Targeted Gene Expression Profiling in Beef Cattle under Finishing Conditions in the South Eastern United States: Identification of regulatory factors involved in metabolic efficiency, energy partitioning, and intramuscular fat deposition

by

Derris Deon Burnett

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Approved by

Werner G. Bergen, Chair, Professor of Biochemistry and Nutrition
Keith A. Cummins, Professor of Dairy Cattle Management and Canine Nutrition
Ya Xiong Tao, Associate Professor of Physiology
Kevin Huggins, Associate Professor of Nutrition and Dietetics

Abstract

Three studies were conducted in order to examine the molecular events associated with metabolic efficiency in specific skeletal muscle and adipose tissue depots in finishing beef cattle. In the first study a total of 15 male cattle (n = 7 Bulls and 8 Steers) were selected from the initial progeny of a Residual Feed Intake (RFI) selection program to determine the relationship between RFI and the transcriptomic signature of performance efficiency in finishing beef cattle. Calves were individually fed twice daily for 84 days and refusals were collected once daily. Feed intake data was collected for each animal and used to determine residual feed intake (RFI). At day 84, skeletal muscle and adipose biopsies were collected for analysis of regulatory gene expression related to protein turnover and lipid metabolism. Overall, the results from this study indicate that RFI appears to be related to genes involved in protein turnover and to a lesser extent, other metabolic genes; however genes involved in AT metabolism did not appear to be related. It is possible that the range of RFI values in bulls and steers was too narrow to ascertain a relationship between fattening and feed efficiency and future studies should utilize more divergent cattle populations with respect to RFI.

In a second study was conducted to determine the effect of days on feed and beta-agonist administration on the expression of regulatory genes in skeletal muscle and adipose tissue of finishing heifers. Seventy-one crossbred heifers were stratified according to height and height and assigned to one of six pens (12 cattle per pen). Cattle had continuous access to automatic water troughs and each pen contained 12 Calan Gates® to allow for individual feed provision

and intake determination. Treatment groups consisted of days on feed (DOF, n= 16 per group) with the following assignments: 79, 100, 121, and 142 DOF. For each DOF group, half (n=8) of the animals were treated with ractopamine hydrochloride (RAC) 300mg/hd/d for the final 35d prior to harvest while the other half served as controls (CON). At slaughter, skeletal muscle and adipose samples were collected to determine regulatory gene expression. These data did not identify coordinated regulation of metabolic pathways in response to RAC administration in any of the DOF groups however temporal patterns of gene expression were observed that are consistent with the order and priority of tissue development in finishing cattle.

The final study looked at the effects of feed restriction and re-feeding on gene expression patterns in muscle and adipose tissue of forage fed beef. Temporal regulation of gene expression was observed between biopsy dates and plane of nutrition differentially regulated growth initially however poor forage growth led to a lack of robustness in the treatment groups as the experiment proceeded and weight gains began to equalize between groups. This led to a lack of coordinated regulation of the selected genes between treatment groups. As a whole, this body of work provides the basis for future studies and has established that transcriptomic/gene expression profiling in beef cattle determined by quantitative RT-PCR of skeletal muscle and adipose tissue samples can be an effective approach when used in conjunction with more elaborate approaches to identify and manipulate the molecular distinctions that are inherent to more efficient animals. This will eventually lead to innovations to improve production efficiency and carcass value by augmenting the growth of specific tissues independently of less valuable ones.

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Chapter 1. Introduction

Feed represents the single largest input cost in most animal production systems comprising about 70% of the input costs for commercial beef cattle operations (Herd at al., 1998; Liu et al., 2000; Arthur et al., 2001; Basarab et al., 2002). A major issue threatening the sustainability of the beef industry is trying to maintain and improve product quality in the midst of increasing feed-costs. For beef cattle, 60 to 75% of the feed provided is used to meet maintenance requirements (Ferrell and Jenkins, 1984; NRC, 1996). There is considerable variation in the cattle maintenance requirements and genetic variation appears to be moderately heritable (Carstens et al., 1989) suggesting an opportunity to improve on this trait by selecting for more efficient cattle.

