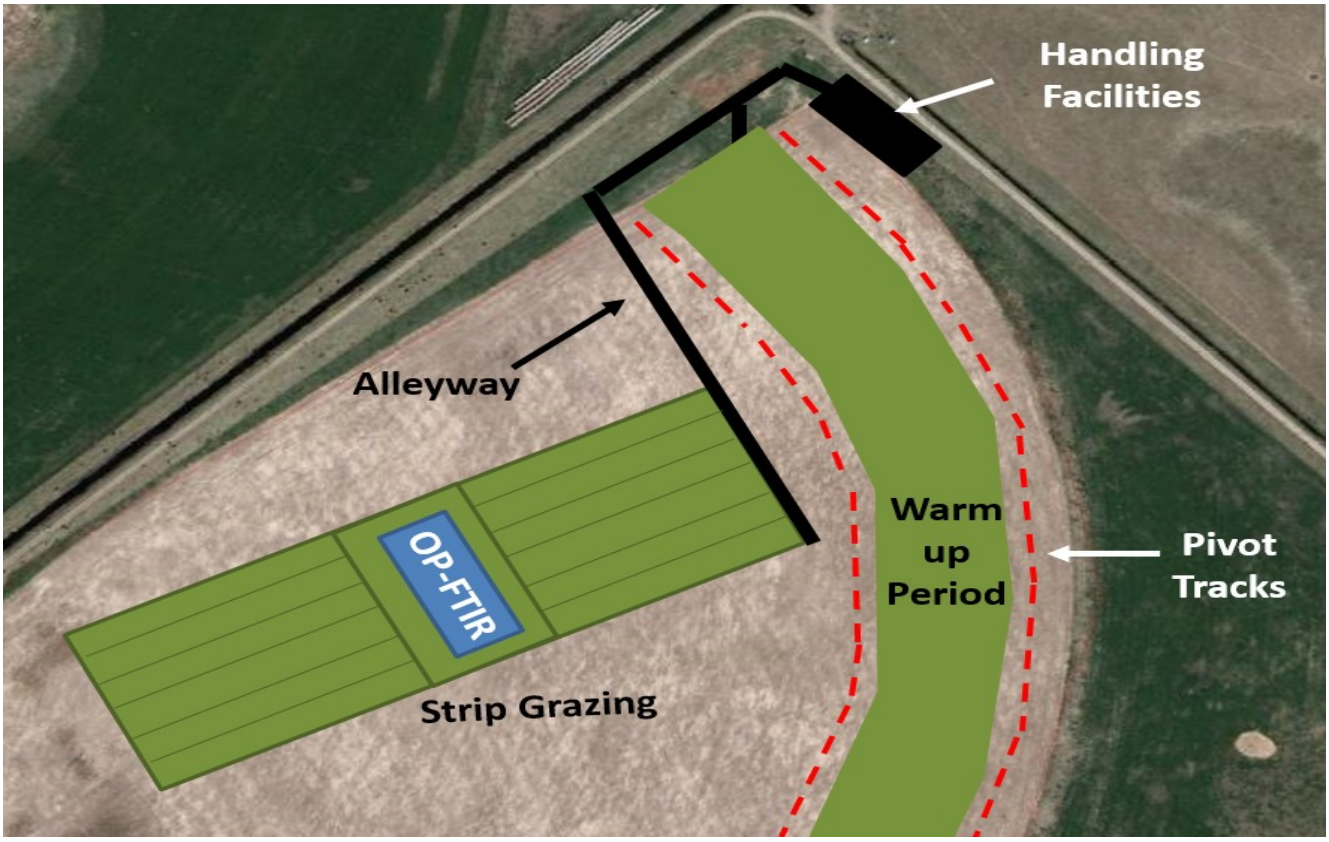

Fig. 1. Pivot 4 at the University of Alberta Mattheis Research Ranch, fenced off and set-up for grazing during the warm-up period and for strip grazing during collection of CH_4 observations. The warm-up area was positioned between pivot tracks to prevent interference with pivot use. Long, fenced off alleyways were used to move cattle between the handling facilities, warm-up area, and the grazing strips. The original image was sourced from Google Earth.

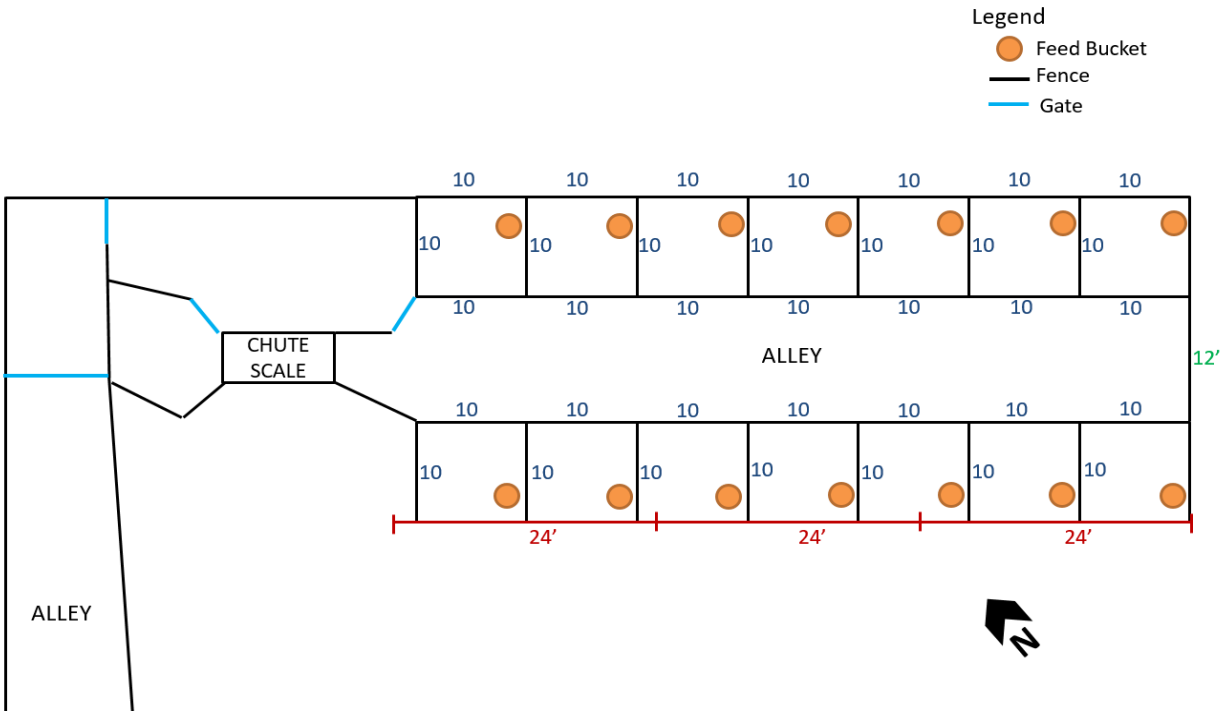

Fig. 2. Layout of individual feeding pens, scale and chute set-up for individual feeding of alkane pellets to heifers and the collection of animal weight and fecal samples during the pasture intake trial on Pivot 4 at the University of Alberta Mattheis Ranch.

