ASSOCIATIONS BETWEEN RFI, AND METABOLITE PROFILES AND FEEDING BEHAVIOR TRAITS IN FEEDLOT CATTLE

A Thesis

by

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ABSTRACT

Objectives of this study were characterize residual feed intake (RFI) in finishing steers to examine relationships with performance, feed efficiency, feeding behavior traits, and blood metabolite profiles and identify biomarkers (feeding behavior traits and blood metabolites) to understand underlying biological mechanisms of RFI. Performance, DMI, and feeding behavior traits were measured for 70 d in Angus crossbred steers (N = 168) using a GrowSafe system. Steers were classified into low (n = 52), medium (n = 64), and high (n = 52) RFI groups based on ± 0.5 SD from the mean RFI of 0.00 (SD = 0.82). Partial least squares (**PLS**; MetaboAnalyst) were used to examine associations between RFI, and feeding behavior traits and metabolites.

Components 1 and 2 of the PLS analysis accounted for 39.1% of between animal variance in RFI, and 4 feeding behavior traits had a variable of importance in projection (VIP) score > 1, which included HD duration, BV duration, HD to meal duration ratio, and bite frequency. Steers with low RFI had 15% greater (P < 0.0001) bite frequency, 34% lower HD duration, 24% lower BV duration, and 24% lower HD:MD ratio than high RFI steers. To examine associations between RFI and blood metabolite profiles to identify RFI biomarkers, blood was collected on day 0 and 70 of the trial for steers with RFI that were ± 1 SD from the mean RFI (0.00 ± 0.82 kg/d), which included 25 low and 24 high-RFI steers. Partial least squares analysis of day 0 metabolite profiles resulted in overfitting of the data (P = 0.264), but day 70 metabolite data was not over-fitted (P = 0.009). Components 1 and 2 of the PLS analysis accounted for 34.2% of between animal

variance in RFI. Of the 44 metabolites detected by ^{1}H-NMR, 5 metabolites had VIP scores > 2, which included glycine, betaine, tyrosine, valine, and leucine. Steers with low-RFI had 54% higher ($P < 0.0003$) concentrations of glycine, and 14% lower ($P < 0.05$) concentrations of betaine, 12% lower ($P < 0.05$) concentration of tyrosine, 9% lower ($P < 0.06$) concentration of valine, and 14% lower ($P < 0.04$) concentration of leucine than high-RFI steers. Results from this study indicate glycine, betaine, tyrosine, valine, and leucine are possible biomarkers for identification of feed-efficient cattle. Further studies are needed to evaluate the repeatability and robustness across breeds, diets, etc. for these metabolites.

DEDICATION

I would like to dedicate this thesis to my family. They have been there to pick me up and keep me going. Thank you for all the love and support through this journey especially my loving wife Nikki.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Improvements in the efficiency of feed utilization by livestock are needed more than ever due to increasing demands for animal-sourced foods by a growing global population in the face of finite availability of natural resources. Selection of livestock with favorable feed efficiency and/or adoption of other technologies that improve feed efficiency will enhance the economic sustainability of livestock operations as the expense of feed inputs represents the largest variable cost of livestock production systems. Along with reducing costs of production, favorable selection for feed efficiency will also decrease the environmental impact of animal agriculture due to reductions in methane emissions and manure excretions.

In recent years, feed efficiency has been well characterized in growing beef cattle (Archer et al., 2001a; Bishop et al., 1991). The most widely used trait to measure feed efficiency is feed conversion ratio (FCR), which has been shown to be moderately heritable and highly correlated in a negative manner with average daily gain (ADG) and body weight (BW; Archer et al., 2001a; Bishop et al., 1991). Consequently, favorable selection for FCR would result in indirect selection for faster growing cattle that are larger in mature size. Thus, FCR is not an ideal trait for use in selection programs to improve genetic merit for feed efficiency.

Residual feed intake (RFI) is an alternative trait to measure feed efficiency that is calculated as the actual intake minus predicted intake, with predicted intake for an individual animal typically computed by regressing feed intake on mid-test $BW^{0.75}$ and ADG. Because RFI is phenotypically independent of variation in body size and growth, selection based on RFI will improve feed efficiency with minimal effects on mature size or level of productivity (Basarab et al., 2003; Lancaster et al 2009a; Lancaster et al., 2009b). Therefore, RFI is an ideal trait for selection of feed efficiency in beef cattle.

Historically, the beef industry has focused on selecting cattle based on output traits, with little emphasis on selection to reduce inputs traits. The heritability of RFI has been well characterized in growing animals (Arthur et al., 2001b; Archer et al., 1997; Schenkel et al., 2004). In young bulls and heifers, RFI has been shown to be moderately heritable trait (≈ 0.30 to 0.45; Arthur et al., 2001b; Archer et al., 1997; Schenkel et al., 2004), suggesting that genetic improvement in RFI will reduce feed inputs independent of variation in body size or level of production.

Sources of Variation in Residual Feed Intake

Genetic variation in feed efficiency in cattle is influenced by many factors. The ability to identify and understand these factors will enable development of improved selection tools for producers to breed more feed-efficient animals. Richardson and Herd (2004) characterized RFI in Angus cross-bred steers, and concluded that differences in body composition, digestion, feeding behavior, protein turnover, tissue metabolism and stress, heat increment of fermentation, activity, and other unknown mechanisms

accounted for 5, 10, 2, 37, 9, 10, and 27% of the inter-animal variation in RFI, respectively.

Numerous studies have examined the associations between composition of growth and RFI in beef cattle (Carstens et al., 2002; Nkrumah et al., 2004; Robinson and Oddy, 2004; Schenkel et al., 2004; Lancaster et al., 2009a). The consensus has developed that feed-efficient (low-RFI) cattle have less propensity to deposit fat than feed-inefficient (high-RFI) cattle. The phenotypic and genetic correlations between back fat thickness (BF) and RFI were weak to moderately positive in beef cattle (Arthur et al., 2001a; Nkrumah et al., 2004; Richardson et al., 2004; Lancaster et al., 2009a; Lancaster et al., 2009b; Schenkel et al., 2004; Shaffer et al., 2011).The longissimus muscle area (LMA) phenotypic and genetic correlations with RFI were more variable and ranged from weakly negative to weakly positive (Arthur et al., 2001a; Basarab et al., 2003; Nkrumah et al., 2004; Schenkel et al., 2004; Lancaster et al., 2009a; Lawrence et al., 2011). In growing cattle, most studies demonstrated minimal associations between intra-muscular (IM) fat and RFI (Carstens et al., 2002; Schenkel et al., 2004; Lancaster et al., 2009b). However, in finishing cattle, some studies have shown that IM fat was weakly correlated in a positive manner with RFI (Nkrumah et al., 2004; Basarab et al., 2003). The positive correlations between RFI and BF depth, means that more efficient cattle are leaner most likely due to increased energetic cost to deposit fat compared to muscle. To minimize the potential detrimental effects of selection for RFI on carcass quality (e.g. reduced marbling), it has been suggested that BF depth be included in the model to compute RFI (Basarab et al., 2003; Lancaster et al., 2009a; Lancaster et al., 2009b).

Although Richardson and Herd (2004) reported that body composition accounted for 5% of inter-animal variation in RFI, several studies have reported higher variances in RFI due to body composition of approximately 8-10% (Lancaster et al., 2009a; Basarab et al., 2003; Arthur et al., 2003).

The association between dry matter digestibility (DMD) and RFI has been evaluated in several studies. McDonald et al. (2010) reported that low-RFI cows had a 19% higher DMD than high-RFI cows. Nkrumah et al. (2006) reported that low-RFI steers tended ($P = 0.10$) to have a greater DMD than high-RFI steers, and Richardson et al. (1996) also found a tendency for low-RFI bulls and heifers to have greater DMD than high-RFI bulls and heifers. In contrast, Cruz et al. (2010) and Fitzsimons et al. (2013) did not find differences in DMD between low- and high-RFI steers or heifers. The inconsistent results reported in these studies may be due to the variable diets fed among studies and the methods used to measure individual animal DMD. More research is needed to fully understand how digestibility influences feed efficiency.

Numerous studies have examined the associations between feeding behavior traits and RFI in beef cattle. Nkrumah et al. (2007b) reported that low-RFI steers had 14% fewer visits to the feed bunk and spent 24% less time at the feed bunk than high-RFI steers. The frequency of feed bunk events has been shown to have weak-to-moderately positive correlation with RFI (Nkrumah et al., 2007b; Kelly et al., 2010a; Kelly et al., 2010b). However, Basarab et al. (2007) found that bunk visit frequency was not correlated with RFI. Bunk visit duration has been found to have a positive correlation with RFI (Nkrumah et al., 2007b; Basarab et al., 2007; Lancaster et al.,

2005). Moderate to strong positive correlations between HD duration and RFI have been reported (Basarab et al., 2007; Nkrumah et al., 2007a; Lancaster et al., 2009a). Likewise, daily meal duration was positively associated with RFI (Basarab et al., 2007; Nkrumah et al., 2007b; Lancaster et al., 2009a). Eating rate was also positively correlated to RFI (Kelly et al., 2010a; Kelly et al., 2010b), but Lancaster et al. (2009a) found that RFI was not correlated with meal eating rate. These results indicate that cattle with divergent RFI phenotypes have distinct feeding behavior patterns associated with consumption of feed. Feeding behavior accounts for inter-animal variation in RFI most likely due to the physical nature of eating. Kayser and Hill (2013) reported feeding behavior accounted for approximately 20% of the inter-animal variation in RFI, which is significantly higher than Richardson and Herd (2004) reported variance of 2%. Feeding behaviors traits identified as being most influential in accounting for inter-animal variation in RFI may have potential as biomarkers for identification of feed-efficient animals.

There is much to be learned about the underlying mechanisms that control feed-efficiency in cattle. Richardson and Herd (2004) reported that 27% of the inter-animal variation in RFI was due to unknown mechanisms, which indicates that other mechanisms yet to be fully identified or quantified (e.g. mitochondria) also contribute to variation in RFI. One of the main barriers to widespread adoption of RFI is the cost of measuring feed intake. Thus, biomarkers or genetic markers would be valuable tools for identification and selection of animals that have improved RFI.

The association between blood metabolites concentrations and RFI has been investigated in numerous beef cattle studies. β-hydroxybutyrate (BHB) was positively

correlated ($P < 0.01$) with RFI in heifers fed grower and finisher diets (Kelly et al., 2010ab). However, Richardson et al. (2004) did not find a significant correlation between RFI and BHB in steers fed a finisher diet. Serum glucose concentrations were not correlated with RFI in heifers fed grower and finisher diets (Kelly et al., 2010a and b), but Richardson et al. (2004) reported a positive correlation ($P < 0.05$) between glucose and RFI. Urea was positively correlated ($P < 0.05$) with RFI in heifers fed grower and finisher diets (Kelly et al., 2010a and b), but Richardson et al. (2004) found no correlation between urea and RFI in steers fed a finisher diet. Blood concentrations of creatinine were found to be greater ($P < 0.01$) in low RFI animals compared to high RFI animals (Lawrence et al., 2011), and Richardson et al. (2004) reported a negative correlation ($P < 0.05$) between creatinine and both RFI and DMI. The metabolite that is most consistently correlated with RFI is BHB, and is a possible biomarker for feed efficiency, while glucose is the metabolite that is the most inconsistently associated with RFI. The variable results reported with metabolites association with RFI could be due to differences in gender and/or diets fed during the studies.

