THE RELATIONSHIP BETWEEN MITOCHONDRIA AND RESIDUAL FEED INTAKE IN FEEDLOT CATTLE

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DEDICATION

This dissertation is dedicated to my wife and sons for the support they have given and sacrifices they have made during the pursuit of this degree.

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CHAPTER I

LITERATURE REVIEW

INTRODUCTION

The goal of a commercial livestock operation is to have a positive return on investment. The profitability of an operation is dependent on both input costs (e.g. feed) and output values (e.g. fat cattle). In recent years, a large emphasis has been placed on the improvement of output values such as carcass quality, growth rate, live weight, and fertility. However, virtually no improvements have been made on feed inputs. This is surprising since feed costs for feedlot cattle have been estimated to be 50 to 70% of the cost of production (Arthur and Herd, 2005). Lack of improvement in feed inputs is the result of difficulty in measuring individual animal feed intake. Recent technological advances now allow for accurate measurement of individual animal feed intake in a grouped pen of cattle. With these technological advances, improvement in profitability, through increased feed efficiency, is now possible.

MEASURES OF FEED EFFICIENCY

Feed efficiency is not a directly measurable trait, and must be computed as a function of feed intake, body weight gain and time (Koch et al., 1963). Feed efficiency is traditionally measured by the ratio of feed consumed to body weight gain during the same period (i.e. feed conversion ratio or FCR). Scientifically, feed efficiency is measured by the inverse of FCR (i.e. gain to feed ratio). This ratio is mathematically more correct because outputs are divided by inputs. However, because these measures only provide a

gross accounting for inputs and outputs, several problems are encountered during their use.

First, while these measures are both genetically and phenotypically correlated with production traits, they do not affect feed intake. For example, milk yield in dairy cows is both phenotypically and genetically correlated with measures of gross efficiency (Buttazzoni and Mao, 1989). Likewise, Brelin and Brannang (1982) have shown genetic correlations (-0.61 to -0.95) between ADG and gross measures of efficiency in beef cattle. However, while Lancaster et al. (2005) found that FCR was highly correlated with ADG, it was not correlated to dry matter intake in growing bulls. Similarly, Mrode et al. (1990), selecting for growth rate or feed conversion ratio in Hereford cattle, found that cattle selected for growth rate had greater improvements in feed conversion ratios than those selected for improved feed conversion ratio.

A second problem encountered when using gross measures of efficiency is that improving gross efficiency will not necessarily improve the profitability of an operation. This is due to the increase in mature body size that occurs when selection is based on feed conversion ratios (Archer et al., 1999). The increased maintenance requirements of the cow would offset any improvement in efficiency that is observed by the calf, since the feed consumed by the cow over her lifetime is much greater than that consumed by her calves. In addition, gross measures of efficiency are based on growth rate; therefore they are not applicable for use in the breeding herd where females are not growing. Finally, FCR is influenced by composition of gain, making comparisons between animals differing in maturity impossible. These problems limit the use of FCR to monitoring the

performance of a group of calves in the feedlot and prevent it from being used as a selection tool for the improvement of feed efficiency.

Another measure of efficiency that has been proposed is maintenance efficiency. This measure is defined as the ratio of body weight to feed intake, while there is no change in body weight. Maintenance efficiency is important in the cow herd since this segment has been estimated to use 65 to 85% of the energy used for beef production (Archer et al., 1999). However, there are difficulties associated with this measurement. Animals must not be growing, and often have to be held at a constant weight for extended periods of time to obtain accurate measurements.

Partial efficiency of growth is another measure of efficiency that has been proposed. This measure is defined as the ratio of weight gain to feed after maintenance requirements have been subtracted, with maintenance requirements being estimated from feeding tables or metabolism studies. Maintenance requirements from feeding tables assume that no variation exists and measurements from metabolic studies are not practical for large number of animals (Archer et al., 1999). While both of these measures are theoretically sound, they are not appropriate for the selection of feed efficiency in a large number of animals.

Residual feed intake (RFI) has also been proposed as a means to measure feed efficiency. Residual feed intake (also referred to as net feed intake or net feed efficiency) does not have the pitfalls of gross measures of efficiency, because RFI describes feed intake independent of growth rate and maintenance requirements. This is done by regressing intake against metabolic mid weight (average body weight of the test period to the 0.75 power) and average daily gain during the test period. This regression is used to

calculate an animal's expected intake. The animals expected intake is subtracted from its actual measured intake to yield an RFI value (Koch et al., 1963). Residual feed intake values that are negative indicate a better than average efficiency and positive values indicate a below average efficiency.

THE EFFECT OF SELECTION BASED ON RESIDUAL FEED INTAKE

A large variation in RFI exists within the beef herd (Archer et al., 2002). A large body of research has also shown that RFI is a moderately heritable trait with estimates ranging from 0.39 to 0.43 (Arthur et al., 2001). Therefore, it is possible to make substantial improvements in RFI values within a few generations, when RFI is used as a selection tool.

Using RFI as a selection tool does not appear to affect performance. When RFI was used as a selection tool, progeny selected to have low RFI consumed less feed and were slightly leaner, but performed similarly to those animals selected to have high RFI (Arthur et al., 2001). Also, no major differences in carcass composition or meat quality were found between the high and low RFI animals (Richardson et al., 2001; McDonagh et al., 2001). Similarly, Lancaster et al. (2005) found no correlation between ultrasonic measures of carcass composition and RFI. However, Carstens et al. (2002) found that high RFI steers had greater amounts of backfat as measured by ultrasound than low RFI steers, but found no difference in longissimus dorsi area or intramuscular fat between the two groups. Basarab et al. (2003) examined the carcass composition of steers selected to have high, medium or low RFI and found no difference in carcass weight, backfat,

marbling score, yield or quality grade or lean meat yield. However, Basarab et al. (2003) found that high RFI steers had increased liver, stomach, small intestine, and kidney fat compared to low RFI steers. No differences were found in the distribution of carcass fat, carcass composition (lean, bone, fat), or distribution of wholesale cuts (round, loin, chuck, etc.). Knott et al. (2005) examined the body composition of 52 crossbred rams at the beginning and end of a RFI test using dual energy x-ray absorptiometry. They found that lean tissue mass only accounted for 3% of the variation in RFI while fat tissue mass accounted for none of the variation. The relationship between meat quality and palatability in Angus steers selected to have high, average or low RFI was examined by Baker et al. (2006). They found no differences between the groups for hot carcass weight, longissimus dorsi muscle area, fat thickness, KPH, USDA yield grade, marbling score, or quality grade. Likewise, calpastatin activity, Warner-Bratzler shear force, and sensory panel tenderness and flavor scores were not different between the RFI groups. However, reflectance color b* scores were greater for steaks from high RFI animals than for those from low RFI animals. Similarly, steaks from high RFI steers had lower off-flavor scores and less cooking loss than the steaks from low RFI steers. The authors concluded that there in no relationship between RFI and meat quality. All of these studies would indicate that RFI selection has little to no impact on carcass and meat quality traits.

In addition, using RFI as a selection tool appears to have little affect on reproductive performance. Arthur et al. (2005) evaluated the effects of RFI selection in a herd of 185 females. The cows were the result of ~1.5 generations of selection for RFI in which RFI differed on average by 0.8 kg per day between the high and low RFI lines.

Cows in the high RFI line were generally fatter than the low RFI line, but this difference was only significant at the beginning of the breeding season. There was no difference in body weight between high and low RFI cows at anytime during the year for the 3 years examined. Reproductive performance was similar between the two RFI lines as assessed by pregnancy rate, calving rate and weaning rate. In addition, no difference was found in maternal productivity traits (milk yield, weight of calf born or calf weaned per cow exposed). However, gestation tended to be five days longer for the low RFI cows compared to high RFI cows. This difference is because 22% of the low RFI calves were sired by natural service compared to 13% of the high RFI calves in this study. As expected, due to the lack of relationship between growth rate, body size and RFI, no difference was found in pre-weaning growth of calves. Researchers studying the relationship between RFI and reproductive performance in poultry and mice have found unfavorable relationships between egg production, litter size and RFI (Hagger, 1994; Hughes and Pitchford, 2004). In summary, based on research to date, selection based on RFI appears to have no negative affects on bovine reproduction. However, RFI selection may negatively affect reproductive performance in mice and poultry. Therefore, further research is needed to ensure that RFI selection does not negatively impact fertility in the cow herd.

POTENTIAL MECHANISMS AFFECTING RESIDUAL FEED INTAKE

Researchers are beginning to examine some of the potential mechanisms affecting RFI. It is unlikely that a single mechanism is responsible for differences in RFI status in

cattle. There are few examples in which the mutation of a single gene was responsible for the observed phenotype, e.g. double-muscling (Herd et al., 2004). Therefore, it is believed that multiple mechanisms cause variation in RFI. Herd et al. (2004) proposed that five major processes contribute to the variation in RFI. They are: 1. Intake of feed; 2. Digestion of feed and associated energy use; 3. Metabolism, including both anabolism and catabolism; 4. Activity; and 5. Thermoregulation.

Differences in feed intake between animals could impact RFI status because as feed intake increases, the amount of energy needed for digestion also increases, thereby increasing the maintenance requirement. This is due to an increased mass of digestive tissues needed for digestion. As the mass of digestive tissues increase, the metabolism of these tissues may be altered, reducing digestive efficiency. However, it is unknown if metabolism of the gastrointestinal tissues is genetically programmed or if behavioral differences cause the shift in metabolism.

Differences in RFI status may also be affected by site of digestion or utilization of digestive products. For example, differences in microbial protein synthesis between animals, which may affect the supply of microbial protein to the small intestine (Herd et al., 2004). Also, the appearance of digestive products in the portal circulation differs between animals, which due to alteration in nutrient supply, could affect tissue metabolism.

Differences in metabolism are thought to be the major factor contributing to differences in feed efficiency. Processes contributing to heat production, such as protein turnover, ion pumping, and proton leakage may be responsible for differences in feed

intake. An increase in activity of any of these would result in an increased amount of heat production, thereby decreasing metabolic efficiency.

Researchers studying RFI in chickens have found that activity level contributes to a substantial proportion of the variation in RFI (Braastad and Katle, 1989). Differences in the ability of animals to retain heat could also contribute to the variation in RFI. It has been shown that hens with lower RFI have smaller nude body areas and are slightly better feathered (Luiting et al., 1994). These factors along with others may also contribute to the variation in RFI in cattle.

There is limited research examining the relationship between specific gene mutations and RFI. Nkrumah et al. (2004) examined the effect of a single nucleotide polymorphism in exon 2 of the leptin gene on a number of phenotypic traits relating to feed intake and feeding behavior. They found no relationship between the three genotypes and feed intake or feeding behavior as measured by feeding frequency and duration. In a second study examining the leptin promoter region, Nkrumah et al. (2005) found that animals with CT and TT genotypes consumed more feed than those with the CC genotype. However, animals with the CT and TT genotypes had greater marbling scores and backfat thicknesses.

The relationship between energy partitioning and RFI was examined by Nkrumah et al. (2006). The authors found that in steers selected to have high, medium or low RFI there was no difference in ADG or metabolic body weight. However, high RFI animals consumed ~ 2 kg/d more dry matter, spent 26 more minutes per day at the bunk, and visited the bunk 18 times more per day compared to low RFI animals. They also found that high RFI animals produce ~ 0.5L per kilogram of metabolic body weight more

methane per day than low RFI steers. Apparent digestibility of DM and CP was 5% greater for the low RFI animals. These same animals were restricted to 2.5x their estimated maintenance requirements while dietary energy flows were measured. There was no difference in energy intake; however DE, ME, and RE were all greater for the low RFI steers. Heat production, and methane energy was greater for the high RFI steers. The authors attributed the difference in heat production to differences in metabolic rate and/or efficiency.

