MITOCHONDRIAL COMPLEX I PROTEIN IS RELATED TO RESIDUAL FEED INTAKE IN BEEF CATTLE

A Dissertation

presented to

the Faculty of the Graduate School

at the University of Missouri-Columbia

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

MARCELO HENTZ RAMOS

Dr. Monty Kerley, Dissertation Supervisor

DECEMBER 2011

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

The dissertation entitled:

MITOCHONDRIAL COMPLEX I PROTEIN IS RELATED TO RESIDUAL FEED INTAKE IN BEEF CATTLE

presented by Marcelo Hentz Ramos,

a candidate for the degree of Doctor of Philosophy,

and hereby certify that, in their opinion, it is worthy of acceptance.

Professor Monty Kerley

Professor James Williams

Professor Duane Keisler

Professor Mark Ellersieck

DEDICATION

Dedicate this dissertation to my dad (Joao Jose Ramos), my mom (Maria Jose Hentz Ramos), my brother (Ricardo Hentz Ramos), grandparents, uncles, cousins and dear friends.

ACKNOWLEDGEMENTS

I would like to thank all my family for their love and support.

I would like to thank Dr. Monty Kerley for providing me with an opportunity on being part of his lab and to share with me important concepts that helped me better understand animal nutrition. Special thanks to Dr. Keisler, Dr. Ellersieck and Dr. Williams for being part of the committee.

In addition, I would like to thank James Porter for all his support on all my lab work. Also I cannot forget all my friends for helping me during difficult times. Thanks also for all my co-workers for helping with my research.

TABLE OF CONTENTS

LIST OF TABLES

MITOCHONDRIAL COMPLEX I PROTEIN IS RELATED TO RESIDUAL FEED INTAKE IN BEEF CATTTLE

Marcelo Hentz Ramos

Monty Kerley, PhD., Dissertation Supervisor

ABSTRACT

Residual feed intake (RFI) is a calculated using DMI as dependent variable and metabolic body weight ($BW^{0.75}$) and ADG as independent variable. Mitochondria complex I (mitochondrial NADH:ubiquinone oxidoreductase) is one of the least understood membrane bound protein complexes. Insulin resistance has been reported in calves, dairy cows, horses and swine. For all four mitochondria experiments, there was a significant difference ($P < 0.05$) between RFI and DMI but no difference ($P > 0.05$) was reported for ADG and MMBW. For experiments one, two and three, CI was greater ($P < 0.05$) for –RFI compared to other treatments. The correlation between CI and RFI was -0.37 ($P = 0.02$), -0.48 ($P = 0.034$), and -0.84 ($P = 0.015$) for experiment one, two and three, respectively. For experiment 4, animals with –RFI had a trend for greater concentration ($P = 0.07$) of Band I (protein S1) than +RFI. Correlation between RFI and Band I was -0.72 ($P = 0.04$). A regression was developed using Band I and RFI with RFI = 6.1715 -0.00015 Band I, $R^2 = 0.85$, RMSE = 0.62. No significant difference ($P > 0.05$) was reported in any variable measured during both the glucose and epinephrine tolerance test. We concluded that mitochondrial function was at least in part responsible for differences among animals in metabolic efficiency. In addition, insulin resistance is not responsible for RFI classification in feedlot animals.

CHAPTER 1

LITERATURE REVIEW

Introduction

A well-established measurement of efficiency in beef cattle is called residual feed intake (RFI). In a contemporary group of beef cattle, a regression could be made using ADG and metabolic body weight (MBW, body weight $^{0.75}$) as independent variables and measured dry matter intake (DMI) as dependent variable. Positive residuals are noted as +RFI (inefficient animals, since they are eating more than the contemporary group to achieve the same ADG) and negative residuals are noted as –RFI (efficient animals, since they are eating less than the contemporary group to achieve the same ADG (Herd and Arthur, 2009).

The major concern with use of RFI phenotype is that ADG and MBW alone do not account for all the variation in RFI, therefore some animals that are considered efficient could potentially be inefficient. However, RFI is considered a better measure of efficiency than gain to feed ratio (GF), since RFI is independent of BW and ADG and selection-using GF usually translates into bigger animals (Herd and Arthur, 2009) .

Using steers and bulls totaling 150 animals, researchers reported correlations between RFI and ADG of -0.03 ($P < 0.05$) and between gain to feed (GF) and ADG of 0.63 ($P < 0.05$) (Arthur et al., 2001b). Those correlations illustrate that RFI is independent of weight and GF is not. In the same experiment, the correlation between

RFI and metabolizable energy intake per unit of metabolic weight (MEI) was 0.83 ($P < 0.05$) while the correlation between GF and MEI was 0.13 ($P < 0.05$). Those numbers support the case for RFI being a measure of true metabolic efficiency (Nkrumah et al., 2004). Using records of 1,180 Angus bulls and heifers that were utilized in performance tests, researchers reported the correlation between GF and ADG of -0.74 ($P < 0.05$) when correlations between RFI and ADG was -0.06 ($P < 0.05$) (Arthur et al., 2001a).

The trait RFI is of low to medium heritability. Genetic heritability of RFI was reported to be 0.16 (Herd and Bishop, 2000), 0.21 to 0.39 (Renand et al., 1998), 0.29 (Liu et al., 2000), 0.39 (Arthur et al., 2001a), 0.39 to 0.43 (Arthur et al., 2001b), 0.07 to 0.23 (Fan et al., 1995), 0.38 (Shenkel et al., 2004), 0.39 (Arthur et al., 2001a), 0.16 (Herd and Bishop, 2000), 0.39 (Arthur et al., 2001b), and 0.43 (Arthur et al., 2001b).

The variation in RFI can be explained by the following biological mechanisms: protein turnover, tissue metabolism and stress (37%), digestibility (10%), activity (10%), heat increment and fermentation (9%), body composition (5%), feeding patterns (2%) and others (27%) (Herd and Arthur, 2009).

Feeding Behavior and RFI

Feeding behavior of 115 Brangus heifers were measured during 70 days, researchers reported –RFI heifers spent more time ($P < 0.001$) with their head down than +RFI animals (152 vs. 124 min/d, respectively)(Bingham et al., 2009). However, analyzing the record of 464 animals, researchers reported animals with –RFI with greater ($P < 0.05$) head down than +RFI (42.3 min/d vs. 30.2 min/d, respectively) (Nkrumah et al., 2007b). In addition, using 176 crossbred steers, researchers reported no difference ($P > 0.05$) in feeding duration (87.0 min/d vs. 87.7 min/d, respectively) between +RFI and -RFI cattle (Basarab et al., 2003).

Researchers reported that feeding frequency of –RFI cattle were greater ($P < 0.05$) than +RFI (31.5 events/d vs. 27.2 events/d) (Nkrumah et al., 2007b). However, head down frequency was greater ($P < 0.001$) for +RFI than –RFI (119 vs. 91 events/d, respectively) (Bingham et al., 2009). In addition, correlation between feeding events and RFI was reported to be 0.45 ($P < 0.05$) (Kelly et al., 2010a). Using crossbred steers, researchers reported no difference ($P > 0.05$) in feeding frequency (9.0 events/d vs. 8.4 events/d, respectively) between +RFI and -RFI cattle (Basarab et al., 2003).

More efficient animals spent less time ($P < 0.05$) at the feed bunk compared to inefficient animals (Schwartzkopf et al., 2002). Greater meal duration for +RFI cattle compared to –RFI cattle was also reported (Paddock et al., 2008). In addition, correlation between feeding duration and RFI was reported to be 0.43 (Lancaster et al., 2005).

From the above review, it was concluded that efficient animals spent less time at the feed bunk. That would be supported by lower DMI of –RFI cattle, which at this point

in science we do not have enough data to establish if less time at the bunk is cause or consequence of being efficient.

IGF-1 and RFI

Insulin like growth factor I (IGF-1) is a basic polypeptide made of 70 amino acids (Rinderknecht and Humbel, 1978), which mediate the actions of growth hormone (Van and Underwood, 1978).

Several researchers have reported higher levels of IGF-1 for –RFI compared to +RFI. When 43 bulls and 55 heifers selected for –RFI and +RFI were monitored for 168 days, researchers reported a tendency ($P < 0.10$) for –RFI to have higher levels of IGF-1 on day 28 of the test compared to +RFI (125 ng/ml vs. 89 ng/ml, respectively) (Bishop et al., 1989). Using 169 steers, researchers reported correlation of RFI and IGF-1 of 0.22 (Brown et al., 2004). When feeding calves ($n = 36$) 80, 90, or 100% of their requirements, researchers reported a positive correlation ($P < 0.05$) between IGF-1 levels and efficiency (Stick et al., 1998). Using 62 bulls, researchers reported correlation of RFI and IGF-1 of 0.38 (Brown et al., 2004).

However, no differences in IGF-1 between –RFI and +RFI cattle has also been reported. Using bulls and heifers selected for high or low IGF-1 concentration during two sets of studies, researchers were unable ($P > 0.05$) to find differences in RFI due to IGF-1 lines (Lancaster et al., 2008). In addition, researchers reported that IGF-1 level were not correlated with RFI (Kelly et al., 2010a).

Although IGF-1 is thought to be the metabolite responsible for increase in efficiency when implants are used in beef cattle (Johnson et al., 1998), its capacity to select between –RFI and +RFI cattle is still not strong.

Glucose, Insulin, Leptin and RFI

Researchers have turned their attention to several hormones or metabolites that could account for a big variation in RFI.

When using 464 steers to measure leptin concentrations, researchers reported that animals with –RFI showed lower ($P < 0.05$) levels of leptin (8.6 vs. 20.3 ng/ml) compared with +RFI cattle (Nkrumah et al., 2007c). However, correlation between cortisol, leptin, T3 and T4 levels and RFI was not significant when measured in steers or in bulls (Brown et al., 2004).

Using 40 steers, researchers reported greater ($P < 0.05$) glucose concentration for +RFI compared to –RFI cattle (87 mg/dl vs. 101 mg/dl, respectively) (Kolath et al., 2006a). However, glucose level was not correlated with RFI in heifers (Kelly et al., 2010a).

Insulin levels were not different ($P > 0.05$) between –RFI and +RFI cattle (9.2 vs. 11.1 ng/ml) (Kolath et al., 2006a). In addition, insulin levels were not correlated with RFI when measured in heifers (Kelly et al., 2010a).

Carcass Characteristics and RFI

Using 189 steers, researchers reported no difference ($P > 0.05$) in HCW and REA between –RFI and +RFI cattle (58.9 cm^2 vs. 60.3 cm^2, respectively) (McDonagh et al., 2001). During two experiments which carcass characteristics were measured, researchers reported no differences ($P > 0.05$) in HCW and REA between –RFI and +RFI (Golden et al., 2008). There was no difference ($P > 0.05$) in HCW and REA between –RFI and +RFI when it was measured in 54 purebred Angus steers (Baker et al., 2006). Using 24 Angus x Hereford steers, researchers reported no difference ($P > 0.05$) in HCW and REA between +RFI and –RFI (Bulle et al., 2007).