Profitability in the beef industry is determined by both inputs (e.g. feed intake) and outputs (e.g. lean meat produced), therefore improvement of the ratio of outputs to input would provide significant economic benefits to beef producers. For years animal scientists and producers have sought to improve the various desirable aspects of meat quality that are determined by consumer preference and minimize or eliminate factors that may cause health concerns. Researchers have focused on improving the efficiency of livestock production while providing a product of acceptable quality in an increasingly global and diverse marketplace. This has included efforts to improve rate of gain, feed efficiency, and manipulating the composition of gain in an economically relevant fashion.

There are some inherent conflicts in obtaining these goals and some factors are often sacrificed at the expense of others. The selection for increasingly lean animals has led to increases in production efficiency and consumer preference, in many cases at the detriment of intramuscular fat (IMF) content in various meat products. Despite some of

the detrimental impacts, intense selection strategies based on animal science research have led to relative improvements in overall production efficiency and decreased environmental impact of beef production.

Profitable beef production in the modern era will require producers to take advantage of emerging technologies and utilization of strategic management practices to minimize operation costs. Compared to other meat-producing animals, beef cattle are the least efficient at converting feed to gain with current estimates indicating that cattle have a feed conversion ratio (FCR) of about 7.7 (unit feed: unit gain). This is compared to a FCR of 1.1, 2.0, and 2.5 to 3.0 in farm raised fish, poultry, and swine, respectively (Bergen, 2008; Roux, 2006). It is estimated that a 5% improvement in feed efficiency has an economic impact equivalent to a 20% improvement in average daily gain (Gibb and McAllister, 1999). Therefore feed efficiency will continue to be one of the primary determinants of profit or loss in beef production systems and improvements in performance that maximize the conversion of feed to usable products will be vital to long-term industry sustainability. Understanding the molecular determinants of efficient feed utilization will provide valuable genetic targets and cues for implementing management strategies to optimize production efficiency.

Increased consumer health awareness has led to efforts to manipulate the amount, quality, and distribution of adipose tissue (AT) in beef cattle and other meat species. The flow of energy into and out of AT has significant economic implications in beef production because the amount and distribution of body fat are primary determinants of beef carcass quality and yield grades according to the USDA grading system (USDA, 1996). Increased IMF can have positive impacts on beef quality and eating

characteristics. On the other hand, wasteful deposition of fat in low-value depots represents an inefficient use of feed energy and has a negative impact on overall production efficiency. Selective enhancement of valuable depots (e.g. IMF) is a promising strategy to improve carcass quality and efficient use of nutrients by directing them into economically valuable depots. However, the knowledge of depot specific adipose tissue metabolism, particularly molecular regulatory components, as related to beef cattle production is currently lacking.

Metabolic modifiers are a group of compounds that improve production efficiency in part by increasing lean protein deposition and decreasing AT accretion. Several of these exogenous agents have been developed with varying efficacies and species specificity. Beta-adrenergic agonists (BAA) are part of a class of metabolic modifiers known as energy repartitioning agents, which increase protein accretion and decrease adiposity in various livestock species. Major hurdles that inhibit the widespread incorporation of many of these useful compounds in commercial operations include consumer acceptance, long withdrawal periods, and variable effectiveness. Determination of specific mechanisms of action may lead to improved compounds that have more specific targets and reduced undesirable effects.

The lack of a comprehensive understanding of the molecular determinants of feed efficiency, energy partitioning, and marbling accumulation have hindered our ability to manipulate the beef carcass beyond the means of traditional genetic selection tools. Livestock production in the post-genomic era is poised to benefit from innovative application of novel technologies originally designed for the sequencing of the human genome. In the current, post-genomic era there are a plethora of tools, technologies, and

genomic information databases that are becoming available and affordable for application to improve livestock production. Identification of measurable regulatory factors will lead to more specific targets for pharmacological intervention and will help optimize management strategies to improve product quality, lower production costs, and reduce the environmental burden of the beef cattle industry.

In the current project we have utilized a targeted transcriptomic approach aimed at mapping the expression patterns of putative and novel regulatory factors involved in energy metabolism, protein turnover, and adipogenesis in beef cattle. These categories relate directly to muscle and fat deposition and overall beef carcass composition and quality. Three independent studies were conducted to observe the gene expression profiles as they relate to beef cattle under finishing conditions in the Southeastern US (SE). In the first study we sought to investigate genes associated with residual feed intake in finishing steers and bulls to identify potential molecular targets to improve this trait. In a second study we investigated the effect of Ractopamine HCl (Optaflexx, Elanco Animal Health) on the expression of genes involved in adipogenesis, lipid metabolism and energy metabolism. In a final study we looked at the effect of compensatory growth and a forage-based finishing system on the expression of key regulatory metabolic genes in loin muscle and subcutaneous adipose tissue using serial biopsies obtained during the finishing phase.