An alteration of the stress response and/or blood parameters may alter RFI status in feedlot cattle. Knott et al. (2004) measured cortisol levels prior to and following an ACTH challenge in rams ranked for feed efficiency based on FCR. They found no difference in basal serum cortisol levels; however, incremental serum cortisol response to ACTH was greater for the inefficient rams. Theis et al. (2002) examined the relationship between serum cortisol levels, white and red blood cell parameters, and RFI. They found that pre- and ACTH induced cortisol levels were not correlated with RFI. In addition, white and red blood cell counts and differentials, hematocrit and hemoglobin also were not correlated with RFI. Similarly, Richardson et al. (2002) examined a number of blood parameters in steers selected for high or low RFI and found no differences between the two groups. Blood parameters were also measured prior to and following transport. The authors observed increased white and red blood cell counts, hemoglobin, hematocrit, and neutrophil counts in both high and low RFI animals following transportation, however the increases were similar for both groups.

Blood metabolites could be used as markers of RFI status or provide insight into the factors which reduce efficiency. Richardson et al. (2004) measured a number of

blood metabolites in steers selected to have either high or low RFI. The authors found that the high RFI group had a greater blood urea level than the low RFI group. The increased blood urea level was most likely the result of greater protein intake by high RFI steers. Low RFI animals had increased triglycerides compared to high RFI animals. Insulin and cortisol concentration was elevated in high RFI steers compared to low RFI steers. Protein metabolism and liver function was similar for both the low and high RFI animals. The authors also measured dry matter digestibility and microbial protein production and found no difference between the high and low RFI steers. In conclusion, blood parameters and metabolites do not appear to be greatly affected by RFI status and do not seem to be good indicators or predictors of RFI status.

SELECTION TOOLS FOR IMPROVING RESIDUAL FEED INTAKE

Some authors have suggested the use of plasma insulin-like growth factor-1 (IGF-1) concentration as an indirect selection tool for growth and feed efficiency traits (Moore et al., 2003; 2005). The heritability of IGF-1 concentration has been estimated to be 0.32 to 0.36 with IGF-1 being genetically correlated (0.31 to 0.56) with RFI (Moore et al, 2003; 2005; Johnston et al., 2001). Similar genetic correlations were found between growth and carcass traits in these studies. It is unknown what effect the selection of IGF-1 concentration has on other economically important traits, such as measures of reproductive performance. A number of simulation studies (Kahi et al., 2003;Wood et al., 2004) have been conducted which show increases in profitability when IGF-1 is used as a selection tool, however, there is no phenotypic data suggesting RFI can be indirectly selected for based on IGF-1 concentrations.

MITOCHONDRIAL STRUCTURE AND FUNCTION

Mitochondria are cytoplamsic, double membrane organelles, whose main role is to synthesize adenosine triphosphate (ATP), the energy source of the cell. Mitochondria produce ATP through a series of electron transfers, called oxidative phosphorylation. For oxidative phosphorylation to occur, a series of five protein complexes, called the electron transport chain (ETC), are required. Mitochondria are unique in that they are the only cellular organelle to have their own genetic system. This system only encodes a small proportion of the proteins present in the mitochondrion; however, mutation of this genetic code can cause severe dysfunction. Since mitochondria produce the majority of energy for the cell, alterations in the efficiency of energy production could reduce feed efficiency in cattle.

Mitochondria are bean or oval shaped structures, ranging in size from 0.5 to 1 μm in length. A unique double-membrane allows for oxidative phosphorylation to occur. The outer membrane is readily permeable to small molecules and ions due to the presence of a family of integral membrane proteins called porins. However, the inner membrane is impermeable to most small molecules including protons (H^+). Passage across this membrane is only possible through molecule-specific transporters. The inner membrane also houses all of the components of the ETC and is folded in order to increase the surface area of the membrane. These folds are called cristae. Cristae allow for greater amounts of electron chain components to be housed in the mitochondria. The area within the inner mitochondrial membrane is called the mitochondrial matrix. The matrix contains the majority of enzymes that make up the pathways for the oxidation of lipids,

carbohydrates and amino acids. Mitochondrial DNA and the enzymes required to produce proteins are also found in the matrix.

The pathways that oxidize lipids, carbohydrates, and amino acids all converge to the citric acid (TCA) cycle. As these substrates are oxidized in the TCA cycle, carbon dioxide, reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide ($FADH_2$) are produced. Electrons are transferred from NADH and $FADH_2$ to complex I and II, respectively. Complex I is an L-shaped protein and has a molecular weight of 850 kDa. Two simultaneous processes occur at this complex. 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 to complex III. Complex II, also called succinate dehydrogenase, 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 140 kDa. Electrons pass from succinate to FAD^+ and then are transferred through complex I to ubiquinone. Complex III transfers electrons from ubiquinone to cytochrome c, along with moving two protons to the intermembrane space. This complex is intermediate in size, between complex I and II, with a mass of 250 kDa. 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 is similar in size to complex III, with a mass of 240 kDa and carries electrons from cytochrome c to molecular oxygen, thereby reducing it to water. For each electron that is transferred to oxygen, one proton from the matrix is pumped into the intermembrane space and one proton is transferred to

the molecular oxygen to form water. The result of this process is that for each pair of electrons transferred from NADH to oxygen, 10 protons are pumped to the intermembrane space, one is transferred to oxygen and a single molecule of water is formed.

The goal of these reactions is the formation of a proton motive force, formed by the pumping of protons into the intermembrane space. The result being a difference in proton concentration and separation of charge between the mitochondrial matrix and inter membrane space. The proton motive force is then used by complex V (also referred to as ATP synthase) to produce ATP. Complex V is composed of two subunits called F_O and F_1. The $_O$ designation refers to the sensitivity of the F_O subunit to the antibiotic oligomycin. The F_O subunit is positioned within the membrane while the F_1 subunit resides within the mitochondrial matrix. The F_1 subunit is responsible for the binding of ADP and ATP, and therefore, ATP formation. The F_O subunit makes up the pore portion through which protons pass from the intermembrane space to the mitochondrial matrix. The passage of protons thru the F_O subunit causes both the F_1 and F_O subunits to rotate and it is through this process that the formation of ATP is catalyzed. The passage of four protons is required for the production of one ATP.

Adenine nucleotide translocase is one transporter required for ATP synthesis. This antiporter moves ADP into the mitochondrial matrix and ATP into the intermembrane space. Since ATP has four negative charges and ADP has only three, the movement of ATP out of the matrix is favored by the proton gradient. A second transporter required is phosphate translocase, a symporter which moves one proton and one phosphate group into the mitochondrial matrix. Once again the movement of both

the phosphate group and proton is favored due to low concentration of protons in the mitochondrial matrix.

The production of ATP is tightly regulated in a number of ways. First, ATP synthesis is limited by the availability of ADP as a substrate. As the concentration of ADP increases due to energy consuming activities, ATP production will increase until the concentration of ADP is reduced to basal levels. Second, high concentrations of ATP inhibit many of the enzymes of glycolysis and the TCA cycle. The inhibition of these enzymes by ATP insures NADH and $FADH_2$ production is tightly coupled to ATP production. In addition, the consumption of oxygen is coupled to ATP production (Erlanson-Albertsson, 2003).

As stated earlier, the oxidation of substrate, consumption of oxygen and the production of ATP are all highly coupled. However, as with any biological system this coupling is not perfect. For example, mitochondria still consume small amounts of oxygen even when the activity of ATP synthase is blocked by oligomycin (Stuart et al., 1999). In addition, the presence of uncoupling protein (UCP) in brown adipose tissue of mammals is an example of the uncoupling of oxidative phosphorylation and ATP production. Uncoupling proteins act to dissipate the proton motive force and as a result heat is produced instead of ATP. This process is required for the survival of the neonate during periods of cold stress. However as animals age, this protein is lost due to the ability of the adult mammal to maintain their body temperature during cold periods.

With advances in molecular biology techniques during the late 1990’s it was discovered that five UCP homologues exist (Jezek, 2002). Uncoupling protein 1 is distributed in the brown adipose tissue, UCP2 is widely distributed throughout the body,

UCP 3 is found primarily in skeletal muscle and UCP 4 and 5 are found in the brain (Erlanson-Albertsson, 2003). The major difference between UCP 1 and UCP 2 and 3 is their level of expression. Uncoupling proteins 2 and 3 are expressed 200 to 700 fold lower than UCP 1 (Erlanson-Albertsson, 2003). Based on the ability of these proteins to reduce the proton motive force, these proteins are thought to play a role in energy expenditure and thermoregulation. Also consistent with this finding is that thyroid hormone increases the expression of UCP 2 and 3 (Harper and Himms-Hagen, 2001). However, studies in mice that lack UCP 2 have shown that these mice have a normal metabolic rate and body mass and are not affected by the loss UCP 2 (Schrauwen and Hesselink, 2002). Similarly, mice lacking UCP 3 were not obese and had a normal metabolic rate (Gong et al., 2000). However, mice overexpressing UCP 3 consumed significantly greater amounts of food compared to control mice, but were not obese, suggesting an increase in metabolic rate and heat production through the uncoupling of oxidative phosphorylation (Clapham et al., 2000). An increased expression of UCP in inefficient animals could therefore explain some of the variation that is found in feed efficiency. Little is known about the role that UCP 4 and 5 play, if any, in the regulation of energy expenditure, as they were only recently discovered.

Mitochondria possess their own genetic system that is believed to be the result of their incorporation into ancestral eukaryotic cells (Ganong, 2001). Much of this genetic information has been transferred to the control of the nuclear genome with 99% of the proteins in the mitochondria being the product of the nuclear genome. However, mitochondrial DNA (mtDNA) still has a significant impact on the functionality of the mitochondria.

Mammalian mtDNA is a relatively small, abundant, circular double stranded molecule of about 16.7 kilobases in length (Taanman, 1999). This structure encodes for only 37 genes, which is insignificant compared to the number of genes that are encoded by the nuclear genome. These 37 genes encode for two ribosomal RNA, 22 transfer RNA, and 13 proteins of the electron transport chain. The information encoded by mtDNA is extremely organized. It does not contain any intronic sequences, and intergenetic and regulatory regions are almost entirely absent (Taanman, 1999).

The bovine mtDNA sequence has been known since 1982 (Anderson et al., 1982), however, only one study has been conducted to date examining the relationship between production traits and mutations of mtDNA (Beitz et al., 1994). The majority of research has focused on using mtDNA for phylogenetic analysis (Hansen et al., 2003; Wu et al., 2000; Steinborn et al., 1998). The lack of research examining the relationship between mtDNA and production traits is because in humans, the majority of mtDNA mutations result in severe mitochondrial dysfunction, due to the impairment of energy production (Leonard and Schapira, 2000).

RELATIONSHIP BETWEEN MITOCHONDRIA AND FEED EFFICIENCY IN POULTRY

The relationship between parameters of mitochondrial function and feed efficiency has been examined in poultry. These studies would indicate mitochondrial dysfunction in inefficient birds; however, no site of dysfunction has yet been identified. In all of the studies mentioned below, the experimental animals were selected based upon gain to feed ratios. There were significant differences in gain to feed ratios between

efficient and inefficient birds (Bottje et al., 2002; Iqbal et al., 2004; 2005; Ojano-Dirain et al., 2004; 2005a; 2005b) however, these differences were the result of differences in growth rate and not feed intake.

The major measures of mitochondrial function are respiratory control ratio (RCR), and adenosine diphosphate: oxygen ratio (ADP: O). These measures assess the coupling of electron transfer to oxidative phosphorylation and the ability of ADP to induce respiration, respectively. Phenotypic differences in feed efficiency may be due to differences in RCR. However, no difference in ADP: O would be expected because differences in this ratio would indicate an impairment of basic mitochondrial function. Both ratios are calculated from the various rates of mitochondrial respiration. The respiration rates alone can also be used to give insight into a treatment's effect(s) on mitochondrial function.

