THE ROLE OF POSTRUMINAL AMINO ACID SUPPLY AND MITOCHONDRIAL FUNCTION ON RESIDUAL FEED INTAKE

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by

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

THE ROLE OF POSTRUMINAL AMINO ACID SUPPLY AND MITOCHONDRIAL FUNCTION ON RESIDUAL FEED INTAKE

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A candidate for the degree of Master of Science

And hereby certify that in their opinion it is worthy of acceptance

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DEDICATION

To my parents Tânia Mara M. Masiero and Paulo José Masiero, my sister Ana Paula M. Masiero and brother Matheus M. Masiero,

who I truly love and admire,
supported me my entire life,
and I know are very proud of what I have accomplished.

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THE ROLE OF POSTRUMINAL AMINO ACIDS SUPPLY AND MITOCHONDRIAL FUNCTION ON RESIDUAL FEED INTAKE

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ABSTRACT

Residual feed intake (RFI), used as a metabolic efficiency measurement, is BW and growth rate independent and also is a moderately heritable trait (Arthur et al., 1999). Previous research reported RFI was correlated to feed activity and behavior, ruminal fermentation nutrient digestion, body composition, blood hormones and metabolites as well as mitochondria function (Herd et al., 2004; Lancaster et al., 2008; Bottje and Carstens, 2009; Herd and Arthur, 2009). Since mitochondria are responsible for producing 90% of the energy for the cell, some of the variations in growth performance and phenotypic expression of feed efficiency might be due to differences or inefficiencies in mitochondria function (Kolath et al., 2006b). Three studies were conducted to understand how AA requirement and mitochondria function influence different RFI phenotypes. The first study had three diets with increasing RUP (LOW, MID and HIGH) fed to

continuous culture fermenters to characterize RUP supplementation using forage-based diet. We hypothesized increasing diet RUP concentration would increase diet AA flow from the rumen without influencing microbial fermentation. We observed RUP supplementation in forage-based diets increased RUP flow from the rumen without influencing microbial fermentation. Reduced RUP for HIGH compared to MID may be due to microbial adaptation to greater RUP levels. The second study evaluated the effects of postruminal AA supply during the GP on growth performance, carcass measurements, RFI, and blood metabolites using roughage-based diet. We hypothesized increasing growing phase diet RUP, using roughage-based diets, would impact feed efficiency, growth performance during growing and finishing phase, carcass characteristics, plasma metabolites, hormones concentrations and nutritional requirement, such as RUP, for -RFI (efficient) would increase relative to energy consumed compared to +RFI (inefficient) animals. We reported no interaction between level of bypass AA during growing phase and RFI, leading to main effects examination. Calves consuming diets with postruminal AA supplied above requirements may respond with greater gain rate, however improved growth was not sustained throughout the feeding period. As RFI decreased, steers consumed less feed and were more efficient with no change in BW during all periods. Steers classified as efficient during growing phase were not necessary in the same category during finishing phase. The last study conducted determined if mitochondria complex I, complex III and subunits (from complex I) differed among RFI phenotypes and if mitochondria measurements could

account for additional differences in DMI. We hypothesized calf DMI was influenced by metabolic efficiency (RFI) and subsequently mitochondrial complex I, complex III and subunits (from complex I) could describe additional animal to animal DMI variation. Steers with -RFI consumed less feed and had improved feed efficiency without any change in growth rate. Examination of lymphocyte mitochondria proteins (complex I, complex I subunits and complex III) found a relationship between mitochondria band I, band VI and RFI. Mitochondrial measurements increased coefficient of determination for intake prediction. These results led to the conclusion that mitochondrial function is in part responsible for animals metabolic efficiency differences.

CHAPTER I

LITERATURE REVIEW

INTRODUCTION

Feed cost is one of the most important profit determinants for beef cattle production. Minimizing cost of gain as well as optimizing production and product quality are the main beef producers objectives. Selection for more efficient animals, or nutritional management approaches are possible tools to achieve feed efficiency improvement while increasing profitability.

Feed conversion ratio (FCR, feed:gain) has been historically used to assess animal production efficiency. Although FCR is timely information about performance, and can provide cost of gain estimates, disadvantages are encountered when animals are selected by FCR because it only provides a gross accounting for input and outputs. Because metabolic mid-weight (MMWT) is correlated to ADG, selecting animals for better FCR would also increase frame size and mature weight of the herd (Nkrumah et al., 2004).

Residual feed intake (RFI) was proposed by Koch et al. (1963) and is independent of size and growth rate. Using a mathematical relationship between measured feed intake (FI), and predicted FI, based on a specific contemporary group, animals are categorized as low (< -0.5 standard deviation from the mean), average (± 0.5 standard deviation from the mean) and +RFI (> 0.5 standard

deviation from the mean). The -RFI is most efficient and +RFI is least efficient. Residual feed intake has been proposed as considered the best measure to use in the context of genetic selection because it is a more direct measure of metabolic efficiency, and is a moderately heritable trait (Arthur et al., 1999).

The development of a more inexpensive and rapid method to predict RFI would allow wider array of cattle to be tested. Previous research reported RFI was correlated to feed activity and behavior, ruminal fermentation and nutrient digestion, body composition, blood hormones and metabolites as well as mitochondria function (Herd et al., 2004; Lancaster et al., 2008; Bottje and Carstens, 2009; Herd and Arthur, 2009).

Mitochondria are responsible for producing 90% of the energy for the cell, some of the variations in growth performance and phenotypic expression of feed efficiency might be due to differences or inefficiencies in mitochondria function (Kolath et al., 2006b).

This review will present relevant research from the literature pertaining to RFI; focusing on answering: What are the physiological basis of RFI?; How RFI interacts with nutrient requirement?; and finally What is the role of mitochondria in the phenotypic expression on feed efficiency?

FEED EFFICIENCY MEASUREMENTS

Predominate measurements of feed efficiency in beef cattle production and scientific enquiry are FCR and RFI. Feed conversion ratio is defined as feed

consumed per unit of weight gained or the inverse (gain efficiency). Scientifically, the inverse (i.e. G:F) is mathematically more correct because outputs are divided by inputs. Residual feed intake is a mathematical relationship of the measured animal FI, to its predicted FI. Residual feed intake is proposed to be a more direct measure of metabolic efficiency .

Feed conversion ratio is the most used measurement in the beef industry, and can provide cost of gain estimates. Since feed costs are estimated to be 50 to 70% of the production cost (Arthur and Herd, 2005), the main goal of cattle feeders and nutritionists is to reduce the feed required per unit of gain. However, because FCR only provide a gross accounting for input and outputs, disadvantages are encountered. Feed conversion ratio is growth rate dependent (Bishop et al., 1991), and selection for this trait can contribute to increased mature size and consequently higher maintenance breeding herd cost (Liu et al., 2000). Nkrumah et al. (2004) reported FCR is correlated to MMWT, and large framed animals will have a greater ADG than small framed animals because these parameters are correlated to MMWT. Therefore, selection for FCR will decrease FI dependency on growth, and is not the best selection parameter to improve production efficiency.

Residual feed intake proposed by Koch et al. (1963) is calculated by regressing DMI against MMWT and ADG over a 63 to 84 d test period, depending on the number of days between BW (Archer et al., 1997; Archer and Bergh, 2000; Wang et al., 2006). Negative RFI indicate an efficiency better than average whereas positive RFI indicate an efficiency below average.

Typically, ADG and MMWT explain over 60% of the phenotypic variation in feed intake (Carstens and Tedeschi, 2006). However, other components such as ultrasound back fat have been incorporated in the RFI calculation to force independence from correlations (Basarab et al., 2003; Crews D. H., 2006). Research data has shown RFI is a moderate heritable trait in beef cattle (Arthur et al., 2001; Robinson and Oddy, 2004; Schenkel et al., 2004), and that it is possible to select cattle for lower RFI without compromising growth, thereby improving feed efficiency. Moore et al. (2009) reported selection for -RFI could potentially result in a reduction of 9 to 10% in maintenance costs for the cow herd, 10 to 12% reduction in feed intake, 25 to 30% reduction in methane emissions (Nkrumah et al., 2006; Hegarty et al., 2007) and 15 to 20% reduction in manure production without affecting mature cow size or ADG (Basarab et al., 2002).

RESIDUAL FEED INTAKE PHYSIOLOGICAL BASIS

Since FE is not a measured trait, and must be computed as a function of FI, ADG and time (Koch et al., 1963), the main selection strategies limitation based on RFI is difficulty and expense of measuring individual animal BW and FI over an approximate 90 d period. This makes development of an easier, faster and less expensive method to predict RFI an attractive alternative to direct measurement on large number of animals.

Several studies report RFI better reflects inherent inter-animal variation in biological processes associated with feed efficiency. There are some major processes by which variation in efficiency can arise such as nutrient digestion, body composition, heat increment of fermentation, energy expenditures associated with basal metabolism, protein turnover, physical activity, thermoregulation and feeding behavior (Richardson and Herd, 2004; Carstens and Kerley, 2009; Herd and Arthur, 2009; Fitzsimons et al., 2014b).

Ion pumping (i.e., Na+/K+ ATPase), mitochondrial proton leak, uncoupling proteins (UCP), thyroid hormones, leptin, IGF-I, lipid metabolism enzymes, or sympathetic activity were suggested by Johnson et al. (2003) as possible physiological processes accounting for variation in energy expenditures. Considering basal energy expenditures, mitochondrial proton leak, Na+/K+ ATPase, and protein turnover contribute each about 20% to the total inter-animal variation (Rolfe and Brown, 1997; Ramsey et al., 2000).

Feed Intake Activity and Behavior

Animals with greater DMI expend more energy digesting, in part because of an increase in digestive organ size, and greater feeding heat increment (Fitzsimons et al., 2014b). The ingestion rate and meal duration have been reported as key factors in determining the energy cost of eating in cattle (Adam et al., 1984). Considering -RFI animals have decreased DMI, feeding related behaviors may contribute to variation in RFI.

Based on a limited number of studies, Richardson and Herd (2004) proposed activity and feeding patterns accounted for approximately 12% of the variation in RFI. Robinson and Oddy (2004) reported genetic variation in three feeding behavior traits were moderately heritable, and were positively correlated with RFI, suggesting +RFI was associated with longer time feeding per day, more eating sessions per day, and faster rate of eating (g/min). Steers with -RFI appeared to quickly settle into a regular feed-intake cycle compared to +RFI steers, which had more variable temporal patterns of feed intake early in the RFI test period (Dobos and Herd, 2008).

Animals with -RFI have shorter daily feeding duration compared to +RFI (Schwartzkopf-Genswein et al., 1999; Nkrumah et al., 2006; Nkrumah et al., 2007; Golden et al., 2008; Gomes et al., 2013; Hafla et al., 2013; Fitzsimons et al., 2014b) however, different studies found no effect of RFI on total feeding duration (Basarab et al., 2003; Dobos and Herd, 2008; Kelly et al., 2010a; Basarab et al., 2011).

Schwartzkopf-Genswein et al. (1999) and Kelly et al. (2010a) fed high concentrate diets, whereas Hafla et al. (2013) and Basarab et al. (2011) fed forage-based diet to beef heifers, and Fitzsimons et al. (2014b) used pregnant cows on forage-based diet. The other data reported used steers and fed high concentrate diets. GrowSafe automated feeding system (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) was used in all the studies except for Gomes et al. (2013), Kelly et al. (2010a), and Fitzsimons et al. (2014b). Calan Broadbent system (American Calan Inc., Northwood, NH, USA), Insentec RIC system

(Hokofarm Group, Voorstertocht, Netherlands) and Griffith Elder Mealmaster Multi Feeder System, (Griffith Elder and Co. Ltd., Suffolk, UK) were used respectively.

The literature is equivocal, therefore these findings suggest that diet type and feeding system used to record the FI may have an effect on the observed differences in feeding related behaviors between high and -RFI animals.

Ruminal Fermentation and Total Feed Digestion

Residual feed intake has been correlated to heat production, methane emissions and diet digestibility (Herd and Arthur, 2009). Digestibility and heat increment of fermentation account for 10 and 9% of the RFI variation respectively (Richardson and Herd, 2004). Feed conversion ratio can be improved by gut environment manipulation using different approaches: improving nutrient digestion, reducing gut energy expenditure due to host-microbe interaction and reduced methane synthesis (Kerley and Hill, 2012). Studies have demonstrated different microbiome profile for low and +RFI animals (Guan et al., 2008; McCann et al., 2014). As FI increases, energy amount needed for digestion also increases, which increases maintenance requirement. The greater energy requirement is due to an increased mass of digestive organs. Increasing digestive organ mass may alter metabolism of these tissue and consequently reduce digestive efficiency. Residual feed intake phenotype differences may be affected by site of digestion, fermentation and utilization of digestive products.

Fitzsimons et al. (2014b) observed decreased ruminal pH in +RFI pregnant beef cows fed forage silage compared to -RFI. However, other studies did not find differences in pH (Lawrence et al., 2011; Lawrence et al., 2013). Physiological factors most likely responsible for digestibility differences among animals are enzyme production and ruminal dilution rate (Kerley and Hill, 2012). Considering ruminal fluid pH fluctuations may arise from passage rate, meal patterns, and organic matter degradation (Allen, 1997) these findings suggest different ruminal kinects and aspects of fermentation exist in different RFI phenotypes.

Guan et al. (2008) reported +RFI steers fed high concentrate diets had decreased total VFA production compared to -RFI. Lawrence et al. (2011) found +RFI pregnant beef heifers, 2 to 4 h after feeding grass silage, had decreased ammonia and propionate and increased acetate:propionate compared to -RFI. Lawrence et al. (2013) observed greater acetate:propionate for +RFI pregnant beef cows fed grass silage, and Fitzsimons et al. (2014b) didn't find differences in acetate:propionate between RFI phenotypes but ammonia concentration was greater for +RFI cows.

Differences in ruminal fermentation may also arise from differing microbial population between RFI phenotypes. Previous research has shown differences in microbial profile and abundance between high and -RFI animals which contribute to methane production and concomitant energetic efficiency (Guan et al., 2008; Zhou and Hernandez-Sanabria, 2009; Carberry et al., 2012; McCann et al., 2014).

As feed intake level relative to maintenance increases, the digestion of feed tends to decrease (Allen, 1996). In calves fed high concentrate diets, -RFI animals had 28% decreased methane energy loss and apparent digestibility was 6% greater than +RFI animals (Nkrumah et al., 2006). Richardson et al. (1996) estimated that difference in digestibility between high and -RFI calves fed a pelleted roughage-based diet, accounted for about 14% of the variation in efficiency.

Differences in intake between RFI phenotypes may influence processes such as ruminal kinects, fermentation, microbial profile, total tract digestion and consequently nutrient availability.

Body Composition

Richardson and Herd (2004) demonstrated body composition accounted for 5% the variation in RFI. Because protein tissue has decreased energy density (more water and minerals), less energy per unit of gain is required (1.24 vs. 9.39 kcal/g) compared to fat. However the partial efficiency of metabolizable energy utilization to deposit protein is lower and more variable resulted due to protein turnover, especially in visceral organs such as liver and heart (Carstens and Kerley, 2009). The partial efficiency of nutrient use for lean gain ranges from 45 to 50% whereas for fat gain efficiency is approximately 70 to 95% (Herd and Arthur, 2009). Therefore it is important to understand how differences inherent to gain and body composition can influence metabolic efficiency.

Research (Arthur et al., 2001; Basarab et al., 2003; Nkrumah et al., 2004; Schenkel et al., 2004; Basarab et al., 2007) found positive correlations between RFI and carcass fat traits in beef bulls, heifers and finishing steers. -RFI steers tended to have less intermuscular fat, significantly less fat in the round and loin and a slower accretion rate of empty body fat than medium and +RFI steers (Basarab et al., 2003). In feedlot steers and heifers, Robinson and Oddy (2004) reported strong genetic correlations between RFI and 12th to13th rib, rump fat depths and intramuscular fat percentage. Nkrumah et al. (2004) found -RFI steers had an improved lean meat yield when compared with +RFI steers. Lancaster et al. (2009) found weak correlation between RFI with gain in back fat and loin eye muscle area, and the variation in carcass ultrasound traits accounted for 9% of the variation in RFI in feedlot steers. In contrast to these studies Gomes et al. (2012) and Fitzsimons et al. (2014a) found no effect of RFI on, or correlations with, carcass fat. Kolath et al. (2006b) reported -RFI crossbred steers did not differ from +RFI crossbred steers in loin eye muscle area, fat thickness and yield grade. A meta-analysis of genetic correlations between RFI and carcass conformation done by Berry and Crowley (2013) observed -RFI animals tends to have improved carcass conformation with superior genetic merit for muscularity and less body fat.

Splanchnic tissue [gastro-intestinal tract (GIT), liver, spleen, pancreas, and mesenteric fat depots], associated connective tissue and blood vessels are approximately 15 to 20% of the total body mass in ruminants (Seal and Parker, 2000). Splanchnic tissue consumes 35 to 60% of the total oxygen consumption

(Seal and Reynolds, 1993), and GIT alone consumes approximately 20% (Cant et al., 1996). Eisemann and Nienaber (1990) reported portal drained viscera consumed 25.4% and liver consumed 20.5% of whole-body oxygen uptake in steers. These findings suggest the importance of cellular and molecular differences in organs that have high metabolic activity such as the GIT and liver between animals of differing feed efficiency.

An increase in ingesta volume and nutrients supply can affect liver GIT weights, primarily through changes in cell number, size and consequently protein turnover (Johnson et al., 1990; Ortigues and Doreau, 1995). Basarab et al. (2003) and Fitzsimons et al. (2014a) working with steers and bulls found -RFI cattle had around 9% lower GIT weight than those with +RFI. In contrast, Richardson et al. (2001), Mader et al. (2009) and Gomes et al. (2012) using steers, found no effect of RFI on the weights of any of the visceral organs measured. More recently, Bonilha et al. (2013) reported 12% lighter kidneys for -RFI than +RFI bulls. Therefore, more research is warranted to determine if differences in proportional visceral organ growth accounts for significant variation between RFI phenotypes.

Blood Hormones and Metabolites

Metabolic hormones such as IGF-I (Stick et al., 1998; Wood et al., 2004; Lancaster et al., 2008), insulin (Richardson and Herd, 2004) and leptin (Nkrumah et al., 2005), have been investigated in order to identify potential physiological markers of FE in cattle. Insulin-like growth factor 1 mediates growth stimulating

action and metabolic activities of growth hormone (Delafontaine et al., 2004). In experiments that IGF-I was correlated with RFI the animals were at a young age i.e. shortly after weaning (Johnston, 2008) and were consuming a lower energy roughage-based diet (Brown, 2005). It could be interpreted that the many genes responsible for IGF-I concentration differed between postweaning and finishing stages of development. In contrast to these studies, plasma concentration of IGF-I was reported not different between the RFI phenotypes by Richardson et al. (1996) and Kelly et al. (2011).

Kelly et al. (2011), observed +RFI bulls had 9.7% lower mean plasma insulin concentration compared to -RFI bulls. They also concluded the lower insulin concentration in the +RFI animals could result in lower glucose and AA uptake by cells, especially muscle cells. In contrast, Brown (2005) and Richardson and Herd (2004) reported +RFI steers tended to have higher insulin concentration than -RFI steers at the end of a feedlot test, whereas Kolath et al. (2006b) saw that plasma insulin concentrations and the ratio of glucose to insulin did not differ between the high and -RFI feedlot steers fed.

Leptin concentration is typically associated with increased fatness in cattle (Brandt et al., 2007; Kelly et al., 2011) and positively correlated with RFI, which was in line with the greater fatness of the less efficient steers. Richardson and Herd (2004) reported positive phenotypic correlations between serum leptin concentration and low-RFI steers. Kelly et al. (2011) found that leptin was related to DMI and FE as measured by FCR but not with RFI. In contrast to the findings

of Richardson and Herd (2004) and Kelly et al. (2011), Brown et al. (2004) reported systemic leptin was unrelated to intake, performance, and FE traits.

Kelly et al. (2011), Fitzsimons et al. (2014a), and Lawrence et al. (2013) didn't find an association between RFI and glucose concentrations. These findings conflict with those of Richardson and Herd (2004), Kolath et al. (2006b) and Davis (2009). Richardson and Herd (2004) observed glucose measured at the start of performance test was correlated with RFI. Kolath et al. (2006b) and Davis (2009) observed +RFI steers had greater plasma glucose concentrations than -RFI steers.

Greater systemic urea concentrations associated with ruminants RFI, may be a function of greater protein intake, greater ruminal passage rate (Hegarty et al., 2007), less protein digestibility, greater body protein degradation rate, or deviation in AA supply in part because of variation in microbial efficiency (Kerley and Hill, 2012). Several studies reported urea to be negatively related to protein content in bulls (Robinson et al., 1992), negatively related to lean growth (Cameron, 1992; Clarke et al., 1996) positively related with backfat in sheep (Clarke et al., 1996), and positively related to genetic and phenotypic measures of RFI in steers (Richardson et al., 1996; Richardson and Herd, 2004). However, Kelly et al. (2011), Fitzsimons et al. (2014a) and Lawrence et al. (2013), didn't find an association between blood urea and RFI.

Creatinine, a product of creatine phosphate breakdown in muscle, was reported positively associated with muscle mass in sheep (Cameron, 1992;

Clarke et al., 1996) and negatively associated with fat depth in sheep (Clarke et al., 1996) and negatively associated with steer RFI (Richardson and Herd, 2004).

Given the many biological mechanisms involved in the variation in RFI phenotypes, it is important to understand the relationship between feed intake and production traits in order to effectively utilize RFI for the main goal, of improvement of production efficiency.

RESIDUAL FEED INTAKE AND NUTRITIONAL REQUIREMENTS

Feed efficiency can be altered by selection for superior genetics, as discussed previously, or dietary manipulation. Considering a population of animals with similar performance with lower feed intake (-RFI compared to +RFI) how does diet formulation need to be adjusted to ensure nutrient adequacy?

Ruminants utilize microbial and dietary protein to provide AA required to support maintenance and growth. To maximize ruminal microbial efficiency, diets need to provide adequate RDP, to supply ammonia and peptides to achieve microbial requirements relative to fermentable carbohydrate supply (Fu et al., 2001). Microbial protein supplies 60 to 80% of the AA absorbed in the ruminant intestine (NRC, 2000). Microbial protein AA profile of is relatively constant across various diets (Kerley, 2010). Methionine, lysine, arginine and histidine are reported as most limiting AA to ruminants (Storm and Ørskov, 1983; Coleman, 1996; Greenwood and Titgemeyer, 2000).

Feeding protein resistant to ruminal degradation, or AA treated to prevent ruminal degradation, are strategies developed to increase the supply of limiting AA for cattle. To maximize animal lean tissue gain, AA available for growth showed to be in proportion to the energy available for growth (AA:energy). The microbial AA contribution from the rumen cannot support high levels of gain, making AA supplied from RUP sources necessary to improve gain in growing animals (Kerley and Hill, 2012). Supplementing ruminants with a slowly degradable protein source can increase small intestine AA flow, which would improve growth and efficiency (Titgemeyer and Loëst, 2001).

Data demonstrating the importance of postruminal AA supply on gain and FCR are reported by Zinn and Owens (1993), Mueller et al. (2004), Awawdeh et al. (2006), Williams et al. (2006), Davis (2009) and Hersom et al. (2009).

Zinn and Owens (1993) evaluated RUP supplementation effects in an 84 d growth study involving 140 feedlot steers. They used four different diets with increasing. Meat and bone meal, blood meal and feather meal were used as RUP sources. The basal diet contained 18% alfalfa hay, 10% sudangrass hay, 61% steam-flaked corn, 2.5% yellow grease, 6% molasses, and 2.5% supplement. Quadratic effect of RUP supplementation on ADG and FE, with the greatest response for 2% RUP, which increased ADG and FE by 13.4 and 8.4%, respectively, over that of the basal diet.

Mueller et al. (2004) used growing crossbred Angus steers to evaluate increasing levels of RUP (6, 9, 12, and 15% blood meal). Diets contained 70% whole shelled corn and a pelleted supplement consisting primarily of blood meal

and ground corn. Average daily gain was not different among RUP treatments however, FCR was highest for the 9% and the lowest for the 6 and 12% treatments.

Ruminally cannulated Holstein steers, looking at the effects of excess AA supply (methionine and leucine) were done by Awawdeh et al. (2006). Supplementation with excess AA improved methionine and leucine use for protein deposition by growing cattle.

Similarly, Williams et al. (2006) conducted a study using Angus Simmental crossbred calves. Steers and heifers were fed increasing levels of corn distillers dried grains with solubles (DDGS) to optimize AA:energy. The control diet consisted of 38% corn, 40% soyhulls and 20% wheat midds. There were three treatments with increasing DDGS levels. The AA:energy was predicted to be balanced when diets consisted of 28% DDGS. Intake varied minimally among treatments, whereas ADG was greater for D2, which resulted in improved FCR when AA:energy was optimized. Optimizing AA:EE improved ADG by 14% and FCR by 6% compared to inadequate or excessive AA:EE.

Hersom et al. (2009) evaluated the effects of level and source of supplemental sulfur-containing AA (SAA) on the performance of growing beef calves. Calves were offered hay and supplements containing 0, 2, 4, and 6 g/d of SAA from corn gluten meal, or 2, 4, 6, or 8 g/d of SAA using 88% 2-hydroxy-4-methylthio butanoic acid (Alimet, Novus International Inc. St. Louis, MO). Greater ADG was observed for calves supplemented with CGM or 2 and 6 g/d from Alimet than for control calves.