Table 1. Summary of n-alkane pellet nutritional composition during the dosing period. All nutritional values are on a dry matter basis (n = 6).

Dry Matter (%)	89.3 (1.83)
Crude Protein (%)	17.5 (0.59)
Acid Detergent Fiber (%)	11.6 (0.85)
Neutral Detergent Fiber (%)	19.0 (0.34)
Total Digestible Nutrients (%)	82.7 (0.52)
Calcium (%)	0.19 (0.01)
Phosphorus (%)	0.54 (0.005)
Magnesium (%)	0.22 (0.004)
Potassium (%)	0.56 (0.01)

Standard deviations are shown in brackets

Table 2. Summary of the nutritional composition of pasture forage oats during the warm-up (n = 9) and sampling periods (n = 6). All values are on a dry matter basis.

	Warm-Up Period (day -8 to -1)	Sampling Period (day 9 to 14)
Dry Matter (%)	20.9 (2.95)	19.5 (2.67)
Crude Protein (%)	30.0 (2.15)	23.5 (0.91)
Acid Detergent Fiber (%)	21.7 (1.65)	24.5 (0.69)
Neutral Detergent Fiber (%)	45.3 (1.82)	48.6 (1.66)
Total Digestible Nutrients (%)	66.9 (1.05)	65.4 (0.89)
Calcium (%)	0.26 (0.15)	0.25 (0.07)
Phosphorus (%)	0.41 (0.03)	0.35 (0.03)
Magnesium (%)	0.24 (0.04)	0.24 (0.02)
Potassium (%)	3.57 (0.18)	4 (0.39)

Standard deviations are shown in brackets

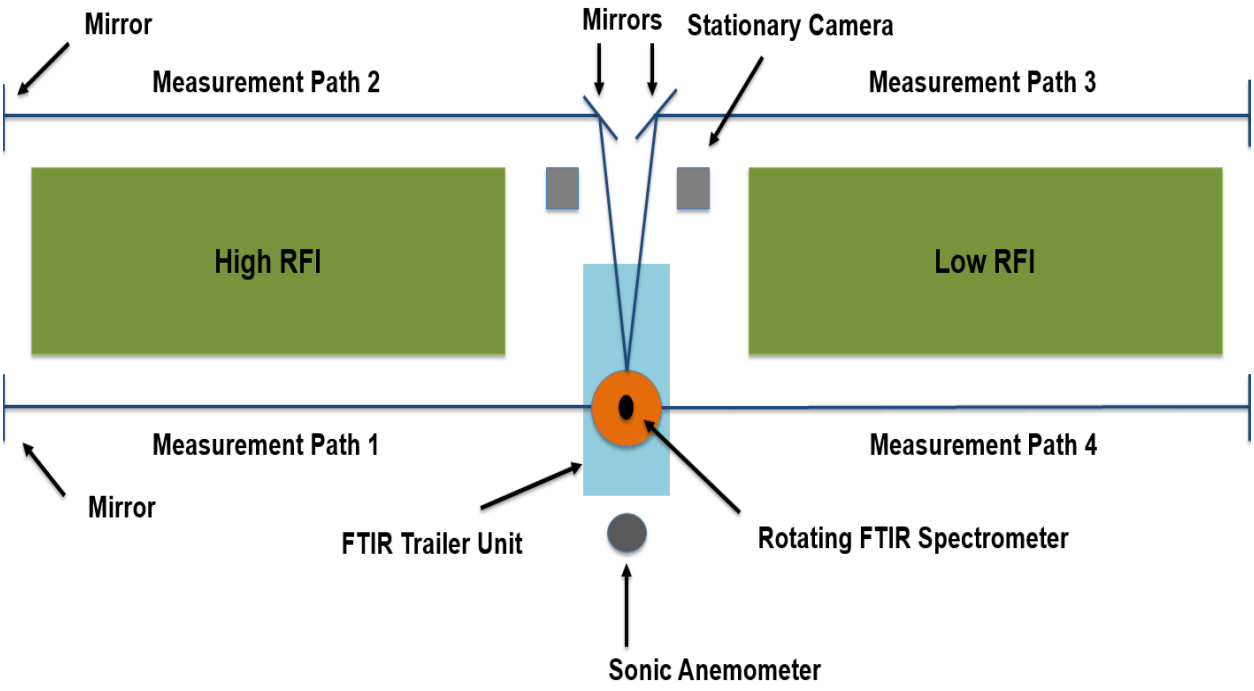

Fig. 3. Pasture CH_4 emissions set-up on Pivot 4 at the University of Alberta Mattheis Research Ranch. Four different measurement paths are depicted, one along either side of the high and low RFI_{FAT} paddocks with a rotating OP-FTIR spectrometer in the center, positioned on a portable OP-FTIR trailer.