The relationship between mitochondrial function and gain to feed ratio were examined by Bottje et al. (2002). These authors observed a greater RCR value for mitochondria isolated from breast or leg skeletal muscle of efficient birds compared to inefficient birds when glutamate/malate was provided as a respiratory substrate. However, mitochondrial respiration rates were similar for efficient and inefficient birds. Iqbal et al. (2005) also studied respiration rates in poultry. They found that inefficient birds had a greater rate of state 4 respiration in mitochondria isolated from the liver. No difference was observed for the other states of respiration between the efficient and inefficient birds. In addition, a greater RCR value was observed for the mitochondria isolated from efficient birds compared to inefficient birds with no difference in ADP/O ratios. Furthermore, in a study conducted by Ojano-Dirain et al. (2004) there was no

difference in rates of mitochondrial respiration, RCR values or ADP: O ratios of duodenal mitochondria isolated from efficient and inefficient birds. While these studies show no clear alteration in mitochondrial function due to efficiency status, they do indicate that tissue specific differences may exist. On the other hand, there may be no relationship between mitochondrial respiration and feed efficiency because of the measure of feed efficiency used to select these birds.

Hydrogen peroxide production is often used to determine the amount of electron leak arising from mitochondrial respiration. Alterations in the amount of hydrogen peroxide production would be indicative of mitochondrial dysfunction due to a defect within the ETC. Ojano-Dirain et al. (2004), using a variety of respiratory substrates, concluded that inefficient birds had greater hydrogen peroxide production than efficient birds. Similarly, when succinate or pyruvate/malate was provided to mitochondria isolated from liver, inefficient birds produced more hydrogen peroxide (Iqbal et al., 2005). Mitochondria isolated from the breast muscle of inefficient birds also produced a greater amount of hydrogen peroxide, however, no difference in hydrogen peroxide production was found in leg muscle (Bottje et al., 2002). The authors also measured protein carbonyls to assess the extent of cellular oxidative stress. It has been observed in a number of different tissue types that inefficient birds have greater protein carbonyls, and hence oxidative stress, compared to efficient birds (Iqbal et al., 2004; 2005; Lassiter et al., 2004; Ojano-Dirain et al., 2005b). Greater hydrogen peroxide production and protein carbonyls in inefficient birds together would support that electron leak may be the cause of the reduction in feed efficiency in these animals.

Measurement of the activity of individual complexes of the ETC can be used to assess the site of potential defects. Bottje et al. (2002) found that in breast muscle, inefficient birds had lower activity in both complex one and two compared to efficient birds. Iqbal et al. (2004; 2005) observed a reduced activity of liver mitochondrial complexes one thru four in inefficient birds. In duodenal mitochondria, there was no difference between the efficient and inefficient birds in the activities of any of the four complexes (Ojano-Dirain et al., 2005a). However, a second study by Ojano-Dirain et al. (2005b) showed lower activity of ETC complexes 1 to 3 and 5 in the duodenal mitochondria of inefficient birds. These studies show no clear site of mitochondrial defect that could be responsible for increased electron leak in inefficient birds. This is similar to what was observed when mitochondrial respiration rates were measured.

Protein expression of the ETC complex subunits was also measured in order to identify the potential site of defect. A reduction in the expression of the mitochondrial DNA encoded subunits ND4, ND6C, and COX II for inefficient birds was observed in duodenal mitochondria (Ojano-Dirain et al., 2005b). These authors also showed that inefficient birds had increased expression of the nuclear encoded subunits 70S, core I, core II, cyt c1, ISP, and ATPase-alpha. In breast muscle, Iqbal et al. (2004) observed an increased protein expression of cyt c1 and core I in inefficient birds. Lassiter et al. (2004) found increased expression of core I, cyt c1, and ATPase-alpha and decreased expression of cox II and CII 30 in the lymphocytes of efficient birds. Similar to the results obtained in measuring mitochondrial complex activity, these results do not show a clear site of defect for the observed decrease in feed efficiency status.

Adenosine nucleotide transporter (ANT) is a mitochondrial transporter responsible for shuttling ADP and ATP across the inner mitochondrial membrane. Defects of ANT could alter mitochondrial respiration and/or function. Iqbal et al. (2004) measured the expression of ANT1 and found an increase in the expression of this protein in the breast muscle of inefficient birds. In a second study examining liver mitochondria, no difference was found in the expression of ANT1 (Iqbal et al., 2005). Further studies will be needed to elucidate if ANT is involved in altering feed efficiency status, as these two studies do not provide conclusive results.

SUMMARY

Virtually no genetic improvement of feed efficiency has been made which is surprising since feed costs make up a 50 to 70% of expenses. There are many measures of feed efficiency; however, RFI is the only measure that can be used to accurately and efficiently rank and select animals. Residual feed intake also does not impact growth rate, carcass composition, meat quality, or other economically important traits in beef cattle.

Mitochondria produce the majority of energy used by the cell. This is accomplished through oxidative phosphorylation by the electron transport chain. Mitochondrial function has been linked to feed efficiency in poultry. This research indicates that increased oxidative stress in inefficient birds may be the cause of the reduction in feed efficiency. However, to date, no specific site of mitochondrial dysfunction has been identified.

Based on these data, we hypothesized that:

1. A relationship exists between mitochondrial function/respiration and residual feed intake status
2. Altered expression of mitochondrial uncoupling protein and/or mutation of mitochondrial DNA would result in altered residual feed intake status
3. Differences in mitochondrial function/respiration can be utilized in a blood assay as markers of residual feed intake status

CHAPTER II

THE RELATIONSHIP BETWEEN MITOCHONDRIAL FUNCTION AND RESIDUAL FEED INTAKE IN ANGUS STEERS

ABSTRACT

The objective of this study was to examine the relationship between mitochondrial function and residual feed intake in Angus steers. Individual feed intakes were recorded for a contemporary group of 40 steers via the GrowSafe feed intake system. Intakes were then used to calculate residual feed intake (RFI), a measure of efficiency. Based on these calculations, nine low (RFI = -0.83) and eight high (RFI = 0.78) residual feed intake (RFI) animals were selected for further study. Blood samples were collected via jugular venipuncture 1 wk before slaughter for the determination of plasma glucose and insulin concentrations. Tissue samples were taken from the LM from both the high and low RFI animals and mitochondria were isolated for measurement of oxygen consumption and hydrogen peroxide production. Average daily gain and carcass composition were not different between the high and low RFI steers; however, ADFI by the high RFI animals was 1.54 kg/d greater ($P < 0.001$) than the low RFI animals. Low RFI steers exhibited a greater ($P < 0.05$) rate of state 2 and 3 respiration, respiratory control ratio, and hydrogen peroxide production than high RFI steers when provided with glutamate or succinate as a respiratory substrate. The acceptor control and adenosine diphosphate:oxygen ratios were not different between the two groups for either substrate. When hydrogen peroxide production was expressed as a ratio to respiration rate there was

no difference between groups, signifying that electron leak was similar for both groups. Plasma glucose concentration was greater ($P < 0.05$) in the high RFI steers than in the low RFI steers; however, plasma insulin concentration was not different ($P = 0.22$) between the two groups. The ratio between plasma glucose and insulin concentration was similar ($P = 0.88$) between the two groups indicating no impairment of glucose metabolism. The increased plasma glucose concentration observed in the high RFI steers was presumed to be the result of a greater feed intake by these animals. It seems that mitochondrial function is not different between the high and low RFI groups but rather the rate of mitochondrial respiration is increased in low RFI steers compared with high RFI steers.

INTRODUCTION

Substantial improvements in the profitability of beef production could be made by improving G:F because feed costs represent a major proportion of production costs (Arthur et al., 1996). Dietary manipulations such as balancing the AA to energy ratio of the diet (Mueller et al., 2004) and removing forage from the diet (Willis and Kerley, 2004) can result in improved G:F; however, the variation in G:F among animals remains (Mueller et al., 2004). Animal selection for superior G:F traits is mandatory if improving G:F in beef cattle is to be realized.

Residual feed intake (RFI) is an index that can be used to calculate an animal's efficiency (Archer et al., 1999) and describes the divergence in intake from that needed for maintenance and growth. Maintenance and growth requirements are not accounted for by G:F making RFI comparisons between animals a better measure of efficiency. A large

genetic variation in RFI exists, and this trait has been found to be moderately heritable (Archer et al., 1998). However, the measurement of individual feed intake and hence RFI is difficult and costly. Determining the physiological mechanism that is responsible for the observed differences in feed intake would provide for a more cost effective method of determining an animal's RFI status.

Mitochondria are the site of energy production in the cell and produce the majority of cellular ATP. Recent work in poultry (Bottje et al., 2002) and rats (Lutz and Stahly, 2003) has provided evidence of a link between inefficient mitochondrial respiration and decreased G:F. These findings led us to hypothesize that mitochondrial function would be altered similarly in beef cattle as has been observed in these species. Therefore, our hypothesis was that inefficient mitochondrial respiration was related to decreased G:F in beef cattle. The objectives of this research were to evaluate the relationship between mitochondrial respiration and hydrogen peroxide production in steers selected for low or high RFI.

MATERIALS AND METHODS

Animal Management

The research protocols used in this study were approved by the University of Missouri Animal Care and Use Committee. Forty Angus steers (average initial BW = 325.4 $\pm$ 23.7 kg) were used to select high and low RFI animals. Steers were obtained from a single herd enrolled in the MFA Health Track Beef Alliance (Columbia, MO), were all of the same sire, and had been previously vaccinated and preconditioned for 45 d before arrival at the University of Missouri Beef Research Farm.

Upon receiving the animals, electronic ID tags (Allflex USA, Inc., Dallas Ft. Worth Airport, TX) were attached to the exterior of the left ear for the measurement of individual feed intake with the GrowSafe feed intake system (Model 4000E; GrowSafe Systems Ltd., Airdrie, AB Canada). Steers were placed on a receiving diet for 14 d to allow for acclimation to the feeding system. After the acclimation period, steers were fed Trendsetter SLR (MFA, Inc., Columbia, MO) at a rate of 25% Trendsetter SLR and 75% whole corn until they reached 454 kg. At 454 kg, the diet was switched to 12.5% Trendsetter SLR and 87.5% whole corn for the remainder of the experiment. All steers had ad libitum access to both feed and water. Steers were weighed every 21 d and RFI values were calculated for the entire feeding period. The expected feed intake was calculated by regressing the actual intake against ADG and metabolic mid weight (Basarab et al., 2003). The RFI value for each animal was calculated as the difference between the actual and expected intake.

Nine low and eight high RFI steers were selected based on their RFI values and were used for the study of mitochondrial respiration. These 17 steers were transported to the University of Missouri Abattoir where the animals were killed to obtain tissue from the LM for mitochondrial isolation. Hot carcass weights were documented for each animal and the carcasses were chilled for 24 h at 5°C. After the 24 h chill, LM area of each carcass was measured to the nearest 0.01 cm^2. Subcutaneous fat thickness at the 12th rib was determined using a USDA preliminary yield grade ruler (USDA, 1997) at an anatomical location perpendicular to the vertebral column and three fourths the distance caudal to the LM. To determine preliminary yield grades, the fat measurements were then adjusted, correcting for any atypical fat distribution.

Mitochondrial Isolation

Mitochondria were isolated from the LM according to the procedures of Bottje et al. (2002) with modifications. Briefly, 5 to 10 g of tissue was collected and placed in 30 mL ice-cold medium I (100 mM sucrose, 100 mM Tris-HCl, 46 mM KCl, 10 mM EDTA, pH 7.4). The tissue was minced with scissors, placed back in medium I and incubated with 8 mg of Protease K (Sigma-Aldrich Co., St Louis, MO) at room temperature for 5 min. The tissue was homogenized (approximately 2 min) in a Potter-Elvenhjem vessel with a Teflon pestle, after which it was incubated on ice for an additional 5 min. The homogenate was centrifuged at 1,000 x *g* for 10 min and the pellet containing cellular debris was discarded. The supernatant was then centrifuged at 10,000 x *g* for 15 min to pellet the mitochondria. The pellet was washed with 10 mL of medium I containing 0.5% BSA. Mitochondria were re-pelleted by centrifuging at 10,000 x *g* for 15 min, suspended in 2 mL of medium II (230 mM mannitol, 70 mM sucrose, 20 mM Tris-HCl, 5 mM KH_2PO_4, pH 7.4) and placed on ice until assays were performed. Mitochondrial protein was determined by the Coomassie Plus protein assay kit (Pierce Biotechnology, Inc., Rockford, IL).