Davis (2009) conducted an experiment to investigate the relationship among diet, production traits and RFI. Calves were fed a traditional growing steer feedlot diet and three other diets which did not contain roughage and had increasing bypass AA levels. Fish and blood meal were used as AA bypass source. Arginine was predicted as the most limiting AA, and treatments were designated as percent of arginine supplied relative to requirement [Low (80%), Medium (100%) and High (120%)]. As postruminal supply of AA in the diet increased, ADG increased and FCR improved to the limit that arginine requirement was met. Arginine supplied above that required did not improve ADG or FCR. Removing roughage from the diet and optimizing AA:energy improved FCR by 18%. The level of arginine maximizing FCR was different within RFI phenotype. The +RFI calves reached the best FCR when diets provided 100% of predicted arginine requirement, medium RFI calves reached the best FCR value when arginine was supplied at 120% of predicted requirement whereas -RFI did not differ in FCR was across diets.

Nutrition management and selection for -RFI are important tools for efficiency improvement. Combining diet formulation for postruminal amino acid supply relative to diet energy density, and selection for favorable RFI, FCR can be improved by at least 22% (Kerley and Hill, 2012).

MITOCHONDRIAL STRUCTURE AND FUNCTION

Mitochondria generate approximately 90% of the total cellular energy (ATP) via oxidative phosphorylation (Nelson et al., 2008) and play important roles in thermogenesis, apoptosis, and cellular calcium homeostasis (Rossignol et al., 2000). There are two membranes present in mitochondria, outer (phospholipid bilayer) and inner. The inner membrane is highly convoluted, forming folds called cristae that greatly increase its surface area and bears the components of the electron transport chain (ETC), or respiratory chain. Respiratory chain consists of five multisubunit enzyme complexes (I, II, III, IV and V) and two electron carriers, Coenzyme Q (CoQ) and cytochrome c (cyt c). Mitochondria are the only organelle with DNA that codes for mitochondrial protein synthesis. Considering mitochondria as cell energy producer, variations in cattle FE phenotypic expression could be explained, in part, by mitochondria function.

Mitochondria matrix contains the majority of enzymes that make up the pathways for lipids, carbohydrates and AA oxidation. Oxidation occurs in the citric acid cycle and produces, carbon dioxide, NADH and reduced flavin adenine dinucleotide ($FADH_2$) (Nelson et al., 2008).

The electron carriers NADH and $FADH_2$ donate electrons for complex I and II, respectively. Two simultaneous processes occur at complex I. The first is the transfer of a hydride ($:H^-$) ion from NADH and a proton from the matrix to ubiquinone. The second is the transfer of four protons from the matrix to the intermembrane space. Ubiquinone or ubiquinol (reduced form) is dispersed in the

inner mitochondrial membrane where it transfers electrons from complex I and II to complex III. Complex III transfers electrons from ubiquinone to cyt c, along with moving two protons to the intermembrane space. Cytochrome c is a soluble protein that resides in the intermembrane space. It accepts a single electron from complex III and transfers it to complex IV. Complex IV carries electrons from cyt c to molecular oxygen, thereby reducing it to water. The goal of these reactions is to form a proton motive force by pumping protons into the intermembrane space. The membrane potential and pH gradient formed provides energy for ATP synthesis as protons flow back into the matrix through complex V (Nelson et al., 2008).

The ATP production is tightly regulated by a number of mechanisms, such as allosteric mechanisms, feedback effects, and substrate concentration. As the concentration of ADP increases, due to energy consuming activities, ATP production will increase until the ADP concentration is reduced to basal levels. High concentrations of ATP inhibit many of the enzymes of glycolysis and the TCA cycle. In addition, the oxygen consumption is coupled to ATP production (Nelson et al., 2008).

All five complexes have unique structure, and molecular weights. Complex I, also called NADH:ubiquinone oxidoreductase, is a L-shaped protein with 46 subunits and a molecular mass of 980 kDa (Hirst et al., 2003). Complex II, also called succinate:ubiquinone oxidoreductase, is unique because it is the only membrane bound enzyme of the TCA cycle. This complex is much smaller than complex I, with a mass of 124 kDa (Sun et al., 2005). Complex III

(ubiquinol:cytochrome c oxidoreductase) is intermediate in size, between complex I and II, with a mass of 450 kDa. Cytochrome c is a soluble protein residing in the intermembrane space. Complex IV (cytochrome c oxidase) and V (ATP synthase or ATPase) has a molecular weight of around 200 and 700 kDa respectively (Abdrakhmanova et al., 2006; Nelson et al., 2008).

Complex I is the point of entry for the major fraction of electrons, is the most intricate membrane-bound enzyme known to date, and seven of the 46 subunits present in this complex are encoded by mitochondrial DNA. Research on complex I is of greater significance since the many human mitochondrial diseases involve structural and functional defects at the level of this enzyme complex. Correlation of complex I to human diseases is related to but not limited to reactive oxygen species (ROS) damage (Hirst et al., 2008). Examples of such diseases are Parkinson disease (Dawson and Dawson, 2003), cancer, diabetes, Alzheimer's (Lambert and Brand, 2009), fatal neonatal mitochondria disease (Saada et al., 2009) and Leigh syndrome (Ugalde et al., 2004).

Complex I is made up of three functional modules; the electron input module (binds NADH and captures electrons from NADH oxidation), electron output module (electron transfer to ubiquinone) and the proton translocation module (binding of ubiquinone and proton pumping) (Lazarou et al., 2009). Electrons from the oxidation of NADH are transferred through complex I via flavin mononucleotide and a series of iron–sulfur clusters to ubiquinone, forming ubiquinol.

Complex I can be dissected into four subcomplexes (Iα, Iβ , Iλ and Iγ) (Carroll et al., 2003). Subcomplex Iα is made up of subunits from both the peripheral arm and a portion of the hydrophobic membrane arm, while subcomplex Iβ consists of subunits that make up the majority of the membrane arm. Subunits not found in either of these subcomplexes are located in subcomplex Iγ. Altering the conditions can further dissociate subcomplex Iα to produce subcomplex Iλ. This subcomplex represents the hydrophilic peripheral arm and contains the 15 subunits that provide all the redox cofactors (Hirst et al., 2003).

MITOCHONDRIAL FUNCTION AND FEED EFFICIENCY

Studies looking at mitochondrial function and FE began with Bottje et al. (2002). Breast and leg muscle were isolated from a single genetic line of male broilers to measure electron transfer efficiency (RCR), ability to carry out oxidative phosphorylation (ADP:O), electron leakage (H_2O_2), and complex activities. Respiratory chain coupling, was greater in high FE breast, and leg mitochondria provided NADH-linked, but not FADH-linked, energy substrates. Adenosine diphosphate to oxygen (ADP:O) was not different among broilers. Electron leak (H_2O_2), was greater in the low FE than in high FE breast mitochondria. Electron leak increased following inhibition of electron transport at complex I and complex III in low FE but not in high FE breast mitochondria. There were no differences in basal electron leak in leg mitochondria between

groups, but H_2O_2 generation was elevated compared to basal values in low FE leg mitochondria after Complex I inhibition. Complexes I and II activities were greater in high FE breast and leg muscle mitochondria compared to those in low FE mitochondria. The authors concluded that lower respiratory chain coupling in low FE muscle mitochondria may be due to lower complexes I and II activities and defects in electron leak and provide insight into cellular mechanisms associated with the phenotypic expression of FE in broilers.

Subsequent studies using broilers were conducted in various tissues [i.e., breast muscle, leg muscle, intestine, heart and lymphocytes (Iqbal et al., 2004; Ojano-Dirain et al., 2004; Tinsley et al., 2004; Iqbal et al., 2005; Ojano-Dirain et al., 2005a; Ojano-Dirain et al., 2005c; Lassiter et al., 2006; Ojano-Dirain et al., 2007)]. Ojano-Dirain et al., 2004, 2005b using duodenal mitochondria from broilers observed greater RCR in high-FE mitochondria after the second addition of ADP with succinate, but not with NADH-linked energy substrates. The ADP:O values were greater in low-FE mitochondria provided NADH linked substrates (malate, pyruvate, or both) (Ojano-Dirain et al., 2004).

Kolath et al. (2006b) and Lancaster et al. (2014) assessed mitochondrial function in steers and heifers with divergent RFI. Mitochondria isolated from skeletal muscle were not different between the two groups for either substrate (glutamate or succinate), however -RFI steers exhibited a greater rate of state 2 and 3 respiration and RCR, than +RFI steers when provided with glutamate or succinate as a respiratory substrate (Kolath et al., 2006b). Lancaster et al. (2014)

reported greater RCR values and state 3 respiration rate in liver mitochondria from -RFI heifers compared with +RFI, however, states 2 and 4 didn't differ.

Greater basal H_2O_2 levels were consistently observed in mitochondria from breast muscle, liver, and duodenum isolated from low-FE broilers with the exception of leg muscle (Bottje et al., 2006). Similarly, Kolath et al. (2006b) observed greater H_2O_2 production in -RFI steers. Grubbs et al. (2013a) more recent study, looking at how RFI impacts mitochondria reactive oxygen species production (ROS) in pigs and observed less ROS production in mitochondria from the white portion of the semitendinosus in the -RFI compared to +RFI, when both NADH and $FADH_2$ energy substrates were used (glutamate and succinate, respectively). Additionally, mitochondria from the red portion of the semitendinosus in the -RFI had less ROS production when succinate was used as an energy substrate.

Findings regarding complex activities are not consistent across studies. Ojano-Dirain et al. (2005a) reported no difference in complex activities between low-FE and high-FE duodenal mitochondria in broilers. In other studies, a general decrease in all complex activities (with one exception in complex IV of the duodenum) was observed in low-FE compared with high-FE mitochondria (Bottje and Carstens, 2009). Sharifabadi et al. (2012) reported sheep with -RFI had greater activity of all five respiratory chain complexes. Sandelin (2005) reported that activities of all five ETC complexes were greater in mitochondria from neck muscle of low-FE steers compared with high-FE steers.

Glutathione (GSH) is a vital defense mechanism of mitochondria against free radical damage (Meister and Anderson, 1983). The oxidized GSH (GSSH) to GSH ratio is an oxidative stress indicator. The GSSH to GSH ratio tended to be greater in duodenal mitochondria from low-FE broilers (Ojano-Dirain et al., 2005a).

Greater H_2O_2 production is consistently observed in high-FE and -RFI mitochondria. Mitochondrial ROS have the potential to oxidize structures such as proteins, DNA, and lipids (Yu, 1994). Protein carbonyls are used as an indicator of protein oxidation (Stadtman and Levine, 2000) and have been associated with aging and certain metabolic diseases (Dalle-Donne et al., 2005). Mitochondria from breast muscle, duodenum, liver, and lymphocyte homogenates from low-FE birds, as well as neck muscle from low-FE steers exhibited greater total protein carbonyls compared to high-FE (Iqbal et al., 2004; Iqbal et al., 2005; Ojano-Dirain et al., 2005b; Sandelin, 2005; Lassiter et al., 2006)

Several studies assessed ETC protein expression in order to better explain feed efficiency differences among animals (Iqbal et al., 2004; Iqbal et al., 2005; Ojano-Dirain et al., 2005b; Kolath et al., 2006a; Davis, 2009; Grubbs et al., 2013b; Ramos and Kerley, 2013). Iqbal et al. (2004) reported higher protein expression in low-FE breast muscle mitochondria for five mitochondrial proteins, [core I, cytochrome c1, cytochrome b (complex III), COX II (cytochrome c oxidase subunit II, complex IV), and adenine nucleotide translocator (ANT1)]. They also found a protein band of 47 kDa expressed at a higher level in low-FE compared with high-FE mitochondria. Instead of breast muscle, the same group

in 2005 used liver. They observed expression of four proteins [mitochondrially encoded NADH dehydrogenase 3 (NAD3, complex I), subunit VII (complex III), COX II, and COX IVb (complex IV)] were higher in low-FE mitochondria and two proteins [flavoprotein (complex II) and a-ATP synthase (complex V)] were higher in high FE birds (Iqbal et al., 2005). Ojano-Dirain et al. (2005c) reported that six out of seven nuclear-encoded respiratory chain subunits [flavoprotein, core I, core II, cytochrome c 1, iron-sulfur protein, and ATPase-α] were higher, whereas three out of six mitochondrial-encoded subunits (mitochondrially encoded NADH dehydrogenase 4, mitochondrially encoded NADH dehydrogenase 6-C, and COXII) were lower in the low FE duodenum mitochondria.

Kolath et al. (2006a) reported no difference between the high- and -RFI animals in their expression of UCP protein 2 or 3 mRNA or protein using LM from steers. Also using steers, complex I protein concentrations in lymphocytes mitochondria tended to be negatively correlated and ratios of complex I to II and complex I to III were negatively correlated with RFI (Davis, 2009). Ramos and Kerley (2013) isolated mitochondria from lymphocytes in four similar experiments. Experiment one, two, and three found greater complex I concentration for -RFI compared to +RFI. Additionally, Exp. 4 reported a tendency for lower concentration of band I in steers with -RFI. Heat shock protein 60 and 70 (inhibits cellular death) were greater for -RFI pigs as well as ATP synthase beta subunit (Grubbs et al., 2013b).

Mitochondria play a vital role in growth and development. Functional and biochemical differences such as electron transfer efficiency, ability to carry out

oxidative phosphorylation, electron leakage, ROS production, mitochondial complexe activities, protein expression and antioxidants status provide clear evidence for the link between mitochondria and production efficiency. Further research is needed to develop tools for identification of efficiency phenotype.

CONCLUSION

Improving feed efficiency through selection for RFI has been proved to be the most accurate method however this method is expensive and time consuming. There is a need for understanding the relationship between feed intake and production traits, since several biological mechanisms are involved. Formulating diets that allow genetic potential of efficiency to be expressed is an important step toward improving feed efficiency. Mitochondria produce the majority of energy used by the cell and mitochondrial function has been linked to feed efficiency in several species. Research suggests mitochondria function related to mitochondria is a potential method for efficiency selection, however more research is needed to assist the development of this approach.

CHAPTER II

EFFECTS OF INCREASING RUMEN UNDEGRADABLE PROTEIN IN DIET AMINO ACID OUTFLOW FROM CONTINUOUS CULTURE FERMENTERS FED FORAGE-BASED DIET.

ABSTRACT

Three diets with increasing RUP (LOW, MID and HIGH), were fed (50 g/d) to continuous culture fermenters to characterize RUP supplementation (porcine blood meal and Aminoplus) using forage-based diet. We hypothesized that increasing diet RUP concentration would increase diet AA flow from the rumen without influencing microbial fermentation. Diets were randomly distributed over fermenters (n = 24), acclimated for 4 d, and sampled over 3 d. Fermenter content was subsampled at 0 h and 4 h post feeding for pH and analyzed for VFA (mM) and ammonia concentration (mM/dL). The pH was greater (P < 0.01) for HIGH, compared to MID, and LOW, which were similar at 0 and 4 h. Ammonia concentration increased as RUP increased (LOW 3.18; MID 5.30; HIGH 8.79) at 0 h, however at 4 h ammonia concentration was greater (P < 0.01) for only HIGH (10.58) compared to MID (6.10) and LOW (4.26) which were similar. Acetate, propionate, valerate, total VFA and acetate:propionate did not differ (P > 0.05) at

0 and 4 h among treatments. Butyrate did not differ (P = 0.37) at 0 h. At 4 h butyrate was greater (P = 0.04) for LOW (16.9) compared to HIGH (15.4), however MID (16.5) did not differ among treatments. Isobutyrate was greater (P < 0.01) for HIGH (1.5) compare to MID (1.2) and LOW (1.1) at 0 h. At 4 h, isobutyrate increased as RUP increased (LOW 1.2; MID 1.4; HIGH 1.6; P < 0.01). Isovalerate was greater (P < 0.01) for HIGH (2.9; 3.0), compared to MID (2.5; 2.6), and LOW (2.2; 2.4), for 0 and 4 h, respectively. Organic matter, NDF and ADF digestibility, microbial efficiency and g of bacterial N outflow/d did not differ (P > 0.05) among treatments. Protein digestibility (%) was greater (P = 0.04) for LOW (47.2) and HIGH (46.0) compared to MID (38.3). As RUP increased in diets AA outflow increased, but outflow was not stoichiometric for HIGH diet compared to MID diet. In conclusion, RUP supplementation in forage-based diets increased RUP flow from the rumen without influencing microbial fermentation. Reduced RUP for HIGH compared to MID may be due to microbial adaptation to greater RUP levels.

INTRODUCTION

The protein reaching small intestine for absorption in ruminants is the sum of microbial protein synthesized in rumen and dietary protein which escapes rumen fermentation. Microbial protein supplies 60 to 80% of the AA absorbed in the intestine (NRC, 2000), however microbial protein AA profile is relatively constant across various diets (Kerley, 2010). For high production rates, microbial

protein alone may be insufficient to meet cattle growth requirements. Therefore, to optimize cattle growth, diets need to provide balanced levels of RDP to maximize microbial growth while providing adequate RUP to meet AA requirement for maximum animal productivity.

Methionine, lysine, arginine, and histidine are reported as the most limiting AA to ruminants fed forage-based diets (Storm and Ørskov, 1983; Coleman, 1996; Greenwood and Titgemeyer, 2000). Supplementing ruminant diets with slowly degradable protein sources can increase the AA flow to small intestine and improve growth and efficiency (Titgemeyer and Loëst, 2001).

The experimental objective was to characterize RUP supplementation in a forage-based diet using three diets with increasing RUP levels (LOW, MID and HIGH). We hypothesized that increasing diet RUP concentration would increase RUP flow from the rumen without influencing microbial fermentation and AA digestion would remain constant at increasing dietary RUP levels.

MATERIAL AND METHODS

Diet formulation

Dietary treatments were composed of differing levels of dried alfalfa haylage, soyhulls, dried distillers grains with soluble (DDGS), corn, Amino-plus, and blood meal. All the ingredients were ground to pass through 2-mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA). Diets were formulated with increasing rumen undegraded AA levels, LOW, MID and HIGH of

the most limiting AA requirement for a 200 Kg steer. The LOW diet was dried alfalfa haylage-based diet with soyhulls, DDGS, corn and amino-plus. Diets MID and HIGH replaced some of the dried alfalfa haylage, soyhulls, and DDGS with Amino-plus and blood meal (Table 2.1).

Continuous Culture

Animals use was approved by University of Missouri Animal Care and Use Committee. Rumen fluid was obtained from ruminally fistulated multiparous lactating Holstein cows housed in free-stall facilities at the University of Missouri-Columbia Foremost Dairy Research Center. Cows were fed a common lactation diet. Rumen fluid was collected, transported from the farm to the lab (estimated travel time 10 min) on closed thermal controlled containers, strained through four layers of cheese cloth and diluted with McDougall's artificial saliva in a 1:2 dilution of rumen fluid to buffer.

Twenty-four single-flow effluent continuous culture polycarbonate vessels (Nalgene, Rochester, NY) were inoculated (1,460 mL) and maintained as described by Meng et al. (1999). Fermenters were continuously flushed with CO^2, stirred with magnetic stir plates, and immersed in a water bath maintained at 39°C using thermostatically controlled heaters. High buffer capacity solution modified by Slyter (1990) from McDougall's artificial saliva (McDougall, 1948), containing 250 mg cysteine-HCl/L was continuously infused into fermenters using peristaltic pumps. Fermenter dilution rates were held constant at 4% ± 0.2%·h^{-1} for all treatments. Effluent collection vessels were kept immersed in ice until

freezing (- 4˚C) after 24 h total collection. Fermenters were randomly assigned to one of the three treatments (*n* = 8) and fed half the daily ration of 50 g/d (as fed) at 0800 h and 1700 h. The experiment was conducted over a 7 d period, with 4 d adaptation and 3 d sampling.

Sampling

One liter subsamples were taken from effluent collected over the previous 24 h and stored at 4˚C. These samples were composited for each fermenter over the 3 d period for later analysis. On the last day of sampling, fermenter contents were collected and stored at 4˚C until analyzed. Further, 0 and 4 h after feeding, pH was measured and a 9 mL sample was taken directly from the fermenter and frozen at - 20˚C. These samples were later composited by hour for each fermenter over the 3 d period and analyzed for ammonia and VFA concentration.

Analyses

Fermenter samples contents were centrifuged at 1,000 x g for 5 min at 4°C to remove feed particles. Supernatant was centrifuged at 27,000 x g for 30 min. The final pellet, containing bacteria, was transferred to plastic cups using deionized distilled water, lyophilized at 10°C (Genesis, Virtis, Gardiner, NY), and ground using a mortar and pestle. Subsamples of effluent (500 mL) were lyophilized at 10°C (Genesis, Virtis, Gardiner, NY), and ground using a mortar and pestle.

Diet, effluent and fermenter contents were analyzed for DM, ash (AOAC, 2006) and nitrogen content (Model FP-428, Leco Co., St. Joseph, MI). Effluent and fermenter samples were analyzed for purine content using the procedure of Zinn and Owens (1986) to determine microbial N, which was used with OM digested to determine microbial efficiency (MOEFF; g microbial N/kg OM truly digested). Diet and effluent samples were analyzed for NDF and ADF using an ANKOM^{200} Fiber Analyzer (ANKOM Technology). Ammonia concentration (mM) was determined colorimetrically by DU-65 spectrophotometer (Beckman, Palo Alto, CA) with the hypochlorite-phenol procedure of Broderick and Kang (1980). Volatile fatty acids concentration (mM) was determined by gas chromatography (Model 3400, Varian, Palo Alto, CA) following procedures outlined by Salanitro and Muirhead (1975). Diets, bacteria and effluent samples were sent to the University of Missouri Experiment Station Chemistry Laboratory for AA analysis (AOAC, 2006).

Statistical Analysis

All statistical analyses were performed using the GLM procedure in SAS (SAS Inst. Inc., Cary, NC) The data were analyzed as a randomized complete design with three treatments. When the F-test was significant ($P \leq 0.05$), means separation was performed using LSMEANS statement.

RESULTS

Fermentation

Fermentation parameters at feeding (0 h) are presented in Table 2.2. Isobutyrate and isovalerate were greater ($P < 0.01$) for HIGH compared to LOW and MID diets. Ammonia increased ($P < 0.01$) as RUP increased in the diet, and pH was greater ($P < 0.01$) for HIGH compared to LOW and MID. Acetate, propionate, butyrate, valerate, total VFA, and acetate:propionate did not differ ($P > 0.05$) among treatments at feeding.

Four hours after feeding, pH and ammonia were greater ($P < 0.01$) for HIGH compared to MID and LOW (Table 2.3). Propionate was greater ($P < 0.02$) for LOW and MID compared to HIGH. Butyrate was greater ($P < 0.04$) for LOW compared to HIGH, however MID did not differ. Isobutyrate increased ($P < 0.01$) as RUP increased in the diet. Isovalerate was greater ($P < 0.01$) for HIGH compared to LOW and MID. Acetate, valerate, total VFA and acetate:propionate did not differ ($P > 0.05$) among treatments 4 h after feeding.

Digestibility

Nitrogen intake increased ($P < 0.01$) as RUP was included in the diet (Table 2.4). Nitrogen digestibility was greater ($P < 0.04$) for LOW and HIGH compared to MID. Dietary nitrogen in the effluent (RUP) increased ($P < 0.01$) as RUP AA sources were included in the diet. Organic matter, NDF and ADF

digestibility, MOEFF and grams of bacterial N/d did not differ ($P > 0.05$) among treatments.

Amino Acid Digestibility and Flow

Dietary digestibility of seven AA (threonine, glycine, cysteine, methionine, isoleucine, tyrosine, tryptophan) did not differ across treatments ($P > 0.05$; Table 2.5). Aspartic acid, serine, alanine, valine, leucine, phenylalanine, lysine, arginine, histidine and total AA had greater ($P < 0.05$) digestibility for LOW compared to MID. Glutamic acid and proline had greater digestibility for LOW and HIGH compared to MID ($P < 0.02$).

Most of the dietary bypass AA flow increased ($P < 0.01$) as bypass sources were included in the diet (aspartic acid, threonine, serine, glycine, alanine, valine, leucine, phenylalanine, lysine, histidine, arginine and total AA; Table 2.6). Cysteine, and tyrosine was greater ($P < 0.02$) for HIGH compared to LOW, however MID did not differ. Glutamic acid, and proline were greater ($P < 0.01$) for MID and HIGH compared to LOW. Tryptophan was greater for HIGH compared to LOW and MID ($P < 0.01$). Methionine and isoleucine flow did not differ among treatments ($P < 0.05$).

DISCUSSION

We hypothesized that increasing diet RUP concentration would increase RUP flow from the fermenter without influencing microbial fermentation and AA digestion would remain constant at increasing dietary RUP level.

Fermentation parameters, such as VFA production (propionate, butyrate, isovalerate, and isobutyrate), pH, and ammonia differed among diets, however microbial efficiency and OM digestibility did not differ across diets. Consistent with the current study, Carrasco et al. (2013) observed decreased molar proportions of propionate while isovalerate increased with RUP inclusion. In addition, there were no treatment effects on duodenal flow of microbial N. However, ruminal pH did not differ, acetate:propionate molar ratio increased, and ruminal OM digestion decreased with RUP inclusion (Carrasco et al., 2013).