Genetic Selection for RFI

Identifying feed-efficient animals before maturity is critical for effective selection decisions. The associations between post-weaning RFI and efficiency of feed use in older animals has been evaluated in several studies (Arthur et al., 2001b; Nieuwhof et al., 1992; Archer et al., 2002; Hafla et al., 2013). Arthur et al. (2001b) measured RFI in the same bulls at 15 and 19 mo of age, and found strong phenotypic (0.85) and genetic (0.95) correlation coefficients. These results suggest that RFI at 15

mo of age is representative for RFI at 19 mo of age for bulls fed the same diet (Arthur et al., 2001b). Similar studies have measured RFI in heifers shortly after weaning and at maturity with the genetic correlations between them ranging from 0.42 to 0.98 (Nieuwhof et al., 1992; Archer et al., 2002; Hafla et al., 2013). These results suggest that measurements of post weaning RFI is a good predictor of efficiency at maturity (Nieuwhof et al., 1992; Archer et al., 2002; Hafla et al., 2013).

Ideally, selection for RFI will have minimal influences on other economically relevant production traits. Several studies reported slightly negative to moderately positive genetic correlations between ADG and RFI, and genetic correlations between feed intake and RFI range from 0.39 to 0.85 (Robinson and Oddy, 2004; Van der Westhuizen et al., 2005; Nkrumah et al., 2007a; Herd and Bishop, 2000; Schenkel et al., 2004; Lancaster et al., 2009a). These genetic correlations indicate that selection for RFI will reduce feed intake with minimal effects on performance (Archer et al., 1999; Archer et al., 2002). Beef producers will be able to select for cattle that will consume less feed with minimal effects on performance and body size, if they include RFI as a trait in breeding selection.

Metabolomics

Recent studies have indicated the use of metabolomics has considerable potential to help decipher the underlying mechanisms associated with inter-animal variation in RFI. Metabolomics is the study of metabolite profiles for comprehensive charactrization of biological mechanisms at the molecular and cellular level (Sun et al., 2015). Metabolites are low-molecular weight compounds that have been processed by animal's

enzymes and transporter proteins, and can be measured in an animal's biofluid such as blood, milk, and rumen fluid (Kühn et al., 2014). An animal's metabolome is considered to be an intermediary phenotype between the whole-animal phenotype and the transcriptome or proteome (Kuhn et al., 2014).

Nuclear Magnetic Resonance (NMR) spectroscopy has been used for more than 30 years to identify numerous metabolites due it being non-biased, does not destroy samples, and can be easily quantified (Wishart, 2008). Spectral patterns measured by NMR are processed and compared to databases of pure compounds to identify metabolites, with an internal standard (usually 4,4-dimethyl-4-silapentane-1-sulfonic acid) used to quantify the metabolites (Wishart, 2008).

Metabolomics has been used to identify biomarkers associated with various disease states, and with phenotypic traits (Chan et al., 2010; Dutta et al., 2012; Kuhn et al., 2014). Combining metabolomics and genomics offer opportunities to identify important pathways and genes associated with economically relevant traits in livestock (Widmann et al., 2015). The metabolomic approach involves the identification of metabolites and subsequent discovery of metabolic pathways, then combining genomics to identify genes that influence economically relevant traits. These 'omics techniques are useful for understanding the etiology of a disease and to better understand biological mechanisms that underlies the phenotypic variation in economically relevant traits (Gieger et al., 2008).

Metabolomic approaches have been used to study rumen fluid, milk, and blood metabolites to understand diet effects and disease states in lactating and transition dairy

cows (Ametaj et al., 2010; Bertram et al., 2009; Boudonck et al., 2009; Maher et al., 2013; Saleem et al., 2012; Sun et al., 2015; Zhao et al., 2014). Saleem et al. (2012) investigated the association between metabolite profiles and metabolic disorders in dairy cows on high grain diets, and discovered several novel metabolites including putrescine, ethanolamine, and short-chain fatty acids as being related to sub-acute rumen acidosis. Hailemariam et al. (2014) used metabolomics to identify 3 metabolites (carnitine, propionyl carnitine, and lysophosphatidylcholine acyl C14:0) that can be used as a biomarkers to identify periparturient diseases in transition dairy cows up to 4 wk before parturition.

To completely comprehend the underlying mechanisms associated with RFI, the genetic component must be understood. Metabolite profiles combined with genetic data can be used to identify and interpret the genetic and metabolic interactions (Weikard et al., 2010; Widmann et al., 2013; Widmann et al., 2015). Weikard et al. (2010) identified novel metabolic pathways associated with genetic variation in fat tissue deposition in cattle using metabolomics. Widmann et al. (2013) combined metabolite analysis and genetic data to understand gene networks that affect growth in cattle. Widmann et al. (2015) combined metabolomics and genomics to identify key genes and gene networks associated with variation in RFI in cattle. Combining metabolomics and genomics will help give insight of why one animal is more efficient than another.

An accurate statistical analytical method is needed to correctly interpret complex metabolite profiles. Various metabolites are often times highly correlated with each other, otherwise known as multi-collinearity, and normal statistical methods such as

multiple linear regression does not account for multi-collinearity. Consequently, the use of multiple linear regression has the potential to inflate the significance due to unmeasured nonlinear terms (Cortina, 1993). Mason and Perreault (1991) used Monte Carlo simulations to investigate the effects of multi-collinearity in multiple linear regression analysis. As multi-collinearity was increased the inaccuracies in estimated coefficients and the percentage of Type II errors also increased (Mason and Perreault, 1991). Karisa et al. (2014) used multiple regression analysis to examine associations between metabolite profiles and RFI. Carnitine, creatine, and hippurate were all found to be associated with RFI in both the discovery and validation populations with partial r-squares for both populations being 0.25 and 0.35 (Karisa et al., 2014). Since metabolites have high multi-collinearity, the results from Karisa et al. (2014) could have possibly inflated the proportion of variance in RFI attributed to these metabolites. There is a need to use a statistical method that accounts for multicollinearity when analyzing metabolite profiles.

Statistical methods such as Partial Least Squares (PLS) and Principal Component Analysis (PCA) are designed to account for multicollinearity, which are both data reduction methods used to identify variables that have the most influence on group differences (Xia and Wishart, 2011). A supervised pattern recognition method such as PLS uses discriminate analysis to determine differences between groups, but PCA is an unsupervised pattern recognition method (Xia and Wishart, 2011). Both methods are useful in identifying variables that are most associated with the dependent variable of interest.

Programs such as MetaboAnalyst (http://www.metaboanalyst.ca; Xia et al., 2015) combine univariate and multivariate statistical methods to aid in the interpretation of metabolite data. MetaboAnalyst includes PLS and PCA methods and score and loading plots to evaluate group differences. It is critical that prior to PLS analysis that data is first normalized and standardized (Xia et al., 2015; Dong et al., 2011).

For this literature review, PLS is the only multivariate analysis discussed. Partial least squares transforms the data into latent components, and these latent components maximize covariance between the predictor and response variables (Hailemariam et al., 2014). Score plots are used to evaluate group separations (Xia and Wishart, 2011). Score plots have the first component as the X-axis and the second component as the Y-axis, and then plots each animal's score for the components (Xia and Wishart, 2011). The variable of importance in projection (VIP) score is used to identify metabolites that account for the most variation between groups, which takes into account the amount of explained y variance of each component using a weighted sum of squares of the PLS loadings (Xia and Wishart, 2011). Permutation and cross validation methods are used to assess whether or not over-fitting of the data by PLS (Westerhuis et al., 2008; Xia and Wishart, 2011). Partial least squares is a valuable tool to identify metabolites that are important for group separation.

CHAPTER II

ASSOCIATIONS BETWEEN RFI AND FEEDING BEHAVIOR TRAITS IN FEEDLOT CATTLE

Introduction

Improvements in the efficiency of feed utilization by livestock are needed more than ever due to increasing demands for animal-sourced foods by a growing global population in the face of finite availability of natural resources. Residual feed intake (RFI) is alternative feed efficiency trait that quantifies inter-animal variation in DMI independent of differences in body size and productivity (e.g. ADG), and has been shown to be favorably linked with variation in metabolic processes (e.g., heat production, digestion) involved with efficient utilization of feed. Thus, RFI is an ideal trait for identification of predictive biomarkers of efficiency.

The ability to identify the underlying mechanisms that contribute to inter-animal variation in feed-efficiency is necessary for selection of these animals. As the ability to collect larger and more complex data sets become easier, accurate statistical methods are essential for correct interpretation. Multiple linear regression (MLR), has been widely used in beef cattle research to identify variables that are associated with economically relevant traits (Karisa et al., 2014; Kelly et al., 2010a; Basarab et al., 2007; Arthur et al., 2003).

Multiple linear regression does not account for multi-collinearity between the variables, and will inflate the significance due to unmeasured nonlinear terms (Cortina,

1993). Statistical method like partial least squares (PLS) that accounts for multi-collinearity are better suited for data sets with this issue (Cramer III, 1993), which will enable more accurate interpretation of the data, and identification of independent variables (feeding behavior traits) associated with the dependent variable (RFI).

The objective of this study is to compare statistical methods (multiple linear regression vs. partial least squares) to identify biomarkers (feeding behavior traits) that are associated with RFI, and the use of divergent subsets of the population to represent the entire population.

Material and Methods

Animals and Experimental Design

All animal care and use procedures were in accordance with the guidelines for use of Animals in Agricultural Teaching and Research and approved by the Texas A&M University Institutional Animal Care and Use Committee.

Angus-crossbred steers (N = 168) from the Rex Ranch (Ashby, NE) with an initial body weight (**BW**) of 274 ± 26 kg and age of 290 ± 16 d were used in this study. Upon arrival at McGregor Research Center (McGregor, TX), cattle were fitted with passive, half-duplex transponder ear tags (Allflex USA Inc., Dallas, TX) and randomly assigned to 1 of 2 pens (46 x 58 m) with each pen containing 10 electronic feed bunks (GrowSafe System LTD., Airdrie, AB, Canada). Steers were adapted to a high grain diet (Table 2.1) for 28 d prior to start of the study. Following the diet adaptation period, the steers were fed ad libitum and individual animal feed intake and feeding behavior data collected for 70 d.

Table 2.1 Ingredient and chemical composition of the experimental diet.