There should be no significant difference in yield grade (YG) between –RFI and +RFI cattle (Baker et al., 2006; Bulle et al., 2007; Golden et al., 2008); dressing percentage (DP) (McDonagh et al., 2001; Bulle et al., 2007); quality grade (QG) (McDonagh et al., 2001; Baker et al., 2006; Bulle et al., 2007; Nkrumah et al., 2007a). Although there are a few experiments reporting no difference ($P > 0.05$) in back fat (BF) between –RFI and +RFI cattle (Basarab et al., 2003; Baker et al., 2006; Bulle et al., 2007; Golden et al., 2008), the current believe is that +RFI cattle have a greater amount of BF than –RFI (Nkrumah et al., 2007a).

Correlation between BF and RFI were reported to be 0.17 (Exton et al., 2003), 0.19 (Nkrumah et al., 2004), 0.12 (Lancaster et al., 2009), 0.35 (Richardson et al., 2001), 0.17 (Shenkel et al., 2004), and 0.22 (Basarab et al., 2003). Correlation data support the fact that +RFI animals have more fat in their carcass compared to -RFI animals (Bulle et al., 2007).

When using 176 crossbred steers, researchers reported a heavier weight for kidney fat (10.2 vs. 8.8 kg, respectively) and Trim (8.1 vs. 7.4 kg, respectively) for +RFI compared with –RFI (Basarab et al., 2003). Protein gain (g/d) measured in 24 Angus x Hereford steers were not different (P = 0.60) between –RFI and +RFI (94 vs. 83, respectively), however there was a tendency (P = 0.07) for +RFI to gain more fat (g/d) than –RFI (719 vs. 494, respectively) (Bulle et al., 2007). Taking measurements on empty body composition from 176 crossbred steers, researchers reported more (P = 0.02) fat (282 vs. 265 g/kg of empty body weight (EBW)) and the same (P = 0.73) amount of protein (167 vs. 167 g/kg of EBW) between +RFI and –RFI, respectively (Basarab et al., 2003). In addition, correlation between RFI and empty body fat (g/d) was reported at 0.26 (Basarab et al., 2003).

Energetically the deposition of fat is more efficient than protein. However, since protein in the muscle is stored with water, lean tissue deposition is four times more efficient than fat tissue deposition (Owens et al., 1995). Since inefficient animals spend more energy depositing fat in the carcass, it can be concluded that even eating the same amount of energy, +RFI cattle is more inefficient.

Maintenance, Methane and RFI

One theory used to explain differences between efficient and inefficient animals is that +RFI cattle have a greater maintenance requirement than –RFI cattle. When using 176 crossbred steers, researchers reported a heavier weight for liver (6.6 vs. 6.0 kg, respectively), kidney fat (10.2 vs. 8.8 kg, respectively), Trim (8.1 vs. 7.4 kg,

respectively), small and large intestine (30 vs. 27 kg, respectively) and stomach and intestine (49 vs. 45 kg, respectively) for +RFI compared with –RFI (Basarab et al., 2003). However, using 24 Angus x Hereford steers, researchers reported no difference (P = 0.21) for ME requirement for maintenance (Mcal/kg $^{-0.75}$/d) between +RFI and –RFI steers (Bulle et al., 2007).

Another possible explanation for differences in efficiency in beef cattle is that +RFI cattle produce more methane than –RFI cattle, therefore wasting energy that could be used for growth. Using the SF6 tracer technique to measure methane production, researchers reported greater (P = 0.01) methane production for +RFI compared with –RFI (190 vs. 142 g/d, respectively). However, when methane was expressed as g/kg of ADG or g/kg of DMI, no difference (P > 0.05) was reported between +RFI and –RFI (Hegarty et al., 2007). In addition, correlation between methane production and RFI was 0.44 (Nkrumah et al., 2006).

Mitochondria

Mitochondria is a membrane bound organelle that ranges from 0.5 to 10 µm in diameter (Henze and Martin, 2003). The outer membrane surrounds the organelle, while the inner membrane creates the matrix. Where the inner membrane folds, it creates structures called cristae (Nelson and Cox, 2000). While proteins for tricarboxylic cycle (TCA) cycle are found in the matrix, all electron transport chain (ETC) proteins are inserted into the inner membrane (Liu et al., 2002). The ETC has the function of transporting electrons and hydrogen atoms across the mitochondrial membrane and is

composed of five protein complexes : complex I, II, III, IV, and V (Parker and Parks, 1994).

The relationship between RFI and mitochondrial protein complexes exist in poultry. All four respiratory chain complexes (I, II, III and IV) showed a greater activity for –RFI broilers compared to +RFI animals (Iqbal et al., 2004). Researchers reported a greater activity of Complex I and III for efficient vs. inefficient poultry (Bottje et al., 2002). Researchers have reported greater ($P < 0.05$) concentration of complex I in high feed efficiency vs. low feed efficiency birds (Tinsley et al., 2010).

To date, the mechanism that links mitochondria protein complexes and RFI is not clear. One hypothesis is that the relationship was related to production of reactive oxygen species (ROS). The formation of superoxide anion radical (O_2^-), the main source for production of H_2O_2 (ROS), occurs at Complex I and Complex III (Turrens, 1997). Production of H_2O_2 was greater ($P < 0.05$) in breast mitochondria from more inefficient compared to efficient broilers (Bottje et al., 2002). In addition, –RFI broilers had lower ($P < 0.05$) electron leak and lower ($P < 0.05$) ROS production than inefficient animals (Bottje et al., 2004).

There is also a relationship between RFI and mitochondria in bovine. Testing the hypothesis that more efficient cattle have reduced proton leak both protein and mRNA expression of uncoupling protein 2 and 3 were not different ($P > 0.05$) between efficient vs. inefficient animals (Kolath et al., 2006b). However, rate of mitochondria respiration (greater rate of state 2 and 3 respiration) was greater ($P < 0.05$) in efficient vs. inefficient cattle (Kolath et al., 2006a). Research is now focused specifically on complex I and RFI.

Mitochondria protein complex I:II and I:III ratios were greater ($P < 0.05$) for –RFI vs. +RFI cattle (Davis, 2009).

Mitochondria Complex I Subunits

Mitochondria complex I (mitochondrial NADH: ubiquinone oxidoreductase) is the least understood membrane bound protein complex compared to the other protein complexes in mitochondria (Videira, 1998; Brandt et al., 2003; Lazarou et al., 2009). Its protein complex is formed by 45 different subunits (Hirst et al., 2003; Sherwood and Hirst, 2006). It has a L-shaped structure, with the hydrophobic arm embedded in the membrane and the hydrophilic part protruding into the mitochondria matrix (Grigorieff, 1998; Guenebaut et al., 1998). The major function of complex I is to oxidize NADH, reduce ubiquinone, and transport protons across the mitochondrial membrane (Yakovlev and Hirst, 2007; Hirst, 2010). When electrons are transferred from NADH to ubiquinone, four protons are translocated across the inner membrane, as shown below (Wikstroem, 1984; Weiss and Friedrich, 1991; Vogel et al., 2007).

$$NADH + H^+ + Q + 4H^+_{matrix} \rightarrow NAD^+ + QH^2 + 4H^+_{intermembrane}$$

With the stoichiometry mentioned above ($4H^+/2e^-$), complex I is responsible for about 40% of the proton motive force that drives ATP synthesis by ATP synthase (Hunte et al., 2011). Although this mechanism is well accepted, the coupling of proton translocation with electron transfer is still not clear (Hinchliffe and Sazanov, 2005). However, the mechanism could be via close proximity of the ubiquinone binding site to proton translocation, or indirectly via conformational changes of the enzyme complex

(Sharpley et al., 2006; Vogel et al., 2007). The NADH, via flavin mononucleotide (FMN), donates two electrons to the chain of Fe-S clusters, which are passed on via terminal cluster N2 to the quinine. This electron transfer results in changes of ionizable residue inside proton channels, leading to translocation of protons (Hirst, 2005; Efremov et al., 2010; Roessler et al., 2010).

Mitochondrial DNA encodes seven complex I subunits (all hydrophilic); the other 38 are encoded by nuclear genes, which are imported into mitochondria after translation (Carroll et al., 2002; Lazarou et al., 2009; Walker et al., 2009). Nuclear gene-encoded subunits are termed NADH dehydrogenase ubiquinone ("NDU") followed by a description of predicted function/location (FS-iron-sulfur protein region, FV-flavoprotein region, FA-subcomplex α, FB-subcomplex β, FC-undefined subcomplex). In addition, mtDNA-encoded subunits are termed NADH dehydrogenase (ND) followed by the subunit number (Lazarou et al., 2009).

Between all the complexes in mitochondria (I, II, III, IV, and V), deficiency in complex I is the most prevalent defect (Ugalde et al., 2004; Lazarou et al., 2009). Dysfunction in complex I is implicated in many human neurodegenerative diseases (Berrisford and Sazanov, 2009). Correlation of complex I to human diseases is related but not limited to ROS damage (Hirst et al., 2008). Examples of diseases are Parkinson disease (Dawson and Dawson, 2003; Lambert and Brand, 2009), cancer (Lambert and Brand, 2009), diabetes (Lambert and Brand, 2009), Alzheimer's (Lambert and Brand, 2009), fatal neonatal mitochondria disease (Saada et al., 2008; Saada et al., 2009; Mckenzie and Ryan, 2010) and Leigh syndrome (Ugalde et al., 2004).

Deficiency in specific subunits of complex I is reported in the literature. Mutations on NDUFS1 have been linked to Leigh syndrome (Benit et al., 2001; Martin et al., 2005), leukoencephalopathy (Benit et al., 2001; Bugiani et al., 2004), mitochondrial encephalopathy (Papa et al., 2009), and leukodystrophy (Bugiani et al., 2004). Using fibroblasts from human patients, researchers reported that mutation in NDUFS1 was associated with reduced level of whole complex I, reduced activity of complex I, and increased accumulation of ROS (Iuso et al., 2006). In addition, mutations on NDUFV1 have also been linked to Leigh syndrome (Benit et al., 2001) and leukoencephalopathy (Schelke et al., 1999; Benit et al., 2001). Hypertrophic cardiomyopathy and encephalopathy were associated with mutations in NDUFS2 (Loeffen et al., 2001) as well.

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen capable of initiating some kind of deleterious reaction (Lambert and Brand, 2009). These include superoxide anion (O_2^-), hydrogen peroxide (H_20_2), hydroxyl radical (HO), peroxyl radical (RO_2), alkoxyl radical (RO), hydroperoxyl radical (HO_2), hypochlorous acid (HOCl), and oxygen (O_2) (Cocheme and Murphy, 2009; Westermann, 2010). Complex I is a major source of reactive oxygen species (ROS) (Balaban et al., 2005; Grivennikova and Vinogradov, 2006). A proposed mechanism for the generation of ROS is that electrons are passed to complex I in pairs and at certain reactions in the hydrophobic arm they are passed singly. These single electrons might react with oxygen instead of passing down the chain and ROS is formed (Kussmaul and Hirst, 2005; Lambert and Brand, 2009). In addition high ratio of NADH/NAD are linked to greater production of ROS (Murphy, 2009).