Mitochondrial Function Measurement

Oxygen consumption was measured in duplicate with a Clark-type oxygen probe (YSI, Inc., Yellow Spring, OH) in a respiration chamber at 37°C under constant stirring (Bottje et al., 2002). Mitochondria (0.5 mL) were added to 3 mL of respiration buffer (220 mM mannitol, 70 mM sucrose, 3 mM KH_2PO_4, 2 mM HEPES, pH 7.0). Glutamate (10 mM) was used to stimulate respiration at complex I and succinate (10 mM) was used for complex II respiration. State 3 respiration was initiated by the addition of 10 μL of a

50 mM solution of ADP. Respiratory control ratio was calculated as the ratio of state 3 respiration to state 4 respiration. Acceptor control ratio was calculated as the ratio of state 3 to state 2 respiration. The adenosine diphosphate to oxygen (ADP:O) ratio was calculated according to the methods of Eastbrook (1967).

Hydrogen Peroxide Measurement

The production of hydrogen peroxide by mitochondria isolated from steers selected to have a high or low RFI was measured using the procedures of Bottje et al. (2002) with modifications. Hydrogen peroxide was measured using the dichloroflourescin diacetate probe (Molecular Probes, Inc., Eugene, OR) in a 96-well plate fluorimeter (Fluoroskan Ascent, Thermo Electron Corporation, Vantaa, Finland). Mitochondria (0.05 to 0.1 mg protein) were incubated with 52 μM dichloroflourescin diacetate, 64 μL buffer (145 mM KCl, 30 mM HEPES, 15 mM KH_2PO_4, 3 mM MgCl, 0.1 mM EGTA, pH 7.4), 10 U superoxide dismutase, and either 10 mM glutamate or succinate. Samples were incubated at 37°C for 40 min with fluorescence measured every 5 min. Hydrogen peroxide production is calculated from a standard curve and is expressed as nanomoles H_2O_2 generated per minute per milligram of mitochondrial protein.

Plasma Glucose and Insulin

Blood was collected by jugular venipuncture 1 wk before slaughter into vacutainers containing EDTA as an anticoagulant (Becton, Dickinson and Company; Franklin Lakes, NJ). Samples were collected in the morning before the animal's first major feeding event. The blood samples were centrifuged at 2,200 x *g* for 15 min, the plasma was decanted and frozen at -20°C until further analysis. Plasma glucose was

determined using a colorimetric glucose oxidase kit (Thermo Electron Corporation, Louisville, CO) according to the manufacture's instructions. Plasma concentrations of insulin were quantified using a specific, double-antibody, equilibrium radioimmunoassay as described by Elsasser et al. (1986) with some modifications. Preparation of bovine insulin (Sigma-Aldrich Co., St Louis, MO) for iodination and for standard curve material was via the method of Sodoyez et al. (1975) for preparation of zinc free insulin. Ten micrograms of zinc free bovine insulin was then solubilized in 50 μl H_2O, combined with 500 μCi ^{125}I-Na, and incubated in the presence of 100 μg of iodogen (Pierce Biotechnology, Inc.) for 6 min with gentle mixing. Recovery of the mono-iodinated 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) as follows. The Sep-Pak C18 Cartridge was initially washed with 10 mL of 50% (v/v) acetonitrile containing 50 mM triethylamine solution (pH adjusted to pH 3 with phosphoric acid), followed by 10 mL of deionized H_2O before addition of the iodination mixture. The cartridge was then washed sequentially with: a) 5 mL of 0.4 M phosphate buffer pH 7.4, b) 10 mL of 29% (v/v) acetonitrile containing 50 mM triethylamine, c) 5 mL of 10% (v/v) acetonitrile containing 0.2 M ammonium acetate, pH 5.5, and finally d) 5 mL of 50% (v/v) acetonitrile containing 0.2 M ammonium acetate, pH 5.5. This final fraction was collected and diluted to 25,000 cpm per 100 μL of assay buffer (0.1% gelatin, 0.01 M EDTA, 0.9% NaCl, 0.01 M PO_4, 0.01% sodium azide, 0.1% Tween-20, pH 7.1). Guinea pig anti-bovine insulin antisera (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 a bovine plasma pool (25 to 300 μl) were added to

assay tubes in quadruplicate and the total volume balanced to 300 μL per tube with assay buffer. All plasma samples (100 μL aliquots) to be analyzed 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 μL of a preprecipitated sheep-anti-guinea pig second antibody. The second antibody complex was then precipitated by centrifugation at 3,000 *g* for 30 min and the supernatant discarded by aspiration. Assay tubes containing the precipitate were counted for 1 min on a LKB1275 (LKB Wallac, Turku, Finland) gamma counter. Standards and plasma aliquots of the bovine plasma pool were linear (log/logit transformation; $r^2 = 0.98$) and parallel over a mass of 0.064 to 40 ng/tube and a plasma volume of 25 to 300 μL. Total specific binding was 38%, the minimum detectable concentration was 0.064 ng/tube, percentage recovery of mass was 98.1%, and the inter- and intraassay CV were 5.2 and 6.8 % respectively.

Statistical Analysis

The data were analyzed using the GLM Procedure (SAS Inst., Inc., Cary, NC) as a completely randomized design. An alpha level of 0.05 was used for the determination of statistical significance.

RESULTS AND DISCUSSION

The growth performance of the high and low RFI steers is shown in Table 2.1. There were no differences ($P = 0.80$) in initial or final BW or ADG between the two groups. However, G:F was decreased and ADFI was greater ($P < 0.001$) for the high RFI steers, which consumed 1.54 kg/d more feed than low RFI steers. Basarab et al. (2003) reported similar data in which ADFI and G:F were greater ($P < 0.0001$) and ADG, and

BW of high and low RFI steers were not different. In contrast, poultry researchers (Bottje et al., 2002; Iqbal et al., 2004; Ojano-Dirain et al., 2004) have observed increased gain with no difference in feed intake between birds of high and low feed efficiency. The lack of differences in feed intake between high and low feed efficient birds may represent an alternative mechanism which alters G:F in poultry. Carcass composition as assessed by LM area, s.c. fat thickness over the 12th rib, HCW, and USDA Yield Grade were not different between the high and low RFI groups. Other investigators (Richardson et al., 2001; Basarab et al., 2003) have reported increased fat deposition in steers selected to have high RFI.

Respiratory function of mitochondria isolated from the LM of high and low RFI steers is shown in Table 2.2. When mitochondria were provided with either glutamate or succinate, there was no difference in acceptor control ratio or ADP:O ratio. These data are in agreement with Bottje et al. (2002) in which acceptor control ratio and ADP:O ratios were not altered in high or low feed efficient birds. Acceptor control ratio is a measure of O_2 consumption in the presence ADP. Lower acceptor control ratio values would be indicative of less control of oxidative phosphorylation by ADP concentration suggesting a mechanism other than ADP concentration controls the rate of O_2 consumption by the mitochondria. The ADP:O ratio explains the relationship between ATP synthesis and oxygen consumption. A decreased ADP:O ratio would signify an impairment in ATP synthesis by the mitochondria. Neither of these conditions were expected nor were observed in this experiment.

The respiratory control ratio of the low RFI steers was greater ($P < 0.05$) than that of the high RFI steers. A greater respiratory control ratio value results from a greater

degree of coupling between respiration and oxidative phosphorylation and suggests an increased efficiency of electron transfer. These data are in agreement with Bottje et al. (2002) in which high feed efficient birds also exhibited a greater respiratory control ratios with glutamate than low feed efficient birds suggesting a greater efficiency in the transfer of electrons through the electron transport chain.

A greater amount of hydrogen peroxide production, which would be indicative of electron leak, would be expected based on the observed lower respiratory control ratio values in high RFI steers in the present study; however this was not the case. High RFI steers produced less ($P < 0.05$) hydrogen peroxide than low RFI steers when either glutamate or succinate was provided as a substrate. These observations are in contrast to the observations of Bottje et al. (2002) in which the isolated mitochondria of low feed efficient birds produced greater amounts of hydrogen peroxide. However, because electron leak is a function of respiration (Chance et al., 1979), we expressed H_2O_2 production as a ratio to respiration rate (state 2). We observed no difference between the high and low RFI steers in the amount of electron leak when it was expressed as a function of respiration rate. Increased electron leak, hydrogen peroxide production, and/or reactive oxygen species would result in the destruction of the mitochondria and ultimately cell death (Fleury et al., 2002) and therefore most likely would not be involved in altering gain efficiency. Based on these data, mitochondrial function was not impaired in high RFI steers, but rather the flux of electrons through the electron transport chain was impaired.

A reduced supply of substrate to the mitochondria could affect mitochondrial respiration rates. We measured plasma glucose and insulin concentrations as an indicator

of glucose metabolism and substrate availability to the mitochondria (Table 2.3). We observed that high RFI steers had greater ($P < 0.05$) plasma glucose concentrations than did low RFI steers. However, plasma insulin concentrations and the ratio of glucose to insulin did not differ between the high and low RFI steers. Plasma insulin values are greater than those reported in the literature (Yambayamba et al., 1996; Hersom et al., 2004) most likely due to the measurement of plasma insulin values with a bovine specific insulin assay. The greater plasma glucose is a result of the greater feed intake of the high RFI steers; however, glucose metabolism does not seem to be altered because the ratios of glucose to insulin were similar between the high and low RFI steers. It seems that glucose metabolism or availability does not alter mitochondrial respiration rates.

Table 2.1. Growth performance of steers with high or low residual feed intake (RFI)

Variable	Low RFI (n = 9)	High RFI (n = 8)	SEM[c]
Initial BW, kg	332.78	330.17	7.16
Final BW, kg	566.77	563.07	10.61
ADG, kg/d	1.48	1.47	0.05
G:F	0.20 [a]	0.16 [b]	0.01
ADFI, kg/d	7.40 [b]	8.94 [a]	2.10
RFI	-0.83 [b]	0.78 [a]	0.10
HCW, kg	352.17	367.61	10.16
LM area, cm^2	76.7	79.74	1.22
Fat thickness over the 12th rib, cm	2.16	1.92	0.15
USDA yield grade	4.27	4.00	0.22

[a,b] Means within a row lacking a common superscript differ ($P < 0.001$).

[c] Pooled standard error of the mean (n = 8)

RFI was calculated from the following equation:

Expected Intake = 0.02967 MMWT + 4.49354 ADG + 0.77721

RFI = Actual Intake – Expected Intake

Table 2.2. Respiratory function of skeletal muscle mitochondria from steers with high or low residual feed intake (RFI)

Variable[1]	Glutamate Low RFI (n = 9)	Glutamate High RFI (n = 8)	Glutamate SEM[c]	Succinate Low RFI (n = 9)	Succinate High RFI (n = 8)	Succinate SEM[c]
State 2 respiration	98.00[a]	62.78[b]	9.53	109.45[a]	77.35[b]	8.05
State 3 respiration	275.17[a]	182.87[b]	27.77	482.90[a]	344.33[b]	44.83
State 4 respiration	84.68	79.72	4.97	155.47	133.67	16.51
ACR	3.11	2.68	0.22	4.62	3.93	0.28
RCR	3.09[a]	2.28[b]	0.25	3.84[a]	2.50[b]	0.19
ADP:O	2.02	1.80	0.11	1.92	1.76	0.06
H_2O_2 production	4.16[a]	2.77[b]	0.46	13.95[a]	6.20[b]	2.25
State 2 respiration / H_2O_2 Production	22.46	20.42	2.37	11.17	9.42	2.81

[1] ACR = acceptor control ratio (State 3 / State 2), RCR = respiratory control ratio (State 3 / State 4), ADP:O = adenosine diphosphate to oxygen consumption ratio, H_2O_2 production is presented as nmol H_2O_2 produced $min.^{-1}$ mg mitochondrial $protein^{-1}$,State 2, 3 and 4 respiration data are presented as nmol O_2 consumed $min.^{-1}$ mg mitochondrial $protein^{-1}$.

[a,b] Means within a row lacking a common superscript differ ($P < 0.05$).