Several studies also evaluated the effect of RUP on rumen fermentation. Legleiter et al. (2005) observed lower acetate, isovalerate and acetate:propionate concentrations, greater propionate and valerate concentrations, and no effect on total VFA concentration for control compared to other treatments with increasing RUP. Greater RUP levels increased MOEFF compared to lower levels of supplementation (Legleiter et al., 2005). Calsamiglia et al. (1992) reported no effect on OM and DM digestion but lower digestion of NDF and ADF when fermenters received diets containing fish meal plus heat treated soybean meal rather than control diet. Although total VFA concentration was not affected by dietary treatment, molar proportion of propionate was greater, and acetate and

acetate:propionate lower for the treatment compared to control diet (Calsamiglia et al., 1992). Koenig and Beauchemin (2013) fed barley-based diets containing 12% CP with non-protein nitrogen alone or combined with RDP and RUP to attain 14% CP. They reported protein sources ruminal degradability did not influence the protein composition flowing to the intestine and site and extent of nutrient digestibility.

Numerous studies have focused on feeding RUP to increase AA flow to the small intestine and consequently increase animal growth. However, experiments haven't provided consistent results. Some have shown increased N and AA flows to the small intestine (Cecava and Parker, 1993; Coomer et al., 1993; Zinn and Owens, 1993), as well as increased growth due to RUP addition (Sindt et al., 1993; Zinn and Owens, 1993; Lehmkuhler and Kerley, 2007), while others have reported no responses to feeding RUP, decreased total N flow to the small intestine, and/or decreased microbial protein synthesis (Loerch and Berger, 1981; Ludden and Cecava, 1995).

Previous work done by Lehmkuhler (2001) suggested that RUP value decreased as the dietary inclusion level of a protein source increased, however Legleiter et al. (2005) reported that levels of protein supplementation did not influence RUP value. Lehmkuhler (2001) reported a decrease in RUP flow as levels of blood meal (BM) increased from 3 to 12% of DM, and this decrease was attributed to microbial degradation. Legleiter et al. (2005) observed that ruminal microorganisms do not adaptively increase proteolytic activity toward RUP sources. At the lower RUP inclusion in the diet (LOW to MID), RUP was

recovered in the effluent flow, however at greater inclusion (MID to HIGH) only 34% of added RUP was recovered in the effluent flow. Although the current study didn't measure proteolytic activity, greater digestibility of N may be due to microbial adaptation to greater RUP levels.

Digestibility and outflow were not consistent across AA. Bach and Stern (1999) reported methionine supplementation could increase cysteine, tyrosine, and isoleucine flows to duodenum using continuous culture system. Carrasco et al. (2013) observed 20% DDGS inclusion had the greatest value of AA leaving the abomasum (methionine, histidine, phenylalanine, threonine, leucine, isoleucine and valine), with the exception for lysine and arginine. Tyrosine, cysteine and threonine are reported as the most resistant AA to microbial fermentation, whereas methionine and lysine as the most degradable (Bach and Stern, 1999). Duodenal AA flow was directly proportional to bypass AA added in the diet (Zinn and Owens, 1993).

CONCLUSION

Feeding increasing levels of RUP did not influence microbial efficiency, therefore the main source of AA absorbed in the intestine, which is microbial protein, was not compromised. In addition, greater dietary RUP increased total bypass AA flow. Based on these results, microbes appeared to adapt and degrade more protein when RUP sources were included at HIGH level.

Table 2.1. Ingredients and nutrient composition of diets fed to continuous culture fermenters

	Treatments		
Item (% DM)	LOW	MID	HIGH
Ingredients			
Dried alfalfa haylage	50.17	46.19	43.23
Soyhulls	22.07	22.69	19.1
Dried distillers grains with soluble	16.71	15.06	14.68
Corn	9.70	9.71	9.72
Aminoplus[1]	0.97	1.94	3.89
Porcine blood meal	0.00	3.97	8.95
Vitamin Mineral Premix[2]	0.38	0.44	0.44
Nutrient Composition			
DM, %	93.69	93.62	93.69
CP, % DM	15.39	17.87	24.85
NDF , % DM	47.59	44.31	41.82

[1]Aminoplus (AGP, Omaha, NE) contained CP 51.14% DM, RUP 72% CP, RDP 28% CP.
[2] Vitamin Mineral Premix contained 8,818,490.5 IU/kg of vitamin A, 1,763,698.1 IU/kg of vitamin D3, 1,102.3 IU/kg of vitamin E, Ca, 2.5% Fe, 2% Mn, 3% Zn, 1% Cu, 100 ppm Se, 500 ppm I, and 100 ppm Co.

Table 2.2. VFA, ammonia concentration and pH at 0 h after feeding

	Treatments				
Item	LOW	MID	HIGH	SEM	*P*-value
Acetate, mM	93.30	91.90	88.80	2.46	0.43
Propionate, mM	24.50	24.20	23.30	0.73	0.49
Butyrate, mM	15.70	15.50	14.80	0.47	0.37
Isobutyrate, mM	1.10[b]	1.20[b]	1.50[a]	0.05	**< 0.01**
Isovalerate, mM	2.20[b]	2.50[b]	2.90[a]	0.08	**< 0.01**
Valerate, mM	2.40	2.40	2.30	0.08	0.51
Total VFA, mM	139.20	137.70	133.80	3.55	0.55
Acetate:Propionate	3.82	3.81	3.83	0.09	0.98
Acetate, mol%	0.67	0.67	0.66	0.004	0.60
Propionate, mol%	0.18	0.18	0.18	0.003	0.90
Butyrate, mol%	0.11	0.11	0.11	0.001	0.44
pH	6.32[b]	6.34[b]	6.46[a]	0.03	**< 0.01**
Ammonia, mM/dL	3.18[c]	5.30[b]	8.79[a]	0.65	**< 0.01**

[a, b, c] least square means within a row with different superscript differ ($P < 0.05$).

Table 2.3. VFA, ammonia concentration and pH at 4 h after feeding

	Treatments				
Item	LOW	MID	HIGH	SEM	*P*-value
Acetate, mM	91.80	91.70	87.20	1.84	0.15
Propionate, mM	25.50[a]	25.10[a]	23.70[b]	0.44	**0.02**
Butyrate, mM	16.90[a]	16.50[ab]	15.40[b]	0.367	**0.04**
Isobutyrate, mM	1.20[c]	1.40[b]	1.60[a]	0.05	**< 0.01**
Isovalerate, mM	2.40[b]	2.60[b]	3.00[a]	0.06	**< 0.01**
Valerate, mM	2.50	2.60	2.50	0.85	0.92
Total VFA, mM	140.40	139.70	133.40	2.63	0.14
Acetate:Propionate	3.60	3.66	3.69	0.03	0.28
Acetate, mol%	0.182	0.179	0.177	0.001	0.09
Propionate, mol%	0.12	0.118	0.116	0.001	0.11
Butyrate, mol%	0.65	0.66	0.65	0.003	0.71
pH	6.24[b]	6.27[b]	6.42[a]	0.03	**< 0.01**
Ammonia, mM/dL	4.26[b]	6.10[b]	10.58[a]	0.78	**< 0.01**

[a, b, c] least square means within a row with different superscript differ ($P < 0.05$).

Table 2.4. Digestibility (Dig) and fermentation measurements of diets fed to continuous culture fermenters

	Treatments				
Item	LOW	MID	HIGH	SEM	*P*-value
OM Dig, %	56.70	55.80	56.00	1.87	0.93
NDF Dig, %	48.30	47.00	52.40	2.85	0.39
DF Dig, %	51.90	50.90	54.80	2.41	0.51
MOEFF[1]	20.20	22.50	21.10	1.17	0.39
Bacteria Nitrogen, g/d	0.50	0.53	0.55	0.04	0.76
Nitrogen intake, g/d	1.16[c]	1.34[b]	1.87[a]		**< 0.01**
Nitrogen Dig, %	47.20[a]	38.30[b]	46.00[a]	2.51	**0.04**
RUP, g/d	0.61[c]	0.83[b]	1.01[a]	0.04	**< 0.01**

[1]Microbial efficiency (g effluent bacterial nitrogen/ kg OM truly digested).

[a, b, c] least square means within a row with different superscript differ ($P < 0.05$).

Table 2.5. Dietary AA digestibility of diet fed to continuous culture fermenters

	Treatments				
Amino Acid[1]	LOW	MID	HIGH	SEM	*P*-value
Taurine	0.76[a]	0.75[a]	0.59[b]	0.03	**0.01**
Aspartic Acid	0.56[a]	0.44[b]	0.49[ab]	0.03	**0.05**
Threonine	0.49	0.43	0.49	0.03	0.11
Serine	0.54[a]	0.46[b]	0.51[ab]	0.02	**0.02**
Glutamic Acid	0.49[a]	0.42[b]	0.50[a]	0.02	**0.02**
Proline	0.58[a]	0.49[b]	0.56[a]	0.02	**0.01**
Glycine	0.47	0.39	0.46	0.03	0.06
Alanine	0.49[a]	0.38[b]	0.45[ab]	0.03	**0.02**
Cysteine	0.46	0.42	0.46	0.03	0.27
Valine	0.54[a]	0.41[b]	0.47[ab]	0.03	**0.01**
Methionine	0.54	0.49	0.53	0.03	0.29
Isoleucine	0.51	0.47	0.54	0.03	0.06
Leucine	0.53[a]	0.41[b]	0.47[ab]	0.03	**0.01**
Tyrosine	0.58	0.56	0.55	0.03	0.48
Phenylalanine	0.52[a]	0.41[b]	0.47[ab]	0.03	**0.02**
Lysine	0.50[a]	0.38[b]	0.46[ab]	0.03	**0.01**
Histidine	0.56[a]	0.38[b]	0.44[b]	0.03	**0.01**
Arginine	0.59[a]	0.48[b]	0.54[ab]	0.02	**0.01**
Tryptophan	0.58	0.56	0.55	0.03	0.48
Total	0.53[a]	0.43[b]	0.49[ab]	0.03	**0.02**

[1]All the AA are expressed in g of digested AA/g of AA fed.

[a, b, c] least square means within a row with different superscript differ ($P < 0.05$).

Table 2.6. Dietary bypass AA flow from continuous culture fermenters fed diets with increasing RUP concentration

	Treatments				
Amino Acids[1]	LOW	MID	HIGH	SEM	*P*-value
Taurine	0.01	0.01	0.01	0.00	0.4
Aspartic Acid	0.28^{c}	0.45^{b}	0.58^{a}	0.03	**< 0.01**
Threonine	0.14^{c}	0.18^{b}	0.20^{a}	0.01	**< 0.01**
Serine	0.13^{c}	0.18^{b}	0.22^{a}	0.01	**< 0.01**
Glutamic Acid	0.45^{c}	0.54^{b}	0.60^{a}	0.02	**0.01**
Proline	0.18^{c}	0.23^{a}	0.25^{a}	0.01	**< 0.01**
Glycine	0.19^{c}	0.26^{b}	0.30^{a}	0.01	**< 0.01**
Alanine	0.23^{c}	0.34^{b}	0.42^{a}	0.02	**< 0.01**
Cysteine	0.23^{c}	0.34^{ab}	0.42^{a}	0.02	**0.02**
Valine	0.19^{c}	0.32^{b}	0.41^{a}	0.02	**< 0.01**
Methionine	0.06	0.06	0.06	0.00	0.22
Isoleucine	0.15	0.16	0.15	0.01	0.56
Leucine	0.32^{c}	0.51^{b}	0.65^{a}	0.03	**< 0.01**
Tyrosine	0.12^{b}	0.15^{ab}	0.16^{a}	0.01	**0.01**
Phenylalanine	0.17^{c}	0.26^{b}	0.34^{a}	0.01	**< 0.01**
Lysine	0.18^{c}	0.30^{b}	0.39^{a}	0.02	**< 0.01**
Histidine	0.08^{c}	0.18^{b}	0.27^{a}	0.01	**< 0.01**
Arginine	0.13^{c}	0.19^{b}	0.23^{a}	0.01	**< 0.01**
Tryptophan	0.03^{b}	0.03^{b}	0.04^{a}	0.00	**0.01**
Total	3.16^{c}	4.43^{b}	5.40^{a}	0.23	**< 0.01**

[1]All the AA are expressed in g/d.
$^{a, b, c}$ least square means within a row with different superscript differ ($P < 0.05$).

CHAPTER III

EFFECTS OF POSTRUMINAL AMINO ACID SUPPLY DURING GROWING PHASE ON GROWTH PERFORMANCE, CARCASS MEASUREMENTS, RESIDUAL FEED INTAKE,AND BLOOD METABOLITES USING FORAGE-BASED DIET

ABSTRACT

This study investigated the effect of increasing potruminal AA supply level in the diet during the growing phase of steers on performance, carcass measurements, residual feed intake, and blood metabolites using roughage-based diets (30% DM-alfalfa haylage). Seventy-two steers were randomly assigned to three roughage-based diets with increasing ruminal undegraded AA; below, equal to and above the requirement (LOW, MID or HIGH) of the most limiting AA during growing phase (GP; 83 d). Steers were fed the same no roughage-based diet during finishing phase (FP; 83 d) and slaughtered when gain cost exceeded gain value. No RFI by bypass AA level interaction was observed ($P > 0.05$). Initial and final BW did not differ ($P > 0.05$) among treatments. Calves fed MID had greater ($P = 0.01$) ADG than LOW during the first 21 d on feed, however this gain rate, was not maintained through the end of

83 d GP (P = 0.50). Average daily gain during FP and overall, DMI during GP and FP, and FCR during GP and overall did not differ (P > 0.05) among treatments, however, MID had greater FCR than HIGH during FP (P = 0.02). Plasma urea nitrogen concentrations were greater (P < 0.01) for HIGH compared to LOW and MID. Leptin plasma concentrations trended to be greater (P = 0.06) for LOW compared to MID while IGF-I trended greater (P = 0.08) for HIGH compared to MID. Plasma concentrations of glucose, creatine and GH did not differ (P > 0.05) among treatments. Carcass characteristics did not differ (P > 0.05) among treatments. Steers were classified as -RFI, AVE and +RFI during growing, finishing and total period (RFI-GP, RFI-FP and RFI-TP respectively). Growing phase RFI was correlated (P < 0.05) with RFI-FP and RFI-TP. Dry matter intake and FCR increased as RFI-GP increased (P ≤ 0.01). Carcass characteristics were not influenced by RFI-GP (P > 0.05). Finishing phase RFI was correlated (P < 0.05) with RFI-TP. Dry matter intake and FCR values increased (P < 0.05) as RFI-FP increased. Longuissimus muscle area trended to decrease (P = 0.07) and intramuscular fat (P = 0.06) trended to increase as RFI-FP increased. Dry matter intake and FCR values increased (P ≤ 0.01) as RFI-TP increased. Longuissimus muscle area (P = 0.09) trended to decrease and intramuscular fat (P = 0.06) trended to increase as RFI-TP increased. In conclusion, calves consuming diets with postruminal AA supplied above requirements may respond with greater compensatory growth, however improved growth was not sustained over the feeding period. As RFI decreased, steers consumed less feed and were more efficient with no change in BW during all

periods. Steers classified as efficient during GP were not necessary in the same category during FP.

INTRODUCTION

Selection for more efficient animal, or nutritional management approaches are some tools to achieve feed efficiency improvement and consequently increase profitability in beef cattle industry. Lean tissue deposition, when calculated on a wet-tissue basis, requires less kilocalories per unit of protein than fat deposition (1.2 vs. 8.3 kcal/g), independent of animal or diet composition (Owens et al., 1995). To maximize lean tissue growth, AA available for growth needs to be in proportion to energy available for growth (AA:energy). If AA available for growth are not balanced compared to that needed for lean tissue growth, then fat will be deposited. Microbial protein supplies 60 to 80% of the AA absorbed in the intestine (NRC, 2000), however cannot support animals high levels of gain, making AA supplied from RUP sources necessary to improve ADG in young growing animals. Previous research conducted in our laboratory demonstrated that adding bypass AA to no roughage diets improved feed efficiency due to increased lean tissue, and decreased intramuscular or back fat (Lehmkuhler, 2001; Mueller, 2004; Davis, 2009). We hypothesized increasing growing phase diet RUP, using roughage-based diets, would improve feed efficiency, impact growth performance during growing and finishing phase (GP, FP respectively) and carcass characteristics.

The rate and extent of growth is impacted not only by nutrition but hormonal factors, with the majority of control being through the actions of the endocrine system. Data suggests, by increasing AA flow of to the intestine from RUP sources could stimulate secretion of hormones and therefore influence growth (Davenport et al., 1990; Guerino et al., 1991; Davenport et al., 1995; Reecy et al., 1996; Ragland-Gray et al., 1997; Richardel, 2004; Sissell, 2007). We hypothesized increasing growing phase diet RUP would affect plasma metabolites and hormones concentrations.

Residual feed intake (RFI) proposed by Koch et al. (1963) is a measure of feed efficiency and describes the divergence in intake from that needed for maintenance and growth. Because animals with similar performance can have intake differences between most and least efficient up to 40% (Kerley and Hill, 2012). We hypothesized that nutritional requirement, such as RUP, for -RFI (efficient) would increase relative to energy consumed compared to +RFI (inefficient) animals.

Therefore, the objective of this experiment was to evaluate the effects of postruminal AA supply during the GP on growth performance, carcass measurements, RFI, and blood metabolites using roughage-based diet.

MATERIAL AND METHODS

Animal Management

The use of animals in this experiment was approved by the University of Missouri Animal Care and Use Committee. Seventy-two spring-weaned yearling crossbred steers were de-wormed (Cydectin, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO), tagged, weighed and vaccinated (Bovi-shield Gold 5, One Shot Ultra 8, Zoetis, Florham Park, NJ) after arrival at the research facility. Three animals were dehorned four days post-arrival and nine animals were castrated (surgical) eleven days post-arrival. Steers were placed in groups of four in one of 18 concrete pens and offered *ad libitum* access to water and receiving diet (Table 3.1) for 14 d before the feeding trial. At the start of the experiment, steers were implanted (Component with Tylan TE-IS, Elanco, Indianapolis, IN) and consecutive 2 d weights were taken to determine initial BW (IBW). Weights were ordered from smallest to largest and treatments LOW, MID and HIGH were randomly assigned to every set of four cattle, producing similar average IBW across treatments. The same procedure was repeated within treatment, assigning pen numbers one to three to produce similar average IBW across pens, resulting in three treatments with six pens per treatment with four animals per pen. All treatments had one steer that had to be dehorned and three castrated during receiving period. Individual feed intake was electronically measured using GrowSafe Systems (Airdrie, Canada).

Individual DMI, ADG and metabolic midweight (MMWT) were used to predict DMI. Predicted DMI was calculated by using DMI as dependent variable and MMWT and ADG as independent variable, using PROC GLM (SAS Inst. Inc., Cary, NC). The model fitted was:

$$Y_i = B_0 + B_1 (ADG) + B_2 (MMWT)$$

Where Y_i = expected daily DMI on animal I; B_0 = the regression intercept; B_1 = partial regression coefficient on actual DMI on ADG; and B_2 = partial regression coefficient of actual DMI on MMWT. To calculate RFI, predicted DMI was subtracted from measured DMI. Three different RFI were calculated, growing phase (RFI-GP), finishing phase (RFI-FP) and total period (RFI-TP). Average and standard deviation of RFI was calculated and one standard deviation above the average was declared +RFI, and one standard deviation below the average was declared -RFI.

Animal weights were taken on days 0, 1, 21, 42, 63, 84, 105, 126, 148, 167 and 168 to determine initial and final BW, ADG and FCR. Steers were slaughtered in a commercial plant when gain cost exceeded gain value.

Diet formulation

During GP steers received alfalfa haylage-based diet. Dietary treatments (Table 3.2) were composed of differing levels of, dried distillers grains with soluble (DDGS), soyhulls, corn, amino-plus, blood meal and MFP (Novus

International, Inc., St. Charles, MO). Diets were formulated with increasing ruminal undegraded AA levels, 80, 100 and 120% of the most limiting AA requirement for a 200 kg steer (figure 3.1). Growing diets were formulated to achieve 1.13 kg ADG and 2.2% BW DMI using growing average BW. Alfalfa haylage and corn stayed constant for all the diets, while DDGS decreased and soybean hulls, amino-plus, blood meal and MFP increased the inclusion across dietary treatments. All diets had similar DM, CP, ME, NDF and ADF. The most limiting AA for LOW (80% of the most limiting AA requirement) was lysine followed by arginine and histidine. Diet MID (100%) and HIG (120%) had arginine as the most limiting followed by methionine and lysine. During finishing phase all the steers were fed a common corn-based diet (Table 3.3) balanced for ruminal undegradable AA. Finishing diet was formulated to achieve 1.8 kg ADG and 2% BW DMI using finishing average BW. All diet samples were analyzed for DM, ash (AOAC, 2006), nitrogen content (Model FP-428, Leco Co., St. Joseph, MI), NDF and ADF [ANKOM Technology, Macedon, NY, (Goering and Van Soest, 1970)].

Blood collection

Blood samples were collected at GP beginning and end (0 d and 83 d) to analyze for plasma hormones (GH, IGF-I and leptin) and metabolites [glucose, plasma urea nitrogen (PUN) and creatine]. Metabolites were analyzed by Veterinary Medical Diagnostic Laboratory, Columbia, MO.

Statistical analysis

Interaction of dietary treatment during the growing phase and continuous random variable RFI-GP, RFI-FP, RFI-TP on production traits and carcass measurements investigated using GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Interaction between RFI and treatment was found not significant ($P > 0.15$) for all traits therefore only main effects of treatment and RFI were investigated in separate models.

Partial correlation coefficients were calculated for RFI measurements, production trait and carcass characteristics using CORR procedures. Steer was the experimental unit for all traits. The variables BW, DMI ADG and FCR were analyzed using the MIXED procedure as a repeated measure analysis among different time intervals on the test. The compound symmetry [CS] covariance model was chosen for DMI, and feed:gain and Autoregressive [AR-1] was chosen for BW, and ADG. The GLM procedure was used to analyze, carcass characteristics and blood metabolites, d 0 was used as covariate for d 84. When the F-test was significant ($P \leq 0.05$), means separation was performed using LSMEANS statement.

RESULTS

The interaction between RFI (during all the phases) and level of bypass AA during growing phase was found not significant ($P > 0.05$) leading to main effects examination.

Dietary Effects on Performance, Blood Metabolites, Hormones and Carcass Characteristics.

Body weight was not affected by dietary treatments. Initial (P = 0.66) and final BW (P = 0.60) did not differ among treatments (table 3.4). Table 3.5 reported DMI, ADG and FCR differences among treatments. Calves fed MID had greater ADG than LOW during the first 21 d on feed (LOW 1.43; MID 1.76; HIGH 1.62; P = 0.01), however this gain rate, was not maintained by the end of 83 d GP (P = 0.5). Average daily gain during FP and overall, DMI during GP and FP, and FCR during GP and overall did not differ among treatments (P > 0.05), however, MID had greater FCR than HIG during FP (LOW 6.61; MID 6.92; HIGH 6.30; P = 0.02). Plasma metabolites and hormones were reported on table 3.6. Plasma urea nitrogen concentrations were greater (LOW 17.36; MID 16.50; HIGH 20.45; P < 0.01) for HIGH compared to LOW and MID. Leptin trended greater (LOW 4.12; MID 3.14; HIGH 3.75; P = 0.06) plasma concentrations for LOW compared to MID while IGF-I trended greater (LOW 202.9; MID 192.4; HIGH 209.8; P = 0.08) plasma concentrations for HIGH compared to MID. Plasma concentrations of glucose, creatine, and GH did not differ (P > 0.05) among treatments. Carcass characteristics did not differ (P > 0.05) among treatments (Table 3.7).

Relationship between Residual Feed Intake, Performance, and Carcass Characteristics.

Growing Phase RFI was correlated ($P < 0.05$) with RFI-FP ($r = 0.34$), RFI-TP ($r = 0.84$), growing phase DMI ($r = 0.56$) and FCR ($r = 0.71$), total period DMI ($r = 0.45$) and FCR ($r = 0.55$), LM area (cm^2/45.4 kg; $r = -0.22$) and intramuscular fat ($r = 0.27$; table 3.8). Body weight (initial, growing and final), total period DMI and ADG were not influenced ($P > 0.05$) by RFI-GP (Table 3.9).

Growing phase DMI increased as RFI-GP increased (-RFI 8.34; AVE 8.96; +RFI 9.89; $P < 0.01$). Total period DMI increased as RFI-GP increased (-RFI 8.96; AVE 9.56; +RFI 10.08; $P < 0.01$). Feed conversion ratio during growing phase (-RFI 4.85; AVE 5.07; +RFI 5.72; $P < 0.01$), finishing phase (-RFI 6.47; AVE 6.31; +RFI 7.10; $P = 0.01$), and total period (-RFI 5.59; AVE 5.66; +RFI 6.35; $P < 0.01$) increased as RFI-GP increased. Growing phase RFI was different among RFI phenotypes (-RFI -0.80; AVE 0.00; +RFI 0.83; $P < 0.01$). Finishing phase RFI (-RFI -0.37; AVE 0.01; +RFI 0.37; $P < 0.01$), and total period (-RFI -0.63; AVE 0.02; +RFI 0.63; $P < 0.01$) increased as RFI-GP increased (Table 3.9). Carcass characteristics was not influenced by RFI-GP ($P > 0.05$; Table 3.10).