Only one experiment has reported a possible link between efficiency in cattle and specific subunits from complex I. Jersey and Limousine crossbred cattle were genotyped for markers related to RFI. Researchers reported NDUFB5 and NDUFA8 were related to RFI (Zulkifli et al., 2009)

Conclusion

Use of RFI instead of feed to gain has the potential to better capture metabolic differences between animals. The understanding of factors that can account for variation in RFI is not yet clear. However, it seems to be accepted that animals with +RFI have a greater amount of BF than –RFI. The use of complex I and complex I subunits as a marker for RFI classification in cattle is new and appears to be a good candidate to explain a large portion of variation in RFI among cattle.

CHAPTER 2

MITOCHONDRIAL COMPLEX I PROTEIN IS CORRELATED TO RESIDUAL FEED INTAKE IN BEEF CATTLE

Abstract

Four experiments were performed to establish a relationship between residual feed intake (RFI) and mitochondrial complex I protein (CI). For experiment one, crossbred Angus steers (initial weight 270 ± 2.0 kg) were assigned to one pen (n = 72). Calves were fed for a total of 170 days. For experiment two, crossbred Braunvieh steers (initial weight 280 ± 3.0 kg) were assigned to one pen (n = 50). Animals were fed for a total of 150 days. For experiment three, crossbred Braunvieh heifers (initial weight 260 ± 3.0 kg) were assigned to one pen (n = 40). Calves were fed for a total of 160 days. For experiment four, crossbred Angus steers (initial weight 290 ± 3.0 kg) were assigned to one pen (n = 40). Calves were fed for a total of 160 days. All cattle in the experiment were fed the same diet. Predicted DMI was calculated using measured DMI as independent variable and metabolic body weight (MMWT) and ADG as dependent variable with PROC GLM (SAS Inst. Inc., Cary, NC). The variable RFI was calculated as the difference between predicted and actual DMI. Blood was drawn, lymphocytes were isolated, and antibody against complex I was used. For experiments one, two and three, complex I quantity was measured using an ELISA commercial kit (Mitosciences, OR). For experiment four, complex I subunits were separated by gel electrophoresis and bands were analyzed for differences in concentration (absorbance). For all four experiments,

there was a significant difference ($P < 0.05$) between RFI and DMI but no difference ($P > 0.05$) was reported for ADG and MMBW. For experiments one, two and three, CI was greater ($P < 0.05$) for –RFI compared to other treatments. The correlation between CI and RFI was -0.37 ($P = 0.02$), -0.48 ($P = 0.034$), and -0.84 ($P = 0.015$) for experiment one, two and three, respectively. For experiment 4, animals with –RFI had a trend for greater concentration ($P = 0.07$) of Band I (protein S1) than +RFI. Correlation between RFI and Band I was -0.72 ($P = 0.04$). A regression was developed using Band I and RFI with RFI = 6.1715 -0.00015BandI, $R^2 = 0.85$, RMSE = 0.62. We concluded that mitochondrial function was at least in part responsible for differences among animals in metabolic efficiency.

Introduction

Residual feed intake (RFI) is calculated using DMI as dependent variable and metabolic body weight ($BW^{0.75}$) and ADG as independent variables. The difference between actual and predicted DMI is defined as RFI. It has been proposed that the variation in RFI can be explained by the following biological mechanisms: protein turnover, tissue metabolism and stress (37%), digestibility (10%), activity (10%), heat increment and fermentation (9%), body composition (5%), feeding patterns (2%) and others (27%) (Herd and Arthur, 2009). Since cell metabolic process can account for more than half the variation in RFI and mitochondria generate the majority of energy used for cell function (Newmeyer and Miller, 2003) a relationship is likely to exist between RFI and mitochondrial function.

Mitochondria complex I (mitochondrial NADH: ubiquinone oxidoreductase) is one of the largest membrane bound protein complexes (Videira, 1998; Brandt et al., 2003; Lazarou et al., 2009). Its protein complex is formed by 45 different subunits (Hirst et al., 2003; Sherwood and Hirst, 2006). It has a L-shaped structure, with the hydrophobic arm embedded in the membrane and the hydrophilic part protruding into the mitochondria matrix (Grigorieff, 1998; Guenebaut et al., 1998).

Research with poultry measured a relationship between RFI and mitochondrial membrane protein (Bottje et al., 2002; Iqbal et al., 2004; Tinsley et al., 2010). Although scarce, research with cattle also measured a relationship between mitochondrial protein complex and RFI (Kolath et al., 2006a; Kolath et al., 2006b; Davis, 2009).

Due to large variations in mechanisms that can explain RFI and limited information on the relationship between RFI and mitochondrial protein complexes, the objective of this research was to determine if a relationship between mitochondrial membrane protein concentration and RFI existed in cattle.

Material and Methods

Feedlot animals

Use of animals in these studies was conducted with approval of University of Missouri-Columbia Animal Care and Use Committee. For experiment one, crossbred Angus steers (initial weight 270 ± 2.0 kg) were assigned to one pen (n = 72). Calves were fed for a total of 170 days. For experiment two, crossbred Braunvieh steers (initial weight 280 ± 3.0 kg) were assigned to one pen (n = 50). Animals were fed for a total of 150 days. For experiment three, crossbred Braunvieh heifers (initial weight 260 ± 3.0 kg)

were assigned to one pen (n = 40). Calves were fed for a total of 160 days. For experiment four, crossbred Angus steers (initial weight 290 ± 3.0 kg) were assigned to one pen (n = 40). Calves were fed for a total of 160 days. All cattle in the experiment were fed the same diet (Table 2.1).

After arrival at the research facility, cattle were de-wormed (Noromectin, Merial, Duluth, GA) and weighed. Cattle was fed once a day in the morning at 0700, allowed ad libitum access to water, and housed in concrete-floored pens with a roof covering approximately one-third of the pen. Individual feed intake was electronically measured using GrowSafe (GrowSafe Systems, Airdrie, Canada) (Wang et al., 2006). Consecutive two-day weights were taken at initiation and at end of feeding and used to calculate average metabolic body weight (body weight$^{0.75}$, MMBW). Sawdust was used as bedding material. Calves were fed until they reached slaughter endpoint (approximately 1 cm of backfat).

All diet samples were analyzed for DM, ash and ether extract (AOAC, 1984), N content (Model FP-428, Leco Co., St. Joseph, MI), and NDF (Goering and Van Soest, 1970). All diets contained 30 mg of monensin/kg (Elanco Animal Health, Greenfield, IN).

Individual DMI, ADG and MMBW 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. Regression R^2 was 0.60, 0.65, 0.64 and 0.67 for experiment one, two, three and four respectively. To calculate RFI, predicted DMI was subtracted from measured DMI (Wang et al., 2006). 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. For experiment one, three –RFI, 26 average and seven +RFI animals were selected. For experiment two, seven –RFI, seven average and six +RFI animals were selected. For experiment three, three –RFI and four +RFI animals were selected. For experiment four, four –RFI and four +RFI animals were selected for mitochondria protein concentration determination.

Lymphocyte isolation

Lymphocytes were isolated from blood (Kolath et al., 2006a). Blood was collected via jugular venipuncture into acid citrate dextrose (ACD) vaccutainer tubes (Becton, Dickinson and Company, Franklin, NJ) and stored at 4°C overnight for further lymphocyte isolation. Fifteen milliliters (ml) 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 40 min at 4°C.

Lymphocyte layers were removed to a 15 ml centrifuge tube (Corning, Corning, NY) and volume brought to 15 ml with phosphate buffered saline (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 10 min at 4°C and the supernatant was removed. Cells were suspended in 1

ml of phosphate buffered saline with Digitonin (200 µg/ml). To disrupt cellular and mitochondrial membranes, lymphocyte homogenate was frozen and thawed with liquid nitrogen five times (Lassiter et al., 2006). Lymphocyte homogenate was held at -80°C until further analysis.

Immunocapture and measurement of mitochondria protein complex I

Protein concentrations of each sample were determined using bicinchoninic acid colorimetric procedures (Pierce Biotechnology, Rockford, IL). All samples were standardized to a protein concentration of 5.5 mg/ml. Immunocapture of mitochondrial protein complex I was done using a microplate assay MS142 (Mitosciences, Eugene, OR). One hundred microliters (µl) of lymphocyte homogenate were incubated with 10 µl of lauryl maltoside stock (200 mM n-dodecyl-B-D-maltopyranoside; Mitosciences MS 910) on ice for 30 min. The lymphocyte homogenate was centrifuged at 13,000 x g for 20 min at 4°C and the pellet containing cellular debris was discarded.

The homogenate was diluted to 1,000 µl with incubation solution (Mitosciences, Eugene, OR) and 200 µl of the solution was added to a microplate well (Mitosciences, Eugene, OR) containing the antibody against complex I. After three hours of incubation, wells were washed twice with 300 µl of buffer solution (Mitosciences, Eugene, OR) and 200 µl of horseradish peroxidase (Mitosciences, Eugene, OR). After one hour, wells were washed three times with 300 µl of buffer solution (Mitosciences, Eugene, OR) and 200 µl of developmental solution (Mitosciences, Eugene, OR) was added to each well. Plates were read at 600 nm using a microplate reader (EL x 808, Biotek, Winooski, VT).

A solution containing two mg of purified bovine heart mitochondrial membranes resuspended in 360 ul of heart mitochondrial resuspension buffer (10 mM Tris-HCL, pH 7.8, 0.25 M sucrose, 0.2 mM EDTA, 1 mM PMSF) was used as a standard for all assays performed. The interassay CV was lower than 5% for all three experiments.

Immunocapture and measurement of mitochondria protein complex I subunits

Protein concentrations of each sample were determined using bicinchoninic acid colorimetric procedures (Pierce Biotechnology, Rockford, IL). All samples were standardized to a protein concentration of 5.5 mg/ml. Three hundred microliters (µl) of lymphocyte homogenate was incubated with 10 µl of lauryl maltoside stock (200 mM n-dodecyl-B-D-maltopyranoside; Mitosciences MS 910) on ice for 30 min. The lymphocyte homogenate was centrifuged at 13,000-x g for 30 min at 4°C and the pellet containing cellular debris was discarded.

Four microliters of protease inhibitor cocktail (P8340, Sigma, St. Louis, MO) and 20 µl of antibody (MS101, Mitosciences, Eugene, OR) was added to the homogenate and allowed for mixing during three hours at room temperature in a shaker (Fisher, Pittsburgh, PA). After mixing, samples were kept overnight at 4°C.

For the elution process, beads were collected by centrifugation during 1 minute 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.

Ten microliters of Tris-Glycine buffer (Jule Inc., Milford, CT) was added to each sample. The mix was heated at 100 °C for five minutes. Samples, protein marker (Broad

Range, Jule Inc., Milford, CT) and bovine mitochondria (Mitosciences, Eugene, OR) 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, Bio-Rad, Hercules, CA) and read (FLA-5000, Fujifilm, Cypress, CA) and analyzed using a software (Multi-gauge, Fujifilm, Cypress, CA).