[c] Pooled standard error of the mean (n = 8)

Table 2.3. Plasma glucose and insulin of steers with high or low residual feed intake (RFI)

Variable	Low RFI (n = 9)	High RFI (n = 8)	SEM[c]
Plasma glucose, mg/dL	86.44[b]	101.12[a]	4.07
Plasma insulin, ng/mL	9.19	11.10	1.07
Ratio of glucose to insulin	10.04	10.32	1.35

[a,b] Means within a row lacking a common superscript differ ($P < 0.05$).

[c] Pooled standard error of the mean (n = 8)

CHAPTER III

THE RELATIONSHIP BETWEEN MITOCHONDRIAL UNCOUPLING PROTEIN 2 AND 3 EXPRESSION, MITOCHONDRIAL DNA SINGLE NUCLEOTIDE POLYMORPHISMS AND RESIDUAL FEED INTAKE IN ANGUS STEERS

ABSTRACT

The objective of this study was to determine the relationships of uncoupling protein 2 and 3 expression and SNP of mitochondrial DNA in Angus steers selected to have high or low residual feed intake (RFI). Individual feed intake was measured via the GrowSafe® feed intake system over a 3-mo period and used to calculate RFI, a measure of efficiency. Based on these calculations, six low (average RFI = -1.57 kg) and six high (average RFI = 1.66 kg) RFI steers were selected for further study. Blood was collected via jugular venipuncture 1 wk before slaughter for the isolation of mitochondrial DNA. The steers were then killed in order to collect LM for the measurement of uncoupling protein 2 and 3 messenger RNA and protein expression. Protein and messenger RNA expression of uncoupling protein 2 and 3 were determined by western blotting and quantitative PCR, respectively. To determine mitochondrial DNA SNP, total DNA was isolated from blood via standard phenol/chloroform extraction; fragments were amplified with PCR and sequenced with an automated nucleotide sequencer. Average daily gain

and carcass composition were not different ($P > 0.13$) between the high and low RFI steers; however, ADFI by the high RFI animals was 3.77 kg greater ($P < 0.001$) than the low RFI animals. No difference ($P > 0.55$) was observed between the high and low RFI animals in their expression of uncoupling protein 2 or 3 messenger RNA or protein. On average 9.8 and 8.9 polymorphisms were found per mitochondrial genome for the low and high RFI steers respectively. None of these polymorphisms were related to efficiency status. It seems that the expression of uncoupling protein 2 and 3 and mitochondrial DNA sequence are not related to RFI status.

INTRODUCTION

Mitochondria are the primary site of cellular energy production and produce the majority of ATP used to drive cellular processes. The electron transport chain is composed of 83 subunits of which 70 and 13 are encoded by the nuclear and mitochondrial genome, respectively (Leonard and Schapira, 2000). Mutations of mitochondrial DNA have been shown to alter mitochondrial energy production in humans and a number of disease states are characterized by the presence of one or more mitochondrial DNA mutations (Wallace, 1999).

Many other nuclear encoded proteins are involved in mitochondrial function including inner membrane transporters such as adenine nucleotide translocator and uncoupling protein 2 and 3 whose functions have yet to be elucidated. One of the hypothesized roles of uncoupling protein 2 and 3 is to "uncouple" oxidative phosphorylation from electron transport by transporting protons back into the mitochondrial matrix. Uncoupling protein 2 or 3 null mice have been shown to have

reduced proton leak (Krauss et al., 2002) and therefore uncoupling proteins could modulate mitochondrial energy production.

Previous work in cattle (Kolath et al., 2006) and poultry (Bottje et al., 2002) has provided evidence of a link between mitochondrial respiration and feed efficiency. We hypothesized that two mechanisms could explain this observation. First, that increased expression of uncoupling protein 2, 3, or both in high RFI steers would uncouple the proton gradient and thereby increase the energy requirements of the animal to produce the same quantity of ATP. Second, polymorphisms of mitochondrial DNA in high RFI steers would reduce the function of the electron transport chain altering the rate of mitochondrial respiration. Therefore, the objective of this study was to determine the relationships between uncoupling protein 2 and 3 expression and mitochondrial DNA sequence in Angus steers selected to have high or low residual feed intake.

MATERIALS AND METHODS

Animal Management

The research protocols used in this study were approved by the University of Missouri Animal Care and Use Committee (No. 3278). Eighty Angus steers (average initial BW = 262.2 $\pm$ 21.75 kg) were used to select high and low RFI animals. Steers were obtained from a single herd enrolled in the MFA Health Track Beef Alliance and had been previously vaccinated and preconditioned for 45 d before arrival at the University of Missouri Beef Research Farm. Upon receiving the animals, electronic identification tags (Allflex USA, Inc., Dallas Ft. Worth Airport, TX) were attached to the exterior of the left ear for the measurement of individual feed intake with the GrowSafe®

feed intake system (GrowSafe® Systems Ltd., Airdrie, AB Canada). The GrowSafe system (model 4000E) consisted of a total of 16 nodes with two nodes per pen. Ten animals were housed in each of the eight pens with five animals per node. Data were collected over the entire feeding period of approximately 160 d. Days in which there was a hardware malfunction, power failure or feed leaks exceeded 3% were removed from the analysis. Average daily leak throughout the experimental period excluding days removed from the analysis was 0.48 $\pm$ 0.66%. Steers were placed on a receiving diet for 14 d to allow for acclimation to the feeding system. The composition of the experimental diet fed for the remainder of the experimental period is shown in Table 4.1. All steers had ad libitum access to both feed and water. Steers were weighed every 28 d and residual feed intake values were calculated for the entire feeding period. Expected feed intake was calculated by regressing actual intake against ADG and metabolic mid weight (Basarab et al., 2003). The RFI value for each animal was calculated as the difference between the actual and expected intake. Six high and six low RFI steers were selected based on their RFI values and were transported to the University of Missouri Abattoir where the animals were killed; tissue was obtained from the longissimus lumborum, frozen in liquid nitrogen, and stored at -80°C until further study. Hot carcass weights were documented for each animal and the carcasses were chilled for a 24-h period at 5°C. After the 24-h chill, a beef LM area dot grid was used to measure LM area of each carcass to the nearest 0.01 cm^2. Fat thicknesses were determined using a USDA preliminary yield grade ruler (USDA, 1997) at an anatomical location perpendicular to the vertebral column and three fourths the distance, caudal the LM. To determine preliminary yield grades, the fat measurements were then adjusted, correcting for any atypical fat distribution. Marbling

scores were identified by an experienced grader using the USDA marbling standards (USDA, 1997; Abundant, Moderately Abundant, Slightly Abundant, Moderate, Modest, Small, Slight, Traces, and Practically Devoid). Maturity scores were also assessed using the USDA standards (USDA, 1997) for animals older than "A" maturity.

RNA Isolation and Quantitative Real Time PCR

Total RNA was isolated using the TRIzol procedure (Invitrogen Life Technologies, Carlsbad, CA). After isolation, RNA was suspended in molecular biology grade H_2O. The RNA concentration of the samples was determined by measuring the absorbance at 260 nm. The purity of the isolated RNA was verified by measuring the ratio of absorbencies between 260/280 nm, and by separating 2.5 μg of RNA through a 0.8 % agarose gel in 0.09 M Tris-borate, 0.002 M EDTA with 0.5 μg/mL ethidium bromide. Total RNA was then reverse transcribed using the Superscript ™ First Strand synthesis system for RT-PCR (Invitrogen Life Technologies). Primers and TaqMan probes for uncoupling protein 2, uncoupling protein 3, and cyclophilin A (which was used as a housekeeping gene) were designed using Primer Express software (Applied Biosystems, Foster City, CA; Table 4.2). Amplification was performed in triplicate using Taqman Universial PCR master mix (Applied Biosystems) and fluorescence was detected with the ABI Prism 7700 sequence detector (Applied Biosystems). The data were analyzed using the Sequence Detection Software (Applied Biosystems) and expression levels were calculated by subtracting the cycle threshold value for cyclophilin A from the gene of interest.

Western Blotting

Frozen tissue samples were homogenized in PBS (137 mM NaCl, 3 mM KCl, 6.5 mM Na_2PO4, and 3.5 mM KH_2PO_4), centrifuged at 500 x *g* for 10 min and the supernatant withdrawn. The protein concentration of the supernatant was determined with a BCA protein assay kit (Pierce Biotechnology, Inc., Rockford, IL). Thirty μg of protein was fractionated on 10% SDS-Page gels and then blotted to polyvinylidene fluoride membranes overnight. An Enhanced NuGlo western blotting kit (Alpha Diagnostics Inc., San Antonio, TX) was utilized for blocking and development of the blots. Antibodies against uncoupling protein 2 and 3 were purchased from Alpha Diagnostics, Inc. The primary antibodies were diluted 1:1000 and 1:750 for uncoupling protein 3 and 2, respectively. The blots were exposed to Hyperfilm ECL (GE Healthcare, Piscataway, NJ) and the density of the each band was determined using 1D Scan EX software (Scanalytics Inc., Fairfax, VA).

Mitochondrial DNA Sequencing

Blood samples were collected via jugular venipuncture from the 12 selected animals into vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ) containing EDTA as an anticoagulant 1 wk before the transport of the steers to the University of Missouri Abattoir for tissue collection. Standard phenol/chloroform extraction was used to extract DNA from the blood samples. The primers used for PCR amplification were based on the GenBank *Bos taurus* mitochondrial genome (GenBank Accession: NC_001567), and were designed with Primer Premier 5 software (Premier Biosoft International, Palo Alto, CA). Twenty-one primer pairs were used to amplify fragments that overlapped adjacent fragments by approximately 100 bp. Eleven

additional single primers were used to resolve regions the original 21 primer pairs could not. Amplified PCR products were verified by agarose gel electrophoresis. Most amplicons were purified using Qiagen QIAquick PCR purification columns (Qiagen, Inc., Valencia, CA). In some cases where byproducts were detected in the agarose gel, the fragment of interest was purified by preparative polyacrylamide gel electrophoresis (Shibuya et al., 1993). A 377A automatic nucleotide sequencer (Applied Biosystems) using the BigDye™ kit (Applied Biosystems) was used to sequence all purified PCR samples. The resulting sequences were edited and assembled using GeneTool 2.0 (BioTools, Inc., Edmonton, AB Canada) to produce a single contiguous ~16,400-bp sequence for each steer. The assembled mitochondrial DNA sequences were compared to one another and to the original sequence in GenBank to discover polymorphic sites using GeneTool 2.0's multi-align feature.

Statistical Analysis

The data were analyzed using the General Linear Model Procedure (SAS Inst., Inc., Cary, NC) as a completely randomized design with animal as the experimental unit and RFI group as a fixed effect. An alpha level of 0.05 was used for the determination of statistical significance.

RESULTS AND DISCUSSION

The performance of the high and low RFI steers is shown in Table 4.3. There were no differences ($P > 0.13$) in initial or final BW or ADG between the two groups. However, G:F was increased ($P < 0.001$) for the low RFI steers and ADFI was greater ($P < 0.001$) for the high RFI steers, which consumed 3.77 kg/d more feed than the low RFI

steers. Basarab et al. (2003) and Kolath et al. (2006) have reported similar data in which feed intake was greater ($P < 0.001$) for the high RFI animals and G:F was increased ($P < 0.001$) in low RFI steers, but ADG and BW of high and low RFI steers were not different ($P > 0.80$). Carcass composition as assessed by LM area, fat thickness over the 12th rib, HCW, USDA yield grade, and marbling score were not different ($P > 0.45$) between the high and low RFI groups. These data agree with previous reports from our laboratory (Kolath et al., 2006) in which carcass composition was not altered by RFI status. Other authors (Richardson et al., 2001; Basarab et al., 2003) have reported increased fat deposition in steers selected to have high RFI.