Finishing phase RFI was correlated ($P < 0.05$) with RFI-TP ($r = 0.78$),growing phase FCR ($r = 0,32$), finishing phase DMI ($r = 0.65$) and FCR ($r = 0.54$), total period DMI ($r = 0.43$) and FCR ($r = 0.54$), LM area (cm^2/45.4 kg; $r = -0.29$) and intramuscular fat ($r = 0.22$; table 3.8). Body weight (initial, growing and final) and ADG were not influenced ($P > 0.05$) by RFI-FP (table 3.11).

Dry matter intake during finishing (-RFI 8.56; AVE 9.97; +RFI 10.63; $P < 0.01$) and total period (-RFI 8.78; AVE 9.47; +RFI 10.12; $P < 0.01$) increased as RFI-FP increased. Feed conversion ratio during growing (-RFI 4.92; AVE 5.12; +RFI 5.50; $P < 0.01$), finishing (-RFI 5.90; AVE 6.37; +RFI 7.41; $P < 0.01$), and total period (-RFI 5.42; AVE 5.71; +RFI 6.32; $P < 0.01$) increased as RFI-FP increased. Finishing phase RFI was different among RFI phenotypes (-RFI -1,14; AVE 0.01; +RFI 0.79; $P < 0.01$). Growing phase RFI (-RFI -0.25; AVE -0.11; +RFI 0.31; $P < 0.01$), and total period (-RFI -0.64; AVE -0.05; +RFI 0.51; $P < 0.01$) increased as RFI-FP increased Ttable 3.11). Longuissimus muscle area (-RFI 4.78; AVE 4.67; +RFI 4.44; $P = 0.05$) decreased as RFI-FP increased. Intramuscular fat and 12th rib backfat depth did not differ ($P > 0.05$) among RFI-FP phenotypes (Table 3.12).

Total period RFI was correlated ($P < 0.05$) with growing phase DMI (r = 0,47) and FCR ($r = 0.61$), finishing phase DMI ($r = 0.51$) and FCR ($r = 0.42$), total period DMI ($r = 0.55$) and FCR ($r = 0.63$), LM area (cm^2/45.4 kg; $r = -0.30$) and intramuscular fat ($r = 0.29$; Table 3.8). Body weight (initial, growing and final) and ADG were not influenced ($P > 0.05$) by RFI-TP (Table 3.13).

Dry matter intake during growing (-RFI 8.28; AVE 8.86; +RFI 9.84; $P < 0.01$), finishing (-RFI 8.82; AVE 9.96; +RFI 10.50; $P < 0.01$) and total period (-RFI 8.62; AVE 9.47; +RFI 10.26; $P < 0.01$) increased as RFI-TP increased. Feed conversion ratio during growing (-RFI 4.77; AVE 5.04; +RFI 5.70; $P < 0.01$), finishing (-RFI 6.25; AVE 6.22; +RFI 7.29; $P < 0.01$), and total period (-RFI 5.44; AVE 5.62; +RFI 6.40; $P < 0.01$) increased as RFI-TP increased. Total period RFI

was different among RFI phenotypes (-RFI -0.80; AVE -0.02; +RFI 0.67; $P < 0.01$). Residual feed intake during growing (-RFI -0.75; AVE -0.04; +RFI 0.67; $P < 0.01$), and finishing phase (-RFI -0.82; AVE -0.03; +RFI 0.67; $P < 0.01$) increased as RFI-TP increased (Table 3.13). Longuissimus muscle area and 12th rib backfat depth did not differ ($P > 0.05$) among RFI-TP phenotypes, however, intramuscular fat was greater for AVE compared to -RFI (-RFI 3.66; AVE 4.07; +RFI 3.99 %; $P = 0.03$; Table 3.14).

DISCUSSION

Dietary Effects on Performance.

Dietary treatments were formulated using a model developed in our laboratory. In order to understand and interpret the data collected from the study, performance data was incorporated into the model. Incorporating the performance data collected into the model, we observed predicted postruminal AA, as a percent of requirement, was greater than expected.

One of the answers why postruminal AA supply was greater might be the intake. Diets are formulated based on a set growth rate and DMI. Intakes were estimated to be 2.2% BW (DMI using average BW), however steers consumed 2.8% BW. Since DMI was greater than expected, postruminal AA supply increased, and dietary treatments, once fixed at 80, 100 and 120% of the most limiting AA, were meeting or exceeding the requirements.

Another answer might be how our diet formulation model works. To determine ruminant's dietary AA needs, microbial AA flow must be estimated. Microbial AA flow from the rumen is a function of how efficiently microbes use the energy generated from fermentation. Our model uses equations developed by Meng et al. (1999) to calculate microbial efficiency (MOEFF). Using continuous culture fermenters, they developed MOEFF equations for fibrous carbohydrate diet, consisted of soybean hulls as a sole ingredient; nonfibrous carbohydrate diet, consisted of 78% ground corn, 14% soybean hulls, and 8% isolated soy protein (DM basis); and protein diet consisted of isolated soy protein as a sole ingredient. The model uses two dilution rate values, one for liquids, and a second for solids, however, with solids particle sizes range, the ingredient used by Meng, (soybean hulls), might not be very representative to the type of fibrous carbohydrate used in the study (alfalfa haylage).

We hypothesized increasing growing phase diet RUP, using roughage-based diets, would improve feed efficiency and impact growth performance during growing and finishing phase. Although BW and DMI were not affected by dietary treatments, ADG had a response during the first 21 days. Feed conversion ratio was not affected when steers received dietary treatments (GP), however during FP when all the steers received the same diet, balanced for postruminal AA, HIGH had better FCR than MID.

Several studies analyze the effect of RUP on performance, however none of them were planned to be isocaloric and isonitrogenous as the current study. Steers with average BW 302 kg and fed a roughage-based diet had a quadratic

response in G:F to protein levels when fed RUP or RDP, however RUP vs. RDP was not different. (Huntington et al., 2001).

Zinn and Owens (1993) using 28% roughage in the diet, and lightweight steers (198 kg), observed increased gain rate and efficiency by 13.4 and 8.4% respectively when fed a combination of blood, feather and meat and bone meal. Knaus et al. (2002) also reported increased ADG when included RUP, resulting in better FCR using about 28% roughage in the diet fed to heavier (251 kg) Holstein steers.

Richardel (2004) and Sissell (2007), both used lightweight Holstein steers fed diets containing 53 and 30% roughage respectively. Feeding diets greater than 16% CP with or without fish meal, did not improve performance in weaned dairy calves, may be due to the low amount of fish meal that did not increase RUP in the diet (Richardel, 2004). Feeding isonitrogenous diets supplemented with sources of RUP did not improve performance in steers at 16% CP with RUP being 45% of CP (Sissell, 2007).

Partial replacement of protein from soybean meal with fish meal did not affect growth rate or efficiency in early weaned rapidly growing lambs fed high concentrate diet (Dabiri and Thonney, 2004). No difference in FCR was observed when steers (395 kg) were fed higher RUP also using high concentrated diets (Wagner et al., 2010).

Steers (205 kg) fed protein restricted roughage-based diet, had postruminal AA flow increased when casein was infused, which increased muscle accretion (Reecy et al., 1996). Growing beef calves (277 kg)

supplemented with sulfur AA from corn gluten meal improved ADG compared to control using roughage-based diets (Hersom et al., 2009).

Lehmkuhler (2001) reported six experiments testing different RUP levels and sources. Increasing the AA:energy ratio, did not significantly affect performance parameters for newly feedlot steers (238 kg) fed a diet containing about 30% hay. Although not statistically different, in the first 21 days on feed, the inclusion of blood and fish meal provided a numerical advantage in ADG without impacting intake for steers (347 kg) fed high concentrate diets. Steers (295 kg) ADG increased during the first 14 d on feed when blood and fish meal was top-dressed at 6 and 12% of the diet DM in comparison to the other three levels (0, 1.5 and 3%). Heavy steers (425 kg) average weight, fed high concentrate, after 56 d on feed was greatest for the 2% blood and fish meal treatment in comparison to the remaining (0, 1, 2, 4% blood and fish meal), reflecting in a cubic trend for G:F. Lehmkuhler (2001) found linear trend for increasing levels of blood meal in overall (growing and finishing) steers G:F (206 kg) fed high concentrate.

Mueller et al. (2004), Davis (2009) and Golden (2013) found RUP responses in performance using roughage free diet. Mueller et al. (2004) testing one diet balanced for postruminal AA and three others exceeding (all isocaloric), found that the balanced diet had better ADG and FCR with no difference in DMI. Davis (2009) reported that increasing bypass AA in the diet improved feed efficiency without altering feed intake. Golden (2013) also observed growth and

feed efficiency improvement when diets were formulated to meet feedlot steers absorbable AA requirements (284 kg).

Studies reporting beef cattle feed efficiency response to increasing postruminal AA supply have been contradictory. Difference in diet type such as high or no inclusion of roughage, growth phase (BW range) and also if the diets were formulated to be isocaloric and isonitrogenous may be some of the factors affecting RUP response. To maximize lean tissue growth, AA available for growth needs to be in proportion to energy available for growth (AA:energy). Which means that improvement on feed efficiency is achieved when nutrients are supplied to match growth potential supported by energy density of the diet consumed.

Dietary Effects on Blood Metabolites

We hypothesized increasing GP diet RUP would affect plasma metabolites and hormones concentrations. Concentrations of PUN were greater for HIGH compared to LOW and MID. Leptin trended greater plasma concentrations for LOW compared to MID while IGF-I trended greater plasma concentrations for HIGH compared to MID. Plasma concentrations of glucose, creatine, and GH were not affected when steers were receiving dietary treatments.

Studies analyzing blood metabolites response to increasing postruminal AA supply in ruminants have been contradictory. Davenport et al. (1990), using heifer calves (233 kg), observed greater blood urea nitrogen when arginine was

infused compared to control. Arginine abomasal infusions did not affect serum glucose and GH amplitude or frequency pulses, however mean and basal GH concentrations were elevated when heifers were infused with higher levels of arginine. Reecy et al. (1996) reported casein and glutamine abomasal infusion increased IGF-I concentrations, and in addition to that, casein infusion tended to increase PUN compared with water infusion when protein restricted hay-based diet was fed to steers. Plasma urea nitrogen were increased when abomasal AA infusions were administrated to Holstein steers (205 kg) eating soybean hulls-based diet (Awawdeh et al., 2006). Arginine infusion increased plasma GH levels, but not IGF-I, while casein infusion increased IGF-I levels, and did not affect GH in Holstein steers calves (159 kg) consuming roughage-based diet (Ragland-Gray et al., 1997).

Bateman II et al. (1999) feeding isonitrogenous diets using different protein sources to lactating Holstein cows, averaging 55 d on lactation, observed similar PUN and glucose plasma concentration across diets. Mature ewes consuming low quality forage had greater serum urea nitrogen on protein supplemented vs. control, however serum glucose and GH concentrations were not influenced by treatment (Swanson et al., 2000).

Knaus et al. (2002) using Holstein steers (251 kg) observed greater PUN concentrations when urea, isolated soy protein or isolated soy protein in addition to blood meal were added in the diet but these three treatments had no effect on glucose. Later, also testing different protein sources, Richardel (2004) reported a

tendency for greater mean GH levels for soybean meal compared to fish meal, however IGF-I and PUN concentrations were not affected.

Holstein steers and angus heifers did not demonstrate a response to protein source on PUN, GH and IGF-I plasma concentrations (Sissell, 2007; Koenig and Beauchemin, 2013). Davis (2009) also did not observe differences in serum glucose, insulin, and IGF-I from steers (385 kg) receiving no roughage diets differing in bypass AA level. However, Lehmkuhler (2001) feeding no roughage diets, with different protein sources (soybean meal, blood meal, and soybean meal in addition to arginine) to steers (227 kg), observed greater IGF-I serum concentrations for blood meal compared to soybean meal plus arginine. Although glucose and GH concentrations were not significant different among treatments, steers receiving blood meal had a numerical 6% increased GH compared to soybean meal. Steers receiving soybean meal treatment responded with greater PUN compared to blood meal, and soybean meal in addition to arginine treatments.

Plasma urea nitrogen was the only metabolite responding significantly to dietary treatments. Steers receiving HIGH treatment had the lowest performance during growing phase. I addition to that, PUN concentration was greater, which suggests that at least a portion of the RUP was not only absorbed, but also catabolized, indicator of excess RUP. MID was predicted having excessing AA, however PUN levels did not differ compared to LOW. This lack of difference cloud be explained by AA been used as energy source. Because AA such as

Arg, Val, His, Met, Iso, Thr and Phe are glucogenic, AA carbon skeletons could be used as energy supply for the cost of urea metabolism.

Dietary Effects on Carcass Characteristics

We hypothesized increasing growing phase diet RUP would impact carcass characteristics in consequence to a difference in performance. Since performance was not affected, carcass characteristics did not respond to higher dietary RUP levels.

Increasing RUP resulted in greater LM area, and trended a decrease in marbling score in yearling heave steers (395 kg), however no difference was found in HCW, dressing percentage or 12th rib backfat depth (Wagner et al., 2010). Previous research from our laboratory have also found response from RUP in carcass characteristics (Lehmkuhler, 2001; Legleiter, 2003; Mueller, 2004; Davis, 2009; Golden, 2013).

Legleiter (2003) fed increasing levels of arginine to steers for 72 days. Diets were isocaloric and had the same RDP, so ammonia nitrogen, AA and peptides were not limiting in the rumen. Steers presented greater LM area when fed 1 and 1.5 times requirements for arginine compared to 0.5 and 2 times requirement. The same study did not observe difference in 12th rib backfat depth among treatments. No differences were noted in HCW, 12th rib backfat depth or intramuscular fat with increasing supply of arginine, however, LM area were greater for steers receiving the highest (115% of the requeriment) supply of arginine (Golden, 2013). Mueller (2004) fed increasing levels of blood meal to

provide greater RUP to steers (317 kg). He observed a linear effect of blood meal inclusion on LM area, with the diet balanced for postruminal AA:energy, having the greater growth. Davis (2009) reported as postruminal AA increased, intramuscular fat and 12th rib backfat depth decreased, while LM area increased. In agreement with the present study, Lehmkuhler (2001) did not observe a response to RUP inclusion in the diet for any carcass characteristics measurements.

Studies has shown postruminal AA supply potential to improve lean tissue growth and change body composition. However, the ability to alter carcass characteristics by altering the supply of postruminal AA was not present in this study.

Relationship between Residual Feed Intake, Performance, and Carcass Characteristics.

In agreement with the current study, RFI has shown correlation with others feed efficiency measurements. Therefore, improvement in RFI will result FCR or G:F improvement also(Arthur et al., 2001; Basarab et al., 2003; Brown, 2005; Nkrumah et al., 2006; Davis, 2009; Lancaster et al., 2009; Kelly et al., 2010b; Gomes et al., 2012; Kayser and Hill, 2013).

Residual feed intake calculated for all periods negative correlated to LM area and positive correlated to intramuscular fat. Contradicting the majority, some studies did not find a correlation between RFI and body composition or carcass characteristics. Some of the reasons for the lack of significance might be

the cattle breed used, such as Nellore, which has a different carcass characteristics from the majority cattle used in RFI tests (Gomes et al., 2012). Davis (2009) also did not observed a correlation. They reported possible discrepancy between their study and others may be due to time ultrasound carcass measurements, which were made at 116 d instead of the last trial day (126 d). If ultrasound was done in the beginning and last day of the trial, differences in body composition change among RFI phenotypes may be significant. Fitzsimons et al. (2014a) in agreement to Davis (2009) and Gomes et al. (2012) reported that contrasting results for carcass fatness traits may arise from different measures of classifying carcass fatness. The different methods cited were EUROP method of allocating carcass fat score, which was used in the study, and the Livestock and Poultry Carcass Grading Regulations, Canada. No differences in ultrasonic measures of body composition and visual muscular scores between RFI groups was explained by Fitzsimons et al. (2013) as a consequence of low energy diet offered, which potentially limited expression of genetic potential for growth and fattening.

Kelly et al. (2010a) found RFI positive correlated to rump and lumbar fat thickness during growing phase of beef heifers, however after finishing phase those results were not significant different and none of the body composition characteristics were correlated to RFI (Kelly et al., 2010b).

Arthur et al. (2001) found 12th rib backfat and rump fat depth to have positive genetic correlations with RFI in beef weanling bulls and heifers. Basarab et al. (2003) observed positive correlations of 12th rib backfat depth and

intramuscular fat with RFI in growing steers. Brown (2005) also reported RFI been positive correlated to 12th rib backfat depth, however, not correlated to intramuscular fat and LM area. Yearling bulls from several beef breeds were reported to have genetic correlations between RFI, scanned 12th rib backfat depth and intramuscular fat (Schenkel et al., 2004). Robinson and Oddy (2004) found strong genetic correlations between RFI, 12th rib backfat and rump fat depth, but weak correlations with intramuscular fat in feedlot steers and heifers. Residual feed intake of growing bulls were positive correlated to 12th rib backfat depth, LM area, but not with intramuscular fat (Lancaster et al., 2009). Positive phenotypic correlations between RFI, 12th rib backfat, lumbar fat and muscle depth have been have been reported in tested pedigree beef bulls (Kelly et al., 2011). Kayser and Hill (2013) found positive correlations between RFI and ultrasound fat thickness, however not to intramuscular fat and LM are using Angus and Hereford bulls.

Pregnant beef cows RFI have been reported positive correlated to ultrasound backfat, rump fat and muscle depth (Fitzsimons et al., 2014b). Lawrence et al. (2011) observed positive correlations between RFI and ultrasound muscle depth, however not to ultrasound fat depth in pregnant beef heifers. Beef steers divergently selected for RFI showed correlation between chemical composition and genetic RFI variation, with steer progeny of -RFI parents having less whole-body fat and more whole-body protein than progeny of +RFI parents (Richardson et al., 2001).

Although all three RFI calculated were correlated, partial correlation coefficient were not similar. Total period RFI had about 7% greater correlation coefficient with growing than with finishing phase RFI. Growing phase RFI was about 60% more correlated to total period than with finishing phase RFI. Steers were fed different diets during growing and finishing phase (roughage vs. corn-based diet), this can be one of the reasons why an steers classified efficient during growing, was not necessary in the same category during finishing phase. In addition different growing period, (growing vs. finishing has been shown to effect RFI). Davis (2009) using feedlot steers, observed a correlation between RFI calculated during growing and finishing phase (r = 0.39). Durunna et al. (2011) investigated if diet type influences feed efficiency ranking, if feed efficiency phenotype changes and the correlation estimates between the two feeding regimens. They observed switching diets as well as feeding period or stage of maturity affects feed efficiency and feed efficiency ranking of steers. The majority of the steers did not maintain previous feed efficiency classes in the second period. The correlation estimates between the two feeding periods for all test groups were low (r = 0.42), but were less for the feed-swap group (r = 0.33). Residual feed intake had greatest correlation between the second period for the majority of the groups.

CONCLUSION

Increasing growing phase diet RUP, using roughage-based diets, may impact feed efficiency during growing and finishing phase. Calves consuming diets with postruminal AA supplied above requirements may respond with greater compensatory growth as occurred in this study, however improved growth was not sustained over the feeding period. Hormones and metabolites were not affected by postruminal AA in this study except plasma urea nitrogen. Postruminal AA requirements was not different among RFI phenotypes.

As RFI decreased, steers consumed less feed and were more efficient with no change in BW during all periods. Residual feed intake calculated for different periods were correlated, however steers classified as efficient during growing phase were not necessary in the same category during finishing phase. Growing phase may be the best period to test efficiency in steers fed roughage-based diet.

Table 3.1. Dietary nutrient composition fed during receiving period

Item	(% AF)
Base diet	
Corn	55.00
Supplement	35.00
Hay	10.00
Supplement Composition	
Corn	31.10
Soybean meal	21.43
Dried Distillers Grains with Soluble	21.43
Blood meal	12.86
Limestone	3.51
Dyna-K[1]	3.14
Choice White Grease	2.29
NaCl	1.43
Vitamin Premix[2]	0.14
Trace Mineral Premix[3]	0.23
Cocci Curb 10x[4]	1.43
Aureomycin 50[5]	0.16
Pellet binder	0.86

[1]Dyna-K (The Mosaic Company, Plymouth, MN) contained 95% potassium chloride, 0.3% sodium chloride 0.2% calcium and magnesium chlorides and sulfates.

[2]Vitamin Premix contained 8,818,490.5 IU/kg of vitamin A, 1,763,698.1 IU/kg of vitamin D3, 1,102.3 IU/kg of vitamin E.

[3]Trace Mineral Premix contained 24% Ca, 2.5% Fe, 2% Mn, 3% Zn, 1% Cu, 100 ppm Se, 500 ppm I, and 100 ppm Co.

[4]Cocci Curb 10x (Nutra Blend, Neosho, MO).

[5]Aureomycin 50 (Zoetis INC., Kalamazoo, MI) contained 111.11 g/kg of chlortetracycline.

Table 3.2. Dietary nutrient composition fed during growing phase

Item (% DM)	Treatments		
	LOW	MID	HIGH
Ingredients			
Haylage-Alfalfa	30.00	29.97	30.00
Dried Distillers Grains with Soluble	31.12	16.22	10.83
Soyhulls	25.45	34.22	31.93
Corn	12.92	12.91	12.93
Aminoplus[1]	0.13	4.28	11.64
Blood meal	0.00	1.98	2.19
MFP-Novus[2]	0.00	0.03	0.11
Vitamin Premix[3]	0.12	0.13	0.13
Trace Mineral Premix[4]	0.15	0.15	0.15
Vitamin E[5]	0.09	0.09	0.09
Rumensin 90[6]	0.01	0.01	0.01
Nutrient Composition			
DM, %	67.87	67.73	67.75
CP, % DM	20.17	21.13	21.18
ME, Mcal/kg DM	2.74	2.69	2.70
NDF, % DM	22.43	23.40	22.84
ADF, % DM	12.38	13.99	13.94

[1]Aminoplus (AGP, Omaha, NE) contained CP 51.14% DM, RUP 72% CP, RDP 28% CP.

[2]MFP (Novus International, INC., ST. Charles, MO) contained 84% methionine activity and 40% rumen bypass.

[3]Vitamin Premix contained 8,818,490.5 IU/kg of vitamin A, 1,763,698.1 IU/kg of vitamin D3, 1,102.3 IU/kg of vitamin E.

[4]Trace Mineral Premix contained 24% Ca, 2.5% Fe, 2% Mn, 3% Zn, 1% Cu, 100 ppm Se, 500 ppm I, and 100 ppm Co.

[5]Vitamin E contained 44,000 IU/kg.

[6]Rumensin 90 (Elanco, Greenfield, IN) Monesin, USP Granulated.

Table 3.3. Dietary nutrient composition fed during finishing phase

Item	(%DM)
Ingredient	
Corn	68.00
Dried Distillers Grains with Soluble	25.71
Aminoplus[1]	1.77
Blood meal	1.57
Urea	0.46
Sodium Chloride	0.11
Limestone	1.96
Tallow	0.08
Vitamin Premix[2]	0.03
Trace Mineral Premix[3]	0.06
Vitamin E[4]	0.23
Rumensin 90[5]	0.01
Nutrient Composition	
DM, %	87.03
CP, % DM	16.49
ME, Mcal/kg DM	3.07
NDF, % DM	16.01
ADF, % DM	4.01

[1]Aminoplus (AGP, Omaha, NE) contained CP 51.14% DM, RUP 72% CP, RDP 28% CP.

[2]Vitamin Premix contained 8,818,490.5 IU/kg of vitamin A, 1,763,698.1 IU/kg of vitamin D3, 1,102.3 IU/kg of vitamin E.

[3] Trace Mineral Premix contained 24% Ca, 2.5% Fe, 2% Mn, 3% Zn, 1% Cu, 100 ppm Se, 500 ppm I, and 100 ppm Co.

[4]Vitamin E contained 44,000 IU/kg.

[5]Rumensin 90 (Elanco, Greenfield, IN) Monesin, USP Granulated.

Table 3.4. Body weight least square means among steers fed different bypass amino acids levels during growing phase

	Treatments				
Days	LOW	MID	HIGH	SEM	*P*-value
IBW[1]	242.65	244.43	246.86	9.56	0.66
21	272.70	281.33	280.67	9.56	0.37
42	312.77	316.10	313.16	9.56	0.73
63	362.77	363.75	359.36	9.56	0.72
84[3]	395.83	390.93	388.47	9.56	0.44
105	431.86	427.24	422.20	9.56	0.31
126	457.55	458.14	454.70	9.56	0.76
148	491.54	486.74	485.38	9.56	0.52
FBW[2]	521.46	515.49	516.40	9.56	0.60

[1] IBW = initial BW.

[2] FBW = final BW.

[3] Day 83 is the end of growing phase and initial of finishing phase.