Statistical analysis

Data were analyzed as a completely random design using the PROC GLM procedure (SAS Inst. Inc., Cary, NC). Treatment (-RFI, average or +RFI) was a fixed effect. Means comparison was made using the LSMEANS statement. Linear regression was performed using PROC REG (SAS Inst. Inc., Cary, NC). PROC CORR (SAS Inst. Inc., Cary, NC) was used to generate correlation between variables. For all variables, P-values less than or equal to 0.05 were declared significant, and values less than or equal to 0.10 were considered tendencies.

Results and Discussion

For all four experiments, correlation between CI and MMBW and CI and ADG were not significant, showing independence of CI and RFI. For experiment one, two and three, the correlation between CI and RFI was -0.37 (P = 0.02), -0.48 (P = 0.034), and

-0.84 (P = 0.015), respectively. For experiment four, the correlation between Band I and RFI was -0.72 (P = 0.04) (Table 2.6).

For experiment one RFI varied from -1.22 to +1.48 kg/d (Table 2.2), for experiment two RFI varied from -2.11 to +3.09 kg/d (Table 2.3), for experiment three RFI varied from -1.8 to 1.6 (Table 2.4), and for experiment four RFI varied from -0.94 to 1.05 kg/d (Table 2.5). The variable DMI was different (P < 0 .05) between RFI groups for all experiments. For experiment one, there was 3.5 kg/d of difference in DMI between –RFI and +RFI group, for experiment two there was 4.6 kg/d of difference in DMI between –RFI and +RFI groups, for experiment three the difference between –RFI and +RFI was 3.4 kg/d of DMI, and for experiment four there was a difference of 1.6 kg between –RFI and +RFI animals. Values for DMI for these experiments are in agreement with values presented in the literature (Zinn et al., 2008; McMeniman et al., 2009)

The variable ADG and MMBW was not different (P < 0.05) between treatments. Residual feed intake is a measure of metabolic efficiency, and therefore independent of ADG and body weight (Arthur et al., 2001a; Nkrumah et al., 2004; Golden et al., 2008; Cruz et al., 2010). For experiment one, ADG varied from 1.86 to 2.01 kg/d. For experiment two, ADG varied from 1.39 to 1.55 kg/d, for experiment three, ADG varied from 1.67 to 1.83, and for experiment four ADG varied from 2.0 to 2.2 kg/d. Values presented here also agree with values reported in the literature (Zinn et al., 2008; Reinhardt et al., 2009).

For all three experiments that measured complex I, its concentration was greater (P < 0.05) for –RFI compared to +RFI cattle. In experiment one, -RFI cattle had a greater (P < 0.05) concentration than average and +RFI calves. Complex I varied from 68.38 to

28.47 µOD/min. In addition, a linear effect ($P < 0.05$) was measured (Table 2.2). For experiment two, CI was greater ($P < 0.05$) for –RFI than the other two treatments. Complex I varied from 14.70 to 28.48 µOD/min and there was a linear effect ($P < 0.05$) between CI and RFI (Table 2.3). Likewise in experiment three CI was greater ($P < 0.05$) for –RFI compared to +RFI.

During experiment four, there was a trend ($P = 0.07$) only for Band I for greater concentration of protein in –RFI compared to +RFI (Table 2.5). The subunit protein in Band I is protein S1, which belongs to complex I. None of the other bands measured was correlated to RFI. Band I was also not correlated with ADG, which makes it a candidate to select for efficiency. Band I was regressed into RFI and an equation was build with RFI = 6.1715 -0.00015 Band I, $R^2 = 0.85$, RMSE = 0.62. The residuals plot against predicted values showed constancy of variance (Kutner et al., 2005).

For energy to be generated inside the cell, electrons flow through four protein complexes that are attached to the inner mitochondrial membrane: complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex III (cytochrome c oxidoreductase) and complex IV (cytochrome oxidase) (Nelson and Cox, 2000). Hydrogen is moved across membranes through the intermembrane space of mitochondria and free energy change is used for ATP synthesis (Boyler, 1997). Since complex I is the protein acceptor for NADH and the first protein in the chain (Walker, 1992; Brandt, 1997), we postulated that a greater concentration of complex I would result in a greater concentration of ATP generated per unit of time.

Research in poultry showed a relationship between mitochondria complex I and efficiency. All four respiratory chain complexes (I, II, III, and IV) showed a greater

activity for –RFI broilers compared to +RFI animals (Iqbal et al., 2004). Researchers have reported greater ($P < 0.05$) concentration of complex I in high feed efficiency vs. low feed efficiency birds (Tinsley et al., 2010).

Mutations on S1 have been linked to Leigh syndrome (Benit et al., 2001; Martin et al., 2005), leukoencephalopathy (Benit et al., 2001; Bugiani et al., 2004), mitochondrial encephalopathy (Papa et al., 2009) and leukodystrophy (Bugiani et al., 2004). Using fibroblasts from human patients, researchers reported that mutation in S1 was associated with: reduced level of whole complex I, reduced activity of complex I, and increased accumulation of reactive oxygen species (ROS) (Iuso et al., 2006). Reactive oxygen species (ROS) are supposed to be the underlined factor of diseases related with complex I (Balaban et al., 2005; Grivennikova and Vinogradov, 2006). A proposed mechanism for ROS production is that electrons are passed to complex I in pairs, at certain reactions in the hydrophobic arm they are passed singly. These single electrons might react with oxygen instead of passing down the chain and ROS is formed (Kussmaul and Hirst, 2005; Lambert and Brand, 2009). Therefore, a second hypothesis was that greater concentration on complex I subunits would allow for less electron leak and less ROS production.

Testing the hypothesis that more efficient cattle have reduced proton leak (low uncoupling protein 2 and 3) researchers concluded that both protein and mRNA expression were not different ($P > 0.05$) between efficient vs. inefficient animals (Kolath et al., 2006b). However, rate of mitochondrial respiration (greater rate of state 2 and 3 respiration) was faster in efficient vs. inefficient steers (Kolath et al., 2006a). In addition,

the correlation between the ratio of complex I/II and RFI was -0.55 ($P < 0.05$) and between the ratio of I/III and RFI was -0.67 ($P < 0.05$) (Davis, 2009).

Production of H_2O_2 was greater ($P < 0.05$) in breast mitochondrial from inefficient poultry compared to efficient birds. In the same experiment, researchers reported a greater activity of Complex I and III for efficient vs. inefficient poultry (Bottje et al., 2002). Using broilers with –RFI and +RFI, researchers concluded that efficient animals reported lower ($P < 0.05$) electron leak and lower ($P < 0.05$) reactive oxygen species (ROS) production than inefficient animals (Bottje et al., 2004). In addition formation of superoxide anion radical (O_2^-), main source for the production of H_2O_2 (ROS), occurs at Complex I and Complex III (Turrens, 1997).

Conclusion

Quantity of complex I protein is greater ($P < 0.05$) for –RFI cattle compared to +RFI cattle. Quantity of S1, a protein of complex I from mitochondria, is greater in –RFI compared to +RFI cattle. Mitochondrial function was at least in part responsible for differences among animals in metabolic efficiency.

Table 2.1 - Ingredients and chemical composition of diets fed to steers

Ingredient	% of DM
Corn	86.9
Blood meal	1.0
Feather meal	1.6
Supplement[1]	2.5
DDGS[2]	8.0
Chemical composition % DM	
DM	89
CP	14.8
NDF	11.7
EE	5.4
ASH	4.4
NFC[3]	63.7

[1]Supplement consisted of 13.6% potassium chloride, 30.0% calcium carbonate, 4.0% sodium chloride, 1% mineral premix (10% Fe, 10% Mn, 2% Cu, 500 mg/kg Co, 1,000 mg/kg I, and 1,500 mg/kg Se), 1% vitamin premix (4,000,000 IU A, 800,000 IU D and 1,200 IU E), and 0.4% Rumensin-80 (Elanco, Greenfield, IL) and 50% corn.
[2]Corn distillers dried grains with soluble
[3]Non-fiber carbohydrate: 100 – (CP + EE + Ash + NDF)

Table 2.2 - Performance and mitochondria complex I protein quantity of animals differing in RFI (Experiment one)

	-RFI	Average	+RFI	SEM	*P-value*	*Linear*
RFI[1]	-1.22[c]	-0.15[b]	1.48[a]	0.147	<0.0001	-
DMI, kg/d	9.71[c]	11.47[b]	13.24[a]	0.608	0.0002	-
ADG, kg/d	2.01	1.86	1.91	0.110	0.6762	-
MMBW[2], kg	79.6	85.7	86.4	3.033	0.3809	-
CI[3], µOD/min	68.38[a]	39.23[b]	28.47[b]	6.52	0.0069	0.0018

N = 36
[1]RFI = Residual feed intake
[2]MMBW = Average metabolic body weight
[3]CI = complex I

Table 2.3 - Performance and mitochondria complex I protein quantity of animals differing in RFI (Experiment two)

	-RFI	Average	+RFI	SEM	*P-value*	*Linear*
RFI	-2.11[a]	-0.02[b]	3.09[c]	0.1886	<0.0001	-
DMI, kg/d	11.3[c]	12.6[b]	15.9[a]	0.3375	<0.0001	-
ADG, kg/d	1.55	1.41	1.39	0.0664	0.1818	-
MMBW[2], kg	92.7	85.3	88.6	2.9885	0.2186	-
CI[3], µOD/min	28.48[a]	15.70[b]	14.70[b]	3.8273	0.0289	0.0167

N = 20
[1]RFI = Residual feed intake
[2]MMBW = Average metabolic body weight
[3]CI = complex I

Table 2.4 - Performance and mitochondria complex I protein quantity of animals differing in RFI (Experiment three)

	-RFI	+RFI	SEM	*P-value*
Rank, kg/d[1]	-1.80[b]	1.61[a]	0.2344	<0.0001
DMI, kg/d	8.0[b]	11.4[a]	0.5453	0.0057
ADG, kg/d	1.83	1.67	0.1561	0.4783
MMBW[2], kg	73.51	73.23	2.1133	0.9239
CI[3] , µOD/min	59.44[a]	52.27[b]	1.1545	0.0054

N = 7
[1]RFI = Residual feed intake
[2]MMBW = Average metabolic body weight
[3]CI = complex I

Table 2.5 - Performance and protein subunits absorbance between –RFI and +RFI cattle.

	-RFI	+RFI	SEM	P-value
RFI, kg/d	-0.94	1.05	0.1739	0.0002
Intake, kg/d	10.9	12.5	0.4586	0.0497
ADG, kd/d	2.2	2.0	0.1312	0.2758
Band I[1]	117,486	79,965	12,058	0.0701
Band II	165,295	157,578	23,866	0.8268
Band III	271,372	178,412	40,679	0.1573
Band IV	110,492	108,497	15,637	0.9311

[1]Protein concentration is expressed in absorbance

Table 2.6 - Pearson's correlation between variables used in complex I protein experiment.

	Intake	ADG	Band I	Band II	Band III	Band IV
RFI	0.63[1]	-0.43	-0.72	-0.26	-0.60	-0.19
	(0.09)	(0.28)	(0.04)	(0.53)	(0.10)	(0.65)
DMI		0.14	-0.25	0.02	-0.15	0.05
		(0.73)	(0.53)	(0.94)	(0.71)	(0.90)
ADG			0.60	0.39	0.60	0.37
			(0.10)	(0.32)	(0.11)	(0.36)
Band I				0.20	0.96	0.06
				(0.62)	(0.0001)	(0.88)
Band II					0.24	0.97
					(0.55)	<0.0001
Band III						0.12
						(0.76)

[1]Pearson's correlation followed by P-value in parenthesis.