Chapter 2. Literature Review

Feed Efficiency

With feed representing the major cost of beef production (Herd et al., 2003), improving the output of beef per unit feed is the primary objective of feed efficiency research and forms the basis for genetic selection for feed efficiency (FE). In the past 50 years the livestock industry, in general, has become more efficient in the face of rising feed costs, increased competition for land and resources, and increased consumer product demand.

Poultry and swine producers have dramatically improved production efficiency. Genetic selection has nearly maximized the biological potential of broiler production and has left little room for further improvement. Havenstein et al. (2003) estimated that today's broilers require less time and less feed to reach a market weight of 1.8kgs. They also concluded that the majority (80-90%) of the improved production efficiency was the result of intense genetic selection for more efficient birds that grew larger faster, while improved nutrition (e.g. vitamin and mineral supplementation) played a nominal role.

Cattle have also experienced substantial improvement in overall efficiency although much of this has come as a result of grain feeding and other nutritional regimens, as well as reproductive and pharmaceutical technologies (Elam and Preston, 2004). While there is considerable variation for FE in beef cattle and this variation appears to be moderately heritable, there has been little improvement in FE or maintenance energy requirements during the past 50 years (Archer et al., 1999).

Gibbs and McAllister (1999) reported that increasing FE by 5% would have an economic impact four times greater than a 5% enhancement in average daily gain (ADG).

With 60% of the total cost to achieve a marketable steer, estimated to be attributed to the feed (Ritchie, 1992), the cumulative effects of improved efficiency over the entire production system would be of economically beneficial to the beef industry as a whole and particularly the pasture based SE beef industry. Furthermore, 70 to 75% of the energy provided in feed is used to meet maintenance energy requirements and this appears to be a moderately heritable trait. The benefits of more efficient cattle include: decreased maintenance requirements, decreased feed intake (FI) with similar weight gain, increased product yield per unit feed, and decreased manure and methane emissions (Nkrumah et al., 2006; Hegarty et al., 2007)

Measures of Feed Efficiency

Feed efficiency can be described as a ratio of some form of inputs to outputs. In beef cattle, feed conversion ratio is defined as units of feed consumed to units of product. While this term is useful for determining the effects of various feeds and management practices on production efficiency, there are some confounding factors that preclude feed conversion ratios (FCR) as a useful trait for genetic improvement. Another measure of efficiency is partial efficiency of gain, which is a ratio of ADG to feed used for growth minus the predicted feed used for maintenance (Arthur et al., 2001). While these measures of efficiency have their merits, they also have their limitations in that many fail to account for various contributing factors such as mature body size.

Residual Feed Intake

Residual feed intake (RFI) was first investigated by Byerly (1941) who examined what he called net efficiency in laying hens. In 1963, Koch et al. observed that there were

differences in the ability to gain and maintain body weight amongst growing beef cattle impacting feed requirements. These factors are related to the net energy requirements for maintenance (NEm) and gain (NEg) which varies between individual animals. They suggested that the feed requirements could be adjusted for body weight (BW) and weight gain by partitioning feed intake into an expected component which is the amount of food that an animal would be expected to eat based on the estimated requirements for a given level of performance, and a residual component which is the actual amount of feed intake above or below the expected intake levels. More efficient animals would show a lower (more negative) RFI value compared to their less efficient counterparts (Koch et al., 1963).

While selection for animals that consume less and maintain a similar body weight and growth rate as their contemporaries is an attractive option to improve sustainability and profitability of beef production, adoption of RFI as a practical efficiency parameter has been met with limited success for various reasons. One of the primary factors that inhibit widespread adoption of RFI is the cost and difficulty of measuring individual feed intake and BW on large numbers of cattle over an extended period of time (Moore et al., 2009). Selection for improved (lower or more negative RFI) may also have some unexpected consequences on the overall efficiency of the beef operation. Shaffer et al (2010) reported that for each 1 unit increase in RFI, a decrease of 7.5 d in age at puberty was observed. They concluded that differences in body fat and rate of metabolism associated with RFI could be responsible for the delay in reproductive maturity. Therefore, it is imperative to understand the long-term impact of selection for RFI on metabolic and reproductive efficiency.