Item	Amount
Ingredient, as-fed	
Dry rolled corn	73.7
Chopped sorghum-sudan hay	6.0
Cottonseed meal	6.0
Cottonseed hulls	6.0
Molasses	5.0
Mineral Premix[a]	2.5
Urea	0.8
Chemical analysis, DM basis	
DM %	90.2
CP	12.6
NDF	20.3
ME, Mcal/kg	3.0

[a]Mineral Premix contained minimum 15.5% Ca, 2800 ppm Zn, 1200 ppm Mn, 12 ppm Se, 14 ppm Co, 30 ppm I, 45.4 KIU/kg Vit-A, 2.3 KIU/kg Vit-D, 726 IU/kg Vit-E, 1200 ppm Monensin, and 400 ppm Tylan.

The GrowSafe system (DAQ 4000E) consisted of feed bunks with load bars to measure feed disappearance and an antenna located within each feed bunk to record animal presence via detection of EID ear tags. Feed intake was assigned to each individual animal based on continuous recordings of feed disappearance during each bunk visit (**BV**) event. Assigned feed disappearance (**AFD**) rates were computed daily for each bunk to assess data quality. Feed intake and feeding behavior data were omitted for 23 d in pen 1 and 5 d in pen 2 due to system failure (equipment malfunction), or low

AFD (< 95%). Average AFD for the remaining days of study was 98.7%. Feeding behavior traits evaluated in this study were based on frequency and duration of BV events. A BV event commences when the EID ear tag of an animal enters the bunk, and ended when the time between the last 2 consecutive EID recordings exceeded 100 s (parameter in the GrowSafe 4000E software [GrowSafe Systems Ltd.]), the EID ear tag was detected at another feed bunk, or the EID ear tag of another animal was detected at the same feed bunk (Mendes et al., 2011). All BV events were recorded regardless of whether or not feed was consumed. Feeding bouts were defined as BV events during which feed was consumed (Table 2.2). Non-feeding intervals (**NFI**) were defined as the interval lengths between BV events. Head-down (**HD**) duration was computed as the sum of the number of times the EID ear tag for an animal was detected each day multiplied by the scan rate of the GrowSafe system (1.0 s). Using R statistical software (R Core Team, 2014), daily time to bunk (**TTB**) was computed daily as the interval between time of feed-truck delivery within pen and each animal's first BV event following feed delivery.

To compute meal data, a 2-pool Gaussian-Weibull distribution model was fit to log-transformed non-feeding interval data, and the intercept of the 2 distributions used to define meal criterion (Bailey et al., 2012; Yeates et al., 2001). Meal criterion was used to compute individual animal meal data (meal frequency and duration, meal length and size, BV per meal, and head down to meal duration ratio). Meal duration was defined as the sum of the lengths of meal events recorded each day, and meal length and size as the average meal event length (min) and size (kg/meal).

Frequency and duration of bite events were derived from continuous scale-weight measurements of feed disappearance recorded during each FB event. To compute bite events, baseline linear regressions were fit to scale-weight data within FB events, and a bite threshold line established at 1 SD above the baseline regression. The 1-s scale-weight values exceeding the bite threshold line were used to compute bite events. For this study, a static criterion of 8 s was used to differentiate bite events. Non-bite intervals ≤ 8 s were considered to be part of a bite event, and those exceeding 8 s used to define the start of the next bite event. Daily bite frequency was defined as number of bite events per FB, and daily bite duration as the sum durations of all bite events for each FB. Bite duration per feeding bout duration is the ratio of bite duration per feeding bout duration (Table 2.2).

Table 2.2. Definition of feeding behavior traits analyzed in this study.

Trait	Definition
BV frequency, events/d	Number of BV events recorded each day
Bunk visit (BV) duration, min/d	Sum of the lengths of all BV events recorded each day
Feeding bout (FB) frequency, events/d	BV events when feed intake > 0
FB duration, min/d	Sum of the lengths of FB events recorded each day
FB eating rate, g/min	DM intake ÷ FB duration
NFI duration, min/d	Sum of the intervals between BV events each day
Meal criterion	Longest NFI interval between BV events that is still part of a meal
Meal frequency, events/d	Average number of meals each day
Meal duration, min/d	Sum of the lengths of meal events recorded each day
Meal length, min	Average meal length
Meal size, kg	Average DM intake per meal
Meal eating rate, g/min	DM intake ÷ meal duration
Bite frequency per FB, events/min	Average number of bites for all feeding bout events
Bite duration, s	Sum of the lengths of all bite events per feeding bout event
Bite duration per feeding bout duration	Ratio of bite duration per FB duration
HD duration, min/d	Number of EID recordings each day multiplied by the scan rate of the GrowSafe[1] system
Time to bunk, min	Length of interval between feed-truck delivery and the first BV event following feed delivery recorded each day
BV frequency per meal event	Ratio of number of BV events per meal events
HD duration per meal duration	Ratio of HD duration per meal duration

[1]GrowSafe Systems Ltd., Airdrie, AB, Canada.

Steers were weighed at 14-d intervals during the study. Diet samples were collected weekly and composited by weight at the end of the study for subsequent chemical analysis. Moisture analysis was conducted by drying in a forced-air oven for 48 h at 105°C and chemical analysis was conducted by an independent laboratory (Cumberland Valley Analytical Services Inc., Hagerstown, MD). Metabolizable energy concentration of the diet was computed using the Large Ruminant Nutrition System (http://www.nutritionmodels.com/lrns.html), which is based on the Cornell Net Carbohydrate and Protein System (Fox et al., 2004).

Statistical Analysis

Individual animal growth rates were modeled by linear regression of serial BW on day of test using the GLM procedure of SAS (SAS Inst., Cary, NC), and regression coefficients used to compute ADG and mid-test $BW^{0.75}$. Moisture analysis of the diet samples were used to compute average daily DMI from feed intake data. PROC GLM procedures were used to derive expected DMI from linear regression of DMI on ADG and mid-test $BW^{0.75}$, and RFI was calculated as actual minus expected DMI (Koch et al., 1963).

Steers were sorted by RFI and classified into 2 divergent RFI groups based on ± 0.5 SD and ± 1 SD from the mean RFI of 0.00 (SD = 0.82); low (n = 52 and 25) and high (n = 52 and 24) RFI groups to examine the effects of RFI classification on performance, feed efficiency, and feeding behavior traits using PROC GLM of SAS.

To examine associations between RFI and feeding behavior traits, PROC REG procedure was used for stepwise selection in multiple linear regression. Pearson

correlations between performance, feed efficiency, and feeding behavior traits were calculated using PROC CORR of SAS.

MetaboAnalyst software (Xia and Wishart, 2011) was used to conduct a multivariate analysis of the data using Partial Least Squares discriminant analysis (PLS-DA), which is a supervised pattern recognition analysis method. Feeding behavior traits were normalized and standardized using Censcale statement of SAS. Score plots were used to graphically display differences between divergent RFI groups based on ± 0.5 and ± 1 SD. The variable of importance in projection (VIP) scores were used to identify feeding behavior traits that accounted for the most variation between RFI groups (Xia and Wishart, 2011). The VIP scores take into account the amount of variance in the dependent variable (RFI) of each component using a weighted sum of squares of the PLS loadings (Xia and Wishart, 2011). Permutation and cross validation analysis were used in MetaboAnalyst to assess whether data were over-fitted by PLS (Westerhuis et al., 2008; Xia and Wishart, 2011). A receiver-operator characteristics (ROC) curve was used in MetaboAnalyst to determine the predictive ability of the identified biomarkers from PLS (Xia et al., 2015). Area under the curve (AUC) is the metric of ROC curve that was used to determine predictive ability, and Hailemariam et al. (2014) guidelines on AUC scores were used for this trial.

Results and Discussion

Summary statistics are presented in Table 2.3 for this trial. Steers had a mean DMI of 9.90 ± 1.04 kg/d, ADG of 1.76 ± 0.21 kg/d, and F:G ratio of 5.62 ± 0.66 kg/d. The average RFI for the trial was 0.00 ± 0.81 kg/d and ranged from -2.60 to 2.31 kg/d,

with a difference between the most and least efficient steer of 4.91 kg/d. Differences between RFI groups based on ± 0.5 SD are presented in Table 2.4. Classification of RFI did not affect ($P > 0.10$) initial BW or ADG, but low-RFI steers consumed ($P < 0.0001$) 7.4 and 16.9% less feed than medium and high-RFI steers, respectively. Low-RFI steers had a 12% lower ($P < 0.0001$) F:G than medium-RFI steers, and medium-RFI steers had a 8% lower ($P < 0.0001$) F:G than high-RFI steers. Performance, feed intake, and feed efficiency traits of the Angus-crossbred steers were similar to previously published studies using growing steers. Nkrumah et al. (2007b) reported means and SE for ADG, DMI, and RFI of 1.46 ± 0.27, 10.45 ± 1.61, and 0.00 ± 0.88 kg/d, respectively, in crossbred steers fed a finisher ration.

Table 2.3. Summary statistics of performance, feed intake, feed efficiency, and feeding behavior traits for Angus-cross steers.

Item	Mean	SD	Minimum	Maximum
Initial age, d	284	9	265	310
Performance and feed efficiency:				
Initial BW, kg	273.9	26.3	219.1	375.5
ADG, kg/d	1.76	0.21	1.07	2.43
DMI, kg/d	9.90	1.04	7.11	12.8
F:G ratio	5.62	0.66	4.12	7.62
Residual feed intake, kg/d	0.00	0.81	-2.60	2.31
Bunk visit (BV) and feeding bout (FB) traits:				
BV frequency, events/d	48.7	11. 2	22.3	76.9
BV duration, min/d	61.9	12.9	36.1	98.6
FB frequency, events/d	41.6	9.07	20.2	63.4
FB duration, min/d	57.3	12.0	33.8	91.3
FB eating rate, g/min	178	0.03	113	296
Non-feeding interval duration, min/d	1324	31.1	1262	1379
Meal traits:				
Meal criterion, min	13.1	8.58	1.92	66.5
Meal frequency, events/d	6.44	2.32	2.55	17.9
Meal duration, min/d	131.5	28.4	67.6	218.1
Meal length, min/event	25.5	11.7	5.58	87.4
Meal size, kg	1.72	0.59	0.13	0.68
Meal eating rate, g/min	78.4	16.9	45.9	130.6
Intensity traits:				
Bite frequency, events per FB	3.43	0.62	2.08	5.31
Bite duration, s per FB	14.2	4.65	6.00	28.5
Bite duration per FB duration, ratio	0.25	0.06	0.11	0.47
Head down duration (HD), min/d	46.3	14.6	18.9	90.7
Time to bunk, min	94.9	35.8	34.7	254.1
BV per meal, ratio	8.22	2.79	2.93	16.8
HD duration per meal duration, ratio	0.36	0.11	0.12	0.68

Table 2.4. Comparison of performance, feed efficiency, and feeding behavior traits for steers with divergent phenotypes for RFI[1].