CHAPTER 3

COMPARISON OF GLUCOSE AND EPINEPHRINE TOLERANCE BETWEEN STEERS WITH HIGH AND LOW RESIDUAL FEED INTAKE PHENOTYPE

Abstract

Two experiments were performed to measure blood glucose and insulin dynamics n –residual feed intake (RFI, efficient) and +RFI (inefficient) feedlot calves. In experiment one, 72 Angus crossbred steers (initial weight 333 ± 8.5 kg) were used and in experiment two, 60 Angus crossbred steers (initial weight 355 ± 7.3 kg) were used. Calves were fed for a total of 70 days and had ad libitum access to a common diet (87% corn, 8% corn distiller's grains, 1% blood meal, 1.6% feather meal and 2.5% mineral and vitamins). Expected feed intake was calculated using DMI as dependent variable and metabolic body weight ($BW^{0.75}$) and ADG as independent variable. The difference between actual and expected DMI was defined as residual feed intake. Two –RFI and two +RFI steers were selected from experiment one for a glucose tolerance test (**GTT1**). Four –RFI and four +RFI steers were selected from experiment two for a glucose tolerance test (**GTT2**) and an epinephrine tolerance test (**ETT**). For GTT1, 150 mg of glucose/kg BW and 30 mU of insulin/kg were administered. Insulin was injected twenty minutes after glucose injection. During GTT2, same dosage of glucose used in GTT1 was injected and 1ug of epinephrine/kg was administered. Body weight and ADG were not different (P >

0.05) between treatments for all tolerance tests. Dry matter intake and RFI were significant different ($P < 0.05$) between treatments for all tolerance tests. No significant difference ($P > 005$) was reported in any variable measured during both the glucose and epinephrine tolerance test. We concluded that insulin resistance is not responsible for RFI classification in feedlot animals.

Introduction

Expected feed intake was calculated using DMI as dependent variable and on metabolic body weight ($BW^{0.75}$) and ADG as independent variable. The difference between actual and expected DMI was defined as residual feed intake. Animals that have a positive residual (+RFI) are considered inefficient and animals that have a negative residue (-RFI) are considered efficient (Kock et al., 1963). Several mechanisms are proposed to account for the majority of the variability in RFI including: mitochondria function and membrane proteins (Kolath et al., 2006a; Davis, 2009); IGF levels (Johnston et al., 2004; Lancaster et al., 2008; Kelly et al., 2010a); feeding behavior (Basarab et al., 2003; Bingham et al., 2009); leptin levels (Nkrumah et al., 2007c), methane levels (Hegarty et al., 2007), and glucose levels (Kolath et al., 2006a). Unfortunately, differences in efficiency are not adequately explained by current physiological mechanisms (Herd and Arthur, 2009).

It is reported that +RFI cattle have a greater amount of back fat than –RFI (Exton et al., 2003; Nkrumah et al., 2004; Lancaster et al., 2009). Epinephrine, a hormone produced by the adrenal gland, will increase lipolysis (Chernick et al., 1986) and inhibits

insulin secretion by the pancreas (Deibert and Defronzo, 1980). When fat accumulates in the cell, glucose metabolism can be impaired; creating what is called insulin resistance (Schinner et al., 2005). Insulin resistance has been reported in calves (Hugi et al., 1997), dairy cows (Chagas et al., 2009), horses (Kronfeld et al., 2005) and swine (Mosnier et al., 2010). However, to our knowledge, no experiment has examined the role of glucose and epinephrine in relation to feed efficiency of cattle. We hypothesized that +RFI cattle are resistant to insulin leading to an inefficiency of glucose utilization. Therefore our objective was to perform glucose and epinephrine tolerance tests in –RFI and +RFI cattle.

Material and Methods

Cattle Management

Use of animals in these studies was conducted with approval of University of Missouri-Columbia Animal Care and Use Committee. In experiment one, 72 Angus crossbred steers (initial weight 333 ± 8.5 kg) were used and in experiment two, 60 Angus crossbred steers (initial weight 355 ± 7.3 kg) were used. All cattle in the experiment were fed the same diet (Table 3.1).

After arrival at the research facility, cattle were de-wormed (Noromectin, Merial, Duluth, GA) and weighed. Cattle was fed once a day in the morning at 0700, allowed ad libitum access to water, and housed in concrete-floored pens with a roof covering approximately one-third of the pen. Individual feed intake was electronically measured using GrowSafe (GrowSafe Systems, Airdrie, Canada) (Wang et al., 2006). Consecutive two-day weights were taken at initiation and at end of feeding and used to calculate

average metabolic body weight (body weight$^{0.75}$, MMBW). Sawdust was used as bedding material. Calves were fed once until they reached slaughter endpoint (approximately 1 cm of backfat).

All diet samples were analyzed for DM, ash and ether extract (AOAC, 1984), N content (ModelFP-428, Leco Co., St. Joseph, MI), and NDF (Goering and Van Soest, 1970). All diets contained 30 mg of monensin/kg (Elanco Animal Health, Greenfield, IN).

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

$$Y_i = B_0 + B_1ADG + B_2MMWT$$

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. Regression R^2 was 0.60 and 0.65 for experiment one and two, respectively. To calculate RFI, predicted DMI was subtracted from measured DMI (Wang et al., 2006). 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. For experiment one, two –RFI and two +RFI steers, were selected for glucose tolerance test (**GTT**). For experiment two, four –RFI, and four +RFI steers were selected for GTT and epinephrine tolerance test (**ETT**).

Glucose and epinephrine tolerance test

Animals were withdrawal from feed the night before the tolerance test was performed. On the morning of the tolerance test, the area around the jugular vein was shaved and disinfected and a sterile nonpryogenic catheter (Abbocath-T, Abbott Laboratories, Abbot Park, IL) was inserted into the jugular vein and glued to the skin. Catheters were flushed with sterile, nonpryogenic saline (Fisher Scientific, Pittsburg, PA). Animals were head-restrained in a head-chute during the experiment.

During GTT1, blood was collected at -10, 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 120, 150, 180, 240, 300, and 360 min relative to the intravenous injection of glucose (50% dextrose solution, 150 mg/kg BW, Vedco, St. Joseph, MO). Twenty minutes after bolus glucose infusion, insulin (30 mU/kg of BW) was bolus infused.

For GTT2 and ETT, blood was collected at -20, -15, -10, -5, 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 min relative to the intravenous injection of glucose (50% dextrose solution, 150 mg/kg BW, Vedco, St. Joseph, MO). Immediately after completion of GTT2, the ETT began using the same animals. Blood was collected at -20, -15, -10, -5, 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 min relative to the intravenous injection of epinephrine (1 ug/kg BW). Glucose, insulin and epinephrine were administered in less than one minute for all the experiments.

Each time blood was collected; 8 ml of blood was drawn into 16 x 100 mm siliconated vaccutainer tubes (Kendall, Mansfield, MA) and allowed to coagulate. Serum was obtained after centrifugation at 2000-x g for 15 min. Serum was then analyzed for glucose concentrations using a colorimetric kit (Thermo Scientific, Middletown, VA).

Ten ul of serum was mixed with 1.5 ml of reagent, incubated in 37 °C for 10 min and then absorbance was read using a spectrophotometer (Beckman, Brea, CA) with wavelength set at 500 nm. Standards were made using a five serial dilution of glucose 200 mg/dl (Thermo Scientific, Middletown, VA).

Insulin concentrations were determined using a specific, double-antibody, equilibrium radioimmunoassay as described by Kolath et al., (2006). Zinc-free bovine insulin was prepared for iodination and for standard curve following the procedures of (Sodoyez et al., 1975). Ten micrograms of zinc-free bovine insulin was then solubilized in 50 ul of H_2O, combined with 500 uCi of ^{125}I-Na, and incubated in the presence of 100 ug of iodogen (Pierce Biotechnology, Rockford, IL) for six min with gentle mixing. A Sep-Pak C18 cartridge was prepared by washing with 10 ml of 50% (vol/vol) acetonitrile containing 50 m*M* triethylamine solution (pH adjusted to pH 3 with phosphoric acid), followed by 10 mL of deionized H_2O before addition of the ^{125}I-bovine insulin. Recovery of the monoiodinated form of ^{125}I-bovine insulin was achieved by differential elution from a 10-mL Sep-Pak C18 cartridge (Mallinckrodt Baker Inc., Phillipsburg, NJ) as previously described by (Deleo, 1994). The cartridge was then washed sequentially with: 1) 5 mL of 0.4 *M* phosphate buffer, pH 7.4; 2) 10 mL of 29% (vol/vol) acetonitrile containing 50 m*M* trimethylamine; 3) 5 mL of 10% (vol/vol) acetonitrile containing 0.2 *M* ammonium acetate, pH 5.5; and 4) 5 mL of 50% (vol/vol) acetonitrile containing 0.2 *M* ammonium acetate, pH 5.5. This final fraction was collected and diluted to 25,000 cpm per 100 ul of assay buffer (0.1% gelatin, 0.01 *M* EDTA, 0.9% NaCl, 0.01 *M* PO4, 0.01% sodium azide, 0.1% Tween-20, pH 7.1). Guinea pig antibovine insulin antiserum (Elsasser et al., 1986) was diluted to a final tube dilution of 1:167,000 in assay buffer.

Standard concentrations of zinc-free bovine insulin (0.064 to 40 ng/tube) and increasing volumes of bovine serum pool (25 to 300 ul) were added to assay tubes in quadruplicate, and the total volume was balanced to 300 ul per tube with assay buffer.

All serum samples (100 ul aliquots) were assayed in triplicate. All components were then incubated at 4°C for 24 h. The antigen-antibody complex was then precipitated following a 15-min, 22°C incubation with 100 ul of a pre-precipitated sheep-anti-guinea pig secondary antibody. The secondary antibody complex was then precipitated by centrifugation at 3,000-× *g* for 30 min, and the supernatant was discarded by aspiration. Assay tubes containing the precipitate were counted for 1 min on an LKB1275 gamma counter (LKB Wallac, Turku, Finland). Standards and serum aliquots of the bovine serum pool were linear (log/logit transformation; r2 = 0.98) and parallel over a mass of 0.064 to 40 ng/tube and a serum volume of 25 to 300 ul. Total specific binding was 38%, the minimum detectable concentration was 0.064 ng/tube, the percentage recovery of mass was 98.1%, and the inter- and intra-assay CV were 5.2 and 6.8 %, respectively.