Molecular Basis of Residual Feed Intake

Genetic and molecular mechanisms that control feed intake and feed efficiency are currently not very well understood. Knowledge of biological contribution to production efficiency is lacking, but the post-genomic era has ushered in new tools that can facilitate novel approaches to understanding feed efficiency and its molecular regulatory mechanisms (Bolormaa et al., 2011; Hill and Azain, 2009; Moore et al., 2009). The ability to predict RFI using genomic tools provides an attractive alternative to circumvent the need for these costly and labor intensive measurements and may lead to more widespread acceptance of RFI (Moore et al., 2009).

Kolath et al. (2006) showed that the rate of mitochondrial respiration is increased in steers with low RFI versus those with high RFI. This is similar to results in poultry and rats, which have identified a relationship between mitochondrial respiration and feed conversion ratio (Bittke et al., 2002; Lutz and Stahly, 2003). Lkhagvadorj et al. (2009) identified common differentially expressed genes in the liver and AT of pigs subjected to caloric restriction (CR) or selected for feed efficiency (RFI). These genes included the transcription factors (TFs) PPAR alpha, PPAR-gamma, and CREB. It is known that several transcription factors are responsible for mediating the physiological response to CR (Desvergne et al., 2006) and this data indicates that a common energy conservation mechanism may exist in CR and low RFI pigs.

While there has been considerable research on defining variation between types of cattle, there has been little progress on identifying the underlying molecular components that regulate metabolic efficiency. Richardson and Herd (2004) listed some sources that

may contribute to variation in RFI including feed intake, digestion, body composition, rate of gain, etc. They estimated that 37% of the variation in RFI can be attributed to tissue metabolism, protein turnover and stress. Even with their comprehensive assessment it can be said that the exact source of a substantial amount of the variation in RFI has yet to be elucidated.

Metabolic Modifiers

Modifiers in Beef Production

Small profit margins are a continuing threat to the long-term stability of the beef cattle industry (Herd, 2003). Feed provision is a major cost; therefore, researchers and producers are constantly looking for ways to improve feed efficiency and increase lean tissue deposition while simultaneously decreasing excessive fat cover in beef cattle. Genetic selection and an increased understanding of various environmental and nutritional factors have led to marked improvements in carcass quality, composition and production efficiency. In addition, several exogenous compounds known as metabolic modifiers have been developed to improve the profitability of animal production (Dikeman, 2007).

Metabolic modifiers are defined as agents that can be fed, injected, or implanted in animals to improve rate of gain, feed efficiency, dressing percent, yield percent, or sensory characteristics of meat (Dikeman, 2007). In the livestock industry, the use of metabolic modifiers is focused on improving the efficiency of meat and milk production, as well as improving the yield and composition of these products (Beerman et al., 2005). These compounds generally work by improving feed efficiency, increasing muscle deposition, decreasing AT accretion, or some combination of the above (Dunshea et al.,

2005). Metabolic modifiers can be separated into various functional categories. These include but are not limited to: anabolic steroids, somatotropins, beta-adrenergic agonists, designer lipids, and other dietary additives such as vitamins and minerals that have been shown to have specific modulatory effects on energy and protein metabolism (Dunshea et al., 2005; Dikeman, 2007).

The United States Food and Drug Administration (FDA) have approved the use of various metabolic modifiers for a number of species. The use of these compounds dates back to 1956 with the introduction of anabolic implants for use in beef cattle (Preston, 1999). There are tangible benefits for the use of metabolic modifiers in beef production and this is evidenced by the widespread use of implants and feed additives in the US beef industry (Beerman et al., 2005). Their advantages include increased production efficiency for producers and increased lean meat yield for packers; translating to the availability of leaner, less expensive meat for consumers. The magnitude of these improvements is influenced by: the dosage and duration of the treatment, the compounds used, and target species (Beermann, 1993; Mersmann, 1998; Moody et al., 2002).