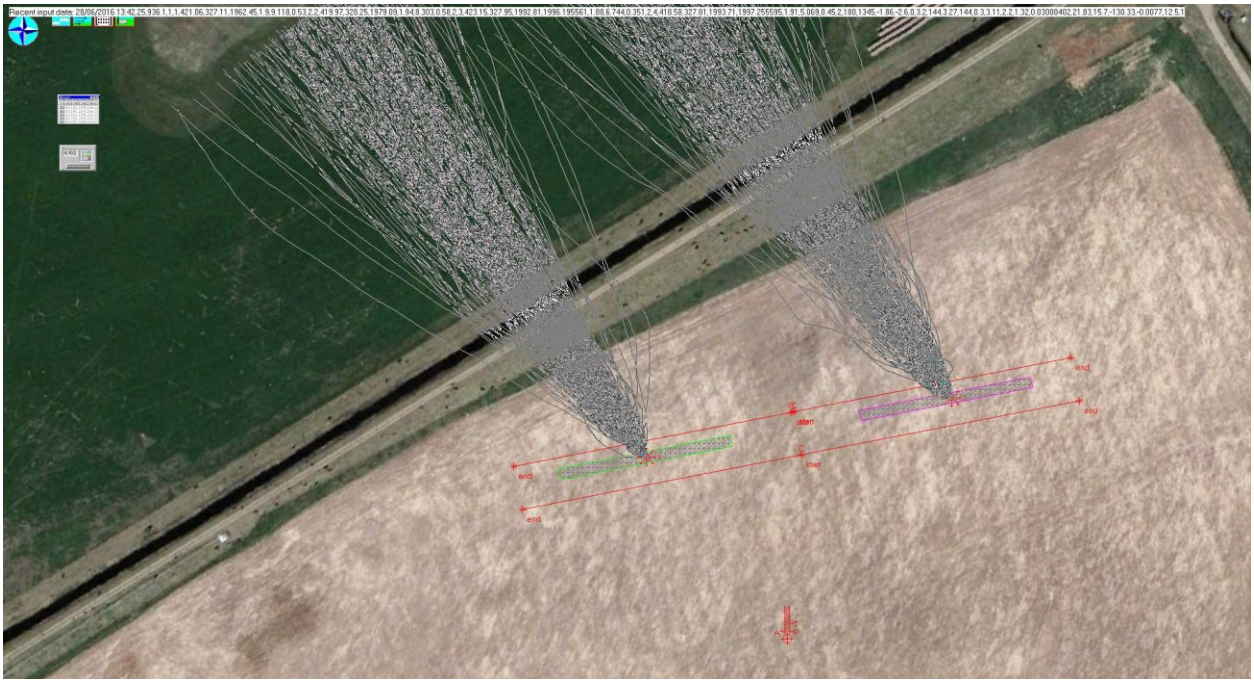

Fig. 4. Image produced by the WindTrax software showing CH_4 emissions emitted from within the high and low RFI_{FAT} grazing strips. The low RFI_{FAT} cattle are grazing the green strip on the left while the high RFI_{FAT} cattle are grazing the red strip on the right. With winds out of the south-east, emissions are nearly perpendicular to the measurement paths, as desired.

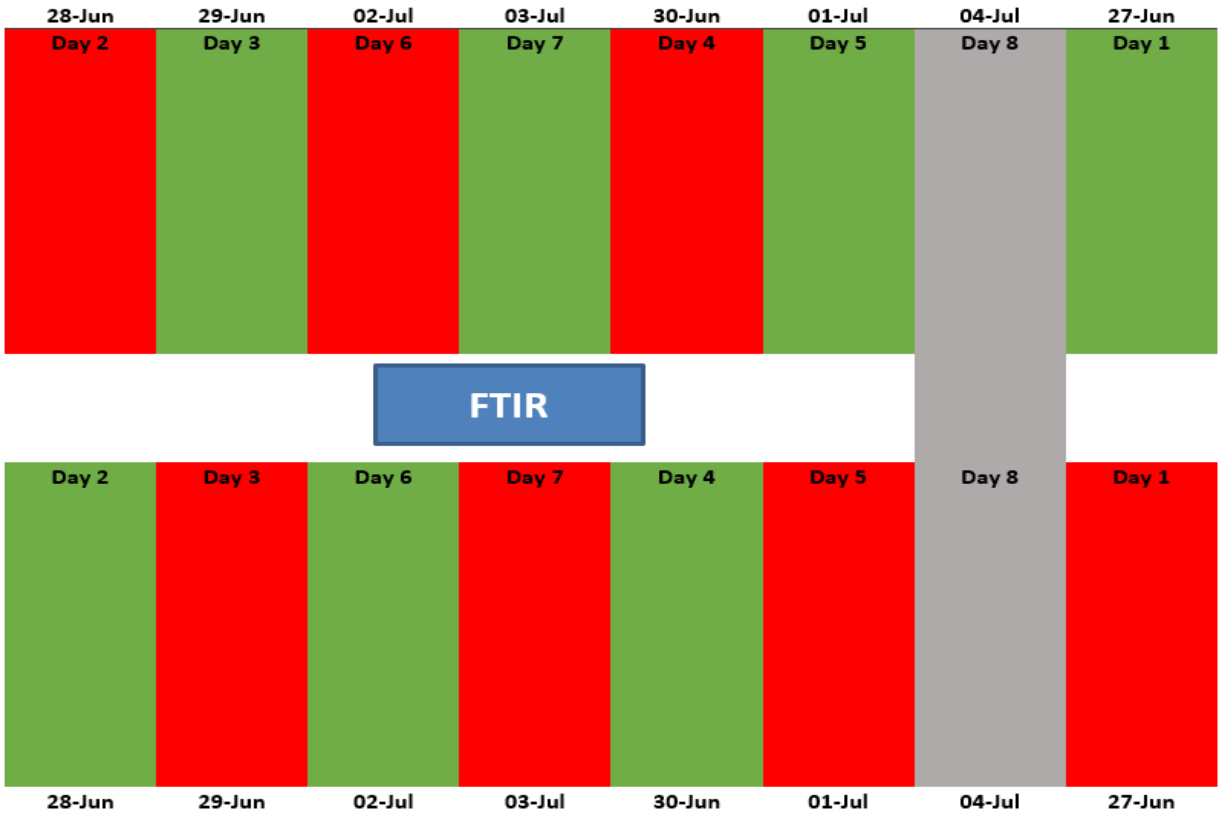

Fig. 5. The schedule of CH_4 observations over an eight day period. Low RFI_{FAT} cattle are depicted by the green strips and high RFI_{FAT} cattle are depicted by the red strips on either side of the OP-FTIR unit. Strips are not shown to scale. Cattle were adjusted to strip-grazing on day one and CH_4 measurements were not collected.

Table 3. Summary ANOVA table for DMI and animal size and condition metrics for each of the high and low RFI_{FAT} heifers while grazing on pasture.

	DMI (kg day^{-1})	
	F-Value	**P-Value**
RFI Group	0.41 (1, 14)	0.533
Sampling Day	31.89 (1, 79)	< 0.0001

	Initial BW (kg)		**Final BW (kg)**	
	F-Value	**P-Value**	**F-Value**	**P-Value**
RFI Group	0.12 (1, 13)	0.76	0.002 (1, 13)	0.96
Age	0.37 (1, 13)	0.56	19.70 (1, 13)	< 0.001

	Initial Backfat (mm)		**Final Backfat (mm)**	
	F-Value	**P-Value**	**F-Value**	**P-Value**
RFI Group	0.17 (1, 13)	0.68	5.41 (1, 13)	0.04
Age	42.93 (1, 13)	< 0.0001	19.04 (1, 13)	< 0.001

Table 4. Summary least square means for DMI and associated animal production metrics of high and low RFI_{FAT} heifers (n=16) while grazing on pasture.