Glucose and insulin calculations

Basal glucose concentration was calculated based on the averaged of times -10 and 0 for GTT1 and -20, -15, -10, -5, and 0 for GTT2. Peak value was at 5 min (highest) post-challenge. Area under the curve (**AUC**) was calculated from 0 to 30 min post-challenge by averaging the concentrations at consecutive time points, subtracting the baseline concentration, and multiplying be the elapsed time between samples : $[([(x_1 + x_2)/2] - x_{bas}) \cdot (t_2 - t_1)]$ (Tilton et al., 1999). Clearance rate (**CR**) was calculated as the slope of the exponential curve, as follow:

CR, %/min = 100 * (ln [t_a] – ln [t_b]) / (t_b – t_a)

Where

[t_a] = concentration of metabolite at time a (t_a) and

[t_b] = concentration of metabolite at time b (t_b).

Half-life (**t** ^{½}) was calculated as follows:

t ^{½} = min = 100 * (ln (2)) / CR

Statistical analysis for glucose and epinephrine tolerance tests

All the statistical analyses were performed using PROC MIXED (SAS Inst. Inc., Cary, NC). Basal and peak values were analyzed using treatment as fixed and animal as random effects. For variables AUC, CR, and $t^{1/2}$, only treatment was used as fixed effect. All P-values less than or equal to 0.05 were considered significant, and values less than or equal to 0.10 were considered tendencies.

Results and Discussion

Body weight and average daily gain were not different (P > 0.05) between treatments for both experiment one and two (Table 3.2 and 3.3). Since cattle used in these studies have the same genetic background, the same frame scores, and were at the same weigh when the research was performed, they also were anticipated to have the same amount of fat in their carcass (Owens et al., 1995). Residual feed intake was different (P < 0.05) between treatments for both studies. In addition, DMI was greater (P < 0.05) for

+RFI compared to –RFI (Tables 3.2 and 3.3). Since cattle were selected to be in this study based on RFI value, the significant difference between RFIs was expected.

There was no significant difference ($P > 0.05$) between treatments for basal levels of glucose in any tolerance test performed (Tables 4 and 5). Values reported here for glucose levels are in agreement with published ones (Mir et al., 1998; Mir et al., 2002). Greater glucose concentration for +RFI compared to –RFI has been reported (Kolath et al., 2006a). However, no difference in glucose levels between +RFI versus –RFI has also been reported (Kelly et al., 2010a; Kelly et al., 2010b). Basal insulin was not significantly different ($P > 0.05$) between treatments for both tolerance tests. Insulin values presented in this publication are greater than values published due to the homologous bovine insulin assay methodology used in this study (Hersom et al., 2004; Vasconcelos et al., 2009).

Peak values for glucose were not statistically different ($P > 0.05$) between treatments for both tolerance tests performed. When beef calves were exposed to a GTT using 150 mg/kg of BW of glucose, researchers reported peak values of glucose varying from 150 mg/dl to 160 mg/dl (Bossaert et al., 2009). Using 300 mg/kg of BW of glucose in steer, researchers reported peak value for glucose of 270 mg/dl to 300 mg/dl. Values reported in this research are in agreement with published ones. Insulin peak values were not different ($P > 0.05$) between treatments for both tolerance tests.

For both tolerance tests there were no differences ($P > 0.05$) in the variable AUC and $t^{1/2}$ for glucose and insulin between –RFI and +RFI. The variable CR for glucose was not different ($P > 0.05$) between treatments for all tolerance tests performed. When researchers infused dairy cows with 250 mg of glucose per Kg of BW, CR varied from 1.6% / min to 2.7% / min (Pires et al., 2007). Clearance rate varied from 1.7% / min to

2.6% / min when researchers performed a GTT using 150 mg of glucose per kg of BW (Hayirli et al., 2001). Since CR for GTT1 and GTT2 was not different between treatments, we speculate that the ability of glucose uptake by cell by +RFI and –RFI are not different. In addition, CR for ETT did not differ between treatments suggesting that glucose release from cell is not affected by RFI status.

Adrenaline is a lipolitic and insulin is a lipogenic factor (Okuda et al., 1966; Kersten, 2000; McCann and Reimers, 1986). Insulin resistance has been reported to exist in feedlot cattle (Eisemann et al.). The main reason for insulin resistance is believed associated with lipid accumulation in the cell (Petersen and Shulman, 2006). The mechanism for insulin resistance has been linked to down regulation of glucose transporter (GLUT4) (Hotamisligil et al., 1993; Kahn and Flier, 2000). As cattle get heavier and closer to mature weigh, lipid will increase in the carcass (Owens et al., 1995). There is a positive correlation between +RFI cattle and back fat (Richardson et al., 2001; Basarab et al., 2003; Exton et al., 2003; Nkrumah et al., 2004; Shenkel et al., 2004; Lancaster et al., 2009). If the high amount of fat in the cell down regulates the expression of GLUT4, more insulin would be necessary to transport the same amount of glucose and thus glucose levels should increase; but this did not occur in our investigations of –RFI and +RFI cattle.

We hypothesized that the +RFI cattle would be more insulin resistant than –RFI. Based on the results on this experiment, we reject this hypothesis. Although no difference in glucose and insulin between +RFI and –RFI has been reported, to our knowledge, no one has performed a GTT or an ETT using these two groups of animals.

Conclusions

We concluded that uptake and release of glucose into the cell is not affected by RFI status of feedlot animals.

Table 3.1 - Diet composition fed to both experiments

Ingredient	% of DM
Corn	86.9
Blood meal	1.0
Feather meal	1.6
Supplement[1]	2.5
DDGS[2]	8.0
Chemical composition %DM	
DM	89
CP	14.8
NDF	11.7
EE	5.4
ASH	4.4
NFC[3]	63.7

[1]Supplement consisted of 13.6% potassium chloride, 30.0% calcium carbonate, 4.0% sodium chloride, 1% mineral premix (10% Fe, 10% Mn, 2% Cu, 500 mg/kg Co, 1,000 mg/kg I, and 1,500 mg/kg Se), 1% vitamin premix (4,000,000 IU A, 800,000 IU D and 1,200 IU E), and 0.4% Rumensin-80 (Elanco, Greenfield, IL) and 50% corn.
[2]Corn dried distillers grains with soluble
[3]Non-fiber carbohydrate: 100 – (CP + EE + Ash + NDF)

Table 3.2 - Body weight, dry matter intake and RFI of animals used during experiment 1

	+RFI	-RFI	SEM	P-value
Body weight, kg	504	504	50.9571	0.9951
DMI, kg	11.2[a]	9.7[b]	0.2151	0.0413
ADG, kg/d	1.17	1.19	0.0226	0.6855
RFI, kg	1.0[a]	-1.1[b]	0.1000	0.0045

n = 2

Table 3.3 - Body weight, dry matter intake and RFI of animals used during experiment 2

	+RFI	-RFI	SEM	P-value
Body weight, kg	575	606	27.60	0.1552
DMI, kg	11.0[a]	9.8[b]	0.1425	0.0101
ADG, kg/d	1.28	1.22	0.0206	0.1758
RFI, kg	-1.4[a]	1.2[b]	0.1190	0.0002

n = 4

Table 3.4 - Glucose values for GTT and ETT

	+RFI	-RFI	SEM	*P-value*
GTT1				
Basal (mg/dl)	97.0	102.0	8.3455	0.6761
Peak (mg/dl)	188.6	203.4	18.5102	0.5925
AUC (mg/dl * min)[1]	2563.1	1240.3	1051.99	0.4678
CR, %/min	2.02	2.23	0.3899	0.7684
$t^{½}$, min	34.29	33.04	5.7662	0.9032
GTT2				
Basal (mg/dl)	102.6	86.2	11.2523	0.3046
Peak (mg/dl)	207.9	234.9	43.8316	0.6696
AUC (mg/dl * min)	1771.6	1743.0	250.60	0.9382
CR, %/min	1.83	3.03	0.8339	0.2083
$t^{½}$, min	48.01	31.10	12.2105	0.2021
ETT				
Basal (mg/dl)	97.1	81.8	9.9671	0.2811
Peak (mg/dl)	123.5	114.1	6.9546	0.3553
AUC (mg/dl * min)	487.5	583.7	172.95	0.7076
CR, %/min	1.63	1.61	0.5169	0.9799
$t^{½}$, min	59.29	46.83	11.3053	0.4925

[1]AUC = area under the curve, CR = clearance rate and $t^{½}$ = half-life

Table 3.5 - Insulin values for GTT2 and ETT

	+RFI	-RFI	SEM	*P-value*
GTT2				
Basal (ng/ml)	27.10	27.38	2.2673	0.9307
Peak (ng/ml)	66.13	72.59	11.9650	0.7078
AUC (ng/ml * min)	781.1	680.2	177.92	0.7022
CR, %/min	3.37	3.62	0.5513	0.7673
$t^{½}$, min	21.40	21.75	3.8855	0.9539
ETT				
Basal (ng/ml)	26.47	29.97	3.6343	0.4973
Peak (ng/ml)	36.27	48.53	8.2215	0.3071
AUC (ng/ml * min)	173.8	53.9	86.05	0.3625
CR, %/min	2.18	2.30	0.5141	0.8620
$t^{½}$, min	32.07	38.42	6.7685	0.5162

[1]AUC = area under the curve, CR = clearance rate and $t^{½}$ = half-life

REFERENCES

AOAC. 1984. Official Methods Analysis (12th Ed.). Association of Official Analytical Chemists, Arlington, VA.

Arthur, P.F., J.A. Archer, D.J. Johnson, R.M. Herd, E.C. Richardson, and P.F. Parnell. 2001a. 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. F., G. Renand, and D. Krauss. 2001b. Genetic and phenotypic relations among different measures of growth and feed efficiency in young Charolais bulls. Livestock Production Science 68: 131-139.

Baker, S. D., J. I. SzasZ, T. A. Klein, P. S. Kuber, C. W. Hunt, J. B. Glaze, D. Falk, R. Richard, J. C. Miller, R. A. Battaglia, and R. A. Hill. 2006. Residual feed intake of purebred Angus steers: Effects on meat quality and palatability. Journal of Animal Science 84: 938-945.

Balaban, R. S., S. Nemoto, and T. Finkel. 2005. Mitochondria, oxidants, and aging. Cell 4: 483-495.

Basarab, J. A., 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.

Benit, P., D. Chretien, N. Kadhom, P. Debeney, V. Daire, A. Cabral, S. Peudenier, R. Pierre, A. Munnich, and A. Rotig. 2001. Large-Scale deletion and point mutations

of the nuclear NDUFV1 and NDUFS1 genes in mitochondrial complex I deficiency. Am. J. Hum. Genet. 68: 1344-1352.

Berrisford, J. M., and L. A. Sazanov. 2009. Structural basis for the mechanism of respiratory complex I. The journal of biological chemistry 284: 29773-29783.

Bingham, G. M., T. H. Friend, P. A. Lancaster, and G. E. Carstens. 2009. Relationship between feeding behavior and residual feed intake in growing Brangus heifers. Journal of Animal Science 87: 2685-2689.

Bishop, M. D., R. C. M. Simmen, F. A. Simmen, and M. E. Davis. 1989. The relationship of insulin-like growth factor-I with postweaning performance in Angus beef cattle. Journal of Animal Science 67: 2872-2880.

Bossaert, P., J. L. M. R. Leroy, S. Campeneere, S. Vliegher, and G. Opsomer. 2009. Differences in the glucose-induced insulin response and the peripheral insulin responsiveness between neonatal calves of the Belgian Blue, Holstein-Friesian, and East Flemish breeds. Journal of dairy science 92: 4404-4411.