[a, b, c] least square means within a row with different superscript differ ($P < 0.05$)

Table 3.5. Feed efficiency and production traits least square means among steers fed different bypass amino acids levels during growing phase

	Treatments				
Item	LOW	MID	HIGH	SEM	*P*-value
	Dry matter intake				
GP[1], DM %	9.27	9.00	8.88	0.26	0.29
FP[2], DM %	9.84	10.11	9.62	0.26	0.19
TP[3], DM %	9.63	9.61	9.33	0.23	0.38
	Average daily gain				
21 d, kg/d	1.43[b]	1.76[a]	1.62[ab]	0.12	**0.01**
42 d, kg/d	1.67	1.71	1.58	0.12	0.27
84 d[4], kg/d	1.82	1.74	1.69	0.05	0.50
105 d, kg/d	1.80	1.74	1.67	0.04	0.27
126 d, kg/d	1.71	1.70	1.65	0.04	0.63
148 d, kg/d	1.98	1.92	1.89	0.05	0.49
167 d, kg/d	1.67	1.62	1.61	0.04	0.29
FP[5], kg/d	1.51	1.50	1.54	0.05	0.57
	Feed:Gain				
GP	5.14	5.17	5.29	0.18	0.57
FP	6.61[ab]	6.92[a]	6.30[b]	0.18	**0.02**
TP	5.80	5.95	5.79	0.13	0.38

[1]GP = growing phase.

[2]FP = finishing phase.

[3]TP = total period.

[4] ADG 84 = ADG from day 0 to 84, which correspond to growing phase.

[a, b, c] least square means within a row with different superscript differ ($P < 0.05$).

Table 3.6. Plasma metabolites and hormones among steers fed different bypass amino acids levels during growing phase

	Treatments				
Item	LOW	MID	HIGH	SEM	*P*-value
Glucose mg/dL	87.27	88.09	88.36	2.29	0.74
Urea[b] mg/dL	17.36[b]	16.50[b]	20.45[a]	0.49	**< 0.01**
Creatine mg/dL	0.96	0.97	0.95	0.02	0.40
Leptin ng/ml	4.12	3.14	3.75	0.36	0.06
IGF-I ng/ml	202.90	192.39	209.79	6.96	0.08
GH ng/ml	17.77	22.66	24.29	3.89	0.27

[a, b, c] least square means within a row with different superscript differ ($P < 0.05$).

Table 3.7. Carcass characteristics least square means among steers fed different bypass amino acids levels during growing phase

	Treatments				
Item[1]	LOW	MID	HIGH	SEM	*P*-value
HCW, kg	308.09	307.78	312.3	5.28	0.54
REA, cm^2/45.4 kg	4.63	4.57	4.70	0.10	0.39
BF, cm	1.12	1.19	1.14	0.08	0.55
IMF, %	3.99	3.89	3.92	0.13	0.58

[1]REA= LM area; BF= 12th rib backfat depth; IMF= Intramuscular fat.

[a, b, c] least square means within a row with different superscript differ ($P < 0.05$).

Table 3.8. Partial correlation coefficients[a] between feed efficiency measures, production traits and carcass characteristics during the growing, finishing and total period of steers

Traits[b]	RFI-GP	RFI-FP	RFI-TP
RFI-FP	**0.34**		
RFI-TP	**0.84**	**0.78**	
IBW	-0.02	0.03	-0.02
BW 84 d	-0.01	-0.03	-0.02
FBW	-0.02	-0.02	-0.01
DMI-GP	**0.56**	0.18	**0.47**
DMI-FP	0.20	**0.65**	**0.51**
DMI-TP	**0.45**	**0.43**	**0.55**
FCR-GP	**0.71**	**0.32**	**0.61**
FCR-FP	0.20	**0.54**	**0.42**
FCR-TP	**0.55**	**0.54**	**0.63**
ADG-GP	0	-0.08	-0.01
ADG-FP	-0.02	0	0.01
ADG-TP	-0.02	-0.05	0
REA	**-0.22**	**-0.29**	**-0.30**
BF	0.02	0.04	0.02
IMF	**0.27**	**0.22**	**0.29**

[a]Correlation coefficients in bold are significant ($P < 0.07$) from 0.

[b]Trait abbreviations: RFI-GP, RFI-FP and RFI-TP = residual feed intake during growing, finishing and total period respectively; IBW = initial BW; BW-84 = BW during growing phase; FBW = final BW; DMI-GP, DMI-FP and DMI-TP = DMI during growing, finishing and total period respectively; FCR-GP, FCR-FP and FCR-TP = feed:gain during growing, finishing and total period respectively; ADG-GP, ADG-FP and ADG-TP = ADF during growing, finishing and total period respectively; REA = LM area; BF = 12th rib backfat depth; IMF = intramuscular fat.

Table 3.9. Production traits least-square means among growing phase residual feed intake (RFI) phenotypes

	RFI-GP [1]				
Items[2]	-RFI	AVE	+RFI	SEM	*P*-value
		Growing Phase			
IBW	250	239	245	4.66	0.07
BW-84	395	388	392	7.21	0.47
DMI	8.34[c]	8.96[b]	9.89[a]	0.25	**<0.01**
ADG	1.72	1.77	1.75	0.05	0.47
FCR	4.85[b]	5.07[b]	5.72[a]	0.11	**<0.01**
RFI-GP	-0.80[c]	0.00[b]	0.83[a]	0.06	**<0.01**
		Finishing Phase			
FBW	518	521	512	8.36	0.39
DMI	9.48	10.03	10.04	0.26	0.13
ADG	1.49[ab]	1.61[a]	1.45[b]	0.05	**0.02**
FCR	6.47[b]	6.31[b]	7.10[a]	0.22	**0.01**
RFI-FP	-0.37[a]	0.01[ab]	0.37[b]	0.16	**0.01**
		Total Period			
DMI	8.96[b]	9.56[a]	10.08[a]	0.23	**<0.01**
ADG	1.61	1.69	1.60	0.03	0.07
FCR	5.59[b]	5.66[b]	6.35[a]	0.11	**<0.01**
RFI-TP	-0.63[c]	0.02[b]	0.63[a]	0.08	**<0.01**

[1]Steers were allocated to RFI group based on RFI calculated using data collected during growing phase. -RFI = RFI was 0.5 SD below the mean; AVE = RFI was ±0.5 SD above or below the mean; +RFI = RFI was 0.5 SD above the mean.

[2]Trait abbreviations: IBW = initial BW; BW-84 = BW during growing phase; FBW = final BW; FCR = feed:gain; RFI-GP = RFI during growing phase; RFI-FP = RFI during finishing phase; RFI-TP = RFI during total period.

[a, b, c] least square means within a row with different superscript differ (P < 0.05).

Table 3.10. Carcass characteristics least-square means among growing phase residual feed intake (RFI) phenotypes

	RFI-GP				
Item[1]	-RFI	AVE	+RFI	SEM	*P*-value
REA, cm^2/45.4 kg	4.7	4.6	4.57	0.13	0.38
BF, cm	1.24	1.04	1.19	0.08	0.07
IMF, %	3.74	4.01	4.03	0.13	0.12

[1]REA= LM area; BF= 12th rib backfat depth; IMF= Intramuscular fat.

[a, b, c] least square means within a row with different superscript differ (P < 0.05).

Table 3.11. Production traits least-square means among finishing phase residual feed intake (RFI) phenotypes

	RFI-FP [1]				
Items[2]	-RFI	AVE	+RFI	SEM	*P*-value
		Growing Phase			
IBW	242	241	250	5.34	0.14
BW-84	392	387	396	8.18	0.35
DMI	8.76	8.85	9.49	0.3	0.07
ADG	1.78	1.74	1.74	0.06	0.56
FCR	4.92^{b}	5.12^{b}	5.50^{a}	0.14	**<0.01**
RFI-GP	-0.25^{b}	-0.11^{b}	0.31^{a}	0.16	**<0.01**
		Finishing Phase			
FBW	513	519	518	9.56	0.65
DMI	8.56^{c}	9.97^{b}	10.63^{a}	0.23	**<0.01**
FCR	5.90^{b}	6.37^{b}	7.41^{a}	0.22	**<0.01**
ADG	1.46	1.59	1.47	0.04	0.09
RFI-FP	-1.14^{c}	0.01^{b}	0.79^{a}	0.09	**<0.01**
		Total Period			
DMI	8.78^{c}	9.47^{b}	10.12^{a}	0.24	**<0.01**
FCR	5.42^{b}	5.71^{b}	6.32^{a}	0.12	**<0.01**
ADG	1.62	1.66	1.61	0.04	0.45
RFI-TP	-0.64^{c}	-0.05^{b}	0.51^{a}	0.11	**<0.01**

[1]Steers were allocated to RFI group based on RFI calculated using data collected during finishing phase. -RFI = RFI was 0.5 SD below the mean; AVE = RFI was ±0.5 SD above or below the mean; +RFI = RFI was 0.5 SD above the mean.

[2]Trait abbreviations: IBW = initial BW; BW-84 = BW during growing phase; FBW = final BW; FCR = feed:gain; RFI-GP = RFI during growing phase; RFI-FP = RFI during finishing phase; RFI-TP = RFI during total period.

$^{a, b, c}$ least square means within a row with different superscript differ ($P < 0.05$).

Table 3.12. Carcass characteristics least-square means among finishing phase residual feed intake (RFI) phenotypes

	RFI-FP				
Item[1]	-RFI	AVE	+RFI	SEM	*P*-value
REA, cm^2/45.4 kg	4.78[a]	4.67[ab]	4.44[b]	0.12	**0.05**
BF, cm	1.14	1.19	1.14	0.1	0.63
IMF, %	3.76	3.92	4.07	0.15	0.12

[1]REA= LM area; BF= 12th rib backfat depth; IMF= Intramuscular fat.

[a, b, c] least square means within a row with different superscript differ ($P < 0.05$).

Table 3.13. Production traits least-square means among total period residual feed intake (RFI) phenotypes

	RFI-TP [1]				
Items[2]	-RFI	AVE	+RFI	SEM	*P*-value
Growing phase					
IBW	245	238	249	4.85	0.07
BW-84	391	386	395	7.69	0.35
DMI	8.28[b]	8.86[b]	9.84[a]	0.25	**< 0.01**
ADG	1.74	1.77	1.74	0.06	0.74
FCR	4.77[b]	5.04[b]	5.70[a]	0.11	**< 0.01**
RFI-GP	-0.75[c]	-0.04[b]	0.67[a]	0.1	**< 0.01**
Finishing Phase					
FBW	511	520	518	8.98	0.44
DMI	8.82[b]	9.96[a]	10.50[a]	0.25	**< 0.01**
ADG	1.44[b]	1.61[a]	1.48[b]	0.06	**0.02**
FCR	6.25[b]	6.22[b]	7.29[a]	0.22	**< 0.01**
RFI-FP	-0.82[c]	-0.03[b]	0.67[a]	0.08	**< 0.01**
Total Period					
DMI	8.62[c]	9.47[b]	10.26[a]	0.22	**< 0.01**
ADG	1.59	1.69	1.61	0.04	0.07
FCR	5.44[b]	5.62[b]	6.40[a]	0.11	**< 0.01**
RFI-TP	-0.80[c]	-0.02[b]	0.67[a]	0.06	**< 0.01**

[1]Steers were allocated to RFI group based on RFI calculated using data collected during total period. -RFI = RFI was 0.5 SD below the mean; AVE = RFI was ±0.5 SD above or below the mean; +RFI = RFI was 0.5 SD above the mean.

[2]Trait abbreviations: IBW = initial BW; BW-84 = BW during growing phase; FBW = final BW; FCR = feed:gain; RFI-GP = RFI during growing phase; RFI-FP = RFI during finishing phase; RFI-TP = RFI during total period.

[a, b, c] least square means within a row with different superscript differ (P < 0.05).

Table 3.14. Carcass characteristics least-square means among total period residual feed intake (RFI) phenotypes

	RFI-TP				
Item[1]	-RFI	AVE	+RFI	SEM	*P*-value
REA, cm^2/45.4 kg	4.83	4.57	4.55	0.13	0.12
BF, cm	1.07	1.19	1.17	0.08	0.33
IMF, %	3.66[b]	4.07[a]	3.99[ab]	0.14	**0.03**

[1]REA= LM area; BF= 12th rib backfat depth; IMF= Intramuscular fat.

[a, b, c] least square means within a row with different superscript differ (P < 0.05).

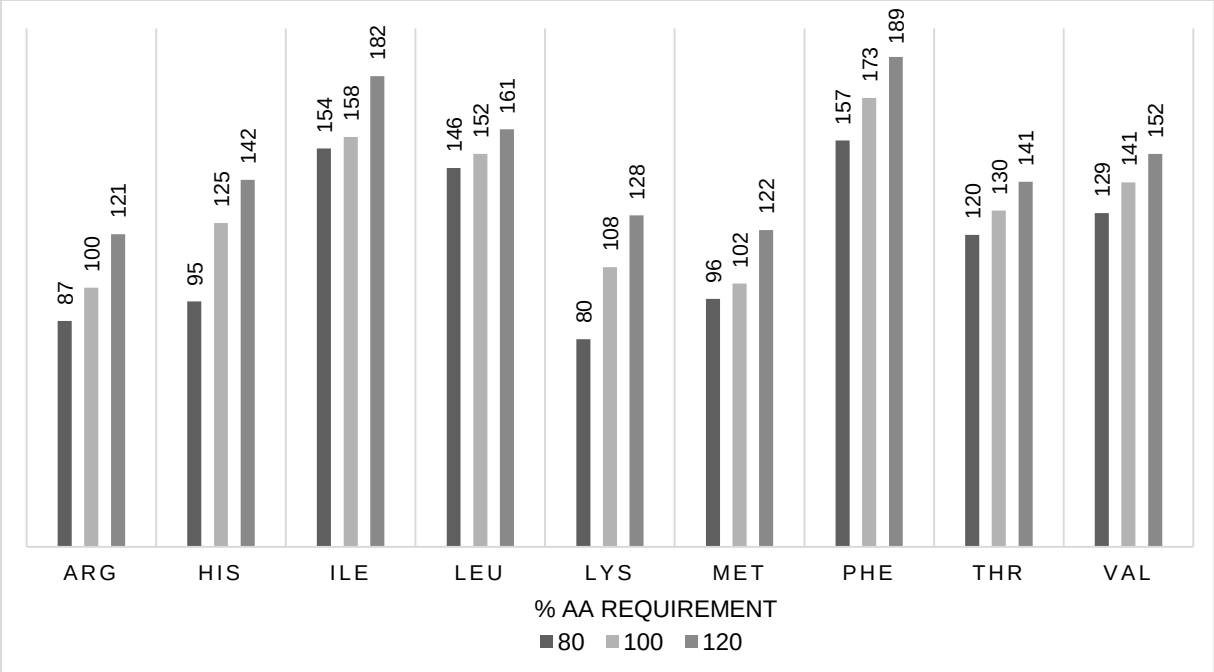

Figure 3.1. Postruminal AA fed during growing phase among treatments

CHAPTER IV

RELATIONSHIP BETWEEN RESIDUAL FEED INTAKE AND MITOCHONDRIAL COMPLEXES

ABSTRACT

Four experiments were performed to determine if residual feed intake (RFI) was related to mitochondrial complexes and protein measurements. Exp. 1 (*n* = 26), crossbred steers (initial BW 230 ± 8 kg) were fed no-roughage corn-based diet for 70 d. Exp. 2 (*n* = 24), crossbred steers (initial BW 300 ± 16 kg) were fed no-roughage corn-based diet for 70 d. Exp. 3 (*n* = 18), crossbred steers (initial BW 255 ± 7 kg) were fed alfalfa-haylage based diet for 83 d during growing phase (GP) and finished (FP) using no-roughage corn-based diets for more 83 d. Exp. 4 (*n* = 8), crossbreed steers (initial BW 300 ± 11 kg) were fed roughage-based diet for 70 d. Residual feed intake was calculated as the difference between predicted and actual DMI for each experiment. Predicted DMI was calculated by regressing DMI on ADG and metabolic midweight (MMWT). Blood was collected, lymphocytes were isolated, and antibody used to capture complex I and III (CI; CIII). Exp. 1, 2, 3, and 4 CI and CIII quantity were measured using ELISA commercial kit (Abcam, MA). Complex I subunits were separated by gel electrophoresis and bands were analyzed for differences in concentration (absorbance) among RFI phenotypes. For all four experiments,

there was a difference ($P < 0.05$) between RFI and DMI but no difference ($P > 0.05$) was observed for ADG and MMWT. Exp. 1, all mitochondrial measurements variables were not different ($P > 0.05$) between RFI phenotypes. Exp. 2, band I was greater ($P < 0.01$) and band II tended ($P = 0.10$) less concentration for +RFI compared to -RFI. Exp. 3, all mitochondrial measurements variables were not different ($P > 0.05$) between RFI phenotypes from different periods. Exp. 4, band VI trended ($P = 0.09$) less concentration for +RFI compared to -RFI. Exp. 1, coefficient of determination was 0.30 for the RFI model, and increased 20% when band I was included in regression as third variable ($R^2 = 0.36$). Exp. 2, coefficient of determination was 0.56 for RFI model, and increased 16% when band I was included in regression as third variable ($R^2 = 0.65$). Exp. 3, during GP coefficient of determination was 0.52 for RFI model, and increased 16% when band III was included in regression as third variable ($R^2 = 0.60$); during FP coefficient of determination was 0.65 for RFI model, and increased 6% when CIII was included in regression as third variable ($R^2 = 0.69$); during TP coefficient of determination was 0.70 for RFI model, and increased 8.5% when band III-GP, as well as 8.5% when complex I or complex I:III was included in regression as third variable ($R^2 = 0.76$). Exp. 4, coefficient of determination was 0.52 for RFI model, and increased 55.8% when band VI was included in regression as third variable ($R^2 = 0.81$). We concluded that mitochondrial function was at least in part responsible for differences among animals in metabolic efficiency.

INTRODUCTION

Residual feed intake (RFI) proposed by Koch et al. (1963) is calculated by regressing DMI against metabolic midweight (MMWT) and ADG. The difference between actual and predicted DMI is defined as RFI. Typically, ADG and MMWT explain over 60% of the phenotypic variation in feed intake (Carstens and Tedeschi, 2006).

Several studies reported RFI reflects inherent inter-animal variation in biological processes associated with feed efficiency. Some major processes by which efficiency variation can arise are: nutrient digestion, body composition, fermentation heat increment, energy expenditures associated with basal metabolism, protein turnover, physical activity, thermoregulation and feeding behavior (Richardson and Herd, 2004; Carstens and Kerley, 2009; Herd and Arthur, 2009; Fitzsimons et al., 2014b). A relationship between RFI and mitochondrial function is likely to exist since RFI variation is explained in part for cell metabolic process and mitochondria generates the majority of energy used for cell function.

Mitochondria generates approximately 90% of the total cellular energy (ATP) via oxidative phosphorylation (Nelson et al., 2008). Respiratory chain consists of five multisubunit enzyme complexes (I, II, III, IV and V) and two electron carriers, Coenzyme Q and cytochrome c. Complex I (CI), also called NADH:ubiquinone oxidoreductase, is a L-shaped protein with 46 subunits and a

molecular mass of 980 kDa (Hirst et al., 2003). Complex III (CIII; ubiquinol:cytochrome c oxidoreductase) have a mass of 450 kDa (Abdrakhmanova et al., 2006; Nelson et al., 2008). Complex I is the point of entry for the major fraction of electrons and CIII is where electrons from CI and CII are combined.

Bottje et al. (2002) reported lower respiratory chain coupling in low fed efficiency (FE) broilers may be due to lower CI and CII activities and defects in electron leak. Kolath et al. (2006b) isolated mitochondria from skeletal muscle and observed no difference in mitochondrial function but greater rate of mitochondrial respiration in -RFI steers compared to +RFI. Lancaster et al. (2014) reported greater respiration control ratio (RCR) values and state 3 respiration rate in liver mitochondria from -RFI heifers compared to +RFI, however, states 2 and 4 did not differ. All five respiratory chain complexes activity were greater for efficient animals compared to inefficient (Bottje and Carstens, 2009; Sharifabadi et al., 2012). Some proteins from all five complexes were different expressed in feed efficiency phenotype for breast muscle, duodenum and liver mitochondria (Iqbal et al., 2004; 2005; Ojano-Dirain et al., 2005c). Kolath et al. (2006a) reported no difference between the +RFI and -RFI animals in their expression of uncoupling protein 2 or 3 mRNA or protein using steers LM. Davis (2009) also using steers, reported CI protein concentrations in lymphocytes mitochondria tended to be negatively correlated and ratios of CI:II and CI:III were negatively correlated with RFI. Ramos and Kerley (2013)

reported greater CI concentration for - RFI compared to +RFI and a tendency for lower concentration of band I (from CI) in steers with -RFI.

Therefore, we hypothesized calf DMI was influenced by metabolic efficiency (RFI) and subsequently mitochondrial CI, CIII and subunits (from CI) could describe additional animal to animal DMI variation. The objective of this study was to determine if mitochondria CI, CIII and subunits (from CI) differed among RFI phenotypes and if mitochondria measurements could account for additional differences in DMI.

MATERIALS AND METHODS

Animal Management

Experimental animals use was approved by the University of Missouri Animal Care and Use Committee. Exp. 1 (*n* = 26), crossbred steers (initial BW 230 ± 8 kg) were fed a no-roughage corn-based diet (Table 4.1) for 70 d. Exp. 2 (*n* = 24), crossbred steers (initial BW 300 ± 16 kg) were fed a no-roughage corn-based diet (Table 4.2) for 70 d. Exp. 3 (*n* = 18), crossbred steers (initial BW 255 ± 7 kg) were fed alfalfa-haylage based diet (Table 4.3) for 83 d during growing phase (GP) and finished (FP) using no-roughage corn-based diets (Table 4.4) for more 83 d. Finally, Exp. 4 used (*n* = 8) crossbreed steers (initial BW 300 ± 11 kg) and fed roughage-based diet (Table 4.5) for 70 d.

Cattle were de-wormed (Cydectin, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO), tagged, weighed and vaccinated (Bovi-shield Gold 5, One Shot

Ultra 8, Zoetis, Florham Park, NJ). On arrival steers were placed in pens and offered *ad libitum* access to water and a receiving diet, at 2% BW, for 14 d before the feeding period. Experimental steers were implanted (Exp. 1, 2 and 4, Revalor-XS, Merck & CO., Summit, NJ; Exp. 3, Component, Elanco, Indianapolis, IN) and consecutive 2 d BW were taken at beginning and end of feeding period and used to calculate average metabolic BW ($BW^{0.75}$). Individual feed intake was electronically measured using GrowSafe (GrowSafe Systems, Airdrie, Canada).

All diet samples were analyzed for DM, ash (AOAC, 2006), nitorgen content (Model FP-428, Leco Co., St. Joseph, MI), and NDF was determined with an ANKOM200 Fiber Analyzer (ANKOM Technology, Macedon, NY, Goering and Van Soest, 1970).

Individual DMI, ADG and MMWT were used to predict DMI. Predicted DMI was calculated by using DMI as dependent variable, MMWT and ADG as independent variable, using PROC GLM (SAS Inst. Inc., Cary, NC). The model fitted was:

$$Y_i = B_0 + B_1\,(ADG) + B_2\,(MMWT)$$

Where Y_i = expected daily DMI on animal I; B_0 = the regression intercept; B_1 = partial regression coefficient on actual DMI on ADG; and B_2 = partial regression coefficient of actual DMI on MMWT. Residual feed intake was calculated during GP of Exp. 1, 2, 3 and 4, with additional calculation during Exp.

3 (FP and total period).Regression *coefficient of determination* were 0.30, 0.56, and 0.52 for Exp. 1, 2,and 4 respectively. Exp. 3 regression *coefficient of determination* were 0.52, 0.65, and 0.70 for GP, FP and total period respectively. To calculate RFI, predicted DMI was subtracted from measured DMI (Wang et al., 2006). Average and SD of RFI was calculated and one SD above the average was declared +RFI, and one SD below the average was declared -RFI. For all the experiments blood was collected to isolate mitochondrial lymphocytes.

Lymphocyte isolation

Lymphocytes were isolated from blood according to procedures of Kolath et al. (2006b) with modifications. Blood was collected via jugular venipuncture into acid citrate dextrose (ACD) vaccutainer tubes (Becton, Dickinson and Company; Franklin Lake, NJ) and stored at room temperature until lymphocyte isolation. Fifteen milliliters of blood was removed from two ACD vaccutainer tubes and placed into one Accuspin tube, which contained 15 ml of ficoll (Sigma-Aldrich, St. Louis, MO). Tubes were centrifuged at 1,000 x g for 20 min at room temperature.

Lymphocyte layers were removed to a 15 ml centrifuge tube (Corning, Corning, NY) and volume brought to 15 ml with phosphate buffered saline (PBS; 0.137 M NaCl, 0.0027 M KCl, 0.0022 M KH_2PO_4, 0.0097 M Na_2HPO_4, pH 7.4). The tube was centrifuged at 300 x g for 15 min at room temperature and supernatant was removed. Cells were suspended in 0.75 ml of PBS and stored at - 80°C until further analysis.

Immunocapture and complex I and III measurement

To disrupt cellular and mitochondrial membranes, lymphocyte homogenate was frozen and thawed with liquid nitrogen four times (Lassiter et al., 2006) and then held on ice. Total cell protein concentrations of each sample were determined using bicinchoninic acid colorimetric procedures (BCA, Pierce Biotechnology, Rockford, IL). Mitochondrial protein CI and CIII were obtained using Immunocapture Kit ab109711 and ab109800 respectively (abcam, Cambridge, MA).