Item	Low RFI	Medium RFI	High RFI	SE	P-value
No. of steers	52	64	52		
Performance and feed efficiency:					
Initial BW, kg	274.6	270.1	277.8	5.1	0.27
ADG, kg/d	1.79	1.72	1.77	0.04	0.15
DMI, kg/d	9.05[a]	9.77[b]	10.89[c]	0.14	0.0001
F:G ratio	5.05[a]	5.68[b]	6.11[c]	0.10	0.0001
Residual feed intake, kg/d	-0.92[a]	0.01[b]	0.91[c]	0.07	0.0001
Bunk visit (BV) and feeding bout (FB) traits:					
BV frequency, events/d	46.2	49.7	50.1	2.2	0.14
BV duration, min/d	54.4[a]	60.2[b]	71.8[c]	2.1	0.0001
FB frequency, events/d	39.5	42.3	42.7	1.3	0.15
FB duration, min/d	50.2[a]	55.5[b]	66.6[c]	1.4	0.0001
FB eating rate, kg/min	186[a]	181[a]	167[b]	0.01	0.007
Non-feeding interval duration, min/d	1334.7[a]	1323.6[b]	1314.2[b]	5.9	0.003
Meal traits:					
Meal criterion, min	13.2	13.3	12.8	1.7	0.95
Meal frequency, events/d	6.44	6.42	6.47	0.45	0.99
Meal duration, min/d	123.6[a]	131.5[ab]	139.3[b]	5.5	0.01
Meal length, min/event	23.6	25.9	26.7	2.3	0.36
Meal size, kg	1.55[a]	1.73[ab]	1.88[b]	0.08	0.01
Meal eating rate, g/min	76.4	77.4	81.5	2.3	0.27
Intensity traits:					
Bite frequency, events per FB	3.62[a]	3.51[a]	3.16[b]	0.08	0.0004
Bite duration, s per FB	12.9[a]	14.0[a]	15.8[b]	0.63	0.005
Bite duration per FB duration, ratio	0.26	0.25	0.24	0.01	0.16
Head down duration (HD), min/d	37.9[a]	44.2[b]	57.4[c]	2.4	0.0001
Time to bunk, min	95.6	99.7	88.5	7.0	0.24
BV per meal, ratio	7.66	8.44	8.50	0.54	0.23
HD duration per meal duration, ratio	0.32[a]	0.35[a]	0.42[b]	0.02	0.0001

[a, b, c]Means in the same row with unlike superscripts are different at $P < 0.05$.

[1]Low, medium, and high RFI phenotypes were based on ± 0.50 SD from mean RFI of 0.00 (SD = 0.82).

Residual feed intake was not correlated with initial BW or ADG, but was correlated ($P < 0.0001$) with F:G (0.67) and DMI (0.78) (Table 2.5). Other studies have

reported similar positive correlations between RFI, and F:G or DMI (Lancaster et al., 2009b; Hafla et al., 2013; Nkrumah et al., 2007a). Dry matter intake was positively correlated ($P < 0.0001$) to initial BW (0.49) and ADG (0.48), which is consistent with previous results from Nkrumah et al. (2007a) and Lancaster et al. (2009b). Feed to gain was negatively correlated ($P < 0.0001$) with ADG (-0.62).

Table 2.5. Pearson correlations among performance and feed efficiency for Angus-cross steers.

Trait[1]	ADG	DM intake	F:G	Residual feed intake
IBW	0.23*	0.49*	0.19*	0.00
ADG		0.48*	-0.62*	0.00
DM intake			0.37*	0.78*
F:G				0.67*

*Correlations differ from zero at $P < 0.05$.

Differences between RFI groups based on ± 0.5 SD for feeding behavior traits are presented in Table 2.4. Low-RFI steers spent 24% less time ($P < 0.0001$) at the bunk (BV duration) than high-RFI steers, and consequently low-RFI steers had greater ($P < 0.003$) duration of non-feeding intervals than high-RFI steers. Previous studies have reported that bunk visit duration was shorter for low- compared to high-RFI steers (Nkrumah et al., 2007b; Lancaster et al., 2005; Basarab et al., 2007). The duration of FB events, which were the BV events during which feed is consumed, was also 25% less ($P < 0.0001$) in low-RFI steers compared to high-RFI steers. The frequencies of FB, BV,

and NFI events, as well as TTB were not affected ($P > 0.10$) by RFI classification in this trial.

Meal criterion and frequency were not affected ($P > 0.10$) by RFI classification. Meal duration was 11% lower ($P < 0.01$) for low- than high-RFI steers, which likely reflects the fact that BV duration was lower in low-RFI steers. Average meal size was 18% less in low-RFI compared to high-RFI steers, which can be attributed to lower DMI of low-RFI steers, as average meal length was not affected by RFI classification. In addition, the rate of DMI consumption during meal events was not affected by RFI classification.

Steers with low RFI had 34% lower ($P < 0.0001$) HD duration than high-RFI steers, which is consistent with previous results from Lancaster et al. (2009b) and Nkrumah et al. (2007b). While both HD duration and duration of meal events were lower in low-RFI steers, the magnitude of the reduction in HD duration was greater such that the ratio of HD duration to meal duration was 24% lower ($P < 0.0001$) in low- compared to high-RFI steers. For this study, we also examined an additional characteristic of feeding behavior patterns associated with the frequency and duration of bite events, which were derived from scale-weight measurements recorded during FB events (Table 2?). Steers with low-RFI phenotypes had 15% greater ($P < 0.001$) frequency of bite events within FB events than high-RFI steers. However, the duration of bite events was 18% less ($P < 0.005$) in low-RFI steers, which likely reflects the fact that the duration of FB events was also lower in low-RFI steers. Consequently, the ratio of bite duration per FB duration was not affected ($P > 0.10$) by RFI classification. The

higher frequency of bite events suggests that low-RFI steers may have been more aggressive in consuming feed during FB events as indicated by the 11% faster ($P < 0.01$) eating rate during FB events exhibited in low- compared to high-RFI steers. These differences in bite-related traits further illustrate that steers with divergent RFI phenotypes have distinctive feeding behavior patterns associated with consumption of feed.

Performance, feed efficiency, and feeding behavior traits for steers with more diverse RFI (± 1.0 SD) are presented in Table 2.6. As expected, the magnitude of the differences between the low- and high-RFI steers was greater between the ± 1.0 SD RFI groups than between the ± 0.5 SD RFI groups. The low-RFI steers consumed 22% less ($P < 0.0001$) DMI and had 34% lower ($P < 0.0001$) duration of BV events than high-RFI steers. In contrast to the comparison between RFI groups based on ± 0.5 SD, the frequencies of BV and FB events were significantly lower for low- compared to high-RFI steers based on ± 1.0 SD. In general, differences in meal patterns between divergent RFI groups were fairly similar for the ± 0.5 SD and ± 1.0 SD RFI groups.

The magnitude of the differences in bite frequency and duration and HD duration between steers with divergent RFI was greater based on ± 1.0 SD RFI groups than the ± 0.5 SD RFI groups. Although the ratio of bite duration to FB duration was not affected by RFI classification when evaluating the ± 0.5 SD RFI groups, this ratio was 17% higher in low- compared to high-RFI steers when the RFI classification was based on ± 1.0 SD.

Table 2.6 Comparison of performance, feed efficiency, and feeding behavior traits for steers with divergent phenotypes for RFI[1].

Item	Low RFI	High RFI	SE	P-value
No. of steers	25	24		
Performance and feed efficiency:				
Initial BW, kg	275.8	276.9	8.7	0.90
ADG, kg/d	1.80	1.81	0.06	0.95
DMI, kg/d	8.77	11.31	0.21	0.0001
F:G ratio	4.88	6.25	0.15	0.0001
Residual feed intake, kg/d	-1.23	1.27	0.12	0.0001
Bunk visit (BV) and feeding bout (FB) traits:				
BV frequency, events/d	45.0	52.3	3.1	0.02
BV duration, min/d	50.5	76.1	3.2	0.0001
FB frequency, events/d	38.8	44.4	2.5	0.03
FB duration, min/d	46.7	70.4	2.9	0.0001
FB eating rate, g/min	193	165	0.01	0.006
Non-feeding interval duration, min/d	1339.8	1316.5	8.0	0.005
Meal traits:				
Meal criterion, min	14.9	11.4	2.6	0.18
Meal frequency, events/d	6.07	7.17	0.70	0.12
Meal duration, min/d	124.6	140.9	8.1	0.04
Meal length, min/event	25.5	24.8	3.3	0.82
Meal size, kg/d	1.61	1.79	0.18	0.32
Meal eating rate, g/min	73.9	83.3	3.6	0.06
Intensity traits:				
Bite frequency, events per FB	3.75	3.08	0.18	0.0006
Bite duration, s per FB	12.8	17.1	1.4	0.003
Bite duration per FB duration, ratio	0.28	0.24	0.02	0.04
Head down (HD) duration, min/d	33.4	63.7	3.6	0.0001
Time to bunk, min	91.1	90.1	9.2	0.91
BV per meal, ratio	8.05	7.97	0.77	0.92
HD duration per meal duration, ratio	0.28	0.46	0.03	0.0001

[1]Low, medium, and high RFI phenotypes based on ± 1 SD from mean RFI 0.00 (SD = 0.82).

The feeding behavior traits that were most highly correlated with RFI for all steers were HD (0.60), BV duration (0.58), HD:MD ratio (0.41), bite frequency (-0.32),

bite duration (0.26), and meal duration (0.25). As would be expected, the magnitude of the correlations increased as the divergence between RFI groups increased (Table 2.7). Positive correlations between RFI and HD duration, BV duration, or meal duration has been previously reported (Nkrumah et al., 2007b; Basarab et al., 2007; Lancaster et al., 2005 and 2009b; Kayser and Hill, 2013).

Table 2.7 Pearson correlations among performance, feed efficiency, feed behavior traits and temperament traits for Angus-cross steers.

Trait	RFI[1]	RFI[2]	RFI[3]
Bunk visit (BV) traits:			
BV frequency	0.16*	0.20*	0.30*
BV duration	0.58*	0.66*	0.74*
NFI duration	-0.23*	-0.27*	-0.33*
Meal traits:			
Meal criterion	0.00	-0.02	-0.10
Meal frequency	0.00	0.02	0.13
Meal duration	0.25*	0.28*	0.32*
Meal length	0.11	0.13	0.06
Intensity traits:			
Bite frequency	-0.32*	-0.40*	-0.47*
Bite duration	0.26*	0.30*	0.37*
Bite duration per FB duration	-0.17*	-0.25*	-0.33*
Head down (HD) duration	0.60*	0.67*	0.77*
Time to bunk	-0.02	-0.03	0.03
BV per meal	0.13	0.14	0.08
HD duration per meal duration ratio	0.41*	0.49*	0.62*

[1]Correlations with RFI for the full population.
[2]Correlations with RFI for ± 0.5 SD RFI groups.
[3]Correlations with RFI for ± 1.0 SD RFI groups.
*Correlations differ from zero at $P < 0.05$. BV frequency = bunk visit frequency; BV duration = bunk visit duration; NFI duration = non-feeding interval; BV per meal = bunk visit per meal; HD duration per meal duration ratio = head down duration per meal duration ratio.