	High RFI_{FAT}		**Low RFI_{FAT}**	
	LSM	**SE**	**LSM**	**SE**
Daily DMI (kg day^{-1})	8.13[a]	0.28	7.88[a]	0.28
Initial BW (kg)	304.6[a]	9.89	309.3[a]	9.99
Final BW (kg)	342.3[a]	10.35	341.6[a]	10.41
Weight Gain (kg)	31.3[a]	2.33	27.9[a]	2.73
Initial Backfat (mm)	3.5[a]	0.29	3.3[a]	0.35
Final Backfat (mm)	4.9[a]	0.34	3.9[b]	0.34

DMI – dry matter intake
BW – body weight

Table 5. Summary ANOVA results for CH_4 production and CH_4 yield of high and low RFI_{FAT} heifers (n=16) while grazing on pasture.

	Daily CH_4 Production (g head^{-1})	
	F-Value	**P-Value**
RFI Group	$0.42_{(1, 194)}$	0.52
3-Hr Time Bin	$3.96_{(1, 194)}$	0.048
	Weight Adjusted CH_4 Production (g kg^{-1} BW day^{-1})	
	F-Value	**P-Value**
RFI Group	$0.43_{(1, 194)}$	0.51
3-Hr Time Bin	$3.79_{(1, 194)}$	0.053
	Weight Adjusted CH_4 Yield (g kg^{-1} DMI day^{-1})	
	F-Value	**P-Value**
RFI Group	$0.65_{(1, 14)}$	0.44
3-Hr Time Bin	$31.90_{(1, 79)}$	< 0.0001

Table 6. Summary of least square means for CH_4 production and CH_4 yield of high and low RFI_{FAT} heifer groups while grazing on pasture.

	High RFI_{FAT}		Low RFI_{FAT}	
	LSM	**SE**	**LSM**	**SE**
CH_4 production (g day^{-1})	203.3[a]	27.46	195.6[a]	27.46
CH_4 production (g kg^{-1} BW)	0.61[a]	0.083	0.58[a]	0.083
CH_4 yield (g kg DM^{-1})	21.7[a]	0.93	20.7[a]	0.93

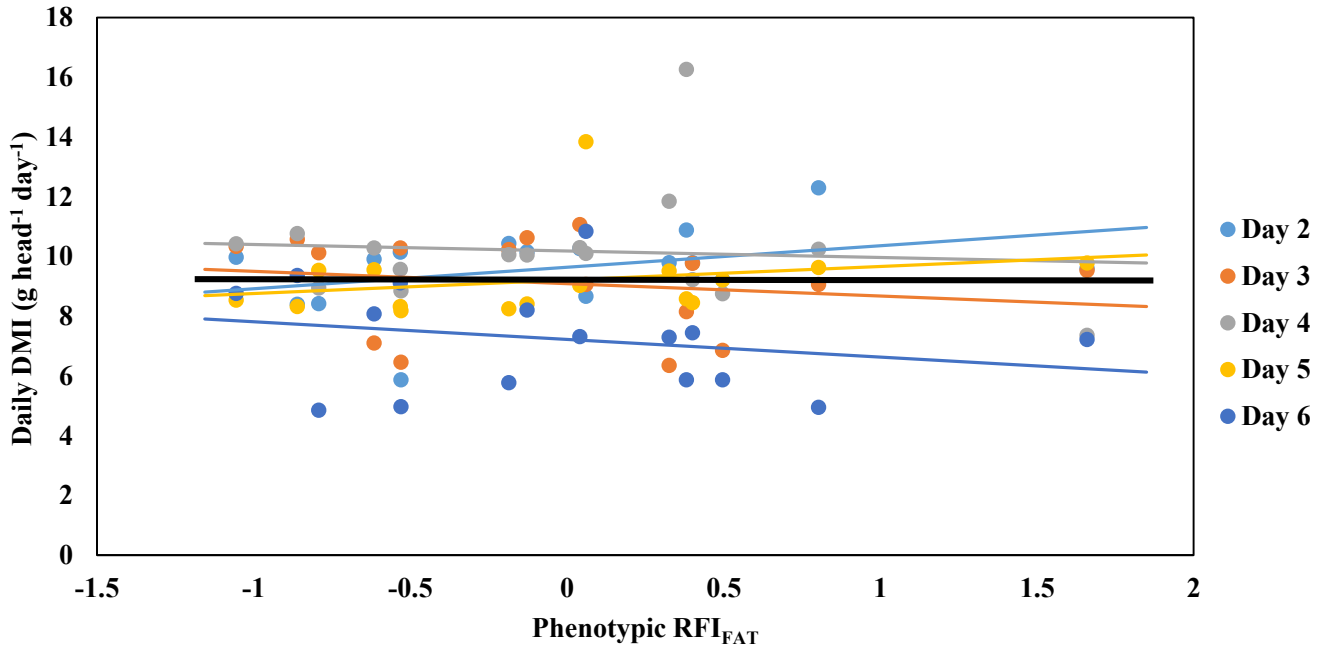

Fig. 6. Regression of daily DMI (g head^{-1} day^{-1}) and individual RFI_{FAT} value for each day of the pasture DMI trial. The black line represents the overall trend of DMI.
Total data: y = 8.97 – 0.03x, R^2 = 0.0001, Adjusted R^2 = - 0.011, P-Value = 0.92.
Day 1: y = 8.93 + 0.96x, R^2 = 0.17, Adjusted R^2 = 0.007, P-Value = 0.11
Day 2: y = 8.17 – 0.05x, R^2 = 0.001, Adjusted R^2 = - 0.07, P-Value = 0.90
Day 3: y = 8.47 – 0.28x, R^2 = 0.06, Adjusted R^2 = - 0.011, P-Value = 0.38
Day 4: y = 8.66 + 0.08x, R^2 = 0.004, Adjusted R^2 = - 0.067, P-Value = 0.81
Day 5: y = 7.27 – 0.09x, R^2 = 0.002, Adjusted R^2 = - 0.069, P-Value = 0.86
Day 6: y = 6.52 – 0.75x, R^2 = 0.096, Adjusted R^2 = 0.031, P-Value = 0.24