Sample solublization: Three hundred microliters of lymphocyte homogenate were incubated with 30 µl of lauryl maltoside (n-dodecyl-ß-D-maltopyranoside; Mitosciences MS910) on ice for 30 min. The lymphocyte homogenate was centrifuged at 13,000 x g for 20 min at 4°C, the pellet containing cellular debris was discarded, protease inhibitor cocktail (P8340, Sigma, St. Luois, MO) was added in the mixture and kept on ice.

Immunoprecipitation: One hundred microliters of immunocapture beads (ab109711 and ab109800) were added in each sample mixed for 3 h by nutator at room temperature and leaved overnight at 4°C.

Elution: After immunopreciptation, beads were collected by centrifugation for 1 min at 1,000 x g. Beads were washed with wash buffer (0.137 M NaCl, 0.0027 M KCl, 0.0022 M KH_2PO_4, 0.0097 M Na_2HPO_4, pH 7.4 plus n-dodecyl-B-D-maltopyranoside) three times.

Elution from beads: Complex was eluted from beads by denaturant SDS (1% sodium dodecyl sulfate). Fifty microliters of SDS buffer elution was used to

suspend beads. After 15 min incubation with frequent agitation using nutator samples were centrifuged for 1 min at 1,000 x g. Supernatant, which have all purified complex, was collected. Complex I and III proteins were measure using BCA procedures.

Measurement of complex I subunits

Ten microliters of Tris-Glycine buffer (Jule Inc., Milford, CT) was added to each sample. The mix was heated at 100°C for 5 min. Samples and protein marker (Benchmark Protein Ladder, Life Technologies, Grand Island, NY) were loaded into a polyacrylamide pre-cast gel (Mini-Protean TGX, Bio-Rad, Hercules, CA). Gel was mounted into a mini vertical unit (mighty small, Hoefer, Holliston, MA) which was attached to a power source (EC 600, E-C Apparatus Corporation, St. Petersburg, FL). Gel was stained (Coomassie Blue G-250, Bio-Rad, Hercules, CA), scanned (FLA-5000, Fujifilm, Cypress, CA) and analyzed using Multi-gauge (Fujifilm, Cypress, CA).

Statistical analysis

Data were analyzed as a completely random design using the GLM procedure (SAS Inst. Inc., Cary, NC). Treatment (- RFI, AVE or +RFI) was a fixed effect. Means comparison was made using the LSMEANS statement. Stepwise regression was performed using DMI as dependent variable. Multiple regressions were performed using REG procedure. CORR procedure was used to generate

correlation between variables and RFI. For all variables, $P < 0.05$ was declared significant, and $P < 0.10$ were considered a tendencies.

RESULTS

For all four experiments, correlation between ADG MMWT and all mitochondrial measurements were not significant, suggesting independence of RFI and mitochondrial measurements. Residual feed intake from all experiments was correlated ($P < 0.05$) with DMI and G:F (Tables 4.6; 4.11; 4.16; 4.21). Band I was positive correlated (0.41; $P < 0.05$; Table 4.6) with RFI for Exp. 2 and Band VI was negative correlated with RFI for Exp. 4 (-0.71; $P < 0.05$; Table 4.21).

Exp. 1, RFI ranged from - 0.88 to 1.12 kg/d (Table 4.7); Exp. 2, RFI ranged from - 1.14 to 0.79 kg/d (Table 4.12); Exp. 3, RFI-GP ranged from - 0.83 to 0.86 kg/d, RFI-FP ranged from - 0.77 to 0.59 kg/d, RFI-TP ranged from - 0.63 to 0.50 kg/d, (Table 4.17); Exp. 4, RFI ranged from - 0.62 to 0.61 kg/d (Table 4.22). Dry matter intake was different ($P < 0.05$) between RFI phenotypes for all experiments. Exp. 1, there was 2.20 kg/d DMI difference between -RFI and +RFI phenotypes; Exp. 2, there was 1.66 kg/d DMI difference between -RFI and +RFI phenotypes; Exp. 3, there was 1.56 (GP), 1.60 (FP), 0.93 (TP) kg/d DMI difference between -RFI and +RFI; Exp. 4, there was 1.34 kg/d DMI difference between -RFI and +RFI phenotypes. Gain:feed was different ($P < 0.05$) between RFI phenotypes for all experiments. Exp. 1, G:F ranged from 0.19 to 0.15 between -RFI and +RFI phenotypes; Exp. 2, G:F ranged from 0.26 to 0.21

between -RFI and +RFI phenotypes; Exp. 3, G:F-GP ranged from 0.21 to 0.17, G:F-FP ranged from 0.16 to 0.14 and G:F-TP ranged from 0.18 to 0.16 between -RFI and +RFI phenotypes; Exp. 4, G:F ranged from 0.22 to 0.20 between -RFI and +RFI phenotypes. Average daily gain and MMWT were not different (P > 0.05) between RFI phenotypes for all experiments.

Exp. 1 all mitochondrial measurements variables were not different (P > 0.05) between RFI phenotypes. Exp. 2, band I was greater (P < 0.01) and band II tended less (P = 0.10) for +RFI compared to -RFI. Exp. 3 all mitochondrial measurements variables were not different (P > 0.05) between RFI phenotypes from different periods. Exp. 4, band VI tended less (P = 0.09) for +RFI compared to -RFI.

Final BW was the only variable included (P < 0.01) for Exp. 1 when DMI was stepwise regressed by all variables measured (R^2 = 0.30; Table 4.9). Exp. 2, FBW, CI:III, IBW, and band I were included (P > 0.14) in DMI stepwise regression however, FBW and IBW were correlated, so those variables cannot be included in regression simultaneously to predict DMI (R^2 = 0.49 to 0.68; Table 4.14). Exp. 3 during GP, MMWT-GP was the only variable included (P < 0.01; R^2 = 0.50; Table; 4.19); during FP, MMWT-FP and CIII were included (P < 0.11; R^2 = 0.61 to 0.68); during TP, MMWT-TP, CI:III-FP, CI-GP and CI:III-GP were included (P < 0.08; R^2 = 0.69 to 0.91) in DMI stepwise regression. Exp. 4, band VI, ADG and band I were included (P < 0.06) in DMI stepwise regression however, band I and VI were correlated, so those variables cannot be included in regression simultaneously to predict DMI (R^2 = 0.46 to 0.17; Table 4.24).

Multiple regression models were used for all experiments. Dry matter intake was predicted using ADG and MMWT, using RFI model and mitochondrial measurements were included one at the time as independent variables. Exp. 1, coefficient of determination was 0.30 for RFI model, and increased 20% when band I was included in regression as third variable (R^2 0.36; Table 4.10). Exp. 2, coefficient of determination was 0.56 for RFI model, and increased 16% when band I was included in regression as third variable (R^2 0.65; Table 4.15). Exp. 3, during GP coefficient of determination was 0.52 for RFI model, and increased 16% when band III was included in regression as third variable (R^2 0.60; Table 4.20); during FP coefficient of determination was 0.65 for RFI model, and increased 6% when CIII was included in regression as third variable (R^2 0.69); during TP coefficient of determination was 0.70 for RFI model, and increased 8.5% when band III-GP, as well as 8.5% when CI or CI:III was included in regression as third variable (R^2 0.76). Exp. 4, coefficient of determination was 0.52 for RFI model, and increased 55.8% when band VI was included in regression as third variable (R^2 0.81; Table 4.25).

DISCUSSION

Residual feed intake is a measure of metabolic efficiency or energy conservation independent of body size and correlated to others efficiency measurements such as FCR (or G:F). Residual feed intake has shown strong positive correlation to DMI, negative correlation to G:F and ADG independence

(2006a; Kolath et al., 2006b; Bottje and Carstens, 2009; Davis, 2009; Moore et al., 2009; Sharifabadi et al., 2012; Grubbs et al., 2013a; Ramos and Kerley, 2013).

Experiments two, three and four had coefficient of determination for RFI close to 0.60, which agrees with Carstens and Tedeschi (2006), suggesting ADG and MMWT explain over 60% of phenotypic variation in feed intake.

Relationship between RFI and mitochondria measurements was observed on Exp. 2 and 4. When DMI was stepwise regressed, Exp. 2, 3 and 4 included mitochondrial measurements as significant variables. Dry matter intake multiple regression models increased coefficient of determination in a range of 6 to 55% when using mitochondrial measurements.

Energy is produced through oxidative phosphorylation operated by protein complexes attached to inner mitochondrial membrane. The electron carriers NADH and $FADH_2$ donate electrons for CI and II, respectively. Complex I transfer of a hydride ($:H^-$) ion from NADH and a proton from the matrix to ubiquinone as well as four protons from the matrix to the intermembrane space. Ubiquinol is dispersed in the inner mitochondrial membrane where it transfers electrons from CI and II to CIII. Complex III transfers electrons from ubiquinol to cytochrome c, along with moving two protons to the intermembrane space. Cytochrome c accepts a single electron from CIII and transfers it to CIV, which carries electrons from cytochrome c to molecular oxygen, thereby reducing it to water. The membrane potential and pH gradient formed provides energy for ATP synthesis as protons flow back into the matrix through CV (Nelson et al., 2008).

Complex I and III were the focus of these experiments because CI is the point of entry for the major fraction of electrons and CIII is where electrons from CI and CII are combined. Those two complexes are also where proton transportation takes place. In addition to that previous research in our laboratory found relationship between these complexes and RFI (Kolath et al., 2006b; Davis, 2009; Ramos and Kerley, 2013). Kolath et al. (2006a) reported no difference between the - RFF and +RFI animals in their expression of uncoupling protein 2 or 3 mRNA or protein using LM from steers. Low RFI exhibited a greater rate of state 2 and 3 respiration and RCR, than +RFI steers when provided with glutamate or succinate as a respiratory substrate (Kolath et al., 2006b). Davis (2009) reported CI protein concentrations in lymphocytes mitochondria tended to be negatively correlated and ratios of CI to II and CI to III were negatively correlated with RFI. Ramos and Kerley (2013) found greater CI concentration for -RFI compared to +RFI and reported a tendency for lower concentration of band I in steers with -RFI. Greater ratio of CI proteins may allow faster shuttling of electrons from NADH into the oxidative phosphorylation and subsequent more rapid return to phosphorylation ratio homeostasis, therefore resulting in more efficient animals. Low RFI animals may achieve satiety more rapid than +RFI animals which result in less feed intake and better efficiency.

Bottje et al. (2002) observed decreased respiratory chain coupling, decreased CI and II activities and increased electron leak in low FE broilers muscle mitochondria. Lancaster et al. (2014) reported greater RCR values and state 3 respiration rate in liver mitochondria from -RFI heifers compared with

+RFI, however, states 2 and 4 did not differ. Sharifabadi et al. (2012) reported sheep with -RFI had greater activity of all five respiratory chain complexes. Generally studies observed decrease in all complex activities in low-FE compared with high-FE mitochondria (Bottje and Carstens, 2009).

Greater basal H_2O_2 levels were observed in mitochondria from breast muscle, liver, and duodenum isolated from low-FE broilers with the exception of leg muscle (Bottje et al., 2006). Grubbs et al. (2013a) observed less reactive oxygen species (ROS) production in mitochondria from the white portion of semitendinosus in -RFI compared to +RFI pigs, when both NADH and $FADH_2$ energy substrates were used (glutamate and succinate, respectively). Greater H_2O_2 production is consistently observed in high-FE and -RFI mitochondria. Mitochondrial ROS have the potential to oxidize structures such as proteins, DNA, and lipids (Yu, 1994). Protein carbonyls are used as an indicator of protein oxidation (Stadtman and Levine, 2000) and have been associated with aging and certain metabolic diseases (Dalle-Donne et al., 2005). Mitochondria from breast muscle, duodenum, liver, and lymphocyte homogenates from low-FE birds, as well as neck muscle from low-FE steers exhibited greater total protein carbonyls compared to high-FE (Iqbal et al., 2004; 2005; Ojano-Dirain et al., 2005b; Sandelin, 2005; Lassiter et al., 2006). Iqbal et al. (2004) reported higher protein expression in low-FE breast muscle mitochondria for five mitochondrial proteins, [core I, cytochrome c1, cytochrome b (complex III), COX II (cytochrome c oxidase subunit II, complex IV), and adenine nucleotide translocator (ANT1)]. They also found a protein band of 47 kDa expressed at a higher level in low-FE

compared with high-FE mitochondria. Using liver samples they observed expression of four proteins [mitochondrially encoded NADH dehydrogenase 3 (NAD3, complex I), subunit VII (complex III), COX II, and COX IVb (complex IV)] were higher in low-FE mitochondria and two proteins [flavoprotein (complex II) and a-ATP synthase (complex V)] were higher in high FE birds (Iqbal et al., 2005). Ojano-Dirain et al. (2005c) reported that six out of seven nuclear-encoded respiratory chain subunits (flavoprotein, core I, core II, cytochrome c 1, iron-sulfur protein, and ATPase-α) were greater, whereas three out of six mitochondrial-encoded subunits (mitochondrially encoded NADH dehydrogenase 4, mitochondrially encoded NADH dehydrogenase 6-C, and COXII) were less in the low FE duodenum mitochondria.

Mitochondria play a vital role in growth and development. Functional and biochemical differences such as electron transfer efficiency, ability to carry out oxidative phosphorylation, electron leakage, ROS production, mitochondrial complexes activities and protein expression provide clear evidence for the link between mitochondria and production efficiency.

CONCLUSION

Steers with -RFI consumed less feed and had improved feed efficiency without any change in growth rate. Examination of lymphocyte mitochondria proteins (CI, CI subunits and CIII) found a relationship between mitochondria band I, band VI and RFI. Mitochondrial measurements increased coefficient of

determination for intake prediction. These results led to the conclusion that mitochondrial function is in part responsible for animals metabolic efficiency differences.

Table 4.1. Experiment one dietary nutrient composition

Item	(% DM)
Ingredients	
Corn	78.59
Dried Distillers Grains with Soluble	9.72
Soyplus[1]	6.25
Wheat Midds	2.65
Limestone	1.50
Urea	0.39
Choice White Grease	0.20
Dyna-K[2]	0.17
Sodium Chloride	0.17
Vitamin Premix[3]	0.17
Trace Mineral Premix[4]	0.17
Rumensin 90[5]	0.01
Nutrient Composition	
DM, %	86.15
CP, % DM	13.55
ME, Mcal/kg DM	3.08

[1]Soyplus (West Central, Ralston, IA) contained CP 46.6% DM, RUP 60% CP, RDP 40% CP.

[2]Dyna-K (The Mosaic Company, Plymouth, MN) contained 95% potassium chloride, 0.3% sodium chloride 0.2% calcium and magnesium chlorides and sulfates.

[3]Vitamin Premix Contained 8,818,490.5 IU/kg of vitamin A, 1,763,698.1 IU/kg of vitamin D3, 1,102.3 IU/kg of vitamin E.

[4]Trace Mineral Premix contained 24% Ca, 2.5% Fe, 2% Mn, 3% Zn, 1% Cu, 100 ppm Se, 500 ppm I, and 100 ppm Co.

[5]Rumensin 90 (Elanco, Greenfield, IN) Monesin, USP Granulated.

Table 4.2. Experiment two dietary nutrient composition

Item	(% DM)
Ingredients	
Corn	75.82
Dried Distillers Grains with Soluble	11.03
Soyplus[1]	7.09
Wheat Midds	3.01
Limestone	1.69
Urea	0.45
Choice White Grease	0.20
Dyna-K[2]	0.17
Sodium Chloride	0.17
Vitamin Premix[3]	0.17
Trace Mineral Premix[4]	0.17
Rumensin 90[5]	0.01
Nutrient Composition	
DM, %	86.31
CP, % DM	14.38
ME, Mcal/kg DM	3.07

[1]Soyplus (West Central, Ralston, IA) contained CP 46.6% DM, RUP 60% CP, RDP 40% CP.

[2]Dyna-K (The Mosaic Company, Plymouth, MN) contained 95% potassium chloride, 0.3% sodium chloride 0.2% calcium and magnesium chlorides and sulfates.

[3]Vitamin Premix contained 8,818,490.5 IU/kg of vitamin A, 1,763,698.1 IU/kg of vitamin D3, 1,102.3 IU/kg of vitamin E.

[4]Trace Mineral Premix contained 24% Ca, 2.5% Fe, 2% Mn, 3% Zn, 1% Cu, 100 ppm Se, 500 ppm I, and 100 ppm Co.

[5]Rumensin 90 (Elanco, Greenfield, IN) Monesin, USP Granulated.

Table 4.3. Experiment three growing phase dietary nutrient composition

Item	(% DM)
Ingredients	
Haylage-Alfalfa	29.97
Dried Distillers Grains with Soluble	16.22
Soyhulls[1]	34.22
Corn	12.91
Aminoplus[2]	4.28
Blood meal	1.98
MFP[3]	0.03
Vitamin Premix[4]	0.13
Trace Mineral Premix[5]	0.15
Vitamin E[6]	0.09
Rumensin 90[7]	0.01
Nutrient Composition	
DM, %	67.73
CP, % DM	21.13
ME, Mcal/kg DM	2.69
NDF, % DM	23.4

[1]Soyplus (West Central, Ralston, IA) contained CP 46.6% DM, RUP 60% CP, RDP 40% CP.

[2]Aminoplus (AGP, Omaha, NE) contained CP 51.14% DM, RUP 72% CP, RDP 28% CP.

[3]MFP (Novus International, Inc., St. Charles, MO) contained 84% methionine activity and 40% rumen bypass.

[4]Vitamin Premix contained 8,818,490.5 IU/kg of vitamin A, 1,763,698.1 IU/kg of vitamin D3, 1,102.3 IU/kg of vitamin E.

[5]Trace Mineral Premix contained 24% Ca, 2.5% Fe, 2% Mn, 3% Zn, 1% Cu, 100 ppm Se, 500 ppm I, and 100 ppm Co.

[6]Vitamin E contained 44,000 IU/kg.

[7]Rumensin 90 (Elanco, Greenfield, IN) Monesin, USP Granulated.

Table 4.4. Experiment three finishing phase dietary nutrient composition

Item	(% DM)
Ingredients	
Corn	68.00
Dried Distillers Grains with Soluble	25.71
Aminoplus[1]	1.77
Blood meal	1.57
Urea	0.46
Sodium Chloride	0.11
Limestone	1.96
Tallow	0.08
Vitamin Premix[2]	0.03
Trace Mineral Premix[3]	0.06
Vitamin E[4]	0.23
Rumensin 90[5]	0.01
Nutrient Composition	
DM, %	87.03
CP, % DM	16.49
ME, Mcal/kg DM	3.07
NDF, % DM	16.01

[1]Aminoplus (AGP, Omaha, NE) contained CP 51.14% DM, RUP 72% CP, RDP 28% CP.

[2]Vitamin Premix contained 8,818,490.5 IU/kg of vitamin A, 1,763,698.1 IU/kg of vitamin D3, 1,102.3 IU/kg of vitamin E.

[3]Trace Mineral Premix contained 24% Ca, 2.5% Fe, 2% Mn, 3% Zn, 1% Cu, 100 ppm Se, 500 ppm I, and 100 ppm Co.

[4]Vitamin E contained 44,000 IU/kg.

[5]Rumensin 90 (Elanco, Greenfield, IN) Monesin, USP Granulated.

Table 4.5. Experiment four dietary nutrient composition

Item	(% DM)
Ingredients	
Soyhulls[1]	49.00
Dried Distillers Grains with Soluble	28.23
Rye Haylage	13.50
Aminoplus[2]	5.20
Blood meal	2.60
MFP[3]	0.07
Sodium Chloride	0.15
Limestone	0.86
Vitamin Premix[4]	0.03
Trace Mineral Premix[5]	0.10
Vitamin E[6]	0.25
Rumensin 90[7]	0.01
Nutrient Composition	
DM, %	70.10
CP, % DM	20.96
ME, Mcal/kg DM	2.85
NDF, % DM	38.70

[1]Soyplus (West Central, Ralston, IA) contained CP 46.6% DM, RUP 60% CP, RDP 40% CP.

[2]Aminoplus (AGP, Omaha, NE) contained CP 51.14% DM, RUP 72% CP, RDP 28% CP.

[3]MFP (Novus International, Inc., St. Charles, MO) contained 84% methionine activity and 40% rumen bypass.

[4]Vitamin Premix contained 8,818,490.5 IU/kg of vitamin A, 1,763,698.1 IU/kg of vitamin D3, 1,102.3 IU/kg of vitamin E.

[5]Trace Mineral Premix contained 24% Ca, 2.5% Fe, 2% Mn, 3% Zn, 1% Cu, 100 ppm Se, 500 ppm I, and 100 ppm Co.

[6]Vitamin E contained 44,000 IU/kg.

[7]Rumensin 90 (Elanco, Greenfield, IN) Monesin, USP Granulated.

Table 4.6. Experiment one partial correlation coefficients[a] between residual feed intake (RFI), production traits and mitochondria complex

Traits[b]	RFI
IBW	0.00
FBW	0.00
DMI	**0.84**
ADG	0.00
MMWT	0.00
G:F	**-0.74**
Complex I	-0.06
Complex III	0.00
Complex I:III	-0.15
Band I	0.29
Band II	-0.26
Band III	0.07

[a]Correlation coefficients in bold are significant ($P < 0.05$) from 0.

[b]Trait abbreviations: IBW = initial BW; FBW = final BW; MMWT = metabolic midweight; Complex I and III = µg of Complex; Band I, II and III = percent in gel total bands area.

Table 4.7. Experiment one residual feed intake (RFI) phenotypes performance differences

Traits[1]	-RFI	AVE	+RFI	SEM	*P* value
IBW	235	226	241	8.19	0.18
FBW	374	368	384	10.30	0.29
DMI	8.91[c]	9.56[b]	11.11[a]	0.24	**< 0.01**
ADG	1.66	1.70	1.70	0.07	0.65
MMWT	72.9	71.6	74.25	1.59	0.23
G:F	0.19[a]	0.18[b]	0.15[c]	0.01	**< 0.01**
RFI	-0.88[c]	-0.12[b]	1.12[a]	0.17	**< 0.01**

[1]IBW = initial BW; FBW = final BW; MMWT = metabolic midweight.

[a, b, c]least square means within a row with different superscript differ ($P < 0.05$).

Table 4.8. Experiment one residual feed intake (RFI) phenotypes mitochondrial characteristics differences

Traits	-RFI	AVE	+RFI	SEM	*P* value
Complex I[1]	5.07	5.18	4.93	0.64	0.78
Complex III	4.00	4.02	4.15	0.65	0.87
Complex I:III	1.53	1.43	1.22	0.22	0.32
Band I[2]	0.42	0.41	0.43	0.02	0.38
Band II	0.38	0.39	0.36	0.03	0.35
Band III	0.20	0.20	0.21	0.01	0.65

[1]µg of Complex I. III.

[2]Band I, II and III % in gel total bands area.

[a, b, c]least square means within a row with different superscript differ ($P < 0.05$).

Table 4.9. Experiment one intake stepwise regression summary

Step	Variable[1]	Partial R^2	Model R^2	*P* value
1	FBW	0.30	0.30	< 0.01

[1]FBW = Final BW.

Table 4.10. Experiment one regression model and coefficient of determination of different residual feed intake (RFI[a]) measurements

Item[a]	Regression model[1]	R^2
RFI	$Y_i = -0.47 + (1.15)ADG + (0.11)MMWT$	0.30
RFI 1	$Y_i = -0.22 + (1.25)ADG + (0.11)MMWT + (-0.04)Complex\ I$	0.30
RFI 2	$Y_i = -0.48 + (1.15)ADG + (0.11)MMWT + (0.01)Complex\ III$	0.30
RFI 3	$Y_i = 0.14 + (1.27)ADG + (0.11)MMWT + (-0.24)Complex\ I{:}III$	0.32
RFI 4	$Y_i = -3.5 + (1.17)ADG + (0.12)MMWT + (5.76)Band\ I$	0.36
RFI 5	$Y_i = 0.54 + (1.29)ADG + (0.12)MMWT + (-3.57)Band\ II$	0.35
RFI 6	$Y_i = -0.77 + (1.20)ADG + (0.11)MMWT + (1.78)Band\ III$	0.30

[1]Y_i = expected daily DMI on animal; MMWT = metabolic midweight.

Table 4.11. Experiment two partial correlation coefficients[a] between residual feed intake, production traits and mitochondria complex

Traits[b]	RFI
IBW	0.00
FBW	0.00
DMI	**0.66**
ADG	0.00
MMWT	0.00
G:F	**-0.74**
Complex I	-0.28
Complex III	0.25
Complex I:III	-0.36
Band I	**0.41**
Band II	0.11
Band III	-0.30

[a]Correlation coefficients in bold are significant ($P < 0.05$) from 0.

[b]Trait abbreviations: IBW = initial BW; FBW = final BW; MMWT = metabolic midweight; Complex I and III = µg of Complex; Band I, II and III = percent in gel total bands area.

Table 4.12. Experiment two residual feed intake (RFI) phenotypes performance differences

Traits[1]	-RFI	AVE	+RFI	SEM	*P* value
IBW	298	302	287	16.85	0.49
FBW	471	465	459	17.38	0.59
DMI	7.74[c]	8.62[b]	9.40[a]	0.44	**< 0.01**
ADG	1.95	1.83	1.93	0.06	0.20
MMWT	101	101	98	3.46	0.59
G:F	0.26[a]	0.21[b]	0.21[c]	0.01	**< 0.01**
RFI	-1.14[c]	0.11[b]	0.79[a]	0.12	**< 0.01**

[1]IBW = initial BW; FBW = final BW; MMWT = metabolic midweight.