Bottje,W., Z.X. Tang, M. Iqbal, D. Cawthorn, 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. G., M. Iqbal, N. R. Pumford, C. O. Dirain, and K. Lassiter. 2004. Role of mitochondria in the phenotypic expression of feed efficiency. Jounal of applied poultry research 13: 94-105.

Boyler, P. D. 1997. The ATP synthase - a splendid molecular machine. Annual Review of Biochemistry 66: 717-749.

Brandt,U. 1997. Proton-translocation by membrane-bound NADH:ubiquinone-oxireductase (complex I) through redox-gated ligand conduction. Biochimica et biophysica acta 1318: 79-91.

Brandt, U., S. Kerscher, S. Droese, K. Zwicker, and V. Zickermann. 2003. Proton pumping by NADH:ubiquinone oxidoreductase. A redox driven conformational change mechanism. The FEBS journal 545: 9-17.

Brown, E.G., G.E. Carstens, J.T. Fox, K.O. Curley, T. M. Bryan, L. J. Slay, T. H. Welsh, R. D. Randel, J. W. Holloway, and D. H. Keisler. 2004. Physiological indicators of performance and feed efficiency traits in growing steers and bulls. Departmental Report - Texas A&M University.

Bugiani, M., F. Invernizzi, and S. Alberio. 2004. Clinical and molecular findings in children with complex I deficiency. Biochimica et biophysica acta 1659: 136-147.

Bulle, F. C. P., P. V. Paulino, A. C. Sanches, and R. D. Sainz. 2007. Growth, carcass quality, and protein and energy metabolism in beef cattle with different growth potentials and residual feed intakes. Journal of Animal Science 85: 928-936.

Carroll, J., R. J. Shannon, I. Fearnley, J. Walker, and J. Hirst. 2002. Definition of the nuclear encoded protein composition of bovine heart mitochondrial complex I. The journal of biological chemistry 277: 50311-50317.

Chagas, L. M., M. C. Lucy, P. J. Back, D. Blache, J. M. Lee, P. J. S. Gore, A. J. Sheahan, and J. R. Roche. 2009. Insulin resistance in divergent strains of Holstein-Friensian dairy cows offered fresh pasture and increasing amounts of concentrate in early lactation. Journal of dairy science 92: 216-222.

Chernick, S.S., P.M. Spooner, M.M. Garrison, and R.O. Scow. 1986. Effect of epinephrine and other lipolytic agents on intracellular lipolysis and lipoprotein lipase activity in 3T3-L1 adipocityes. Journal of Lipid Research 27: 286-294.

Cocheme, H.M., and M. P. Murphy. 2009. Mitochondria as a source of reactive oxygen species. Biochem. J.: 1-4.

Cruz, G. D., J. A. R. Sanches, J. W. Oltjen, and R. D. Sainz. 2010. Performance, residual feed intake, digestibility, carcass traits, and profitability of Angus-Hereford steers housed in individual or group pens. Journal of Animal Science 88: 324-329.

Davis, M. P. 2009. Influence of diet, blood parameters and ETC proteins on RFI, University of Missouri, Columbia.

Dawson, T. M., and V. L. Dawson. 2003. Molecular pathways of neurodegeneration in Parkinson's Disease. Science 31: 819-822.

Deibert, D. C., and R. A. Defronzo. 1980. Epinephrine-induced insulin resistance in man. The Journal of Clinical Investigation 65: 717-721.

Deleo, D. 1994. Structure and function of the insulin receptor: Its role during lactation and fetal development. Ph.D. Dissertation, Curtin University of Technology, Bentley, Australia.

Efremov, R. G., R. Baradaran, and L. A. Sazanov. 2010. The architecture of respiratory complex I. Nature 465: 441-447.

Eisemann, J. H., G. B. Huntington, and D. R. Catherman. 1997. Insulin sensitivity and responsiveness of portal-drained viscera, liver, hindquarters, and whole body of beef steers weighing 275 ot 490 kilograms. Journal of animal science 75: 2084-2091.

Elsasser, T. H., A. C. Hammond, T. S. Rumsey, and R. Fayer. 1986. Perturbed metabolism and hormonal profiles in calves infected with *Sarcocystis cruzi*. Domestic Animal Endocrinology 3: 277-287.

Exton, S. C., R. M. Herd, and P. F. Arthur. 2003. Identifying bulls superior for net feed intake, intramuscular fat and subcutaneous fat. Animal Production in Australia 25: 57-60.

Fan, L. Q., D. R. C. Bailey, and N. H. Shannon. 1995. Genetic parameter estimation of postweaning gain, feed intake, and feed efficiency for Hereford and Angus bulls fed two different diets. Journal of Animal Science 73: 365-372.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Agric. Handbook No. 379 ARS, USDA, Washington, DC.

Golden, J. W., M. S. Kerley, and W. H. 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.

Grigorieff, N. 1998. Three-dimensional structure of bovine NADH:ubiquinone oxido-reductase (complex I) at 22 A in ice. Journal of Molecular Biology 277: 1033-1046.

Grivennikova, V. G., and A. D. Vinogradov. 2006. Generation of superoxide by the mitochondrial complex I. Biochimica et biophysica acta 1757: 553-561.

Guenebaut, V., A. Schlitt, H. Weiss, K. Leonard, and T. Friedrich. 1998. Consistent structure between bacterial and mitochondrial NADH:ubiquinone oxidoreductase (complex I). Journal of Molecular Biology 276: 105-112.

Hayirli, A., D. R. Bremmer, S. J. Bertics, M. T. Socha, and R. R. Grummer. 2001. Effect of chromium supplementation on production and metabolic parameters in periparturient dairy cows. Journal of dairy science 84: 1218-1230.

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

Henze, K., and W. Martin. 2003. Evolutionary biology: Essence of mitochondria. Nature 426: 127-128.

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

Herd, R. M., and S. C. Bishop. 2000. Genetic variation in residual feed intake and its association with other production traits in British Hereford cattle. Livestock Production Science 63: 111-119.

Hersom, M. J., R. P. Wettemann, C. R. Krehbiel, G. W. Horn, and D. H. Keisler. 2004. Effect of live weight gain of steers during winter grazing: III. Blood metabolites and hormones during feedlot finishing. Journal of animal science 82: 2059-2068.

Hinchliffe, P., and L. A. Sazanov. 2005. Organization of Iron-Sulfur Clusters in Respiratory Complex I. Science 309: 771-774.

Hirst, J. 2005. Energy transduction by respiratory complex I - an evaluation of current knowledge. Biochemical Society 33:525-529.

Hirst, J. 2010. Towards the molecular mechanism of respiratory complex I. Biochem. J. 425: 327-339.

Hirst, J., J. Carroll, I. Fearnley, R. Shannon, and J. Walker. 2003. The nuclear encoded subunits of complex I form bovine heart mitochondria. Biochimica et biophysica acta 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.

Hotamisligil, G. S., N. S. Shargill, and B. M. Spiegelman. 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259: 87-91.

Hugi, D., R. M. Bruckmaier, and J. W. Blum. 1997. Insulin resistance, hyperglycemia, glucosuria, and galactosuria in intensively milk-fed calves: dependency on age and effects on high lactose intake. Journal of animal science 75: 469-482.

Hunte, C., V. Zickermann, and U. Brandt. 2011. Functional modules and structural basis of conformational coupling in motochondrial complex I. Science 329: 448-451.

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.

Iuso, A., S. Scacco, and C. Piccoli. 2006. Dysfunctions of cellular oxidative metabolism in patients with mutations in the NDUFS1 and NDUFS4 genes of complex I. The journal of biological chemistry 281: 10374-10380.

Johnson, B. J., N. Halstead, M. E. White, M. R. Hathaway, A. DiConstanzo, and W. R. Dayton. 1998. Activation state of muscle satellite cells isolated from steers

implanted with combined trenbolone acetate and estradiol implant. Journal of Animal Science 76: 2779-2786.

Johnston, D. J., R. M. Herd, A. Reverter, and V. H. Oddy. 2004. Heritability of IGF-1 in beef cattle and its association with growth and carcass traits. Proc. Assoc. Advmt. Breed. Genet. 14: 163-166.

Kahn, B. B., and J. S. Flier. 2000. Obesity and insulin resistance. Journal of Clinical Investigation 106: 473-481.

Kelly, A. K., M. McGee, D. H. Crews, 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. K., M. McGee, D. H. Crews, 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.

Kersten, S. 2000. Mechanisms of nutritional and hormonal regulation of lipogenesis. European Molecular Biology Organization Reports 2: 282-286.

Kock, R. M., L. A. Swiger, D. Chambers, and K. E. Gregory. 1963. Efficiency of feed use in beef cattle. Journal of animal science 22: 486-494.

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

Kolath, W. H., M. S. Kerley, J. W. Golden, S. A. Shahid, and G. S. Johnson. 2006b. 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.

Kronfeld, D. S., K. H. Treiber, T. M. Hess, and R. C. Boston. 2005. Insulin resistance in the horse: Definition, detection, and dietetics. Journal of animal science 83: E22-31.

Kussmaul, L., and J. Hirst. 2005. The mechanism of superoxide production by NADH:ubiquinone oxidoreductase (complex I) from bovine heart mitochondria. PNAS 103: 7607-7612.

Kutner, M. H., C. J. Nachtsheim, J. Neter, and W. Li. 2005. Applied linear statistical models. Fifth ed. McGraw-Hill International Edition.

Lambert, A. J., and M. D. Brand. 2009. Reactive oxygen species production by mitochondria. Mitochondrial DNA, methods and protocols 554: 165-181.

Lancaster, P. A., G. E. Carstens, D. H. Crews, T. H. Welsh, T. D. A. Forbes, D. W. Forrest, L. O. Tedeschi, R. D. Randel, and F. M. Rouquette. 2009. Phenotypic and genetic relationships of residual feed intake with performance and ultrasound carcass traits in Brangus heifers. Journal of Animal Science 87: 3887-3896.

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

Lancaster, P. A., G. E. Carstens, and S. A. Woods. 2005. Evaluation of feed efficiency traits in growing bulls and relationships with feeding behavior and ultrasond carcass estimates. Journal of Animal Science 83 (Suppl. 2): 121.

Lassiter, K., C. O. 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 lymphocyte of male broilers with low and high feed efficiency. Poultry Science 85: 2251-2259.

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 1793: 78-88.

Liu, M. F., 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.

Liu, Y., G. Fiskum, and D. Schubert. 2002. Generation of reactive oxygen species by the mitochondrial electron transport chain. Journal of Neurochemitry 80: 780-787.

Loeffen, J., O. Elpeleg, and J. Smeitink. 2001. Mutations in the complex I NDUFS2 gene of patients with cardiomyopathy and encephalomyopathy. Ann Neurol 49: 195-201.

Martin, M. A., A. Blazquez, and L. G. Gutierrez. 2005. Leigh syndrome associated with mitochondrial complex I deficiency due to a novel mutation in the NDUFS1 gene. Arch Neurol 62: 659-661.