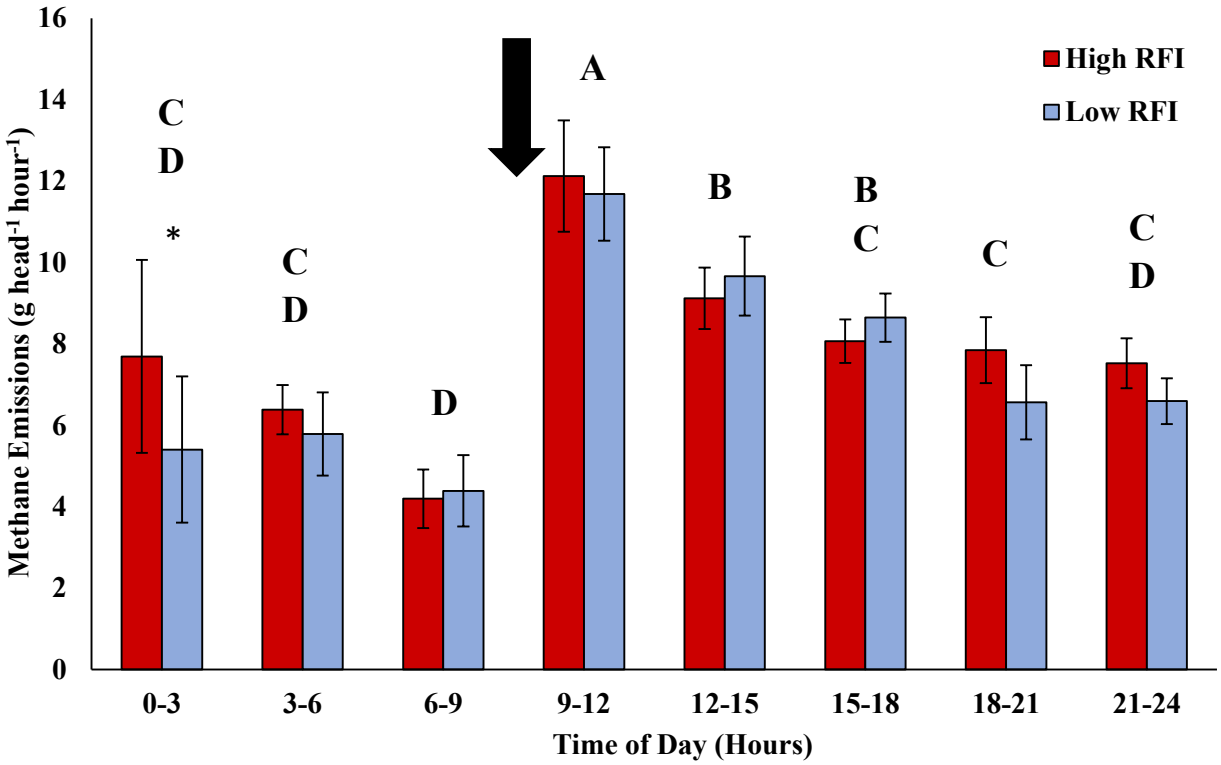

Fig 7. Mean diurnal pattern of CH_4 production (g head^{-1} hour^{-1}) for high and low RFI_{FAT} heifers grazing on pasture over a 24-hour period of time, separated into three hour time bins. Arrow indicates the timing of pasture entry.
* Indicates significant differences in CH_4 production between high and low RFI_{FAT} groups ($P<0.05$). Upper case letters indicate overall differences among sampling times ($P<0.05$).

Chapter 5: Synthesis

RESEARCH CONCLUSIONS

A commercial herd of Hereford-Angus cross cows with molecular breeding values (MBVs) for high and low RFI was separated into three groups based on their predicted RFI values. Cows with MBVs for low RFI were bred to bulls with low RFI phenotypes, cows with MBVs for high RFI were bred to bulls with high RFI phenotypes and cows with MBVs for medium RFI were bred to bulls with medium RFI phenotypes. Production metrics were collected and analyzed for all the cows and calves over a summer grazing period. The results from the trials in the study revealed that selection for cattle with divergent MBV for RFI on pasture does not have a negative impact on the performance of those cattle. More specifically, cows with MBV's for high and low RFI did not differ in their ability to gain weight or put on backfat over the summer grazing period. It also did not affect their ability to become pregnant as the proportion of cows that became pregnant in the first and second breeding cycles, as well as the proportion of open cows, was similar among all three predicted RFI groups. Additionally, calves born to dams with MBV's for high and low RFI did not differ in average daily gain (ADG), growth or body weight following the summer grazing period. The results suggest that using MBV's as a means of increasing a herd's feed efficiency on pasture will not compromise the performance of those cattle.

Eighteen replacement heifers, born to dams with MBVs for high and low RFI were assessed for drylot feed intake and CH_4 emissions. Dry matter intake was measured using GrowSafe Systems technology which automatically monitors individual feed intake. Additionally, CH_4 emissions were monitored using a GreenFeed emissions monitoring system. Individual feed intake observations of the high and low RFI_{FAT} (RFI adjusted for backfat)

replacement heifers, revealed that high RFI_{FAT} heifers consumed significantly more feed than low RFI_{FAT} heifers. Despite the significant difference in feed intake, high and low RFI_{FAT} heifers had similar body weights and similar weight gain over the trial period. Although the mechanism is unclear, low RFI cattle have the ability to gain the same amount of weight using less energy and ultimately fewer resources. Even though there was a significant difference in dry matter intake (DMI) overall methane (CH_4) production did not differ between the high and low RFI_{FAT} heifers. It was hypothesized that CH_4 production would differ, especially considering a significant difference in DMI. It is likely that CH_4 yield played a role in the results of CH_4 production as the low RFI_{FAT} heifers had significantly greater CH_4 yields. There is a possibility that the high CH_4 yields experienced by low RFI_{FAT} heifers contributed to the similarity in overall CH_4 production. Although not tested in the current study, it's possible that the low RFI_{FAT} heifers had significantly greater CH_4 yields as a result of increased digestibility and increased rumen retention times, resulting in more hydrogen available for CH_4 production.

A subset of 18 replacement heifers, previously tested for individual feed intake in drylot were assessed for feed intake and CH_4 production on pasture. Feed intake was assessed using an n-alkane marker, while group CH_4 emissions were monitored using an open-path Fourier transform infrared (OP-FTIR) spectrometry system. The results rejected the hypotheses that high RFI_{FAT} heifers would have greater DMI and greater CH_4 production. Although there were no significant differences, there was a slight tendency for greater DMI and greater CH_4 production in the high RFI_{FAT} heifers, a trend that was expected and supported by literature. It is possible that DMI values were not significantly different, resulting from a low number of observation days. Feed intake observations in drylot are often collected over a period of at least 35 days to

capture accurate results. Because observations were only collected for five days it is possible that DMI values were affected by the low number of days in which observations were collected. It is also possible that CH_4 production did not differ as a result of similarities in DMI.