Beta-Adrenergic Agonists

One class of modifiers that has received much attention is the beta-adrenergic agonists (BAAs) also known as phenethanolamine repartitioning compounds**.** β-adrenergic agonists bind to and, activate the β-adrenergic receptor (BAR), which is expressed in various tissues and cell types across mammalian and avian species (Lefkowitz, 2007; Mersmann, 1995; Coutinho et al., 1992). The physiological BAAs are epinephrine and norepinephrine and numerous synthetic compounds have also been produced to stimulate or block the activity of BAR. Because the receptors they activate

control a wide array of physiological functions, much of the interest in these compounds has been driven by potential biomedical applications as bronchodilators, modulators of cardiovascular function and other therapeutic purposes. The physiological activity of a BAA is dependent on several factors including receptor activity, rate of absorption, rate of metabolism, and rate of elimination as well as receptor distribution with respect to target tissues (Smith, 1998).

Beta-Adrenergic Receptors

Beta-adrenergic receptors (BAR) are ubiquitous in all cells of the body and across all mammalian species. BAR are membrane bound proteins that belong to the seven-transmembrane class of cell surface receptors also known as G-protein coupled receptors (GPCRs). These receptors represent the largest family of plasma membrane receptors with over 1000 genes, and play a role in nearly every mammalian physiological process (Lefkowitz, 2007). They control a wide array of physiological and metabolic functions, ranging from heart-rate regulation to lipolysis and these properties have peaked pharmacological interests in these receptors for decades making them the most common targets of currently prescribed drugs (Lefkowitz, 2007; Ma and Zimmel, 2002; Mersmann, 1998).

A physiological response is induced when a BAA binds to a BAR and the degree and effect of this response is dependent on a combination of factors. The mechanism of BAR action is mediated through the G-stimulatory (Gs) protein-signaling pathway. Once bound by a suitable agonist, Gs protein activity causes activation of adenylate cyclase, which leads to the production of cyclic adenine nucleotide monophosphate (cAMP), a well-characterized intracellular second messenger. cAMP activates protein kinase

resulting in the subsequent phosphorylation of various downstream target proteins and modulation of metabolic pathways.

There are several considerations that account for the overall response to a BAA including dosage duration, receptor subtype targeted, and the specific milieu of downstream effectors that are modulated in response to receptor activation. Together these reasons, along with age, genetics, and environmental factors are responsible for the various physiological effects seen when using different BAA within and across species. Therefore, it cannot be assumed that a given agonist will induce a prototypical response across species or within a species under different conditions and treatment regimens (Mersmann, 1998).

Beta-Adrenergic Receptor Subtypes

There are at least three BAR subtypes that have been identified and these are B1-AR, the B2-AR, and the B3-AR. Beta-Adrenergic receptors are distributed universally on mammalian cells across species and the distribution of these subtypes differs between tissues and across species (Strosberg, 1997; McNeel and Mersmann, 1999; Mersmann, 1996). The rat heart contains more than 90% B1-AR while the rat adipocytes contain greater than 90%B3-AR. Porcine adipocytes in contrast contain less than 10% B3-AR (McNeel and Mersmann, 1999). While these tissues represent extremes with respect to subtype composition, other tissues have more equal distribution of the B1-AR and B2-AR receptor subtypes. The B3-AR subtype shows a more limited distribution being restricted primarily to the gut and AT. In addition, the various subtypes exhibit variable responses to different BAA. In addition to across species and across-tissue differences, the distribution and expression of the BAR subtypes changes with growth/age of the

animal, which can influence the response to a given BAA in vivo (Mersmann, 2002; Feeve et al., 1991).

Although there is considerable homology among the subtypes across species, amino acid sequence variation imparts another layer of complexity that causes differential ligand-binding and physiological responses to a given agonist. The amino acid sequence homology between subtypes is 80-90% with the transmembrane segments sharing many conserved residues (Mills and Mersmann, 1995). BAR subtypes were originally classified based on the ability of agonists or antagonists to stimulate or inhibit a physiological response when bound to the receptor. In addition, these subtypes originally appeared to be localized in a specific tissue. It has since been determined that this specificity is relative and not absolute, and that agonist and antagonist can bind to multiple receptor subtypes making the classification system somewhat ambiguous (Mersmann, 1995).