Multiple linear regression analysis of the full population revealed that HD, bite duration per feeding bout duration ratio, BV frequency, and bite duration were significantly associated with RFI, and accounted for 44.3% of the phenotypic variation in RFI (Table 2.8). When MLR analysis was performed with the ± 0.5 SD RFI groups, there were only 2 feeding behaviors (HD duration and bite duration per feeding bout duration ratio) that were significant, which accounted for 50.1% of the phenotypic variation in RFI. The same 2 feeding behavior traits were significant when analysis was performed with the ± 1 SD RFI groups, and as expected the amount of variance explained was higher at 66.2%.

Table 2.8 Feeding behavior traits associated with RFI using multiple regression.

Population	Feeding behavior traits:	P-value	Partial R-square	Model R-square
Full	HD duration	0.0001	0.356	0.356
	Bite duration: FB duration	0.0003	0.048	0.404
	BV frequency	0.01	0.022	0.426
	Bite duration	0.03	0.017	0.443
± 0.5 SD	HD duration	0.0001	0.454	0.454
	Bite duration: FB duration	0.003	0.047	0.501
± 1 SD	HD duration	0.0001	0.587	0.587
	Bite duration: FB duration	0.003	0.074	0.662

HD duration = head down duration; bite duration: FB duration = bite duration per feeding bout duration; BV frequency = bunk visit frequency.

The variance inflation factor (VIF) is used to assess the degree of multi-collinearity between independent variables, with VIF > 10 providing strong evidence for

multi-collinearity (Ott and Longnecker, 2015; Mason and Perreault, 1991). Mason and Perreault (1991) suggested one method to handle multi-collinearity was to remove variables with VIF > 10. As shown in Table 2.9, only 5 out of 16 feeding behavior traits had VIF values less than 10. Moreover, the VIF values were considerably higher as the divergence of population used in the analysis increased.

Table 2.9 Variance inflation factors (VIF) for feeding behavior traits for contemporary groups.

Trait	VIF[1]	VIF[2]	VIF[3]
Bunk visit (BV) traits:			
BV frequency	16.8	28.7	40.5
BV duration	29.6	36.1	55.7
NFI duration	3.74	4.04	7.16
Meal traits:			
Meal criterion	18.3	20.1	33.5
Meal frequency	10.4	12.4	14.9
Meal duration	25.9	42.3	43.8
Meal length	49.8	112.7	173.9
Intensity traits:			
Bite Frequency	8.61	9.10	10.3
Bite duration	46.7	40.1	51.8
Bite duration per FB duration	31.6	28.3	31.1
Head down	38.4	44.1	68.8
Time to bunk	1.36	1.54	1.99
BV per meal	27.6	62.7	85.7
HD duration per meal duration ratio	33.9	37.1	40.8

[1]Correlations with RFI for the full population.
[2]Correlations with RFI for ± 0.5 SD RFI groups.
[3]Correlations with RFI for ± 1.0 SD RFI groups.
NFI duration = non-feeding interval; BV per meal = bunk visit per meal; HD duration per meal duration ratio = head down duration per meal duration ratio.

Partial least squares analysis of the full population revealed some degree of separation between the low and high-RFI groups based on score plots (Figure 2.1). Of the 16 feeding behavior traits measured in this study, 4 had a variable of importance in projection (VIP) score > 1, which included HD (2.10), BV duration (2.08), HD to meal duration ratio (1.56), and bite frequency (1.31; Figure 2.1 and Table 2.10). These 4 feeding behavior traits accounted for 39.1% (components 1 and 2) of the inter-animal variance in RFI.. For the ± 0.5 SD group the PLS score plot revealed very similar pattern of separation between the low and high-RFI groups as was seen with all the steers (Figure 2.2). Of the 16 feeding behavior traits measured in this study, 5 had a VIP score > 1, which included BV duration (2.10), HD (2.08), HD to meal duration ratio (1.45), bite frequency (1.13), and NFI duration (1.03) (Figure 2.2 and Table 2.10). These 5 feeding behavior traits accounted for 43.9% (components 1 and 2) of the inter-animal variance in RFI. The AUC for the receiver-operator characteristic curve based on the PLS model that used these 4 feeding behavior traits with highest VIP scores was 0.864; Figure 2.4), which based on Hailemariam et al. (2014) AUC guide has good predictive ability. Steers with low-RFI had 34% lower HD duration, 24% lower BV duration, 24% lower HD:MD ratio, and 15% more bites per minute (bite frequency) than high RFI steers (Table 2.4).

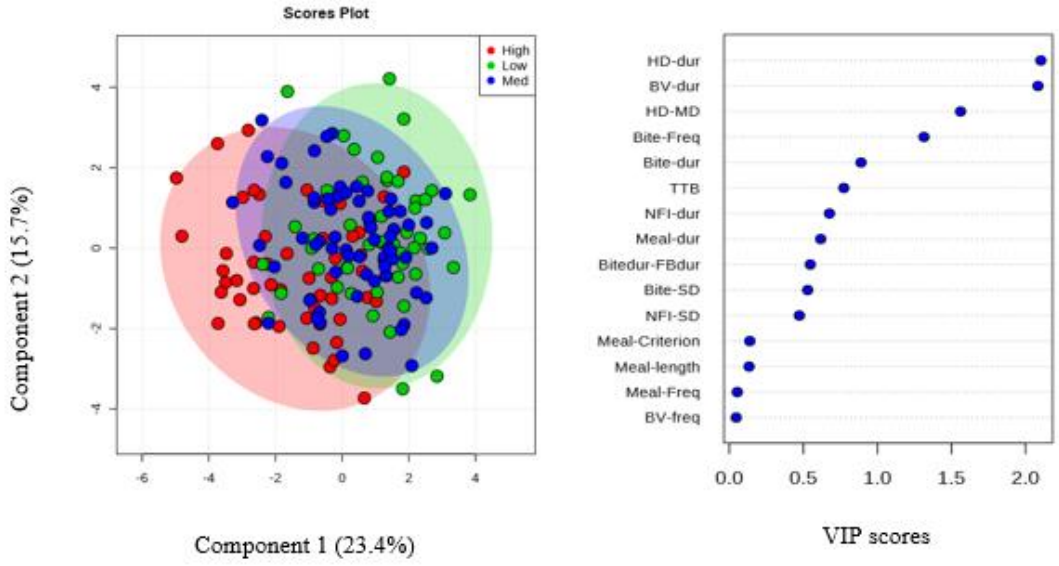

Figure 2.1 Score plots between first and second components on the left panel and variable of importance in projection scores (PLS) on the right panel for feeding behavior traits for all steers (N = 168). Variance in RFI (shown in brackets) explained based on 2-component PLS analysis. HD-dur = head down duration; BV dur = bunk visit duration; HD-MD = head down duration per meal duration ratio; Bite-Freq = bite frequency; Bite-dur = bite duration; TTB = time to bunk; NFI-dur = non-feeding interval duration; Meal-dur = meal duration; Bitedur-FBdur = bite duration per feeding bout duration ratio; Meal-Freq = meal frequency; BV-freq = bunk visit frequency.

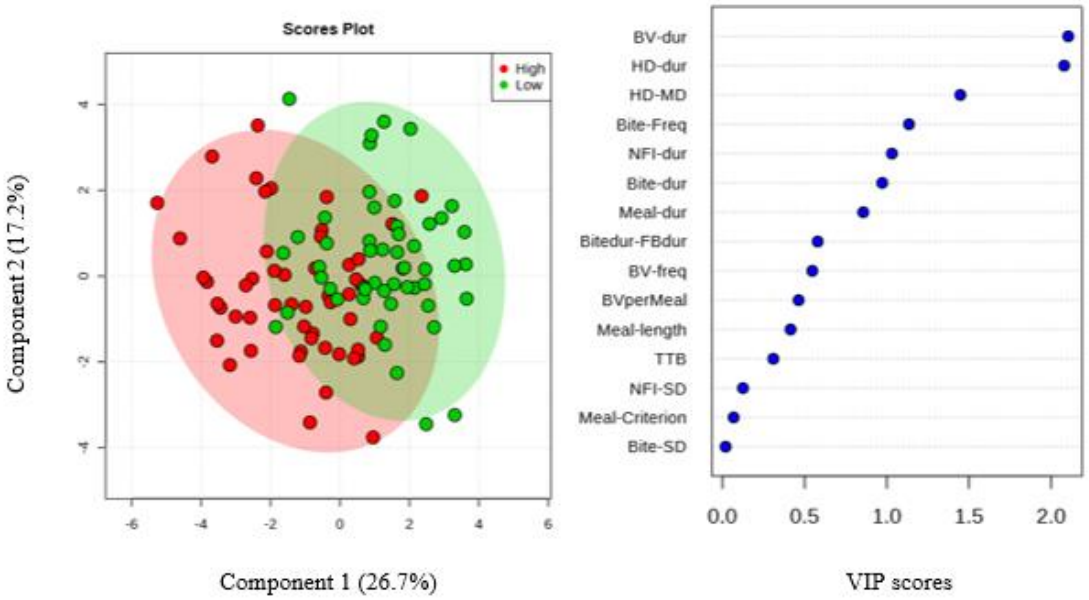

Figure 2.2 Score plots between first and second components on the left panel and variable of importance in projection scores (PLS) on the right panel for feeding behavior traits for ± 0.5 SD group (N = 104). Variance in RFI (shown in brackets) explained based on 2-component PLS analysis. HD-dur = head down duration; BV dur = bunk visit duration; HD-MD = head down duration per meal duration ratio; Bite-Freq = bite frequency; Bite-dur = bite duration; TTB = time to bunk; NFI-dur = non-feeding interval duration; Meal-dur = meal duration; Bitedur-FBdur = bite duration per feeding bout duration ratio; Meal-Freq = meal frequency; BV-freq = bunk visit frequency.

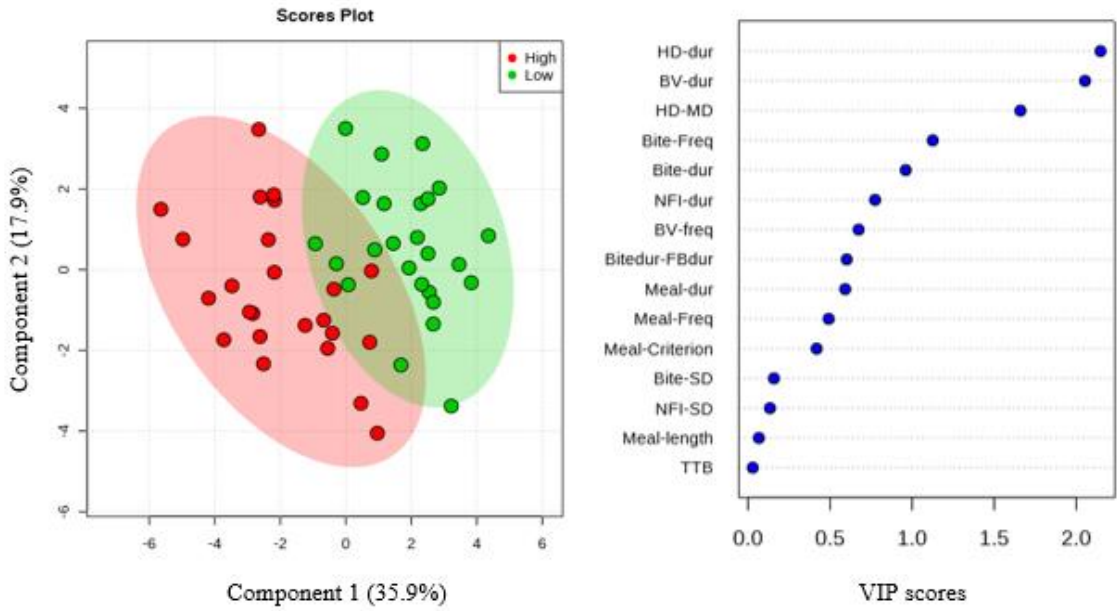

Figure 2.3 Score plots between first and second components on the left panel and variable of importance in projection scores (PLS) on the right panel for feeding behavior traits for ± 1 SD group (N = 49). Variance in RFI (shown in brackets) explained based on 2-component PLS analysis. HD-dur = head down duration; BV dur = bunk visit duration; HD-MD = head down duration per meal duration ratio; Bite-Freq = bite frequency; Bite-dur = bite duration; TTB = time to bunk; NFI-dur = non-feeding interval duration; Meal-dur = meal duration; Bitedur-FBdur = bite duration per feeding bout duration ratio; Meal-Freq = meal frequency; BV-freq = bunk visit frequency.