INDUSTRY IMPLICATIONS

The results from the research conducted in the current study reveal that selection for RFI in cattle that are raised in a pasture environment, using MBV's, should not have an effect on the performance and overall productivity of those cattle. From a producer's perspective this a desirable outcome as it indicates that cattle can be more efficient without compromising their ability to maintain a healthy weight or healthy backfat cover, both of which are necessary for rebreeding. The results also indicate that the low RFI cows, which tend to eat significantly less than their high RFI counterparts, are still able to produce calves with similar weaning weights. Ultimately this means that producers would be able to produce the same amount of beef using fewer resources. Not only does this allow a producer to be more profitable, it also allows them to reduce their carbon footprint as the need for land or feed resources decreases.

Results from the replacement heifers indicate that heifers with low RFI or RFI_{FAT} values consume less feed and are more likely to produce less CH_4 in drylot. This means that beef operations which retain low RFI replacement heifers will require fewer feed resources. Such operations are also more likely to produce fewer CO_2 emissions, and possibly also fewer CH_4 emissions, meaning that their contribution to overall greenhouse gas (GHG) emissions is reduced. Although results of pasture feed intake and CH_4 emissions were not significantly different between the high and low RFI_{FAT} heifers, there was a trend to suggest that low RFI_{FAT} heifers consume less feed and produce less CH_4. These results indicate that replacement heifers

which were identified as low RFI_{FAT} in drylot, tended to also consume less feed and emit fewer CH_4 emissions on pasture. By selecting for and retaining low RFI replacement heifers on pasture or in drylot, it is likely that producers can reduce their overall feed costs allowing for greater profitability.

FUTURE RESEARCH

Due to the lack of RFI-related research with cow-calf herds on pasture, future studies should look to validate the results from the current study. More specifically, future research should focus on understanding whether differences in grazing behaviors between high and low RFI cattle contribute to their efficiency and/or their productivity. Future research should also seek to validate the pasture feed intake and CH_4 emissions results. Very few studies have been published in which high and low RFI cattle were monitored for their emissions on pasture using and OP-FTIR system, future studies will be necessary to further validate the results from the current study. Additionally, subsequent studies should aim to study a larger number of replacement heifers to reflect more realistic herd sizes. Examining rumen retention times, alongside CH_4 and feed intake measurements, would also be beneficial to help explain differences in overall CH_4 production and yield measurements.

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Appendix A:
Evaluating Molecular Breeding Values

APPENDIX A: Genomic Molecular Breeding Value and Breed Composition Evaluations

Molecular Breeding Values for RFI

In order to evaluate molecular breeding values (MBV) for RFI, tissue samples were collected on all 450 cows, using Typifix (Gene Check, Inc., Greeley, CO, USA) ear tags. Samples were genotyped using a BovineSNP50 Beadchip (Illumina Inc., San Diego, CA) 50K panel. MBVs for RFI were assigned by running a genetic evaluation using an animal model with a genomic relationship matrix, known as genomic best linear unbiased prediction (GBLUP). VanRaden et al. (2009) explained that the GBLUP method is a linear method that assigns all markers the same weight, suggesting they are normally distributed, rather than assigning more weight to more significant markers. GBLUP disregards the true genetic determinism of a trait. GBLUP models are most beneficial for polygenic traits (Boichard et al. 2016), traits which are influenced by several genes (Hermisson et al. 2002), such as RFI. A reference population (i.e. a population with both genotypes and phenotypes) of purebred Angus and Hereford-Angus crossbred cattle in the Phenomic Gap dataset (McKeown et al. 2013) as well as purebred Hereford cattle from the Olds College dataset (Olds, AB, Canada) were used, Individual animal solutions were taken as breeding values for RFI. Accuracy of the MBVs were calculated using the following formula:

$$1 - \sqrt{\frac{\text{PEV}}{\text{Var}_{\text{gen}}}}$$

, where PEV= prediction error variance and Var_{gen}=genetic variance. RFI values were significantly different ($P < 0.0001$) between groups of high, medium and low gRFI cattle. Tukey's HSD tests revealed significant differences in RFI among all three groups. The average gRFI values for each group, along with their accuracies and ranges, are shown in Table A1.

Genomic-based breed composition and retained heterozygosity

Genomic-based breed composition was predicted using 43,172 SNPs distributed across the 29 autosomes from the Illumina Bovine 50K SNPs with ADMIXTURE software (Alexander et al. 2009) to account for stratification due to breed effects in the association analyses. A larger dataset (n=7845) of purebred animals of different breeds was used as a reference population. Additionally, the heterosis effect was accounted for in the association analyses, by calculating the genomic-based retained heterozygosity (RH) for each individual according to (Dickerson 1973) as follows:

$$\mathrm{RH} = 1 - \sum_{k=1}^{n} P_i^2$$

where P is the fraction of breed i from each of the n breeds. Average RH and breed composition for high medium and low gRFI cows is summarized in Table A2.

Table A1. Mean parameters of gRFI groups of cows

	Predicted RFI Group		
	High	**Medium**	**Low**
n =	86	287	77
RFI Value	0.074^{a} $(0.028)^{Z}$	-0.017^{b} (0.030)	-0.108^{c} (0.036)
Accuracy	0.34 (0.059)	0.39 (0.060)	0.41 (0.081)
RFI Range	$0.14 \leq x \geq 0.042$	$0.042 < x \geq -0.07$	$-0.071 \leq x \geq -0.213$

a, b, c – indicate significant differences among groups within a row
Z – Standard deviations in parentheses

Table A2. Average retained heterozygosity and breed composition distribution of high, medium and low gRFI cows.

		Predicted RFI Group		
		Low	**Medium**	**High**
Mean Retained Heterozygosity		0.233 (0.186)[Z]	0.293 (0.212)	0.323 (0.199)
Retained Heterozygosity Range		0.0003 - 0.525	0.0003 - 0.701	0.018 - 0.652
Number of each Breed gRFI Group^{-1}	Angus	0	0	6
	Hereford	29	87	5
	Crossbred	6	81	30

Z – Standard deviations in parentheses.

Appendix B: Analytical Procedure References

APPENDIX B: Analytical Procedure References (Cumberland Valley Analytical Services)

Nitrogen/Protein Concentration

Association of Official Analytical Chemists (AOAC) International. 2000. Protein (crude) in animal feed (990.03). Official Methods of Analysis, 17th edition, MD, USA.

- **Specification:** Analysis conducted using a Leco FP-528 Nitrogen Combustion Analyzer (Leco, 3000 Lakeview Avenue, MI, USA)

Acid Detergent Fiber

AOAC International. 2000. Fiber (Acid Detergent) and Lignin in Animal Feed (973.18). Official Methods of Analysis, 17th edition, MD, USA.

- **Modifications:** Whatman 934-AH glass micro-fiber filters with 1.5um particle retention was used in place of fritted glass crucible.

Neutral Detergent Fiber

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for Dietary Fiber, Neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Science 74:3583-3597.