[a, b, c]least square means within a row with different superscript differ ($P < 0.05$).

Table 4.13. Experiment two residual feed intake (RFI) phenotypes mitochondrial characteristics differences

Traits	-RFI	AVE	+RFI	SEM	*P* value
Complex I[1]	4.90	3.21	3.60	1.20	0.33
Complex III	1.87	2.55	2.37	0.30	0.15
Complex I:III	2.52	1.23	1.63	0.60	0.11
Band I[2]	0.36[b]	0.38[ab]	0.40[a]	0.00	**0.01**
Band II	0.26	0.29	0.27	0.00	0.35
Band III	0.38	0.34	0.33	0.00	0.10

[1]µg of Complex I. III.

[2]Band I, II and III % in gel total bands area.

[a, b, c]least square means within a row with different superscript differ ($P < 0.05$).

Table 4.14. Experiment two intake stepwise regression summary

Step	Variable[1]	Partial R^2	Model R^2	*P* value
1	FBW	0.49	0.49	< 0.01
2	Complex I:III	0.09	0.58	0.05
3	IBW	0.04	0.62	0.14
4	Band I	0.06	0.68	0.07

[1]FBW = Final BW; IBW = Initial BW.

Table 4.15. Experiment two regression model and coefficient of determination of different residual feed intake (RFI[a]) measurements

Item[a]	Regression model[1]	R^2
RFI	$Y_i = -0.47 + (1.15)ADG + (0.11)MMWT$	0.56
RFI 1	$Y_i = -0.22 + (1.25)ADG + (0.11)MMWT + (-0.04)Complex\ I$	0.60
RFI 2	$Y_i = -0.48 + (1.15)ADG + (0.11)MMWT + (0.01)Complex\ III$	0.59
RFI 3	$Y_i = 0.14 + (1.27)ADG + (0.11)MMWT + (-0.24)Complex\ I{:}III$	0.62
RFI 4	$Y_i = -3.5 + (1.17)ADG + (0.12)MMWT + (5.76)Band\ I$	0.65
RFI 5	$Y_i = 0.54 + (1.29)ADG + (0.12)MMWT + (-3.57)Band\ II$	0.56
RFI 6	$Y_i = -0.77 + (1.20)ADG + (0.11)MMWT + (1.78)Band\ III$	0.60

[1]Y_i = expected daily DMI on animal; MMWT = metabolic midweight.

Table 4.16. Experiment three partial correlation coefficients[a] between residual feed intake (RFI), production traits and mitochondria complex

Traits[b]	RFI-GP	RFI-FP	RFI-TP
IBW	0.03	0.05	-0.01
FBW	0.01	0.00	-0.02
Growing Phase			
DMI	**0.69**	0.15	**0.58**
ADG	0.00	-0.07	-0.03
G:F	**-0.76**	-0.19	**-0.67**
MMWT	0.00	0.13	0.03
RFI	1.00	0.10	**0.82**
Complex I	-0.17	-0.20	-0.19
Complex III	-0.09	-0.07	-0.04
Complex I:III	0.28	0.13	0.35
Band I	0.09	0.29	0.16
Band II	-0.23	-0.33	-0.28
Band III	0.39	0.32	0.41
Finishing Phase			
DMI	-0.01	**0.59**	0.34
ADG	-0.18	0.00	0.03
G:F	-0.21	**-0.58**	-0.31
MMWT	-0.04	0.00	-0.03
RFI	0.10	1.00	**0.60**
Complex I	-0.40	-0.15	-0.43
Complex III	-0.22	-0.29	-0.33
Complex I:III	-0.44	-0.06	-0.41
Band I	0.20	-0.06	0.17
Band II	0.05	-0.01	-0.06
Band III	-0.22	0.05	-0.05
Total Period			
DMI	0.43	0.39	**0.54**
ADG	-0.12	-0.04	0.00
G:F	**-0.65**	**-0.55**	**-0.65**
MMWT	-0.03	0.06	0.00
RFI	**0.82**	**0.60**	1.00

[a]Correlation coefficients in bold are significant ($P < 0.05$) from 0.

[b]Trait abbreviations: IBW = initial BW; FBW = final BW; MMWT = metabolic midweight; Complex I and III = µg of Complex; Band I, II and III = percent in gel total bands area.

Table 4.17. Experiment three residual feed intake (RFI) phenotypes performance differences

Traits[1]	-RFI	+RFI	SEM	*P* value
		Growing Phase		
DMI	8.41[b]	9.97[a]	0.35	**< 0.01**
ADG	1.76	1.71	0.09	0.76
MMWT	76.81	76.2	1.35	0.66
G:F	0.21[a]	0.17[b]	0.01	**0.04**
RFI	-0.83[b]	0.86[a]	0.12	**< 0.01**
		Finishing Phase		
DMI	8.65[b]	10.25[a]	0.39	**< 0.01**
ADG	1.36	1.41	0.11	0.65
MMWT	98.62	100.25	2.71	0.23
G:F	0.16[a]	0.14[b]	0.01	**< 0.01**
RFI	-0.77[b]	0.59[a]	0.14	**< 0.01**
		Total Period		
IBW	256	255	7.31	0.87
FBW	407	400	12.24	0.65
DMI	9.05[b]	9.98[a]	0.36	**< 0.01**
ADG	1.58	1.57	0.07	0.87
MMWT	89.27	88.16	1.96	0.68
G:F	0.18[a]	0.16[b]	0.01	**0.05**
RFI	-0.63[b]	0.50[a]	0.12	**< 0.01**

[1]IBW = initial BW; FBW = final BW; MMWT = metabolic midweight.

[a, b, c]least square means within a row with different superscript differ ($P < 0.05$).

Table 4.18. Experiment three residual feed intake (RFI) phenotypes mitochondrial characteristics differences

Traits[1]	-RFI	+RFI	SEM	*P* value
Growing Phase				
Complex I	8.94	6.63	2.22	0.47
Complex III	4.52	5.03	3.25	0.30
Complex I:III	1.49	2.85	0.81	0.25
Band I	0.38	0.40	0.05	0.77
Band II	0.50	0.41	0.08	0.44
Band III	0.12	0.19	0.03	0.16
Finishing Phase				
Complex I	4.78	3.37	1.22	0.40
Complex III	5.50	2.65	1.24	0.11
Complex I:III	1.11	1.17	0.24	0.85
Band I	0.44	0.43	0.01	0.52
Band II	0.20	0.20	0.03	0.88
Band III	0.37	0.37	0.03	0.85

[1]µg of Complex I and III; Band I, II and III % in gel total bands area.

[a, b, c]least square means within a row with different superscript differ ($P < 0.05$).

Table 4.19. Experiment three intake stepwise regression summary for each period

Step	Variable[1]	Partial R^2	Model R^2	*P* value
		Growing Phase		
1	MMWT	0.50	0.50	< 0.01
		Finishing Phase		
1	MMWT	0.61	0.61	< 0.01
2	Complex III	0.06	0.68	0.11
		Total Period [a]		
1	MMWT	0.69	0.69	< 0.01
2	Complex I:III (FP)	0.07	0.76	0.08
3	Complex I (GP)	0.09	0.86	0.02
4	Complex I:III (GP)	0.05	0.91	0.03

[1]MMWT = metabolic midweight.

[a]For Total Period, growing and finishing phase mitochondria measurements were used in regressions.

Table 4.20. Experiment three regression model and coefficient of determination of different residual feed intake (RFI[a]) measurements

Item[a]	Regression model[1]	R^2
	Growing Phase	
RFI	Y_i = -5.15 + (0.51)ADG + (0.18)MMWT	0.52
RFI 1	Y_i = -4.01 + (0.61)ADG + (0.16)MMWT + (-0.03)Complex I	0.54
RFI 2	Y_i = -3.76 + (0.29)ADG + (0.16)MMWT + (-0.02)Complex III	0.51
RFI 3	Y_i = -4.27 + (-0.11)ADG + (0.17)MMWT + (0.15)Complex I:III	0.57
RFI 4	Y_i = -5.50 + (0.31)ADG + (0.18)MMWT + (0.65)Band I	0.53
RFI 5	Y_i = -4.63 + (0.12)ADG + (0.18)MMWT + (-1.05)Band II	0.55
RFI 6	Y_i = -5.16 + (0.20)ADG + (0.17)MMWT + (4.11)Band III	0.60
	Finishing Phase	
RFI	Y_i = -3.41 + (1.15)ADG + (0.11)MMWT	0.65
RFI 1	Y_i = -3.91 + (0.83)ADG + (0.13)MMWT + (-0.05)Complex I	0.66
RFI 2	Y_i = -4.41 + (0.69)ADG + (0.13)MMWT + (-0.08)Complex III	0.69
RFI 3	Y_i = -3.30 + (1.13)ADG + (0.11)MMWT + (-0.07)Complex I:III	0.65
RFI 4	Y_i = -2.97 + (1.15)ADG + (0.11)MMWT + (-0.90)Band I	0.65
RFI 5	Y_i = -3.43 + (1.14)ADG + (0.11)MMWT + (-0.09)Band II	0.65
RFI 6	Y_i = -3.93 + (1.08)ADG + (0.12)MMWT + (0.74)Band III	0.65
	Total Period[b]	
RFI	Y_i = -5.86 + (-1.01)ADG + (0.19)MMWT	0.70
	Growing Phase	
RFI 1	Y_i = -4.85 + (-0.72)ADG + (0.18)MMWT + (-0.02)Complex I	0.72
RFI 2	Y_i = -4.99 + (-0.95)ADG + (0.18)MMWT + (-0.01)Complex III	0.70
RFI 3	Y_i = -5.30 + (-1.65)ADG + (0.19)MMWT + (0.12)Complex I:III	0.75
RFI 4	Y_i = -5.86 + (-0.81)ADG + (0.19)MMWT + (0.71)Band I	0.71
RFI 5	Y_i = -4.73 + (-0.47)ADG + (0.17)MMWT + (-0.88)Band II	0.73
RFI 6	Y_i = -5.09 + (-0.05)ADG + (0.16)MMWT + (3.20)Band III	0.76
	Finishing Phase	
RFI 1	Y_i = -6.00 + (-0.77)ADG + (0.19)MMWT + (-0.09)Complex I	0.76
RFI 2	Y_i = -5.92 + (-0.61)ADG + (0.19)MMWT + (-0.07)Complex III	0.74
RFI 3	Y_i = -4.88 + (-1.02)ADG + (0.19)MMWT + (-0.44)Complex I:III	0.76
RFI 4	Y_i = -6.99 + (-1.12)ADG + (0.20)MMWT + (2.11)Band I	0.71
RFI 5	Y_i = -6.02 + (-1.13)ADG + (0.20)MMWT + (-0.44)Band II	0.71
RFI 6	Y_i = -5.35 + (-0.89)ADG + (0.19)MMWT + (-0.63)Band III	0.71

[1]Y_i = expected daily DMI on animal; MMWT = metabolic midweight.

[b]For Total Period, growing and finishing phase mitochondria measurements were used in regressions.

Table 4.21. Experiment four partial correlation coefficients[a] between residual feed intake (RFI), production traits and mitochondria complex

Traits[b]	RFI
IBW	0.01
FBW	0.02
DMI	**0.70**
ADG	0.00
G:F	**-0.89**
MMWT	0.01
Complex I	0.08
Band I	0.37
Band II	0.41
Band III	0.11
Band IV	0.38
Band V	-0.61
Band VI	**-0.71**

[a]Correlation coefficients in bold are significant ($P < 0.05$) from 0.

[b]Trait abbreviations: IBW = initial BW; FBW = final BW; MMWT = metabolic midweight; Complex I = µg of Complex; Band I, II, III, IV, V and VI = percent in gel total bands area.

Table 4.22. Experiment four residual feed intake (RFI) phenotypes performance differences

Traits[1]	-RFI	+RFI	SEM	*P* value
IBW	305	294	11.52	0.52
FBW	440	433	12.21	0.72
DMI	8.35[b]	9.69[a]	0.40	**< 0.01**
ADG	1.83	1.89	0.09	0.65
MMWT	84.70	83.22	1.95	0.61
G:F	0.22[a]	0.20[b]	0.01	**< 0.01**
RFI	-0.62[b]	0.61[a]	0.15	**< 0.01**

[1]IBW = initial BW; FBW = final BW; MMWT = metabolic midweight; RFI = residual feed intake.

[a, b, c]least square means within a row with different superscript differ ($P < 0.05$).

Table 4.23. Experiment four residual feed intake (RFI) phenotypes mitochondrial characteristics differences

Traits[1]	-RFI	+RFI	SEM	*P* value
Complex I[1]	12.58	14.33	3.56	0.74
Band I[2]	0.23	0.24	0.01	0.43
Band II	0.33	0.35	0.03	0.72
Band III	0.13	0.17	0.04	0.49
Band IV	0.16	0.17	0.02	0.57
Band V	0.06	0.03	0.02	0.24
Band VI	0.10	0.04	0.02	0.09

[1]µg of Complex I.

[2]Band I, II, III, IV, V and VI % in gel total bands area.

[a, b, c]least square means within a row with different superscript differ ($P < 0.05$).

Table 4.24. Experiment four intake stepwise regression summary for each period

Step	Variable	Partial R^2	Model R^2	*P* value
1	Band VI	0.46	0.46	0.06
2	ADG	0.34	0.80	0.03
3	Band I	0.17	0.97	0.01

Table 4.25. Experiment four regression model and coefficient of determination of different residual feed intake (RFI[a]) measurements

Item[a]	Regression model[1]	R^2
RFI	$Y_i = -5.28 + (3.90)ADG + (0.08)MMWT$	0.52
RFI 1	$Y_i = -5.27 + (3.82)ADG + (0.08)MMWT + (0.01)Complex\ I$	0.52
RFI 2	$Y_i = -0.83 + (4.09)ADG + (-0.01)MMWT + (8.06)Band\ I$	0.59
RFI 3	$Y_i = -4.86 + (3.75)ADG + (0.07)MMWT + (8.70)Band\ II$	0.66
RFI 4	$Y_i = -6.15 + (3.80)ADG + (0.05)MMWT + (14.76)Band\ III$	0.52
RFI 5	$Y_i = -5.88 + (3.85)ADG + (0.09)MMWT + (1.21)Band\ IV$	0.59
RFI 6	$Y_i = 2.52 + (3.82)ADG + (0.01)MMWT + (-16.21)Band\ V$	0.76
RFI 7	$Y_i = 1.47 + (3.61)ADG + (0.02)MMWT + (-13.03)Band\ VI$	0.81

[1]Y_i = expected daily DMI on animal; MMWT = metabolic midweight.

LITERATURE CITED

Abdrakhmanova, A., K. Zwicker, S. Kerscher, V. Zickermann, and U. Brandt. 2006. Tight binding of NADPH to the 39-kDa subunit of complex I is not required for catalytic activity but stabilizes the multiprotein complex. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1757: 1676-1682.

Adam, I., B. Young, A. Nicol, and A. Degen. 1984. Energy cost of eating in cattle given diets of different form. Animal Production Science 38: 53-56.

Allen, M. S. 1996. Physical constraints on voluntary intake of forages by ruminants. Journal of Animal Science 74: 3063-3075.

Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. Journal of Dairy Science 80: 1447-1462.

AOAC. 2006. Complete amino acids. Chp 45.3.05 in Official Methods of Analysis. AOAC Int., Gaithersburg, MD.

Archer, J., and L. Bergh. 2000. Duration of performance tests for growth rate, feed intake and feed efficiency in four biological types of beef cattle. Livestock Production Science 65: 47-55.

Archer, J. A., P. Arthur, R. Herd, P. Parnell, and W. S. Pitchford. 1997. Optimum postweaning test for measurement of growth rate, feed intake, and feed efficiency in British breed cattle. Journal of Animal Science 75: 2024-2032.

Arthur, P., J. A. Archer, D. J. Johnston, R. M. Herd, E. C. Richardson and P. F. Parnell. 2001. Genetic and phenotypic variance and covariance components for feed intake, feed efficiency, and other postweaning traits in Angus cattle. Journal of Animal Science 79: 2805-2811.

Arthur, P., J. Archer, E. Richardson, and R. Herd. 1999. Potential for selection to improve efficiency of feed use in beef cattle: a review. Australian Journal of Animal Research 50: 147-161

Arthur, P., and R. Herd. 2005. Efficiency of feed utilisation by livestock-Implications and benefits of genetic improvement. Canadian Journal of Animal Science 85: 281-290.

Awawdeh, M., E. Titgemeyer, G. Schroeder, and D. Gnad. 2006. Excess amino acid supply improves methionine and leucine utilization by growing steers. Journal of Animal Science 84: 1801-1810.

Bach, A., and M. D. Stern. 1999. Effects of different levels of methionine and ruminally undegradable protein on the amino acid profile of effluent from continuous culture fermenters. Journal of Animal Science 77: 3377-3384.

Basarab, J., M. G. Colazo, D. J. Ambrose, S. Novak, D. McCartney, and V. S. Baron. 2011. Residual feed intake adjusted for backfat thickness and feeding frequency is independent of fertility in beef heifers. Canadian Journal of Animal Science 91: 573-584.

Basarab, J., D. McCartney, E. Okine, and V. Baron. 2007. Relationships between progeny residual feed intake and dam productivity traits. Canadian Journal of Animal Science 87: 489-502.

Basarab, J. M. A. Price, J. L. Aalhus, E. K. Okine, W. M. Snelling, and K. L. Lyle. 2003. Residual feed intake and body composition in young growing cattle. Canadian Journal of Animal Science 83: 189-204.

Basarab, J., M. Price, and E. Okine. 2002. Commercialization of net feed efficiency. In: Proc. 23rd Western Nutr. Conf., September. p 19-20.

Bateman II, H., J. Spain, M. Kerley, R. Belyea, and R. Marshall. 1999. Evaluation of ruminally protected methionine and lysine or blood meal and fish meal as protein sources for lactating holsteins. Journal of Dairy Science 82: 2115-2120.

Berry, D., and J. Crowley. 2013. CELL BIOLOGY SYMPOSIUM: Genetics of feed efficiency in dairy and beef cattle. Journal of Animal Science 91: 1594-1613.

Bishop, M., M. Davis, W. Harvey, G. Wilson, and B. VanStavern. 1991. Divergent selection for postweaning feed conversion in Angus beef cattle: I. Mean comparisons. Journal of Animal Science 69: 4348-4359.

Bonilha, E. R. H. Branco, S. F. M. Bonilha, F. L. Araujo, E. Magnani and M. E. Z. Mercadante. 2013. Body chemical composition of Nellore bulls with different residual feed intakes. Journal of Animal Science 91: 3457-3464.

Bottje, W. Z. X. Tang, M. Iqbal, D. Cawthon, R. Okimoto, T. Wing, and M. Cooper. 2002. Association of mitochondrial function with feed efficiency within a single genetic line of male broilers. Poultry Science 81: 546-555.

Bottje, W., N. R. Pumford, C. Ojano-Dirain, M. Iqbal, and K. Lassiter. 2006. Feed efficiency and mitochondrial function. Poultry Science 85: 8-14.

Bottje, W. G., and G. E. Carstens. 2009. Association of mitochondrial function and feed efficiency in poultry and livestock species. Journal of Animal Science 87: E48-63.

Brandt, M., D. Keisler, D. Meyer, T. Schmidt, and E. Berg. 2007. Serum hormone concentrations relative to carcass composition of a random allotment of commercial-fed beef cattle. Journal of Animal Science 85: 267-275.

Broderick, G., and J. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. Journal of Dairy Science 63: 64-75.

Brown, E. G. E. Carstens, J. T. Fox, K. O Curley,. T. M. Bryan, L. J. Slay, T. H. Welsh, R. D. Randel, J. W. Holloway, D. H. Keisler, 2004. Physiological indicators of performance and feed efficiency traits in growing steers and bulls. Journal of Animal Science 82: 54.

Brown, E. G. 2005. Sources of biological variation in residual feed intake in growing and finishing steers, Texas A&M University.

Calsamiglia, S., M. Stern, and B. Crooker. 1992. Effects of diets formulated to contain different amounts of rumen non-degradable protein on microbial fermentation and nutrient flow from a continuous culture system. Animal Feed Science and Technology 39: 239-252.

Cameron, N. 1992. Correlated physiological responses to selection forcarcass lean content in sheep. Livestock Production Science 30: 53-68.

Cant, J. P., B. W. McBride, and W. Croom. 1996. The regulation of intestinal metabolism and its impact on whole animal energetics. Journal of Animal Science 74: 2541-2553.

Carberry, C. A., D. A. Kenny, S. Han, M. S. McCabe, and S. M. Waters. 2012. Effect of phenotypic residual feed intake and dietary forage content on the rumen microbial community of beef cattle. Applied and Environmental Microbiology 78: 4949-4958.

Carrasco, R., A. Arrizon, A. Plascencia, N. Torrentera, and R. Zinn. 2013. Comparative feeding value of distillers dried grains plus solubles as a partial replacement for steam-flaked corn in diets for calf-fed Holstein steers: Characteristics of digestion, growth performance, and dietary energetics. Journal of Animal Science 91: 1801-1810.

Carroll, J., I. M. Fearnley, R. J. Shannon, J. Hirst, and J. E. Walker. 2003. Analysis of the subunit composition of complex I from bovine heart mitochondria. Molecular & Cellular Proteomics 2: 117-126.

Carstens, G. E., and M. Kerley. 2009. Biological basis for variation in energetic efficiency of beef cattle. In: Proc. Beef Improvement Federation 41st Annual Research Symposium, Sacamento, CA. p 124-131.

Carstens, G. E., and L. O. Tedeschi. 2006. Defining feed efficiency in beef cattle. Proc. BIF 38th Annu. Res. Sym. Annu. Meet., Choctaw, MS, April: 18-21.

Cecava, M., and J. Parker. 1993. Intestinal supply of amino acids in steers fed ruminally degradable and undegradable crude protein sources alone and in combination. Journal of Animal Science 71: 1596-1605.

Clarke, J., D. B. Binnie, J. L. Dobbie, K. R. Jones, C. M. Mowat, R. W. Purchas, A. E. Uljee, 1996. Repeatabilities of blood plasma metabolites and their associations with leanness in genotypes showing a wide divergence in carcass composition. In: Proceedings-New Zealand Society Of Animal Production Science. p 180-183.

Coleman, S. A. 1996. Rumen protected amino acids for growing cattle, University of Missouri-Columbia.

Coomer, J., H. Amos, M. Froetschel, K. Ragland, and C. Williams. 1993. Effects of supplemental protein source on ruminal fermentation, protein degradation, and amino acid absorption in steers and on growth and feed efficiency in steers and heifers. Journal of Animal Science 71: 3078-3086.

Crews D. H., J. 2006. The Genetics of Feed Efficiency in Beef Cattle Proc. BIF 38th Annu. Res. Symp. Annu. Meet: 22-31.

Dabiri, N., and M. Thonney. 2004. Source and level of supplemental protein for growing lambs. Journal of Animal Science 82: 3237-3244.

Dalle-Donne, I., A. Scaloni, D. Giustarini, E. Cavarra, G.Tell, G. Lungarella, R. Colombo, R. Rossi, A. Milzani, 2005. Proteins as biomarkers of oxidative/nitrosative stress in diseases: the contribution of redox proteomics. Mass spectrometry reviews 24: 55-99.

Davenport, G., J. Boling, and K. Schillo. 1990. Nitrogen metabolism and somatotropin secretion in beef heifers receiving abomasal arginine infusions. Journal of Animal Science 68: 1683-1692.

Davenport, G. M., K. A. Cummins, and D. R. Mulvaney. 1995. Abomasal nitrogen flow affects the relationship between dietary nitrogen and insulin-like growth factor-I in growing lambs. The Journal of Nutrition 125: 842-850.

Davis, M. P. 2009. Influence of diet, production traits, blood hormones and metabolites, and mitochondrial complex protein concentrations on residual feed intake in beef cattle, University of Missouri-Columbia.

Dawson, T. M., and V. L. Dawson. 2003. Molecular pathways of neurodegeneration in Parkinson's disease. Science 302: 819-822.

Delafontaine, P., Y.-H. Song, and Y. Li. 2004. Expression, regulation, and function of IGF-1, IGF-1R, and IGF-1 binding proteins in blood vessels. Arteriosclerosis, Thrombosis, and Vascular Biology 24: 435-444.

Dobos, R., and R. Herd. 2008. Spectral analysis of feeding patterns of steers divergent in residual feed intake. Animal Production Science 48: 843-846.

Durunna, O. N., F. D. N. Mujibi, L. Goonewardene, E. K. Okine, J. A. Basarab, Z. Wang, and S. S. Moore. 2011. Feed efficiency differences and reranking in beef steers fed grower and finisher diets. Journal of Animal Science 89: 158-167.

Eisemann, J., and J. A. Nienaber. 1990. Tissue and whole-body oxygen uptake in fed and fasted steers. British Journal of Nutrition 64: 399-411.

Fitzsimons, C., D. Kenny, M. Deighton, A. Fahey, and M. McGee. 2013. Methane emissions, body composition, and rumen fermentation traits of beef heifers differing in residual feed intake. Journal of Animal Science 91: 5789-5800.