Table 2.10 Feeding behavior traits associated with RFI using partial least squares.

Population	Feeding behavior traits:	VIP score	Accounted variance	AUC
Full	HD duration	2.10	39.1	
	BV duration	2.08		
	HD per meal duration	1.56		
	Bite frequency	1.31		
± 0.5 SD	HD duration	2.10	43.9	0.864
	BV duration	2.08		
	HD per meal duration	1.45		
	Bite frequency	1.13		
	NFI duration	1.03		
± 1 SD	HD duration	2.15	53.8	0.958
	BV duration	2.05		
	HD per meal duration	1.66		
	Bite frequency	1.13		

HD duration = head down duration; BV duration = bunk visit duration; HD per meal duration = head down duration per meal duration; NFI duration = non-feeding interval duration.

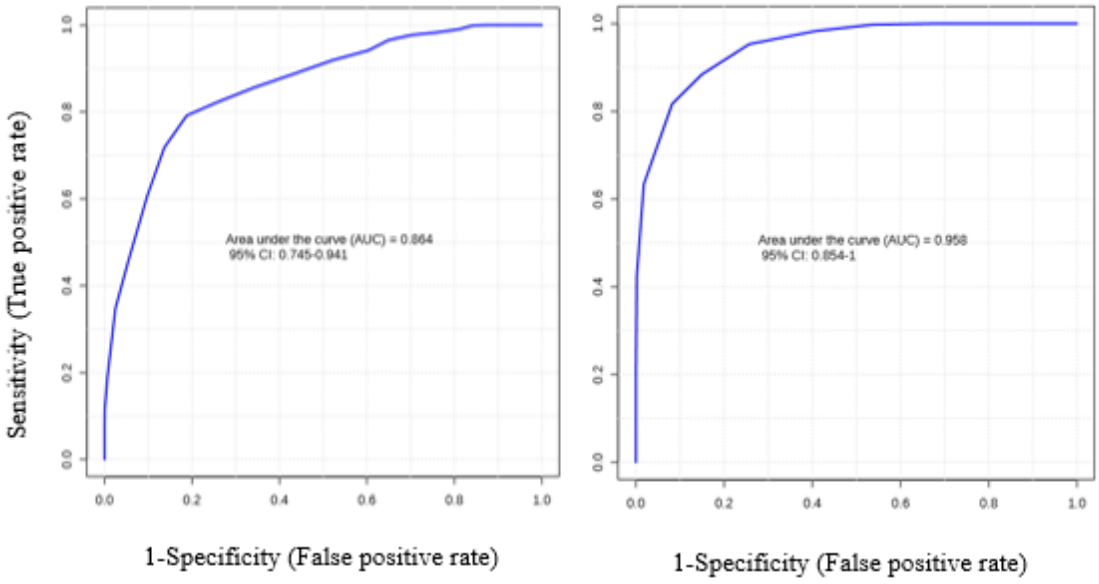

Figure 2.4 Receiver-operator characteristics curve of the top 4 feeding behavior traits identified by VIP scores for ± 0.5 SD group (N = 104) on the left panel and ± 1 SD group (N = 49) on the right panel.

Table 2.11 Feeding behavior traits excluding bite-related traits associated with RFI using partial least squares.

Population	Feeding behavior traits:	VIP score	Accounted variance	AUC
Full	HD duration	2.03	31.7	
	BV duration	2.01		
	HD per meal duration	1.51		
± 0.5 SD	BV duration	1.98	37.3	0.859
	HD duration	1.96		
	HD per meal duration	1.37		
± 1 SD	HD duration	2.03	62.8	0.942
	BV duration	1.94		
	HD per meal duration	1.57		

HD duration = head down duration; BV duration = bunk visit duration; HD per meal duration = head down duration per meal duration; NFI duration = non-feeding interval duration.

Partial least squares analysis was also performed after excluding the bite-related traits to further assess their contributions in accounting for inter-animal variance in RFI. A PLS score plot for all steers revealed separation between the low and high-RFI groups with the medium-RFI group overlaying both (data not shown). Of the 12 feeding behavior traits considered in the analysis, 3 had VIP scores > 1, which included HD (2.03), BV duration (2.01), and HD to meal duration ratio (1.51; Table 2.11), and accounted for 31.7% of inter-animal variance in RFI. For the ± 0.5 SD groups, PLS score plot displayed a similar pattern of separation between the low- and high-RFI steers. Of the 12 feeding behavior traits measured in this study, 3 had VIP scores > 1, which included BV duration (1.99), HD (1.96), and HD to meal duration ratio (1.37; Table 2.11), and accounted for 37.3% of inter-animal variance in RFI. These results suggest bite-related traits should be included in PLS analysis to help account for more inter-animal variance in RFI.

As expected, the ± 1 SD group revealed similar separation between the low and high-RFI groups (Figure 2.3). Of the 16 feeding behavior traits measured in this study, 4 had VIP scores > 1, which included HD (2.15), BV duration (2.05), HD to meal duration ratio (1.66), and bite frequency (1.13) (Figure 2.3). These 4 feeding behavior traits accounted (components 1 and 2) for 53.8% of inter-animal variance in RFI. Steers with low-RFI had 48% lower HD duration, 34% lower BV duration, 39% lower HD:MD ratio, and 22% more bites per minute (bite frequency) than high RFI steers (Table 2.6).

The PLS score plot for ± 1 SD group with all feeding behaviors with the exception of bite-related traits displayed separation between the low and high-RFI groups. Of the 12 feeding behavior traits measured in this study, 3 had VIP scores > 1, which included HD (2.03), BV duration (1.94), and HD to meal duration ratio (1.57; Table 2.11), and accounted for 62.8% of inter-animal variance in RFI. The ± 1 SD group PLS analysis without bite-related traits accounts for more inter-animal variance in RFI, which is the opposite of the full population and the ± 0.5 SD group PLS analysis. Further research is needed to fully understand this change.

Partial least squares analysis for the different RFI groups all had similar patterns of separation between the low- and high-RFI steers. As expected, the ± 1 SD RFI groups had the clearest separation between the low- and high-RFI groups and accounted for the most inter-animal variation in RFI. The feeding behavior traits identified as contributing the most towards the inter-animal variation in RFI were the same for all the RFI groups with the exception of NFI duration also being identified in the ± 0.5 SD RFI groups. The ROC curve using these 4 feeding behavior traits have an AUC of 0.864, which suggests

good predictability as biomarkers. These results support the theory more divergent groups are able to be used for identification of biomarkers for the entire population, but will increase the amount of accounted variance in RFI as the group becomes more divergent. There is one study to the author's knowledge that has used PLS analysis to study feed efficiency and feeding behavior traits in beef cattle research (Montanholi et al., 2010).

The same feeding behavior traits identified by PLS were also the ones highly correlated with RFI, but MLR did not recognize those same feeding behavior traits. The amount of accounted variance were similar between PLS and MLR for each contemporary group, but due to feeding behavior traits that have very high VIF, this suggests that MLR has inflated r-squared values with possible artificial significance (Cortina, 1993; Ott and Longnecker, 2015). Based on these results, PLS is a better method for identifying associations between feeding behaviors and RFI.

Conclusion

Results from this study demonstrated that steers with divergent RFI phenotypes have distinctive feeding behavior patterns associated with consumption of feed. Bite-related traits were also shown to help account for more inter-animal variance in RFI. The results also demonstrated the use of multiple linear regression to quantify inter-animal variance in RFI associated with feeding behavior patterns has limitations due the high degree of multi-collinearity between independent variables. This was illustrated by the fact that 11 out of the 16 feeding behavior traits had a high VIF scores. Partial least squares is resistant to multi-collinearity, and will provide more accurate interpretation

than multiple regression. The results also showed more divergent RFI groups could be used to represent all the steers, and bite-related traits are valuable in identifying differences in feeding behavior patterns between divergent RFI groups. Based on partial least squares HD, BV duration, HD to meal duration ratio, and bite frequency are possible predictive biomarkers for RFI. These need to be validated with further trials, and could be used for identification of efficient cattle.

CHAPTER III

ASSOCIATIONS BETWEEN RFI AND BLOOD METABOLITE PROFILES IN FEEDLOT CATTLE

Introduction

The largest variable expense associated with the production of beef is the cost of feed inputs, thus, strategies that seek to improve efficiency of feed utilization is key to improving profitability of beef cattle systems (Arthur et al., 2004). Residual feed intake (RFI) is a feed efficiency trait that quantifies inter-animal variation in DMI independent of differences in BW and ADG, and has been shown to be favorably linked with variation in metabolic processes (e.g., heat production, digestion) involved with efficient utilization of feed. Thus, RFI is an ideal trait for identification of predictive biomarkers for feed efficiency. The cost of measuring individual-animal feed intake remains the largest barrier to widespread adoption of technology to improve feed efficiency in beef cattle. Thus, there is a need to identify genomic markers or phenotypic biomarkers for identification of feed-efficient cattle to reduce the cost associated with selection for feed efficiency.

The recent advances in metabolomics have provided opportunities for discovery of biological mechanisms responsible for inter-animal variation in economically relevant traits like RFI. Technology such as nuclear magnetic resonance (NMR) and gas chromatography mass spectrometry (GC-MS) are becoming more widely used in research to identify and quantify metabolite concentrations. The metabolome, or the

entire set of metabolites present within an animal's biological fluid (blood, urine) or tissue is comprised of numerous low-molecular-weight molecules (metabolites) processed by enzymes and transporter proteins, and is considered to be an intermediary phenotype between the whole-animal phenotype and the transcriptome or proteome (Kuhn et al., 2012).