The locations of SNP in the mitochondrial DNA sequence of high and low RFI steers are shown in Tables 4.4 to 4.8. On average, 9.8 and 8.9 mutations in the mitochondrial DNA sequence of high and low RFI steers, respectively, were found compared to the Genbank *Bos taurus* mitochondrial complete genome (GenBank Accession: NC_001567). At three locations (587, 9682, and 13,310 bp) all 12 steers differed from the published sequence indicating a possible error in the Genbank sequence. Multiple polymorphisms were found in the D-loop region in both the high and low RFI animals with the majority of the polymorphisms being found in at least one steer of both the high and low RFI groups. Only one steer in the low RFI group contained a given polymorphism found in the genes of cytochrome c oxidase subunits I and III, NADH dehydrogenase subunit 4L, cytochrome B, and the serine transfer RNA. None of the animals in the high RFI group contained a mutation in these genes. One steer in the high RFI group contained a polymorphism in the leucine transfer RNA. At least one steer in both the high and low RFI groups contained a mutation in the following genes:

NADH dehyrdrogenase subunits 1, 2, 4, 5, and 6, cytochrome c oxidase subunit 2, ATP synthase F0 subunit 6 and both ribosomal RNA. Only two of the 13 protein genes, NADH dehydrogenase subunit 3 and ATP synthase subunit 8 were not found to contain any polymorphisms. Nineteen of the transfer RNA genes also did not contain any polymorphisms. The lack of mutations across animals in either group indicated that polymorphisms of mitochondrial DNA are not related to the RFI status in a contemporary group of Angus steers.

No difference ($P > 0.55$) was observed in the expression of uncoupling protein 2 and 3 messenger RNA or protein between the high or low RFI groups (Table 4.9). This result would indicate no difference in the amount of uncoupling of oxidative phosphorylation and electron transport by uncoupling protein 2 and 3. Recent evidence (Krauss et al., 2005) indicated that these proteins have roles in modulating reactive oxygen species production rather than an uncoupling role. Echtay et al. (2002) have shown that uncoupling protein 2 and 3 increase proton leak when superoxide is present thereby protecting the cell from rampant superoxide production. Also, the 100-fold lower expression of uncoupling protein 2 and 3 compared to uncoupling protein 1 would point to a limited role in altering energy expenditure (Pecquer et al., 2001). This evidence along with the lack of differences in superoxide production (Kolath et al., 2006) between high and low RFI animals would indicate that uncoupling protein 2 and 3 do not play a role in altering RFI status.

Table 3.1. Ingredient composition of the experimental diet

Ingredient	Inclusion rate, % as fed basis
Corn	60.5
Soyhulls	20
Grass hay	8
Dried distillers grains + solubles	5
Soybean meal	4.5
Limestone	0.7
Vitamin ADE premix [a]	0.6
Urea	0.5
Salt	0.25
Mineral premix [b]	0.02
Rumensin 80	0.02
Chemical composition[c]	
CP, %	14.7
ME, Mcal/kg	2.99

[a] Contained (as-fed basis) 10% Fe, 10% Mn, 10% Zn, 2% Cu, 1,500 ppm Se, 1,000 ppm I, and 500 ppm Co.
[b] Contained (as-fed basis) 4,000,000 IU of Vitamin A, 800,000 IU of Vitamin D, and 1,200 IU of Vitamin E/kg.
[c] Calculated using tabular values from NRC (1996).

Table 3.2. Primer and probe sequences for bovine uncoupling protein 2, uncoupling protein 3, and cyclophilin A

Item	Accession number	Sequence
Uncoupling protein 2	AF127029	
Forward primer		CCC TCA CCA TGC TCC AGA AG
Reverse primer		AGG ATC CCA AGC GGA GAA A
Probe		FAM-ACC CCA AGC CTT CTA CAA AGG GTT CAT G-TAMRA
Uncoupling protein 3	NM_174210	
Forward primer		TCA AGG AAA AGC TGC TAG ACT ACC A
Reverse primer		GCT CCA AAG GCA GAG ACG AA
Probe		FAM-TCA CCG ACA ACT TCC CCT GCC-TAMRA
Cyclophilin A	NM_178320	
Forward primer		TTA TAA AGG TTC CTG CTT TCA CAG AA
Reverse primer		CCA TTA TGG CGT GTG AAG TCA
Probe		FAM-CAA AGC CAA CAA AGA AAT CTT AGA CGT AAG CAT ACG-TAMRA

Table 3.3. Performance and carcass measurements of steers with high or low residual feed intake (RFI)

Variable	Low RFI (n = 6)	High RFI (n = 6)	SEM
Initial BW, kg	261	263	10.4
Final BW, kg	496	515	11.8
ADG, kg	1.4	1.5	0.05
G:F	0.17[a]	0.13[b]	0.001
ADFI, kg	7.9[b]	11.7[a]	0.30
Residual feed intake	-1.57[b]	1.66[a]	0.09
HCW, kg	334	339	10.9
LM area, cm^2	73.0	73.2	6.03
Fat thickness over the 12th rib, cm	1.4	1.6	0.22
USDA yield grade	3.6	3.8	0.44
Marbling score[c]	58.3	59.2	7.48

[a,b] Means within a row lacking a common superscript differ ($P < 0.001$).

[c] Marbling Scores: Modest = 50.0 - 59.9.

RFI was calculated from the following equation:

Expected Intake = 0.08674 MMWT + 2.84144 ADG – 1.53042

RFI = Actual Intake – Expected Intake

Table 3.4. Locations of single nucleotide polymorphisms in the mitochondrial DNA sequence of high or low residual feed intake (RFI) steers

		Region	D-Loop								12S rRNA				16S rRNA	
		Position	106	169	173	221+	222	363	363+	364-	587+	639	809	1132	1481	1600
ID	RFI Group	Published sequence	T	A	A	-	T	C	-	-	-	T	C	G	G	A
997	Low			G	G				CC		C					-
7112	Low		C	G		C					C					-
9009	Low							-		G	C					
7058	Low					C	-	-		G	C					
45	Low				G						C					-
7092	Low				G			-		G	C		T			
7055	High			G		C					C			A		
7043	High		C	G		C		-		G	C					-
4	High				G			-		G	C		T			
7010	High							-		G	C	C			A	-
43	High			G		C		-			C					
8007	High					CC					C					

Table 3.5. Locations of single nucleotide polymorphisms in the mitochondrial DNA sequence of high or low residual feed intake (RFI) steers

		Region[a]	ND1		ND2				COX1			COX2	ATPase 6			
		Position	3343	3875	4901	5138	5144	5156	5716	7123	7135	7931	8382	8405	8710	8916
ID	RFI Group	Published sequence	A	T	T	T	T	G	C	A	G	G	C	G	C	C
997	Low						C		T	G				A		
7112	Low											A				
9009	Low		G												T	
7058	Low															
45	Low					C					A		T			
7092	Low			C	C											
7055	High							A								
7043	High											A				
4	High			C	C											
7010	High															
43	High															T
8007	High				C											

[a] ND1: NADH dehydrogenase subunit 1, ND2: NADH dehydrogenase subunit 2, COX1: cytochrome c oxidase subunit I, COX2: cytochrome c oxidase subunit II, ATPase 6: ATP synthase F0 subunit 6.

Table 3.6. Locations of single nucleotide polymorphisms in the mitochondrial DNA sequence of high or low residual feed intake (RFI) steers

		Region[a]	COX3			ND4L	ND4				tRNA-Ser	tRNA-Leu	ND5			
		Position	9074	9116	9682	10347	10576	10600	11083	11899	12036	12058	12165	12730	12801	12923
ID	RFI Group	Published sequence	T	A	G	A	G	G	T	T	G	T	T	G	G	A
997	Low				C			A			A					
7112	Low				C					C						T
9009	Low				C								C	A		
7058	Low		C	C	C	G										
45	Low				C											
7092	Low				C											
7055	High				C											
7043	High				C					C						T
4	High				C				C							
7010	High				C				C							
43	High				C										A	
8007	High				C		A					C				

[a] COX3: cytochrome c oxidase subunit III, ND4L: NADH dehydrogenase subunit 4L, ND4: NADH dehydrogenase subunit 4, ND5: NADH dehydrogenase subunit 5.

Table 3.7. Locations of single nucleotide polymorphisms in the mitochondrial DNA sequence of high or low residual feed intake (RFI) steers

		Region[a]	ND5						ND6	CYT B		tRNA-Pro	D-Loop			
		Position	13310	13374	13518	13554	13689	13899	14063	14906	15635	15740	15934	15961	16000	16022
ID	RFI Group	Published sequence	A	C	C	G	G	C	C	C	C	G	C	G	T	G
997	Low		C		T								T			
7112	Low		C	T					T							
9009	Low		C							T	T					
7058	Low		C									A			C	
45	Low		C			A								A		
7092	Low		C													
7055	High		C				A	T								
7043	High		C	T					T							
4	High		C													
7010	High		C													
43	High		C													A
8007	High		C													

[a] ND5: NADH dehydrogenase subunit 5, ND6: NADH dehydrogenase subunit 6, CYT B: cytochrome b.

Table 3.8. Locations of single nucleotide polymorphisms in the mitochondrial DNA sequence of high or low residual feed intake (RFI) steers

ID	RFI Group	Region	D-Loop											
		Position	16042	16057	16085	16109	16122	16135	16141	16231	16232	16247	16255	16302
		Published sequence	T	G	T	T	T	T	T	C	C	C	T	G
997	Low													
7112	Low					C							C	
9009	Low											T		
7058	Low				C				C					
45	Low			C										
7092	Low		C							T	T			
7055	High													A
7043	High					C							C	
4	High		C							T	T			
7010	High													
43	High						C	C						
8007	High		C											

Table 3.9. Expression of uncoupling protein 2 and 3 messenger RNA and protein of high or low residual feed intake (RFI) steers

	High RFI (n = 6)	Low RFI (n = 6)	SEM
Messenger RNA expression			
Uncoupling protein 2	2.79	3.19	0.46
Uncoupling protein 3	2.06	1.88	0.47
Protein expression			
Uncoupling protein 2	167.5	168.0	6.78
Uncoupling protein 3	103.5	105.0	8.86

CHAPTER IV

THE DEVELOPMENT AND EVALUATION OF A BLOOD ASSAY TO PREDICT RESIDUAL FEED INTAKE IN BEEF CATTLE

ABSTRACT

Concluded from research was that parameters of mitochondrial function or respiration rate could be used as predictors of RFI status. The objective of this experiment was to develop and evaluate a blood assay to predict RFI status in cattle. One hundred and ninety two steers underwent a 70 d test to determine RFI status. A blood assay was developed that measured ATP and hydrogen peroxide production in isolated lymphocytes. No difference in ATP production was found between the low and high RFI steers. Hydrogen peroxide production by the low RFI steers was greater than that of the high RFI steers. Residual feed intake was found to be correlated to hydrogen peroxide production. However, hydrogen peroxide production was not able to repeatedly predict the RFI of individual animals. Further research is needed to improve the sensitivity of the assay or develop another method of measuring mitochondrial respiration rate.

INTRODUCTION

Previous research has shown that measures of mitochondrial function and/or respiration rate are related to feed efficiency (Bottje et al., 2002; Kolath et al., 2006). From this research parameters of mitochondrial function/respiration could be used as markers of feed efficiency status. However, this work was conducted using skeletal

muscle tissue. The quantities of muscle tissue that are required for these measurements necessitate obtaining the tissue post-mortem. In addition, the isolation of mitochondria from skeletal tissue is both time consuming and labor intensive, preventing its use in a commercial setting.

Due to the minimal invasiveness of blood collection, lymphocytes are an ideal source of mitochondria. The use of permeablized lymphocytes to measure mitochondrial parameters requires less cellular material than what is required when mitochondria are isolated from skeletal muscle. Furthermore, the use of lymphocytes to measure mitochondrial ATP production has previously been validated (Marriage et al., 2003; Manfredi et al., 2001). Therefore, lymphocytes are an ideal tissue source for the measurement of mitochondrial respiration.

The objective of this experiment was to develop and validate a blood assay using lymphocyte mitochondria to predict RFI status of feedlot cattle. We hypothesized, based on prior research, that lymphocytes isolated from low RFI animals would produce greater amounts of hydrogen peroxide and/or ATP compared to high RFI animals.