McCann, J.P., and T.J. Reimers. 1986. Effects of obesity on insulin and glucose metabolism in cyclic heifers. Journal of animal science 62: 772-782.

McDonagh, M.B., R.M. Herd, E.C. Richardson, V.H. Oddy, J. A. Archer, and P. F. Arthur. 2001. Meat quality and the calpain system of feedlot steers following a single generation of divergent selection for residual feed intake. Australian Journal of Experimental Agriculture 41: 1013-1021.

Mckenzie, M., and M. T. Ryan. 2010. Assembly factors of human mitochondrial complex I and their defects in disease. Life 62: 497-502.

McMeniman, J. P., P. J. Defoor, and M. L. Galyean. 2009. Evaluation of the national research council (1996) dry matter intake prediction equations and relationships between intake and performance by feedlot cattle. Journal of Animal Science 87: 1138-1146.

Mir, P. S., G. J. Mears, C. M. Ross, S. D. Husar, W. M. Robertson, S. D. M. Jones, and Z. Mir. 1998. Insulin responses during glucose tolerance tests in steers with increasing Wagyu genetic influence. Canadia Journal of Animal Science 78: 233-235.

Mir, P. S., Z. Mir, P. S. Kuber, C. T. Gaskins, E. L. Martin, M. V. Dodson, J. A. E. Calles, K. A. Johnson, J. R. Busboom, A. J. Wood, G. J. Pittenger, and J. J. Reeves. 2002. Growth, carcass characteristics, muscle conjugated linoleic acid (CLA) content, and response to intravenous glucose challenge in high percentage Wagyu, Wagyu x Limousin, and Limousin steers fed sunflower oil-containing diets. Journal of Animal Science 80: 2996-3004.

Mosnier, E., N. L. Floch, M. Etienne, P. Ramaekers, B. Seve, and M. C. Pere. 2010. Reduced feed intake of lactating primiparous sows is associated with increased insulin resistance during the peripartum period and is not modified through supplementation with dietary tryptophan. Journal of animal science 88: 612-625.

Murphy, M. P. 2009. How mitochondria produce reactive oxygen species. Biochem. J. 417: 1-13.

Nelson, D. L., and M. M. Cox. 2000. Lehninger principles of biochemistry. Third edition Ed. Worth.

Newmeyer, D. D., and S. F. Miller. 2003. Mitochondria: Releasing power for life and unleashing the machineries of death. Cell 112: 481-490.

Nkrumah, J. D., 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., J. A. Basarab, Z. Wang, C. Li, M. A. Price, E. K. Okine, D. H. Crews, and S. S. Moore. 2007a. Genetic and phenotypic relationships of feed intake and measures of efficiency with growth and carcass merit of beef cattle. Journal of Animal Science 85: 2711-2720.

Nkrumah, J. D., D. H. Crews, J. A. Basarab, M. A. Price, E. K. Okine, Z. Wang, C. Li, and S. S. Moore. 2007b. 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. D., D. H. Keisler, D. H. Crews, J. A. Basarab, Z. Wang, C. Li, M. A. Price, E. K. Okine, and S. S. Moore. 2007c. Genetic and phenotypic relationships of serum leptin concentration with performance, efficiency of gain, and carcass merit of feedlot cattle. Journal of Animal Science 85: 2147-2155.

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

Okuda, H., I. Yanagi, and S. Fujii. 1966. The mechanism of in vitro stimulation of lipolysis by adrenaline. The Journal of Biochemistry 59: 438-442.

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

Paddock, Z. D., G. E. Carstens, P. A. Lancaster, L. R. McDonald, and S. Williams. 2008. Evaluation of feed efficiency and feeding behavior traits in Angus and Red Angus growing bulls. Journal of Animal Science 86: 286.

Papa, S., V. Petruzzella, and S. Scacco. 2009. Pathogenetic mechanisms in hereditary dysfunctions of complex I of the respiratory chain in neurological diseases. Biochimica et biophysica acta 1787: 502-517.

Parker, W. D., and J. Parks. 1994. Electron transport chain defects in Alzheimer's disease brain. Neurology 44: 1040-1052.

Petersen, K. F., and G. I. Shulman. 2006. Etiology of insulin resistance. The american journal of medicine 119: 10S-16S.

Pires, J. A. A., A. H. Souza, and R. R. Grummer. 2007. Induction of hyperlipidemia by intravenous infusion of tallow emulsion causes insulin resistance in Holstein cows. Journal of Dairy Science 90: 2735-2744.

Reinhardt, C. D., W. D. Busby, and L. R. Corah. 2009. Relationship of various incoming cattle traits with feedlot performance and carcass traits. Journal of Animal Science 87: 3030-3042.

Renand, G., M. N. Fouilloux, and F. Menissier. 1998. Genetic improvement in beef production traits by performance testing beef bulls in France. Proceedings of 6th World Congress of Genetics 23: 77-80.

Richardson, E. C., 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. Australian Journal of Experimental Agriculture 41: 1065-1072.

Rinderknecht, E., and R. E. Humbel. 1978. The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. Journal of Biological Chemistry 253: 2769-2776.

Roessler, M. M., M. S. King, A. J. Robinson, F. A. Armstrong, J. Harmer, and J. Hirst. 2010. Direct assignment of EPR spectra to structurally defined iron-sulfur clusters in complex I by double electron-electron resonance. PNAS 107: 1930-1935.

Saada, A., S. Edvardson, and M. Rapoport. 2008. C60RF66 is an assembly factor of mitochondrial complex I. Am. J. Hum. Genet. 82: 32-38.

Saada, A., R. O. Vogel, and S. J. Hoefs. 2009. Mutations in NDUFAF3 (C3ORF60), encoding an NDUFAF4 (C60RF66)-interacting complex I assembly protein, cause fatal neonatal mitochondrial disease. Am. J. Hum. Genet. 84: 718-727.

Schelke, M., J. Smeitink, and E. Mariman. 1999. Mutant NDUFV1 subunit of mitochondrial complex I causes leukodystrophy and myoclonic epilepsy. Nat Genet. 21: 260-261.

Schinner, S., W. A. Scherbaum, S. R. Bornstein, and A. Barthel. 2005. Molecular mechanisms of insulin resistance. Diabetic Medicine 22: 674-682.

Schwartzkopf, G. K. S., S. Atwood, and T. A. McAllister. 2002. Relationships between bunk attendance, intake and performance of steers and heifers on varying feeding regimes. . Applied Animal Behaviour Science 76: 179-188.

Sharpley, M. S., R. J. Shannon, F. Draghi, and J. Hirst. 2006. Interactions between Phospholipids and NADH:Ubiquinone Oxidoreductase (Complex I) from Bovine Mitochondria. Biochemistry 45: 241-248.

Shenkel, F. S., S. P. Miller, and J. W. 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.

Sherwood, S., and J. Hirst. 2006. Investigation of the mechanism of proton translocation by NADH:ubiquinone oxidoreductase (complex I) from bovine heart mitochondria: does the enzyme operate by a Q-cycle mechanism ? Biochem. J. 400: 541-550.

Sodoyez, J. C., F. Sodoyez-Goffaux, M. M. Goff, A. E. Zimmerman, and E. R. Arquilla. 1975. [127-I]- or carrier-free [125-I]monoiodoinsulin. Journal of Biological Chemistry 250: 4268-4277.

Stick, D. A., M. E. Davis, S. C. Loerch, and R. C. 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.

Tilton, S. L., P. S. Miller, A. J. Lewis, D. E. Reese, and P. M. Ermer. 1999. Addition of fat to the diets of lactating sows: II. Effects on hormone-sensitive lipase activity, energy mobilization in response to epinephrine, and plasma insulin and glucose concentrations. Journal of animal science 77: 2501-2509.

Tinsley, N., M. Iqbal, N. R. Pumford, K. Lassiter, C. O. Dirain, T. Wing, and W. Bottje. 2010. Investigation of mitochondrial protein expression and oxidation in heart muscle in low and high feed efficient male broilers in a single genetic line. Poultry Science 89: 349-352.

Turrens, J. F. 1997. Superoxide production by the mitocondrial respiratory chain. Bioscience reports 17: 3-8.

Ugalde, C., R. J. Janssen, L. P. Heuvel, J. 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.

Van, W. J. J., and L. E. Underwood. 1978. The somatomedins and their actions. Academic Press, New York.

Vasconcelos, J. T., J. E. Sawyer, L. O. Tedeschi, F. T. McCollum, and L. W. Greene. 2009. Effects of different growing diets on performance, carcass characteristics, insulin sensitiviy, and accretion of intramuscular and subcutaneous adipose tissue of feedlot cattle. Journal of animal science 87: 1540-1547.

Videira, A. 1998. Complex I from the fungus Neurospora crassa. Biochimica et biophysica acta 1364: 89-100.

Vogel, R. O., J. A. Smeitink, and L. G. Nijtmans. 2007. Human mitochondrial complex I assembly: A dynamic and versatile process. Biochimica et biophysica acta 1767: 1215-1227.

Walker, J., J. Carroll, M. C. Altman, and I. Fearnley. 2009. Mass Spectrometric characterization of the thirteen subunits of bovine respiratory complexes that are encoded in mitochondrial DNA. Methods in Enzymology 456: 111-131.

Walker, J. E. 1992. The NADH:ubiquinone oxidoreductase (complex I) of respiratory chains. Quarterly reviews of biophysics 25: 253-324.

Wang, Z., J. D. Nkrumah, C. Li, J. A. Basarab, L. A. Goonewardene, E. K. Okine, D. H. Crews, 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.

Weiss, H., and T. Friedrich. 1991. Redox-linked proton translocation by NADH-ubiquinone reductase (complex I). J. Bioen. Biomem. 23: 743-754.

Westermann, B. 2010. Mitochondrial fusion and fission in cell life and death. Nature 11: 872-884.

Wikstroem, M. 1984. Two protons are pumped from the mitochondrial matrix per electron transferred between NADH and ubiquinone. The FEBS journal 169: 300-304.

Yakovlev, G., and J. Hirst. 2007. Transhydrogenation reactions catalyzed by mitochondrial NADH-Ubiquinone oxidoreductase (Complex I). Biochemistry 46: 14250-14258.

Zinn, R. A., A. Barreras, F. N. Owens, and A. Plascencia. 2008. Performance by feedlot steers and heifers: daily gain, mature body weight, dry matter intake, and dietary energetics. Journal of Animal Science 86: 2680-2689.

Zulkifli, N. A., M. Naik, W. S. Pitchford, and C. D. K. Bottema. 2009. Cattle residual feed intake candidate genes. Proc. Assoc. Advmt. Breed. Genet. 18: 668-671.

VITA

Marcelo Hentz Ramos was born in Jales-São Paulo-Brazil in 1978. The son of João José Ramos e Maria José Hentz Ramos. After completing high school at Fundação Indaiatubana de Educação e Cultura in 1997 he attended Veterinary School at Federal University of Lavras, Minas Gerais. He graduated with vet degree in 2001. He then started graduate school at University of Wisconsin-Madison and got his Master degree in 2007. In 2011 he finished his PhD work at University of Missouri and now lives in Brazil.