There is only limited knowledge of the specific BAR subtypes on skeletal muscle (SM) and AT of meat producing species. These are the primary tissues that are responsive to administration of oral BAA (Mersmann, 1995). Tissue distribution can be determined by using selected agonists and antagonists (Coutinho et al., 1991; Mersmann, 2002). Using this approach Coutinho et al. (1991) determined that 45% of the BAR present in adipocytes isolated from crossbred barrows showed high affinity for the B1-AR selective agonists ICI 89,406 and thus were classified as B1-ARs. Low affinity binding sites comprised 55% of the total, and were tentatively classified as B2-AR.

Transcripts for the B1-AR, B2-AR, and B3-AR have been identified on bovine and porcine adipocytes (Mills et al., 2002; Mersmann, 1998). In terms of BAR

distribution, 75% of the BAR in SM and AT of beef cattle have been identified as the BR2 subtype. This is in contrast to pigs where BR1 is the predominant subtype (Mills, 2002). The actual expression patterns and functionality of these receptor subtypes is not conclusive in all tissues. Because the BARs are distributed on almost all mammalian cell types, there are complex mechanism of actions depending on the BAA used, the BAR-subtype population, and the distribution of BAA to various tissues.

Beta-Adrenergic Agonist's Effects in Skeletal Muscle and Adipose Tissue

It is known that the hydrolysis of stored triglycerides in AT is regulated by endogenous catacholamines that bind the BAR to activate the lipolytic enzymes. Binding of the BAA to the BAR causes an increase in cAMP levels resulting in protein kinase activation and subsequent activation of hormone sensitive lipase. The liberated TAG can then be exported and oxidized in other tissues to meet energy requirements and protein can be spared for lean deposition. In this manner carcass adiposity can be decreased and leanness increased with the use of BAA. Adipocytes express all three of the BAR subtypes on their cell surface, although the role for each subtype is not clearly understood (Mills et al., 2003). In pigs, the predominant subtype is the B1-AR, which comprises nearly 80% of the total.

One of the more pronounced responses to oral BAA administration is the rapid and preferential increase in SM mass. In beef animals this translates to more dissectible lean and can lead to more efficient production of meat products. Beerman (2002) summarized the effects of BAA on skeletal muscle growth in laboratory and farm animals. The anabolic effects of BAA administration in SM include hypertrophy of muscle fibers, changes in muscle fiber type, and differential rates of nucleic acid and

protein accretion. Bergen et al. (1989) reported that the effects of BAA on SM hypertrophy could be attributed to changes in the muscle hypertrophy and fractional protein accretion rates in finishing pigs. Grant et al. (1993) studied the effects of Ractopamine HCl on the expression of SM alpha-actin and insulin-like growth factor I mRNA in crossbred barrows. Their results indicated that the BAA-induced growth of skeletal muscle was the result of increased myofibrillar gene expression including strong induction of SM alpha actin, and that the response is maximal with short-term administration of the agonist. This is similar to data reported by Gunawan et al. (2007) who found that myosin heavy chain genes are differentially regulated with BAA treatment and that the effects of BAA on muscle hypertrophy may be mediated through changes in muscle fiber type-specific gene expression patterns. Studies have also shown that metabolic maturation of SM tissues may be necessary for maximum efficacy of BAA; therefore, receptor distribution and density are important issues.

Insulin sensitivity has been shown to be decreased in adipocytes from pigs (Liu and Mills, 1990) and rats (Hausman et al., 1989) treated with RAC, while no effect was reported on SM sensitivity in the hindquarters of lambs despite a 50% decrease in circulating insulin concentrations (Beerman, 1987). This appears to indicate opposing effects on insulin sensitivity in AT and SM which may contribute to the effects seen with BAA administration. Clenbuterol, a potent BAA, has also been shown to induce transient and chronic insulin insensitivity in cattle (Sternbauer et al., 1998). Other studies using endocrine altered animals have provided evidence that opposes the involvement of indirect endocrine mechanisms acting on SM. Theil et al. (1987) conducted a study using hypophysectomized rats and showed that SM growth was significantly increased in

animals receiving BAA treatment compared to controls. Similarly, in animals with a genetic growth hormone deficiency, BAA was still capable of augmenting SM growth. While stimulation of growth may also be a result of indirect action on SM tissue involving various endocrine mechanisms, the contribution of indirect mechanisms appears to be negligible (Beerman, 2001).