MATERIALS AND METHODS

Animal Management

The research protocols used in this study were approved by the University of Missouri Animal Care and Use Committee. One hundred and ninety two steers (average initial BW = 316.67 $\pm$ 34.65 kg) were used as a source from which to select animals to develop and validate the blood assay. These steers were obtained from four herds enrolled in the MFA Health Track Beef Alliance (Columbia, MO), and had been

previously vaccinated and preconditioned for 45 d prior to arrival at the University of Missouri Beef Research Farm.

Upon receiving the animals, electronic ID tags (Allflex USA, Inc., Dallas Ft. Worth Airport, TX) were attached to the exterior of the left ear for the measurement of individual feed intake with the GrowSafe feed intake system (Model 4000E; GrowSafe Systems Ltd., Airdrie, AB Canada). Steers were placed on a receiving diet for 14 d to allow for acclimation to the feeding system. After the acclimation period, steers were fed Superbeef 32 (MFA, Inc., Columbia, MO) at a rate of 13% Superbeef 32 and 87% whole corn. All steers had ad libitum access to both feed and water. Consecutive two day weights were taken on day 0 and 70 of the test period and RFI values were calculated for the entire feeding period. Expected feed intake was calculated by regressing the actual intake against ADG and metabolic mid weight (Basarab et al., 2003). The RFI value for each animal was calculated as the difference between actual and expected intake.

Blood Collection and Lymphocyte Isolation

Blood was collected via jugular venipuncture into ACD vacutainers (Becton, Dickinson and Company; Franklin Lakes, NJ) and stored at room temperature until analysis. Six milliliters of blood was removed from the ACD vacutainer and layered onto an Accuspin tube (Sigma-Aldrich Co., St Louis, MO). The tubes were centrifuged at 1,000 *x g* for 40 min at 18 to 25°C. The lymphocyte layer was removed to a 15 mL centrifuge tube (Corning Inc., Corning, NY) and the volume was brought up to 15 mL with phosphate buffered saline (0.137 M NaCl, 0.0027 M KCl, 0.0022 M KH_2PO4, 0.0097 M Na_2HPO_4, pH 7.4). The tube was centrifuged at 300 *x g* for 15 min to pellet the lymphocytes, the supernatant was removed, and cells were washed with 10 mL of

phosphate buffered saline. The tube was centrifuged at 300 *x g* for 10 min and the supernatant was removed. The cells were suspended into incubation buffer (150 mM KCl, 25 mM Tris, 2 mM EDTA, 10 mM KH_2PO_4, pH 7.4 with 0.1% BSA and 200 ug/mL Digitonin) for subsequent analysis. Protein concentration was measured using a BCA protein assay kit (Pierce Biotechnology, Inc., Rockford, IL) to normalize samples.

Measurement of ATP Production

Lymphocytes were suspended into 2.5 mL incubation buffer which included 1mM ADP. The cell suspension was divided into five 500 uL aliquots and 20 uL of 100 mM succinate was added to each aliquot. The lymphocytes were incubated at $37^{O}C$ for 0, 5, 15, 20, or 30 min. At the end of each incubation period, 17.5 uL of 1.6 M perchloric acid was added to the tube to stop ATP production. The tube was centrifuged at 10,000 *x g* to pellet any cellular debris and the supernatant was removed to a 2 mL centrifuge tube. The pH of the sample was adjusted to 7.8 using 24.6 uL of 1.6 M NaOH and the sample was then diluted 1:100 in ddH_2O. One hundred microliters of sample and 100 uL of ATP assay mix (Sigma-Aldrich) was added to a 96 well plate and luminescence was measured with a 96 well plate luminometer (1450 Microbeta Trilux, Wallac, Turku, Finland). The concentration of ATP for each sample was determined in triplicate with appropriate blanks and standards. The rate of ATP production was determined by plotting the ATP concentration as a function of time.

Measurement of Hydrogen Peroxide Production

Hydrogen peroxide was measured using the dichloroflourescin diacetate probe (Molecular Probes, Inc., Eugene, OR) in a 96-well plate fluorimeter (Fluoroskan Ascent, Thermo Electron Corporation, Vantaa, Finland). Lymphocytes were suspended into 1

mL of incubation buffer. Lymphocytes (92 uL) were incubated with 52 µM dichloroflourescin diacetate, 10 U superoxide dismutase, and 20 mM succinate. Samples were incubated at 37°C for 40 min with fluorescence measured every 5 min. Hydrogen peroxide production was calculated from a standard curve and is expressed as nanomoles H_2O_2 generated per minute per milligram of protein.

Statistical Analysis

The data were analyzed using the General Linear Model Procedure (SAS Inst., Inc., Cary, NC) as a completely randomized design with animal as the experimental unit and RFI group as a fixed effect. Correlations between hydrogen peroxide production and RFI were determined with the Correlation Procedure. An alpha level of 0.05 was used for the determination of statistical significance.

RESULTS AND DISCUSSION

The blood assay was able to measure both hydrogen peroxide production and ATP production rates of isolated lymphocytes. No difference was found in the rate of ATP production by lymphocytes isolated from high or low RFI steers (Table 4.1). This result would indicate that ATP concentration is most likely tightly regulated within the cell, and differences in mitochondrial respiration rates are not detectable when ATP concentration is measured. However, the blood assay was able to detect differences in rates of hydrogen peroxide production (Table 4.1). Similar to results obtained from mitochondria isolated from skeletal muscle, the mitochondria from low RFI steers produced greater ($P < 0.05$) amounts of hydrogen peroxide compared to high RFI steers. A significant correlation was found between RFI and hydrogen peroxide production rate which ranged from -0.82 to -0.38. However, hydrogen peroxide production rate could

not be used to predict the RFI status of an individual animal (Figure 4.1). When hydrogen peroxide production was plotted against RFI, the coefficient of determination was 0.1444 indicating very limited prediction of RFI status by hydrogen peroxide production rates. Further research is needed to improve the sensitivity of the blood assay and to develop other mitochondrial parameters that could be used as markers of RFI status in cattle.

Table 4.1. Rate of hydrogen peroxide and ATP production of isolated lymphocytes from steers selected to have high, average or low residual feed intake (RFI)

	High RFI	Average RFI	Low RFI	SEM	Correlation[1]	P- Value
H_2O_2 Production[2]						
Experiment 1 (n = 6)[*]	23.21[b]		34.66[a]	2.49	-0.82	0.05
Experiment 2 (n = 24)[*]	0.17[b]	0.20[a,b]	0.23[a]	0.01	-0.40	0.05
Experiment 3 (n = 6)[#]	1.13[b]		1.47[a]	0.11	-0.75	0.09
ATP Production	0.112		0.115	0.01		

[1] Correlation of RFI and H_2O_2 production

[2] Experiment 1 was not corrected for protein concentration. This caused the values to be higher than experiments 2 and 3.

[a,b] Means within a row lacking a common superscript differ ([*] $P < 0.05$, [#] $P < 0.10$).

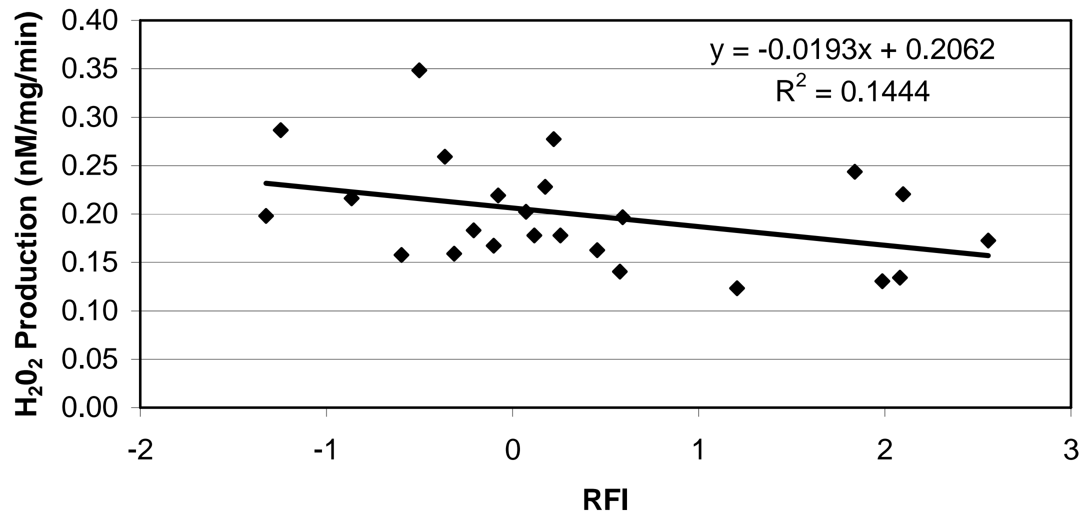

Figure 4.1. The relationship between hydrogen peroxide production and residual feed intake (RFI) in steers with high, average or low RFI.

LITERATURE CITED

Anderson, S., M. H. L. de Bruijn, A. R. Coulson, I. C. Eperon, F. Sanger, and I. G. Young. 1982. Complete sequence of bovine mitochondrial DNA. J. Mol. Biol. 156:683-717.

Archer, J. A., P. F. Arthur, R. M. Herd, and E. C. Richardson. 1998. Genetic variation in feed efficiency and it's component traits. Proc. of the 6th World Congress on Genetics Applied to Livestock Production, Armidale, Australia. 25:81-84.

Archer, J. A., E. C. Richardson, R. M. Herd, and P. F. Arthur. 1999. Potential for selection to improve efficiency of feed use in beef cattle: a review. Aust. J. Agric. Res. 50:147-61.

Archer, J. A., A. Reverter, R. M. Herd, D. J. Johnston,, and P. F. Arthur. 2002. Genetic variation in feed intake and efficiency of mature beef cows and relationships with postweaning measurements. Proc. 7th World Congress Genet. Appl. Livestock Prod. 31:221-224.

Arthur, P. F. and R. M. Herd. 2005. Efficiency of feed utilization by livestock – Implications and benefits of genetic improvement. Can. J. Anim. Sci. 85:281-290.

Arthur, P. F., R. M. Herd, J. F. Wilkins, and J. A. Archer. 2005. Maternal productivity of Angus cows divergently selected for post-weaning residual feed intake. Aust. J. Exp. Agric. 45:985-993.

Arthur, P. F., R. M. Herd, J. Wright, G. Xu, K. Dibley, and E. C. Richardson. 1996. Net feed conversion efficiency and its relationship with other traits in beef cattle. Proc. Aust. Soc. Anim. Prod. 21:107-110.

Arthur, P. F., G. Renand and D. Krauss. 2001. Genetic and phenotypic relationships among different measures of growth and feed efficiency in young Charolais bulls. Livest. Prod. Sci. 68:131-139.

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

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 steers. Can. J. Anim. Sci. 83:189-204.

Beitz, D. C., A. E. Freeman, M. M. Schutz, and G. L. Lindberg. 1994. Association of bovine mitochondrial DNA with traits of economic importance. US Patent #5,292,639.

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

Braastad, B. O. and J. Katle. 1989. Behavioural differences between laying hen populations selected for high or low efficiency of food utilization. Brit. Poul. Sci. 30:533-544.

Brelin, B., and E. Brannang. 1982. Phenotypic and genetic variation in feed efficiency of growing cattle with their relationship with growth rate, carcass traits and metabolic efficiency. Swed. J. Agric. Res. 12:29-34.

Buttazzoni, L. and I. L. Mao. 1989. Genetic parameters of estimated net energy efficiencies for milk production, maintenance and body weight change in dairy cows. J. Dairy Sci. 72:671-677.

Carstens, G. E., C. M. Theis, M. B. White, T. H. Welsh Jr., B. G. Warrington, R. D. Randel, T. D. A. Forbes, H. Lippke, L. W. Green, and D. K. Lunt. 2002. Residual feed intake in beef steers: I. Correlations with performance traits and ultrasound measures of body composition. J. Anim. Sci. 80 (Suppl. 2):121. (Abstr.)

Chance, B., H. Sies, and A. Boveris. 1979. Hydroperoxide metabolism in mammalian organs. Physiol. Rev. 59:527-605.