Weikard et al. (2010) used a metabolomics approach to identify novel metabolic pathways associated with genetic variation in fat tissue deposition in cattle. Metabolomics has also been used in cattle to identify biomarkers predictive of feed efficiency in beef steers (Karisa et al., 2014), and metabolic diseases in transition dairy cows (Hailemariam et al., 2014). Combining metabolomics with genomics, Widmann et al. (2015) identified key genes and gene networks associated with variation in residual feed intake (efficiency) in cattle.

Few studies have examined the association between serum metabolite profiles and RFI, and to our knowledge no one has used partial least squares to account for multi-collinearity to examine associations between serum metabolite profiles and RFI. The objective of this study was to use partial least squares analysis to identify metabolites contributing the most to inter-animal variation in RFI, and to identify metabolic pathways associated with RFI.

Material and Methods

Animals and Experimental Design

All animal care and use procedures were in accordance with the guidelines for the use of Animals in Agricultural Teaching and Research and approved by the Texas A&M University Institutional Animal Care and Use Committee.

A detailed description of the animals and management can be found in chapter 2 of Miller et al. (2016). In brief, Angus-crossbred steers (N = 168) from the Rex Ranch (Ashby, NE) with an initial BW of 274 ± 26 kg and age of 290 ± 16 d were used in this study. The feed intake and feeding behavior traits were measured daily using the GrowSafe system (DAQ 4000E) for 70 d. Individual animal growth rates were modeled by linear regression of BW using the general linear model of SAS (SAS Inst., Cary, NC), and regression coefficients were used to compute ADG, initial and mid-test $BW^{.75}$. Moisture analysis of the diet samples was used to compute average daily dry matter intake (**DMI**) from feed intake data. RFI was calculated by using PROC GLM (SAS Institute Inc., Cary, NC) as the difference between actual and expected DM intake from linear regression of ADG, DM intake, and mid-test $BW^{0.75}$ (Koch et al., 1963).

Blood Samples

Blood samples were collected on days 0 and 70 of the trial from steers with RFI that were ± 1 SD from the mean RFI (0.00 ± 0.82 kg/d), which included 25 low and 24 high-RFI steers. Samples were collected via jugular vein in evacuated blood tubes (7 mL), and stored on ice until centrifuged at 3000 g for 20 min. Serum samples were harvested and stored at -20ºC for subsequent metabolite analysis. Serum metabolite

concentrations were analyzed using ^{1}H-NMR spectroscopy (Bruker 600-MHz AVANCE III solution NMR spectrometer) at Montana State University (Bozeman, Montana). Each sample was independently fitted for NMR spectral patterns using Chenomx small-molecule library for 600 MHz magnetic field strength NMR (Chenomx NMR software Version 8.1), with 4,4-dimethyl-4-silapentane-1-sulfonic acid used as an internal standard to quantify identified metabolites. Forty-four metabolites were identified based on ^{1}H-NMR spectroscopy for this study.

Statistical Analysis

MetaboAnalyst software (Xia and Wishart, 2011) was used to conduct a multivariate analysis of the data using Partial Least Squares (PLS) procedures, which is a supervised pattern recognition analysis method. Prior to PLS analysis, the metabolite data were first normalized by median (metabolite data divided by the median of all the metabolites for that animal) and then standardized using auto-scaling (mean centered and divided by SD) so that variances of all metabolites equal 1 (Craig et al., 2006; Van den Berg et al., 2006; Ametaj et al., 2010; Hendriks et al., 2007). Separation between the low and high RFI animals was determined using score plots. The variable of importance in projection (VIP) score were used to determine metabolites that accounted for the most variation between RFI groups (Xia and Wishart, 2011). The VIP score takes into account the amount of explained y variance of each component using a weighted sum of squares of the PLS loadings (Xia and Wishart, 2011). Permutation and cross validation analysis were used in MetaboAnalyst to assess over-fitting of the data by PLS (Westerhuis et al., 2008; Xia and Wishart, 2011). A receiver-operator characteristics (ROC) curve was used

in MetaboAnalyst to determine the predictive ability of the identified biomarkers from PLS (Xia et al., 2015). Area under the curve (AUC) is the metric of ROC curve that was used to determine predictive ability, and Hailemariam et al. (2014) guidelines on AUC scores were used for this trial. The Pathway analysis module of MetaboAnalyst was utilized to identify metabolite-associated pathways for RFI. The pathways associated with metabolite profiles for the high and low RFI groups were identified by a P-value $\leq$ 0.05 and pathway impact value $\geq$ 0.30 (Xia and Wishart, 2011).

The effects of RFI classification on performance, feed efficiency, and serum metabolite concentrations were evaluated using PROC GLM of SAS. Pearson correlations between performance, feed efficiency, and serum metabolite concentrations were calculated using PROC CORR of SAS.

Results and Discussion

Summary statistics are presented in Table 3.1 for this trial. Steers had a mean DMI of 9.90 ± 1.04 kg/d, ADG of 1.76 ± 0.21 kg/d, and F:G ratio of 5.62 ± 0.66 kg/d. The average RFI for the trial was 0.00 ± 0.81 kg/d and ranged from -2.60 to 2.31 kg/d, with a difference between the most and least efficient steer of 4.91 kg/d. Performance, feed intake, and feed efficiency traits of the Angus-crossbred steers were similar to previously published studies using growing steers. Nkrumah et al. (2007b) reported means and SE for ADG, DMI, and RFI of 1.46 ± 0.27, 10.45 ± 1.61, and 0.00 ± 0.88 kg/d, respectively, in crossbred steers fed a finisher ration. Basarab et al. (2003) also reported means and SE for DMI and ADG of 8.52 ± 1.02 and 1.52 ± 0.22 kg/d, respectively, in crossbred steers fed a finisher ration.

Table 3.1. Summary statistics of performance, feed intake, and feed efficiency for Angus-cross steers.

Item	Mean	SD	Minimum	Maximum
Initial age, d	284	9	265	310
Performance and feed efficiency:				
Initial BW, kg	273.9	26.3	219.1	375.5
Final BW, kg	397.2	33.2	333.6	505.5
ADG, kg/d	1.76	0.21	1.07	2.43
DMI, kg/d	9.90	1.04	7.11	12.8
F:G ratio	5.62	0.66	4.12	7.62
G:F ratio	0.17	0.02	0.12	0.24
Residual feed intake, kg/d	0	0.81	-2.60	2.31

Table 3.2. Pearson correlations among performance and feed efficiency for Angus-cross steers.

Trait[1]	ADG	DM intake	F:G	Residual feed intake
IBW	0.23*	0.49*	0.19*	0.00
ADG		0.48*	-0.62*	0.00
DM intake			0.37*	0.78*
F:G				0.67*

*Correlations differ from zero at $P < 0.05$.

Residual feed intake was not correlated with initial BW or ADG, but was correlated ($P < 0.0001$) with F:G (0.67) and DMI (0.78) (Table 3.2). Similarly, other studies reported non-significant correlations between RFI and initial BW or ADG, and

positive correlations between RFI and F:G or DMI (Lancaster et al., 2009b; Hafla et al., 2013; Nkrumah et al., 2007a). Dry matter intake was positively correlated ($P < 0.0001$) to initial BW (0.49) and ADG (0.48), which is consistent with previous results from Nkrumah et al. (2007a) and Lancaster et al. (2009b). Feed to gain was negatively correlated ($P < 0.0001$) to ADG (-0.62).

Table 3.3. Comparison of performance and feed efficiency for steers with divergent phenotypes for RFI[1].

Item	Low RFI	High RFI	SE	P-value
No. of steers	25	24		
Performance and feed efficiency:				
Initial BW, kg	275.8	276.9	8.7	0.90
ADG, kg/d	1.80	1.81	0.06	0.95
DMI, kg/d	8.77	11.3	0.21	0.0001
F:G ratio	4.88	6.25	0.15	0.0001
G:F ratio	0.19	0.15	0.00	0.0001
Residual feed intake, kg/d	-1.23	1.27	0.12	0.0001

[1]Low and high-RFI phenotypes based on ± 1 SD from mean RFI of 0.00 (SD = 0.82).

The ± 1 SD group is made up of the top 15% most (n = 25) and least (n = 24) efficient steers selected to have serum metabolite profiles analyzed. Differences between the RFI groups are presented in Table 3.3. As expected, RFI classification did not affect ($P > 0.05$) initial BW or ADG, but low-RFI steers consumed 22% less ($P < 0.0001$) DMI than high-RFI steers. Steers with low RFI phenotypes had 22 % lower ($P < 0.0001$) F:G ratio compared to high-RFI steers.

Table 3.4. List of metabolites identified and quantified by ^{1}H-NMR spectroscopy.

Item	Metabolite
Alcohols	Ethanol
Amino acids	Alanine, Arginine, Asparagine, Aspartate, Betaine, Creatine, Creatine phosphate, Cysteine, Glutamate, Glutamine , Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Sarcosine, Serine, Threonine, Tryptophan, Tyrosine, Valine
Carbohydrates	Glucose, Glycerol
Dialkylamines	Dimethylamine
Imidazoles	Allantoin, Creatinine, Imidazole
Ketones	Acetone
Nucleic acids	Thymine
Organic acids	3-Hydroxybutyrate, 3-Hydroxyisobutyrate, Acetate, Caprylate, Formate, Lactate, Malonate, Pyruvate, Succinate
Sulfones	Dimethyl Sulfone
Urea	Urea

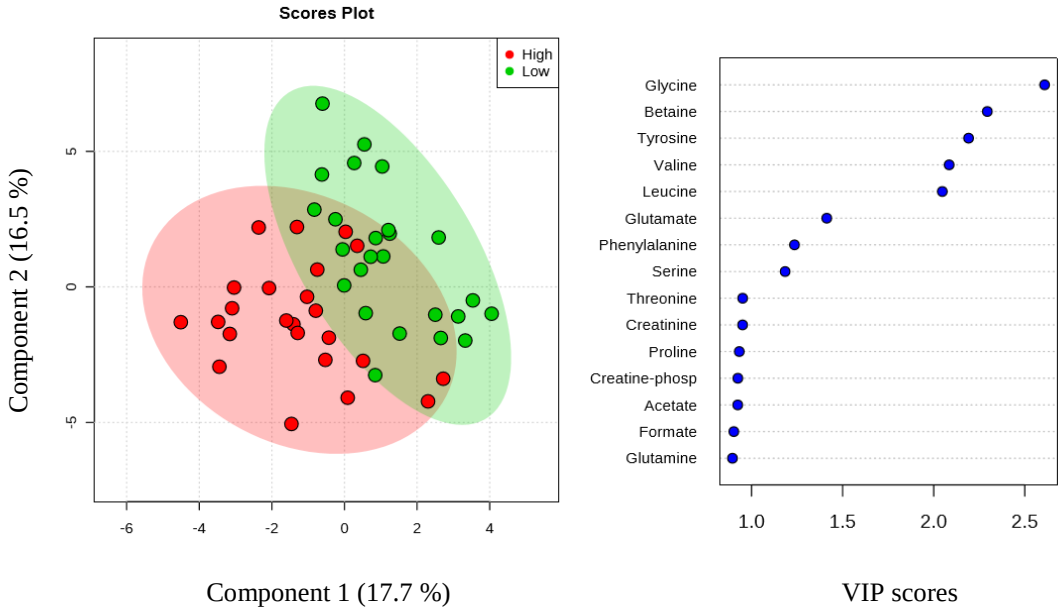

Figure 3.1 Score plots between first and second components on the left panel and variable of importance in projection scores (PLS) on the right panel for serum metabolite profiles for divergent population (n = 49). Variance in RFI (shown in brackets) explained based on 2-component PLS analysis. Creatine-phosp = creatine phosphate.