- **Modification:** Whatman 934-AH glass micro-fiber filters were used with 1.5um particle retention.

Metals and Minerals

AOAC International. 2000. Metals and Other Elements in Plants (985.01). Official Methods of Analysis, 17th edition, MD, USA.

- **Modifications:** Ash 0.35g sample for 1 hr at 535°C, then digest in open crucibles for 20 min in 15% nitric acid on a hotplate. Samples were diluted to 50ml and analyzed using an inductively coupled plasma (ICP) device (Perkin Elmer 5300 DV ICP, PerkinElmer, CT, USA).

Appendix C:
N-Alkane Pellet Preparation

APPENDIX C: N-Alkane Pellet Preparation

Extruded pellets containing Beeswax and C_{32} were used as alkane markers and fed to replacement heifers to determine DMI on pasture. Feeding of C_{32} n-alkane pellets was approved by the University of Alberta Animal Care and Use Committee (AUP00001284). Pellets were prepared at the Food Science and Technology Centre (Brooks, Alberta, Canada) in accordance with standard operating procedures developed by researchers at the AAFC Lacombe Research Centre and the Food Science and Technology Centre. Pellets were prepared in 2015 and stored in large tote bags until the trial in 2016. All ingredients were supplied by Dr. John Basarab at the AAFC Lacombe Research Centre (Lacombe, Alberta, Canada).

1. The pellet ingredient composition is listed in Table C1. The canola meal, ground wheat, ground barley and corn dried distillers grains and solubles (DDGS) were sieved with a 9-14 mm screen to remove contaminants and large grain kernels before weighing. Canola oil was mixed by hand into the canola meal prior to weighing. Weights of all of the ingredients in one batch are listed in Table C2. Each batch had a total weight of 215.6 kg.
2. Beeswax, shredded using a stainless steel cheese grater, was stored at -20°C. Following freezing, the beeswax was crumbled by hand, 1.54 kg was hand-mixed with 16.60 kg of barley flour to create a premix, which was immediately stored in the freezer until final batch blending.
3. A synthetic alkane marker, C_{32} dotriacontane, was added to the feed blend at a concentration of 0.04%. Due to its low inclusion rate, a C_{32} premix was made using barley flour as a diluter. The premix included 4.3 kg barley flour and 0.088 kg C_{32}, for a total of 4.40 kg of C_{32} premix added to each feed batch.
4. A fluidizing paddle blender (Model FPB-20, American Process Systems, Gurnee, Illinois, USA) was used to mix the ingredients at 60 Hz for 2 minutes.
5. Pellet extrusion (i.e. the process of forming pellets) was done using a co-rotating, intermeshing twin-screw extruder (Model ZSK-57, Werner and Pfleiderer, Ramsey, New Jersey, USA) with a barrel diameter of 57 mm and length to diameter ratio of 24:1; the extruder had eight different barrels. Feed

ingredients were supplied to the second barrel and moved throughout subsequent barrels before being pressed through the die. Temperature was independently controlled for three different sets of barrels and for the die:

a. Two and three: 51ºC
b. Four, five and six: 90ºC to 80ºC when moving from barrel four to barrel six
c. Seven and eight: 132ºC
d. Die: 145ºC

6. Feed ingredients were pressed through a circular 2.5 mm diameter die and cut at the die with a rotating knife to approximately 1.9 cm. Extrudates were then dried and cooled in a gas fired fluidized bed dryer (Carrier, Louisville, Kentucky, USA).

7. Dried pellets (48.75 kg) were coated with 1.25 kg canola oil using a garden sprayer and blended with a fluidizing paddle blender at a speed of 50 Hz to ensure even coating.

8. Coated pellets were packed into tote bags with a total net weight of 200-250 kg per bag. Bags were stored indoors at the AAFC Lacombe Research Centre until used.

Table C1. Percentage of ingredients present in the n-alkane pellet feed blend.	
Barley Grain (%) [Z]	55
Wheat Grain (%) [Z]	20
Canola Meal (%)	16
Corn DDGS (%)	5.3
Canola Oil (%)	1
Beeswax (%) [X]	0.7
C_{32} (%) [Y]	0.04
Canola Oil Coating (%) [W]	
Total	*98.04*

Z: finely ground ingredients, X: natural marker, Y: synthetic marker
W: added after extrusion at a rate of approximately 2.5% of the total weight

Table C2. Ingredient preparation by batch.	
Barley Grain (kg)	100
Wheat Grain (kg)	44
Canola Meal (kg)	35.2
Corn DDGS (kg)	11.66
Canola Oil (kg)	2.2
Beeswax Premix (kg)	18.14
C_{32} Premix (kg)	4.4
Total (kg)	*215.6*

Appendix D:
Paddock Layout and Forage Management

APPENDIX D: Paddock Layout and Forage Management

The north half of Irrigation Pivot 4, located at the University of Alberta Mattheis Research Ranch, was seeded to a monoculture of forage oats to allow for the assessment of feed intake and methane production from heifers on pasture. The seeded paddock was fenced in long narrow strips to allow for strip-grazing, adjacent to a large warm-up area at the perimeter of the seeded paddock (Fig. D1). Both the warm-up area (used for pre-conditioning animals to their new diet) and grazing strips were in close proximity to a cattle handling facility and the entire seeded area was relatively flat with few fluctuations in topography.

Forage Management

A grazing paddock, covering half the area of Pivot 4 (19.02 ha) was seeded to CDC Baler forage oats, and irrigated following seeding. Oats were seeded May 3, 2016 with a Bourgalt 5700 Air Hoe Drill at a rate of 78.46 kg ha^{-1} with a seed row spacing of 24.13 cm. In addition, 8.97 kg ha^{-1} of Italian Ryegrass was broadcasted with a Valmar after the oats were drilled in. Ammonium phosphate fertilizer (11-52-0) was incorporated with the seed at a rate of 50.44 kg ha^{-1} along with 68.13 kg ha^{-1} of urea (46-0-0) in midrow (i.e. the fertilizer was placed between 30 cm seed rows). The crop was sprayed once (June 3, 2016) with Curtail® to eliminate undesirable broadleaf weeds; specifically targeting Canada thistle, dandelion, lambs-quarter's, and wild buckwheat. Grazing restrictions and intervals were obeyed to ensure that cattle did not graze the paddock within seven days of herbicide application. Following seeding, and prior to the start of the grazing trials, the paddock was irrigated at least once every two weeks to ensure adequate forage growth by the start of grazing on June 13.