Clapham, J. C., J. R. Arch, H. Chapman, A Haynes, C. Lister, G. B. Moore, V. Piercy, S. A. Carter, I. Lehner, S. A. Smith, L. J. Beeley, R. J. Godden, N. Herrity, M. Skehel, K. K. Changani, P. D. Hockings, D. G. Reid, S. M. Squires, J. Hatcher, B. Trail, J. Latcham, S. Rastan, A. J. Harper, S. Cadenas, J. A. Buckingham, M. D. Brand, and A. Abuin. 2000. Mice overexpressing human uncoupling protein – 3 in skeletal muscle are hyperphagic and lean. Nature 406:415-418.

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

Eastbrook, R. W. 1967. Mitochondrial respiratory control and the polarographic measurement of ADP:O ratios. Methods Enzymol. 10:41-47.

Echtay, K. S., D. Roussel, J. St-Pierre, M. B. Jekabsons, S. Cadenas, J. A. Stuart, J. A. Harper, S. J. Roebuck, A. Morrison, S. Pickering, J. C. Clapham, and M. D. Brand. 2002. Superoxide activates mitochondrial uncoupling proteins. Nature 415:96-99.

Elsasser, T. H., A. C. Hammond, T. S. Rumsey, and R. Fayer. 1986. Perturbed metabolism and hormonal profiles in calves infected with *sarcocystis cruzi*. Domest. Anim. Endocrinol. 3:277-287.

Erlanson-Albertsson, C. 2003. The role of uncoupling proteins in the regulation of metabolism. Acta Physiol. Scand. 178:405-412.

Fleury, C., B. Mignotte, and J. L. Vayssiere. 2002. Mitochondrial reactive oxygen species in cell death signaling. Biochimie 9:131-141.

Ganong, W. F. 2001. Pages 10-11, 271-290 in Review of Medical Physiology. 20th Edition. McGraw-Hill, New York, NY.

Gong, D. W., S. Monemdjou, O. Gavrilova, L. R. Leon, B. Marcus-Samuels, C. J. Chou, C. Everett, L. P. Kozak, C. Li, C. Deng, M. E. Harper, and M. L. Reitman. 2000. Lack of obesity and normal response to fasting and thyroid hormone in mice lacking uncoupling protein 3. J. Biol. Chem. 275:16251-16257.

Hagger, C. 1994. Relationships between income minus feed costs and residual feed consumption in laying hens. Poult. Sci. 73:1341-1344.

Hansen, C., J. N. B. Shrestha, R. J. Parker, G. H. Crow, P. J. McAlpine, and J. N. Derr. 2003. Genetic diversity amoung Canadienne, Brown Swiss, Holstein, and Jersey cattle based on mitochondrial D-loop sequence variation. Can. J. Anim. Sci. 83:39-44.

Harper, M. E. and J. Himms-Hagen. 2001. Mitochondrial efficiency: Lessons learned from transgenic mice. Biochim. Biophys. Acta 1504:159-172.

Herd, R. M., V. H. Oddy, and E. C. Richardson. 2004. Biological basis for variation in residual feed intake in beef cattle. 1. Review of potential mechanisms. Aust. J. Exp. Agric. 44:423-430.

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. J. Anim. Sci. 82:2059-2068.

Hughes, T. E., and W. S. Pitchford. 2004. Does pregnancy and lactation affect efficiency of female mice divergently selected for postweaning net feed intake. Aust. J. Exp. Agric. 44:501-506.

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. Poult. Sci. 83:474-484.

Iqbal, M., N. R. Pumford, Z. X. Tang, K. Lassiter, C. Ojano-Dirain, T. Wang, 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. Poult. Sci. 84:933-941.

Jezek, P. 2002. Possible physiological roles of mitochondrial uncoupling protein – UCPn. Intr. J. Biochem. Cell Bio. 34:1190-1206.

Johnston, D. J., R. M. Herd, A. Reverter, and V. H. Oddy. 2001. Heritability of IGF-1 in beef cattle and its association with growth and carcass traits. Proc. Aust. Assoc. Anim. Breed. Genet. 14:163-166.

Kahi, A. K., S. A. Barwick, and H-U Graser. 2003. Economic evaluation of Hereford cattle breeding schemes incorporating direct and indirect measures of feed intake. Aust. J. Agric. Res. 54:1039-1055.

Knott, S. A., B. J. Leury, L. J. Cummins, and F. R. Dunshea. 2005. Importance of body composition in explaining variation in feed conversion efficiency and residual feed intake between sheep. Asia Pac. J. Clin. Nutr. 14 (Suppl.):S78.

Knott, S. A., B. J. Leury, F. D. Brien, L. J. Cummins, and F. R. Dunshea. 2004. Less efficient sheep are more responsive to an ACTH induced stress challenge. Asia Pac. J. Clin. Nutr. 13(Suppl.):S89.

Koch, R. M., L. A. Swiger, D. Chambers, and K. E. Gregory. 1963. Efficiency of feed use in beef cattle. J. Anim. Sci. 22:486-494.

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

Krauss, S., C. Zhang, and B. B. Lowell. 2002. A significant portion of mitochondrial proton leak in intact thymocytes depends on expression of UCP2. Proc. Natl. Acad. Sci. USA 99:118-122.

Krauss, S., C. Zhang, and B. B. Lowell. 2005. The mitochondrial uncoupling-protein homologues. Nature Reviews 6:248-261.

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 ultrasound carcass estimates. J. Anim. Sci. 83 (Suppl. 2):121. (Abstr)

Lassiter, K., M. Iqbal, N. R. Pumford, C. Orjano-Dirain, N. Tinsley, and W. Bottje. 2004. Protein expression in feed efficiency. Poult. Sci. 83(Suppl. 1):100. (Abstr.)

Lehninger, A.L., D. L. Nelson, and M.M. Cox. 2000. Pages 659 – 688 in Principles of Biochemistry. 3rd Edition. Worth Publishers, New York, NY.

Leonard, J. V., and A.H.V. Schapira. 2000. Mitochondrial respiratory chain disorders I: mitochondrial DNA defects. Lancet 355:299-304.

Luiting, P., E. M. Urff, and M. W. A. Verstegen. 1994. Between animal variation in biological efficiency as related to residual feed consumption. Neth. J. Agric. Sci. 42:59-67.

Lutz, R.T., and T. S. Stahly. 2003. Quantitative relationship between mitochondrial bioenergetics and efficiency of animal growth. J. Anim. Sci. 81(Suppl. 1):141. (Abstr.)

Manfredi, G., A. Spinazzola, N. Checcarelli, and A. Naini. 2001. Assay of mitochondrial ATP synthesis in animal cells. Methods Cell Biol. 65:133-145.

Marriage, B. J., M. T. Clandinin, I. M. MacDonald, and D. Moira Glerum. 2003. The use of lymphocytes to screen for oxidative phosphorylation disorders. Anal. Biochem. 313:137-144.

McDonagh, M. D., 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. Aust. J. Exp. Agric. 41:1013-1021.

Moore, K. L., D. J. Johnston, R. M. Herd, and H-U Graser. 2003. Genetic and non-genetic effects on plasma insulin-like growth factor-1 (IGF-1) concentration and production traits in Angus cattle. Proc. Aust. Assoc. Anim. Breed. Gene. 158:222-226.

Moore, K. L., D. J. Johnston, H-U Graser, and R. Herd. 2005. Genetic and phenotypic relationships between insulin-like growth factor-1 (IGF-1) and net feed intake, fat, and growth traits in Angus beef cattle. 56:211-218.

Mrode, R. A., C. Smith, and R. Thompson. 1990. Selection for rate and efficiency of lean gain in Hereford cattle. 1. Selection pressure applied and direct responses. Anim. Prod. 51:23-34.

Mueller, A. L., W. H. Kolath, J. W. Golden, and M. S. Kerley. 2004. Evaluation of RUP amino acid levels on lean tissue growth and carcass composition of beef steers fed a roughage-free diet. J. Anim. Sci. 83(Suppl. 1):116. (Abstr.)

NRC. 1996. Nutrient Requirements of Beef Cattle. 7th ed. Natl. Acad. Press, Washington, DC.

Nkrumah, J. D., C. Li, J. B. Basarab, S. Guercio, Y. Meng, B. Murdoch, C. Hansen, and S. S. Moore. 2004. Association of a single nucleotide polymorphism in the bovine leptin gene with feed intake, feed efficiency, growth, feeding behavior, carcass quality and body composition. Can. J. Anim. Sci. 84:211-219.

Nkrumah, J. D., 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. J. Anim. Sci. 83:20-28.

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. Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. J. Anim. Sci. 84:145-153.

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. Poult. Sci. 83:1394-1403.

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. Poult. Sci. 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. Poult. Sci. 84:1926-1934.

Pecquer, C., M. Alves-Guerra, C. Gelly, C. Levi-Meyrueis, E. Couplan, S. Collins, D. Ricquier, F. Bouillaud, and B. Miroux. 2001. Uncoupling protein 2, in vivo distribution, induction upon oxidative stress, and evidence for translational regulation. J. Biol. Chem. 276:8705-8712.

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 or against residual feed intake. Aust. J. Exp. Agric. 41:1065-1072.

Richardson, E. C., R. M. Herd, I. G. Colditz, J. A. Archer, and P. F. Arthur. 2002. Blood cell profiles of steers progeny from parents selected for and against residual feed intake. Aust. J. Exp. Agric. 42:901-908.

Richardson, E. C., R. M. Herd, J. A. Archer, and P. F. Arthur. 2004. Metabolic differences in Angus steers divergently selected for residual feed intake. Aust. J. Exp. Agric. 44:441-452.

Schrauwen, P. and M. Hesselink. 2002. UCP2 and UCP3 in muscle controlling body metabolism. J. Exp. Biol. 205:2275-2285.

Shibuya, H., D. J. Nonneman, T. H. M. Huang, V. K. Ganjam, F. A. Mann, and G. S. Johnson. 1993. Two polymorphic microsatellites in a coding segment of the canine androgen receptor gene. Animal Genetics 24:345-348.

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 J. Biol. Chem. 250:4268-4277.

Steinborn, R., M. Muller, and G. Brem. 1998. Genetic variation in functionally important domains of the bovine mtDNA control region. Biochim. Biophys. Acta 1397:295-304.

Stuart, J. A., K. M. Brindle, J. A. Harper, and M. D. Brand. 1999. Mitochondrial proton leak and the uncoupling proteins. J. Bioenerg. Biomem. 31:517-525.

Taanman, J. 1999. The mitochondrial genome: structure, transcription, translation and replication. Biochim. Biophys. Acta 1410:103-123.

Theis, C. M., G. E. Carstens, R. J. Hollenbeck, M. W. Kurtz, T. M. Bryan, L. J. Slay, R. D. Randel, and T. H. Welsh Jr. 2002. Residual feed intake in beef steers: II. Correlations with hematological parameters and serum cortisol concentrations. J. Anim. Sci. 80 (Suppl. 2):88. (Abstr.)

USDA. 1997. USDA Official United States standards for grading of carcass beef: Agric. Marketing Serv. USDA Washington, DC.

Wallace, D. C. 1999. Mitochondrial diseases in man and mouse. Science 283:1482-1488.

Wood, B. J., J. A. Archer, and J. H. 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. Live. Prod. Sci. 91:69-81.

Willis, C. A., and M. S. Kerley. 2004. Effect of removing roughage from the diet on digestibility and fermentation characteristics in vivo. J. Anim. Sci. 82(Suppl. 2): 95. (Abstr.)

Wu, J., R. K. Smith, A. E. Freeman, D. C. Beitz, B. T. McDaniel, and G. L. Lindberg. 2000. Sequence heteroplasmy of D-loop and rRNA coding regions in mitochondrial DNA from Holstein cows of independent maternal lineages. Biochem. Genet. 38:323-335.

Yambayamba, E. S. K., M. A. Price, and G. R. Foxcroft. 1996. Hormonal status, metabolic changes, and resting metabolic rate in beef heifers undergoing compensatory growth. J. Anim. Sci. 74:57-69.

VITA

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