Metabolites identified and quantified using ^{1}H-NMR spectroscopy are presented in Table 3.4. Partial least squares analysis of day 0 metabolite profiles resulted in over fitting of the data (P = 0.264), and consequently results were not presented (Westerhuis et al., 2008; Xia and Wishart, 2011). The PLS analysis of the day 70 metabolite data were not over-fitted (P = 0.009), and the PLS score plot revealed clear separation between the low- and high-RFI groups (Figure 3.1). The 1st 2 components of PLS analysis accounted for 34.2% of between animal variance in RFI. Of the 44 metabolites detected by ^{1}H-NMR, 5 metabolites had VIP scores > 2, which included glycine (2.6), betaine (2.29), tyrosine (2.19), valine (2.08), and leucine (2.04) (Figure 3.1). Steers with

low-RFI had 54% higher (P < 0.0003) concentrations of glycine, and 14% lower (P < 0.05) concentrations of betaine, 12% lower (P < 0.05) concentration of tyrosine, 9% lower (P < 0.06) concentration of valine, and 14% lower (P < 0.04) concentration of leucine than high-RFI steers (Table 3.5).

Table 3.5. Concentrations of serum metabolites identified as having high VIP scores for steers with divergent phenotypes for RFI[1] as determined by using ^{1}H-NMR spectroscopy.

Item[2]	Low RFI	High RFI	SE	P-value
No. of steers	25	24		
Metabolites:				
Glycine, mM	0.043	0.028	0.004	0.0003
Betaine, mM	0.077	0.09	0.007	0.05
Tyrosine, mM	0.036	0.041	0.002	0.05
Valine, mM	0.159	0.175	0.008	0.06
Leucine, mM	0.104	0.121	0.008	0.04

[1]Top 15% low (n = 25) and high (n = 24) RFI phenotypes.

The ratio of serum metabolite concentrations per DMI was used to determine if the lower concentrations of metabolites in the low-RFI steers were associated with lower feed intakes (Table 3.6). The low-RFI steers have a higher ratio of serum metabolite concentration per DMI for glycine, betaine, tyrosine, valine, and leucine, which provides evidence that lower feed intake by the low-RFI steers was not the reason for lower concentrations of those metabolites (Table 3.5 and 3.6). With only one time point for blood collection, there could be a diurnal fluctuation of metabolites so further research is needed to help discern metabolite variations.

Table 3.6. Ratio of serum metabolites concentrations to DMI for metabolites identified as having high VIP scores for steers with divergent phenotypes for RFI[1] as determined by using ^{1}H-NMR spectroscopy.

Item	Low RFI	High RFI	SE	P-value
Metabolites:				
Glycine, mM/kg DMI	0.49	0.25	0.00	0.0001
Betaine, mM/kg DMI	0.88	0.80	0.00	0.19
Tyrosine, mM/kg DMI	0.42	0.36	0.00	0.06
Valine, mM/kg DMI	1.84	1.55	0.00	0.005
Leucine, mM/ kg DMI	1.20	1.08	0.00	0.14

[1]Top 15% low (n = 25) and high (n = 24) RFI phenotypes.

Karisa et al. (2014) used multiple regression to examine the associations between plasma metabolites and RFI in beef cattle. Carnitine, creatine, and hippurate were all found to be associated with RFI in both the discovery and validation populations with partial r-squares for both populations being 0.25 and 0.35 (Karisa et al., 2014). As shown in Chapter 2, multiple linear regression does not account for multi-collinearity, which can potentially inflate the variance associated with independent variables of interest (Cortina, 1993; Ott and Longnecker, 2015). For this reason, PLS was used in this study as it is more resilient to multi-collinearity (Cramer III, 1993). The metabolites identified as being significantly associated with RFI included glycine, betaine, tyrosine, valine, and leucine. This is interesting to note that all of the significant metabolites associated with RFI were amino acids. Likewise, Karisa et al. (2014) also identified glycine, betaine, and tyrosine as being associated with RFI in beef cattle.

A possible theory for these amino acids being significantly associated with RFI is that steers with low RFI phenotypes may have a lower protein turnover rate in muscle.

Richardson et al. (2004) and McDonagh et al. (2001) speculated that more feed-efficient (low-RFI) cattle have a lower protein turnover rate than feed-inefficient (high-RFI) cattle. Protein turnover is costly process energetically with an average cost of 20% of the basal metabolic rate (Richardson et al., 2004; Rauw et al., 2002). Since the metabolite concentrations are not influenced by intake, then the higher concentrations of betaine, tyrosine, valine, and leucine in the high-RFI steers may have been associated with higher protein turnover rates. Therefore more of the energy the high-RFI steers consume will be used towards protein turnover.

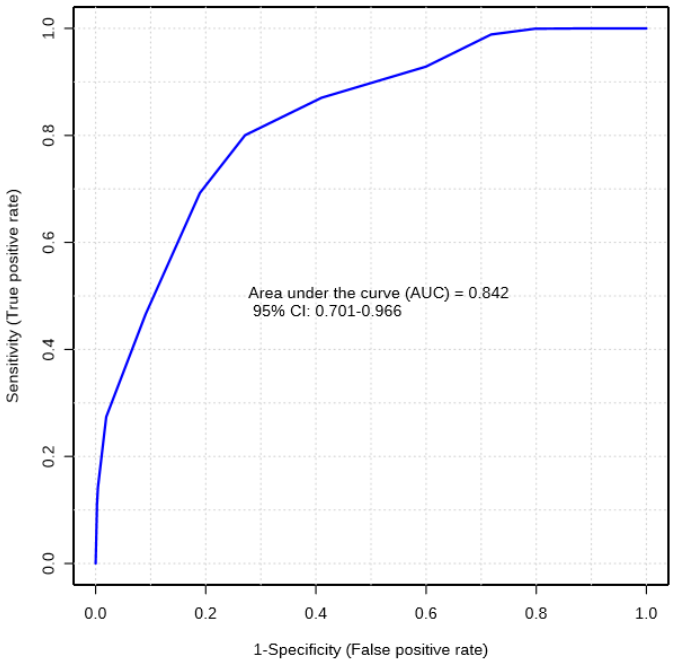

Figure 3.2 Receiver-operator characteristics curve of low (n = 25) and high (n = 24) RFI steers for the top 5 serum metabolites.

The AUC for the receiver-operator characteristic curve based on the PLS model that used the 5 metabolites with highest VIP scores was 0.842 (Figure 3.2). As recommended by Hailemariam et al. (2014), AUC values of 0.9 to 1.0, 0.8 to 0.9, and 0.7 to 0.8 will have excellent, good, and fair predictability. The 5 metabolites have good predictive ability based on the AUC. The pathways associated with the metabolites with high VIP scores included valine, leucine, and isoleucine biosynthesis, phenylalanine, tyrosine, and tryptophan biosynthesis, and, glycine, serine, and threonine metabolism (Table 3.7). The identification of these amino acid biosynthesis pathways as being associated with inter-animal variation in RFI, supports the idea that differences in energetic costs of protein turnover may be contributing to variance in RFI. Further studies will identify the genetic markers such as single nucleotide polymorphisms (SNPs) that are regulating the metabolite-associated pathways.

Table 3.7. Pathways identified to be associated with RFI.

Pathways	Total compounds	P-value	FDR	Impact
Valine, leucine and isoleucine biosynthesis	11	0.0005	0.005	0.66
Phenylalanine, tyrosine, and tryptophan biosynthesis	4	0.004	0.004	0.50
Glycine, serine and threonine metabolism	32	0.00002	0.0002	0.29

Conclusion

Results from this study suggest that glycine, betaine, tyrosine, valine, and leucine may be possible biomarkers for the prediction of RFI. While the AUC for the receiver-operator characteristic curve for prediction of RFI was relatively good at 0.84, the repeatability of these biomarkers could not be evaluated as the PLS model over-fit the metabolite data collected on day 0 of the trial. Moreover, as the associations between metabolite profiles and RFI was only evaluated in 1 trial, the robustness of these biomarkers for the prediction of RFI could not be assessed in this study. If these biomarkers are validated then an accurate, cost effective, and quick assay needs to be created for identification of efficient animals. The metabolite-associated pathways identified were valine, leucine, and isoleucine biosynthesis, phenylalanine, tyrosine, and tryptophan biosynthesis, and, glycine, serine, and threonine metabolism. These results suggest low-RFI cattle have a lower protein turnover rate in muscle compared to high-RFI cattle. Further studies are needed to evaluate the repeatability and robustness across breeds, diets, etc. for these metabolites.

CHAPTER IV

CONCLUSIONS

Results from this study demonstrated that steers with divergent RFI phenotypes have distinctive feeding behavior patterns associated with consumption of feed. Bite-related traits were also shown to help account for more inter-animal variance in RFI. The results also demonstrated the use of multiple linear regression to quantify inter-animal variance in RFI associated with feeding behavior patterns has limitations due the high degree of multi-collinearity between independent variables. This was illustrated by the fact that 11 out of the 16 feeding behavior traits had a high VIF scores. Partial least squares is resistant to multi-collinearity, and will provide more accurate interpretation than multiple regression. The results also showed more divergent RFI groups could be used to represent all the steers, and bite-related traits are valuable in identifying differences in feeding behavior patterns between divergent RFI groups. Based on partial least squares HD, BV duration, HD to meal duration ratio, and bite frequency are possible predictive biomarkers for RFI. These need to be validated with further trials, and could be used for identification of efficient cattle.

Results from this study suggest that glycine, betaine, tyrosine, valine, and leucine may be possible biomarkers for the prediction of RFI. While the AUC for the receiver-operator characteristic curve for prediction of RFI was relatively good at 0.84, the repeatability of these biomarkers could not be evaluated as the PLS model over-fit the metabolite data collected on day 0 of the trial. Moreover, as the associations between metabolite profiles and RFI was only evaluated in 1 trial, the robustness of these

biomarkers for the prediction of RFI could not be assessed in this study. If these biomarkers are validated then an accurate, cost effective, and quick assay needs to be created for identification of efficient animals. The metabolite-associated pathways identified were valine, leucine, and isoleucine biosynthesis, phenylalanine, tyrosine, and tryptophan biosynthesis, and, glycine, serine, and threonine metabolism. These results suggest low-RFI cattle have a lower protein turnover rate in muscle compared to high-RFI cattle. Further studies are needed to evaluate the repeatability and robustness across breeds, diets, etc. for these metabolites.

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