Fitzsimons, C., D. Kenny, and M. McGee. 2014a. Visceral organ weights, digestion and carcass characteristics of beef bulls differing in residual feed intake offered a high concentrate diet. Animal 8: 949-959.

Fitzsimons, C., D. A. Kenny, A. G. Fahey, and M. McGee. 2014b. Feeding behavior, ruminal fermentation, and performance of pregnant beef cows differing in phenotypic residual feed intake offered grass silage. Journal of Animal Science 92: 2170-2181.

Fu, C., E. Felton, J. Lehmkuhler, and M. Kerley. 2001. Ruminal peptide concentration required to optimize microbial growth and efficiency. Journal of Animal Science 79: 1305-1312.

Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analyses (apparatus, Reagents, Procedures, and Some Applications). U.S. Agricultural Research Service.

Golden, J., M. Kerley, and W. Kolath. 2008. The relationship of feeding behavior to residual feed intake in crossbred Angus steers fed traditional and no-roughage diets. Journal of Animal Science 86: 180-186.

Golden, J. W. 2013. Feedlot Cattle Response to Rumen Undegradable Protein and Diet Fiber Level of High Concentrate Diets, University of Missouri-Columbia.

Gomes, R. C., R.D.Sainz, S.L.Silva, M.C.Cesar, M.N.Bonin, P.R.Leme. 2012. Feedlot performance, feed efficiency reranking, carcass traits, body composition, energy requirements, meat quality and calpain system

activity in Nellore steers with low and high residual feed intake. Livestock Science 150: 265-273.

Gomes, R. C., R. D. Sainz, and P. R. Leme. 2013. Protein metabolism, feed energy partitioning, behavior patterns and plasma cortisol in Nellore steers with high and low residual feed intake. Revista Brasileira de Zootecnia 42: 44-50.

Greenwood, R., and E. Titgemeyer. 2000. Limiting amino acids for growing Holstein steers limit-fed soybean hull-based diets. Journal of Animal Science 78: 1997-2004.

Grubbs, J. A. N. Fritchen, E. Huff-Lonergan, J. C. M. Dekkers, N. K. Gabler and S. M. Lonergan. 2013a. Divergent genetic selection for residual feed intake impacts mitochondria reactive oxygen species production in pigs. Journal of Animal Science 91: 2133-2140.

Grubbs, J. K., A. N. Fritchen, E. Huff-Lonergan, N. K. Gabler, and S. M. Lonergan. 2013b. Selection for residual feed intake alters the mitochondria protein profile in pigs. Journal of Proteomics 80: 334-345.

Guan, L. L., J. D. Nkrumah, J. A. Basarab, and S. S. Moore. 2008. Linkage of microbial ecology to phenotype: correlation of rumen microbial ecology to cattle's feed efficiency. FEMS Microbiology Letters 288: 85-91.

Guerino, F., G. Huntington, R. Erdman, T. Elsasser, and C. Reynolds. 1991. The effects of abomasal casein infusions in growing beef steers on portal and hepatic flux of pancreatic hormones and arterial concentrations of somatomedin-C. Journal of Animal Science 69: 379-386.

Hafla, A. G. E. Carstens, T. D. A. Forbes, L. O. Tedeschi, J. C. Bailey, J. T. Walter and J. R. Johnson. 2013. Relationships between postweaning residual feed intake in heifers and forage use, body composition, feeding behavior, physical activity, and heart rate of pregnant beef females. Journal of Animal Science 91: 5353-5365.

Hegarty, R., J. Goopy, R. Herd, and B. McCorkell. 2007. Cattle selected for lower residual feed intake have reduced daily methane production. Journal of Animal Science 85: 1479-1486.

Herd, R., and P. Arthur. 2009. Physiological basis for residual feed intake. Journal of Animal Science 87: E64-E71.

Herd, R., V. Oddy, and E. Richardson. 2004. Biological basis for variation in residual feed intake in beef cattle. 1. Review of potential mechanisms. Animal Production Science 44: 423-430.

Hersom, M., M. Vázquez-Añón, K. Ladyman, M. Kerley, and J. Arthington. 2009. Effect of methionine source and level on performance of growing beef calves consuming forage-based diets. The Professional Animal Scientist 25: 465-474.

Hirst, J., J. Carroll, I. M. Fearnley, R. J. Shannon, and J. E. Walker. 2003. The nuclear encoded subunits of complex I from bovine heart mitochondria. Biochimica et Biophysica Acta (BBA)-Bioenergetics 1604: 135-150.

Hirst, J., M. S. King, and K. R. Pryde. 2008. The production of reactive oxygen species by complex I. Biochemical Society Transactions 36: 976-980.

Huntington, G., M. Poore, B. Hopkins, and J. Spears. 2001. Effect of ruminal protein degradability on growth and N metabolism in growing beef steers. Journal of Animal Science 79: 533-541.

Iqbal, M., N. R. Pumford, Z. X. Tang, K. Lassiter, C. Ojano-Dirain, T. Wing, M. Cooper, and W. Bottje. 2005. Compromised liver mitochondrial function and complex activity in low feed efficient broilers are associated with higher oxidative stress and differential protein expression. Poultry Science 84: 933-941.

Iqbal, M., N. R. Pumford, Z. X. Tang, K. Lassiter, T. Wing, M. Cooper, and W. Bottje. 2004. Low feed efficient broilers within a single genetic line exhibit higher oxidative stress and protein expression in breast muscle with lower mitochondrial complex activity. Poultry Science 83: 474-484.

Johnson, D., C. Ferrell, and T. Jenkins. 2003. The history of energetic efficiency research: Where have we been and where are we going? Journal of Animal Science 81: E27-E38.

Johnson, D. E., K. A. Johnson, and R. L. Baldwin. 1990. Changes in liver and gastrointestinal tract energy demands in response to physiological workload in ruminants. The Journal of Nutrition 120: 649-655.

Johnston, D. 2008. Technical Update NFI & IGF-I. Beef Tech. Note March 2007. Anim. Breed. Genet. Unit, Univ. New England, Armidale, Australia.

Kayser, W., and R. Hill. 2013. Relationship between feed intake, feeding behaviors, performance, and ultrasound carcass measurements in growing purebred Angus and Hereford bulls. Journal of Animal Science 91: 5492-5499.

Kelly, A., M. McGee, D. H. Crews, Jr., A. G. Fahey, A. R. Wylie and D. A. Kenny. 2010a. Effect of divergence in residual feed intake on feeding behavior, blood metabolic variables, and body composition traits in growing beef heifers. Journal of Animal Science 88: 109-123.

Kelly, A., M. McGee, D.H. Crews Jr., C.O. Lynch, A.R. Wylie, R.D. Evans, D.A. Kenny. 2011. Relationship between body measurements, metabolic hormones, metabolites and residual feed intake in performancetested pedigree beef bulls. Livestock Science 135: 8-16.

Kelly, A., M. McGee, D. H. Crews, Jr., T. Sweeney, T. M. Boland and D. A. Kenny. 2010b. Repeatability of feed efficiency, carcass ultrasound, feeding behavior, and blood metabolic variables in finishing heifers divergently selected for residual feed intake. Journal of Animal Science 88: 3214-3225.

Kerley, M. S. 2010. Potential for nutritional imbalance in high-quality forages. In: 4th Grazing Livestock Nutrition Conference. p 125.

Kerley, M. S., and R. Hill. 2012. Nutrition and feed efficiency of beef cattle. Feed Efficiency in the Beef Industry: 75.

Knaus, W., D. H. Beermann, L. O. Tedeschi, M. Czajkowski, D. G. Fox, J. B. Russell. 2002. Effects of urea, isolated soybean protein and blood meal on growing steers fed a corn-based diet. Animal Feed Fcience and Technology 102: 3-14.

Koch, R. M., L. Swiger, D. Chambers, and K. Gregory. 1963. Efficiency of feed use in beef cattle. Journal of Animal Science 22: 486-494.

Koenig, K., and K. Beauchemin. 2013. Nitrogen metabolism and route of excretion in beef feedlot cattle fed barley-based finishing diets varying in protein concentration and rumen degradability. Journal of Animal Science 91: 2310-2320.

Kolath, W., M. Kerley, J. Golden, S. Shahid, and G. Johnson. 2006a. The relationships among mitochondrial uncoupling protein 2 and 3 expression, mitochondrial deoxyribonucleic acid single nucleotide polymorphisms, and residual feed intake in Angus steers. Journal of Animal Science 84: 1761-1766.

Kolath, W. H., M. S. Kerley, J. W. Golden, and D. H. Keisler. 2006b. The relationship between mitochondrial function and residual feed intake in Angus steers. Journal of Animal Science 84: 861-865.

Lambert, A. J., and M. D. Brand. 2009. Reactive oxygen species production by mitochondria Mitochondrial DNA. p 165-181. Springer.

Lancaster, P., G. E. Carstens, J. J. Michal, K. M. Brennan, K. A. Johnson and M. E. Davis. 2014. Relationships between residual feed intake and hepatic mitochondrial function in growing beef cattle. Journal of Animal Science jas. 2013-7409.

Lancaster, P., G. E. Carstens, F. R. B. Ribeiro, M. E. Davis, J. G. Lyons and T. H. Welsh, Jr. 2008. Effects of divergent selection for serum insulin-like growth factor-I concentration on performance, feed efficiency, and ultrasound measures of carcass composition traits in Angus bulls and heifers. Journal of Animal Science 86: 2862-2871.

Lancaster, P., G. Carstens, F. Ribeiro, L. Tedeschi, and D. Crews. 2009. Characterization of feed efficiency traits and relationships with feeding behavior and ultrasound carcass traits in growing bulls. Journal of Animal Science 87: 1528-1539.

Lassiter, K. C. Ojano-Dirain, M. Iqbal, N. R. Pumford, N. Tinsley, J. Lay, R. Liyanage, T. Wing, M. Cooper, and W. Bottje. 2006. Differential expression of mitochondrial and extramitochondrial proteins in lymphocytes of male broilers with low and high feed efficiency. Poultry Science 85: 2251-2259.

Lawrence, P., D. Kenny, B. Earley, D. Crews, and M. McGee. 2011. Grass silage intake, rumen and blood variables, ultrasonic and body measurements, feeding behavior, and activity in pregnant beef heifers differing in phenotypic residual feed intake. Journal of Animal Science 89: 3248-3261.

Lawrence, P., D. Kenny, B. Earley, and M. McGee. 2013. Intake of conserved and grazed grass and performance traits in beef suckler cows differing in phenotypic residual feed intake. Livestock Science 152: 154-166.

Lazarou, M., D. R. Thorburn, M. T. Ryan, and M. McKenzie. 2009. Assembly of mitochondrial complex I and defects in disease. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research 1793: 78-88.

Legleiter, L., A. Mueller, and M. Kerley. 2005. Level of supplemental protein does not influence the ruminally undegradable protein value. Journal of Animal Science 83: 863-870.

Legleiter, L. R. 2003. Responses to Rumen Undegradable Protein as a Source of Essential Amino Acids in Feedlot Cattle, University of Missouri-Columbia.

Lehmkuhler, J., and M. Kerley. 2007. Blood meal and fish meal as supplements to increase the amino acid to energy ratio in steer receiving diets. The Professional Animal Scientist 23: 253-259.

Lehmkuhler, J. W. 2001. Feedlot cattle responses to ruminally undegradable protein, University of Missouri-Columbia.

Liu, M., L. A. Goonewardene, D.R.C. Bailey, J. A. Basarab, R. A. Kemp, P. F. Arthur, E. K. Okine, and M. Makarechian. 2000. A study on the variation of feed efficiency in station tested beef bulls. Canadian Journal of Animal Science 80: 435-441.

Loerch, S., and L. Berger. 1981. Feedlot performance of steers and lambs fed blood meal, meat and bone meal, dehydrated alfalfa and soybean meal as supplemental protein sources. Journal of Animal Science 53: 1198-1203.

Ludden, P., and M. Cecava. 1995. Supplemental protein sources for steers fed corn-based diets: I. Ruminal characteristics and intestinal amino acid flows. Journal of Animal Science 73: 1466-1475.

Mader, C. Y., R. Montanholi, Y. J. Wang, S. P. Miller, I. B. Mandell, B. W. McBride, and K. C. Swanson 2009. Relationships among measures of growth performance and efficiency with carcass traits, visceral organ mass, and pancreatic digestive enzymes in feedlot cattle. Journal of Animal Science 87: 1548-1557.

McCann, J. C., L. M. Wiley, T. D. Forbes, F. M. Rouquette Jr, and L. O. Tedeschi. 2014. Relationship between the Rumen Microbiome and Residual Feed Intake-Efficiency of Brahman Bulls Stocked on Bermudagrass Pastures. PloS one 9: e91864.

McDougall, E. 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. Biochemical Journal 43: 99.

Meister, A., and M. E. Anderson. 1983. Glutathione. Annual Review of Biochemistry 52: 711-760.

Meng, Q., M. Kerley, P. Ludden, and R. Belyea. 1999. Fermentation substrate and dilution rate interact to affect microbial growth and efficiency. Journal of Animal Science 77: 206-214.

Moore, K., D. Johnston, H. Graser, and R. Herd. 2005. Genetic and phenotypic relationships between insulin-like growth factor-I (IGF-I) and net feed intake, fat, and growth traits in Angus beef cattle. Crop and Pasture Science 56: 211-218.

Moore, S., F. Mujibi, and E. Sherman. 2009. Molecular basis for residual feed intake in beef cattle. Journal of Animal Science 87: E41-E47.

Mueller, A., W. Kolath, J. Golden, and M. Kerley. 2004. Evaluation of RUP amino acid levels on lean tissue growth and carcass composition of beef steers fed a roughage-free diet. In: Journal of Dairy Science. p 116-117.

Mueller, A. L. 2004. The ability of empirical equations based on dilution rate to predict microbial efficiency and amino acid flow post ruminally, University of Missouri-Columbia.

Nelson, D. L., A. L. Lehninger, and M. M. Cox. 2008. Lehninger principles of biochemistry. Macmillan.

Nkrumah, J. J. A. Basarab, M. A. Price, E. K. Okine, A. Ammoura, S. Guercio, C. Hansen, C. Li, B. Benkel, B. Murdoch, and S. S. Moore. 2004. Different measures of energetic efficiency and their phenotypic relationships with growth, feed intake, and ultrasound and carcass merit in hybrid cattle. Journal of Animal Science 82: 2451-2459.

Nkrumah, J. D. H. Crews Jr. J. A. Basarab, M. A. Price, E. K. Okine, Z. Wang, C. Li, and S. S. Moore. 2007. Genetic and phenotypic relationships of feeding behavior and temperament with performance, feed efficiency, ultrasound, and carcass merit of beef cattle. Journal of Animal Science 85: 2382-2390.

Nkrumah, J. C. Li, J. Yu, C. Hansen, D. H. Keisler, and S. S. Moore. 2005. Polymorphisms in the bovine leptin promoter associated with serum leptin concentration, growth, feed intake, feeding behavior, and measures of carcass merit. Journal of Animal Science 83: 20-28.

Nkrumah, J. E. K. Okine, G. W. Mathison, K. Schmid, C. Li, J. A. Basarab, M. A. Price, Z. Wang, and S. S. Moore. 2006. Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. Journal of Animal Science 84: 145-153.

NRC. 2000. Nutrient Requirements of Beef Cattle: Seventh Revised Edition: Update 2000. The National Academies Press.

Ojano-Dirain, C., M. Iqbal, T. Wing, M. Cooper, and W. Bottje. 2005a. Glutathione and respiratory chain complex activity in duodenal mitochondria of broilers with low and high feed efficiency. Poultry Science 84: 782-788.

Ojano-Dirain, C. N. R. Pumford, M. Iqbal, T. Wing, M. Cooper, and W. G. Bottje. 2005b. Biochemical evaluation of mitochondrial respiratory chain in duodenum of low and high feed efficient broilers. Poultry Science 84: 1926-1934.

Ojano-Dirain, C., N. B. Tinsley, T. Wing, M. Cooper, and W. G. Bottje. 2007. Membrane potential and H2O2 production in duodenal mitochondria from broiler chickens (Gallus gallus domesticus) with low and high feed efficiency. Comparative biochemistry and physiology. Part A, Molecular & Integrative Physiology 147: 934-941.

Ojano-Dirain, C. P. M. Iqbal, D. Cawthon, S. Swonger, T. Wing, M. Cooper, and W. Bottje 2004. Determination of mitochondrial function and site-specific defects in electron transport in duodenal mitochondria in broilers with low and high feed efficiency. Poultry Science 83: 1394-1403.

Ortigues, I., and M. Doreau. 1995. Responses of the splanchnic tissues of ruminants to changes in intake: absorption of digestion end products, tissue mass, metabolic activity and implications to whole animal energy metabolism. In: Annales de Zootechnie. p 321-346.

Owens, F. N., D. R. Gill, D. S. Secrist, and S. Coleman. 1995. Review of some aspects of growth and development of feedlot cattle. Journal of Animal Science 73: 3152-3172.

Ragland-Gray, K. K., H. E. Amos, M. A. McCann, C. C. Williams, J. L. Sartin, C. R. Barb, and F. M. Kautz. 1997. Nitrogen metabolism and hormonal responses of steers fed wheat silage and infused with amino acids or casein. Journal of Animal Science 75: 3038-3045.

Ramos, M. H., and M. S. Kerley. 2013. Mitochondrial complex I protein differs among residual feed intake phenotype in beef cattle. Journal of Animal Science 91: 3299-3304.

Ramsey, J. J., M.-E. Harper, and R. Weindruch. 2000. Restriction of energy intake, energy expenditure, and aging. Free Radical Biology and Medicine 29: 946-968.

Reecy, J. M., J. E. Williams,, M. S. Kerley, R. S. MacDonald, W. H. Thornton, Jr., and J. L. Davis1996. The effect of postruminal amino acid flow on muscle cell proliferation and protein turnover. Journal of Animal Science 74: 2158-2169.

Richardel, P. T. 2004. Effects of dietary protein level and fish meal on growth and hormonal status of weaned dairy calves, Faculty of the Louisiana State University and Agricultural and Mechanical College In partial fulfillment of the Requirements for the degree of Master of Science in The Interdepartmental Program in Animal and Dairy Sciences by Patricia Terrell Richardel BS, Louisiana State University.

Richardson, E., and R. Herd. 2004. Biological basis for variation in residual feed intake in beef cattle. 2. Synthesis of results following divergent selection. Animal Production Science 44: 431-440.

Richardson, E. R.M. Herdb, P.F. Arthura, J. Wrighta, G. Xu A, K. Dibleya And V.H. Oddy. 1996. Possible physiological indicators for net feed conversion efficiency in beef cattle. In Proceedings-Australian Society Of Animal Production: 21, 103-106.

Richardson, E. R. M. Herd, V. H. Oddy, J. M. Thompson, J. A. Archer and P. F. Arthur. 2001. Body composition and implications for heat production of Angus steer progeny of parents selected for and against residual feed intake. Animal Production Science 41: 1065-1072.

Robinson, D., C. McDonald, K. Hammond, and J. Turner. 1992. Live animal measurement of carcass traits by ultrasound: assessment and accuracy of sonographers. Journal of Animal Science 70: 1667-1676.

Robinson, D., and V. Oddy. 2004. Genetic parameters for feed efficiency, fatness, muscle area and feeding behaviour of feedlot finished beef cattle. Livestock Production Science 90: 255-270.

Rolfe, D., and G. C. Brown. 1997. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiological Reviews 77: 731-758.

Rossignol, R., T. Letellier, M. Malgat, C. Rocher, and J. Mazat. 2000. Tissue variation in the control of oxidative phosphorylation: implication for mitochondrial diseases. Biochemistry. J 347: 45-53.

Saada, A. Rutger O. Vogel, Saskia J. Hoefs, Marie¨l A. van den Brand, Hans J. Wessels, Peter H. Willems, Hanka Venselaar, Avraham Shaag, Flora Barghuti, Orit Reish, Mordechai Shohat, Martijn A. Huynen, Jan A.M. Smeitink, Lambert P. van den Heuvel, and Leo G. Nijtmans. 2009. Mutations in NDUFAF3 (C3ORF60), encoding an NDUFAF4 (C6ORF66)-interacting complex I assembly protein, cause fatal neonatal mitochondrial disease. The American Journal of Human Genetics 84: 718-727.

Salanitro, J., and P. Muirhead. 1975. Quantitative method for the gas chromatographic analysis of short-chain monocarboxylic and dicarboxylic acids in fermentation media. Applied Microbiology 29: 374-381.

Sandelin, B. A. 2005. Association of mitochondrial biochemistry and electron transport chain protein expression with feed efficiency in Angus cattle. University of Arkansas

Schenkel, F., S. Miller, and J. Wilton. 2004. Genetic parameters and breed differences for feed efficiency, growth, and body composition traits of young beef bulls. Canadian Journal of Animal Science 84: 177-185.

Schwartzkopf-Genswein, K., C. Huisma, and T. McAllister. 1999. Validation of a radio frequency identification system for monitoring the feeding patterns of feedlot cattle. Livestock Production Science 60: 27-31.

Seal, C., and D. Parker. 2000. Influence of gastrointestinal metabolism on substrate supply to the liver. Ruminant Physiology, Digestion, Metabolism, Growth and Reproduction. PB Cronje, ed. CABI Publ., Wallingford, UK: 131-148.

Seal, C., and C. Reynolds. 1993. Nutritional implications of gastrointestinal and liver metabolism in ruminants. Nutrition Research Reviews 6: 185-208.

Sharifabadi, H. R., M. J. Zamiri, E. Rowghani, and W. G. Bottje. 2012. Relationship between the activity of mitochondrial respiratory chain complexes and feed efficiency in fat-tailed Ghezel lambs. Journal of Animal Science 90: 1807-1815.

Sindt, M., R. Stock, T. Klopfenstein, and D. Shain. 1993. Effect of protein source and grain type on finishing calf performance and ruminal metabolism. Journal of Animal Science 71: 1047-1056.

Sissell, C. A. 2007. Effects of protein sources on growth and hormonal status of weaned dairy calves, Louisiana State University.

Slyter, L. 1990. Buffers used in the artificial rumen. In: Proc. Continuous Culture Fermenters: Frustration or Fermentation. Northeast ADAS-ASAS Regional Meeting.

Stadtman, E. R., and R. L. Levine. 2000. Protein oxidation. Annals of the New York Academy of Sciences 899: 191-208.

Stick, D., M. Davis, S. Loerch, and R. Simmen. 1998. Relationship between blood serum insulin-like growth factor I concentration and postweaning feed efficiency of crossbred cattle at three levels of dietary intake. Journal of Animal Science 76: 498-505.

Storm, E., and E. Ørskov. 1983. The nutritive value of rumen micro-organisms in ruminants. British Journal of Nutrition 50: 463-470.

Sun, F., H. Xia, Z. Yujia, W. Aojin, X. Jianxing, S. Dan, B. Mark, and R. Zihe. 2005. Crystal structure of mitochondrial respiratory membrane protein complex II. Cell 121: 1043-1057.

Swanson, K. C., J. S. Caton, D. A. Redmer, V. I. Burke, and L. P. Reynolds. 2000. Influence of undegraded intake protein on intake, digestion, serum hormones and metabolites, and nitrogen balance in sheep. Small Ruminant Research 35: 225-233.

Tinsley, N., M. Iqbal, N. Pumford, K. Lassiter, C. Ojano-Dirain, T. Wing, M. Cooper, and W. Bottje. 2004. Expression of proteins in cardiac tissue in broilers with low and high feed efficiency. Poult. Sci 83: 188.

Titgemeyer, E., and C. Loëst. 2001. Amino acid nutrition: Demand and supply in forage-fed ruminants. Journal of Animal Science 79: E180-E189.

Ugalde, C., R. J. Janssen, L. P. van den Heuvel, J. A. Smeitink, and L. G. Nijtmans. 2004. Differences in assembly or stability of complex I and other mitochondrial OXPHOS complexes in inherited complex I deficiency. Human molecular genetics 13: 659-667.

Wagner, J., T. Engle, and T. Bryant. 2010. The effect of rumen degradable and rumen undegradable intake protein on feedlot performance and carcass merit in heavy yearling steers. Journal of Animal Science 88: 1073-1081.

Wang, Z., J. D. Nkrumah, C. Li, J. A. Basarab, L. A. Goonewardene, E. K. Okine, D. H. Crews Jr., and S. S. Moore. 2006. Test duration for growth, feed intake, and feed efficiency in beef cattle using the GrowSafe System. Journal of Animal Science 84: 2289-2298.

Williams, J., F. Farias, J. Wilson, and M. Kerley. 2006. Optimal level of corn distillers dried grains in a no roughage diet for pre-conditioned calves. In: Journal of Animal Science. p 218-218.

Wood, B., J. Archer, and J. Van Der Werf. 2004. Response to selection in beef cattle using IGF-1 as a selection criterion for residual feed intake under different Australian breeding objectives. Livestock Production Science 91: 69-81.

Yu, B. P. 1994. Cellular defenses against damage from reactive oxygen species. Physiological reviews 74: 139.

Zhou, M., and E. Hernandez-Sanabria. 2009. Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies. Applied and Environmental Microbiology 75: 6524-6533.

Zinn, R., and F. Owens. 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. Canadian Journal of Animal Science 66: 157-166.

Zinn, R., and F. Owens. 1993. Ruminal escape protein for lightweight feedlot calves. Journal of Animal Science 71: 1677-1687.