Strip Grazing Layout and Calculations

A section of the paddock was fenced off in long narrow strips, with enough strips to allow the heifers to access a new strip each day; strips were large enough to provide adequate feed for all the heifers within a group (8-9 head) for one day. The following calculations were done to determine the appropriate length and width of the strips. Calculations were based on daily feed utilization of 7% of the heifers' body

weight (Manafiazar et al. 2015), in which 2.5% was necessary to meet feed intake requirements (NRC 1996) and an additional 4.5% accounted for feed wastage and trampling; it also ensured that both groups of heifers (high and low RFI) consumed mostly leafy portions of the grass sward in an effort to maintain consistency of the diet.

1. Three individual 0.5m^2 areas of pasture were clipped right to the base of the sward on June 29, 2016, one day prior to the start of the strip-grazing period. Areas were randomly selected as to represent the entire grazing area. Each of the three samples was weighed on an as-fed basis. Samples were collected shortly before grazing.
    - Sample 1 (collected on June 29, 2016): 1 151.62 g/m^2
    - Sample 2 (collected on June 29, 2016): 1 319.94 g/m^2
    - Sample 3 (collected on June 29, 2016): 1 206.32 g/m^2
2. Weights from the three samples were averaged and forage weight was calculated per square metre.

$$\frac{1151.62 + 1319.94 + 1206.32}{3} = 1\ 225.78\ \text{g/m}^2$$

$$\text{X} = 1225.78\ \text{g/m}^{\ 2} \qquad \text{X} = 1.226\ \text{kg/m}^2$$

3. Average weights of the heifers taken on June 21, 2016 were used to determine average estimated weights of the heifers on June 28, 2016 which were multiplied by 7% to determine the amount of feed necessary in each strip, for one day. (The estimated weight was slightly higher than the actual weight, to ensure that enough feed would be available).

$$351.47\ \text{kg}\ x\ 0.07 = 24.60\ \text{kg/animal/day}$$

4. The size of grazing strips needed was then determined based on the total amount of feed necessary for one day of grazing for all animals within each herd (initially 9, then 8 head per group).

$$24.60\ \text{kg}\ x\ 9\ \text{animals} = 221.43\ \text{kg/group}$$

5. All strips had a width of 5m to accommodate adequate measurements of laser methane emission from cattle. Length of the strips were adjusted to ensure a sufficient abundance of feed as well as appropriate length to capture methane observations. The amount of total feed within the area of the strips was calculated as follows:

$$5 \text{ m wide x } 80 \text{ m long} = 400 \text{ m}^2$$

$$1.226 \text{ kg } x \text{ } 400 \text{ } m^2 = \text{ } 490.40 \text{ kg/strip}$$

Each group of nine heifers (later reduced to eight) had access to a new strip of forage each day, containing approximately 490 kg of dry matter biomass. Although each group of heifers only required approximately 221 kg each day, the abundance of forage allowed for about 45% maximum utilization (including feed intake and wastage). This ensured that heifers had *ad-libitum* access to feed and that they were able to select mostly leafy (i.e. high quality) components of the plant, rather than the stems, allowing for consistency in diet from one day to the next.

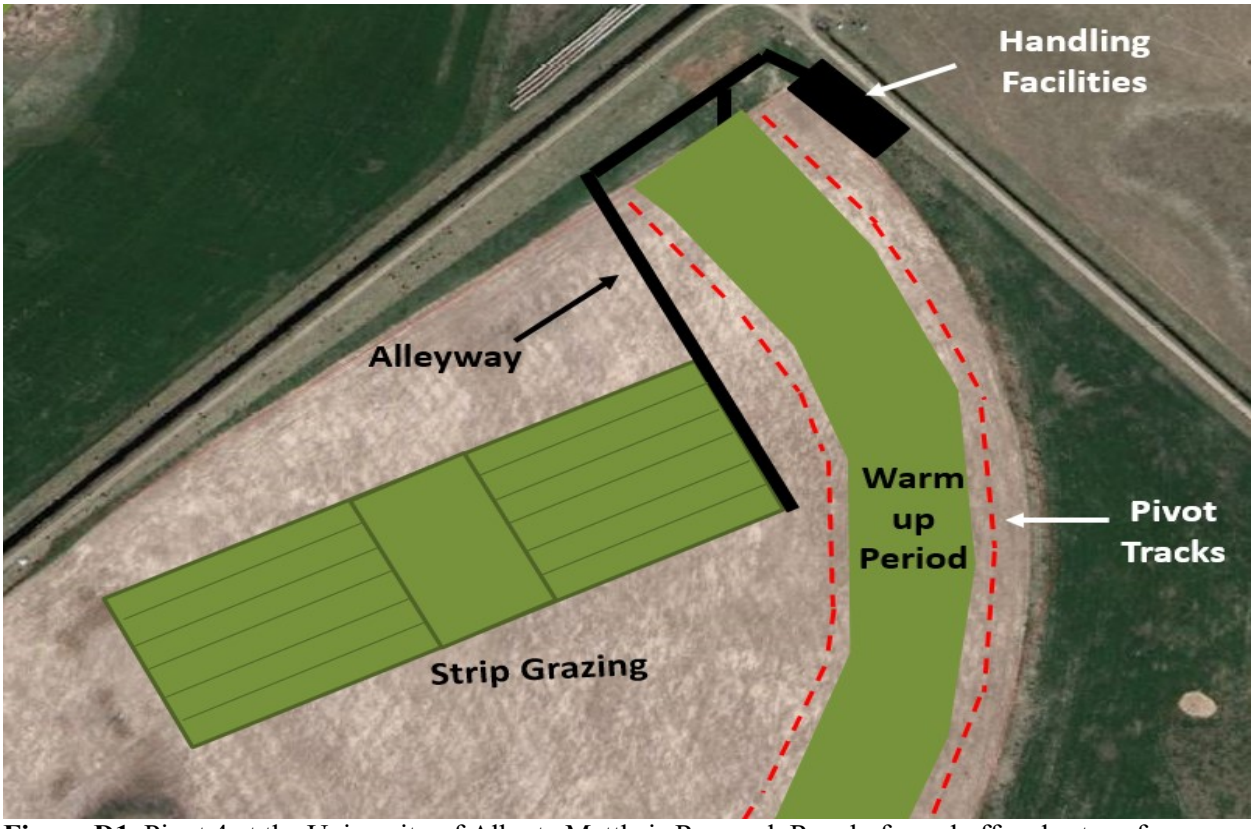

Figure D1. Pivot 4 at the University of Alberta Mattheis Research Ranch, fenced off and set-up for grazing during the warm-up (acclimation) period and for strip grazing during the subsequent collection of methane observations. The warm-up area was positioned between pivot tracks to prevent interference with pivot use. Long, fenced off alleyways were used to move cattle between the handling facilities, warm-up area, and the grazing strips. The original image was sourced from Google Earth.