Beta-Adrenergic Agonists in Livestock Production

Because of the perpetually high cost of feed, considerable attention must be paid to input cost of beef production in order to maintain profitability (Herd et al., 2003). Reduction of input cost via improved feed efficiency and effective nutrient partitioning are the primary economic incentives of BAA use in livestock. BAA are the most recent compounds to gain approval for use in feedlot cattle (Winterholler et al., 2007) and these compounds have been shown to effectively increase ADG, Gain: feed, and hot carcass weight (Laudert et al., 2004). BAA's specifically enhance lean protein deposition (Bergen et al., 1989; Grant et al., 1993) and decreases AT accretion by modifying metabolic flux such that there is a decrease in nutrients directed towards adipogenesis and an increase in lean growth (Lopez-Carlos et al., 2011; Bergen, 2001; Anderson et al., 1991). The net result of administering BAA includes some combination of: increased rate of gain, improved feed efficiency, increased leanness, and increased dressing percentage (Moody et al., 2000).

There are some limitations and drawbacks to the use of BAA in livestock. These compounds are generally only effective for the final 20 to 40 DOF as the effects begin to plateau and additional exposure does not render additional benefit. There are also disparate responses to various BAA between livestock species making it difficult to

extrapolate across species and across studies (Mersman, 1995). Cattle and sheep show greater responses and chickens are the least responsive (Moody et al., 2000). Turkeys and pigs appear to be intermediate. One possible explanation may be the fact that chickens have been under such intense selection pressure that they may be close to reaching their maximal biological potential for growth rate and as such have much less room for improvement via exogenous compounds compared to less efficient species. Elucidation of the molecular factors responsible for BAA action will be critical to understanding these differential responses and effectively tailoring management strategies to maximize the benefit of these compounds.

Ractopamine Hydrochloride

The use of Ractopamine HCl (RAC) in farm animals has been studied extensively for the last 20 years. Mills (2002) provides an excellent summary characterizing the use of RAC in these species. Ractopamine hydrochloride is a phenethanolamine derivative that was originally approved for use in swine in 1999 and subsequently for use in beef cattle in 2003. RAC was developed and patented by Eli Lily in 1986 and is currently patented by Elanco Animal Health (Greenfield, Indiana) and marketed under the trade names Paylean and Optaflexx for the swine, and beef cattle formulations, respectively. RAC is classified as a phenethanolamine agent, which is structurally or functionally similar to the endogenous catecholamine epinephrine and norepinephrine (Beerman, 2002; Hancock et al., 2006), and like other BAA, act as a repartitioning agent with the ability to increase lean protein and decrease adiposity in pigs and cattle.

Structure and Function

The biological activity of a given BAA is a function of its specific chemical composition. The first description of BAA mechanism of action comes from Easson and Stedman (1933) who proposed that BAA interact with the BAR at three contact points: the β-hydroxyl group, the aliphatic positively-charged nitrogen group, and the aromatic ring. Substitution or alteration of these groups can have profound effects on binding and activity of BAA. In addition to these common groups, there is a bulky substituent that confers specificity for the BAR (Carlstrom et al., 1973; Weiner, 1980). These are elements that are common to all BAA. While they share some general characteristics, specific substitutions change the activity and efficacy of the BAA compounds (Figure 4.1). In addition, rate of clearance, uptake, and tissue specificity are determined by the specific substitutions about the aforementioned chemical groups. These are chemical elements also contained in the physiological BAAs epinephrine and norepinephrine with the exception of the bulky group on the aliphatic nitrogen (Smith, 1998).

Stereochemistry of BAA is important in determining biological activity due to the presence of multiple chiral centers on these molecules. RAC is sold as mixture of its component stereoisomers that arise as a result of 2 chiral centers, one provided by the beta-hydroxyl group and a second that arises in the R-group (See Figure 4.1). This chirality produces four stereoisomers two of which (RR and RS) are leveratory and 2 of which (SR and SS) are dextrorotary. Ricke et al. (1999) determined that the RR stereoisomer is responsible for the biological activity of RAC. In terms of BAR subtypes, RAC binds the B1-AR with higher affinity than the B2-AR (Hancock et al., 2006). Some agonists can bind both alpha and BAR. In addition, an agonist for one receptor subtype

may function as an antagonist for another subtype adding yet another level of complexity. This is not the case with RAC, which binds almost exclusively to the B1-AR.

Effectiveness of Ractopamine and other Beta-Adrenergic Agonists in Livestock

Halsey et al. (2011) investigated the effect of RAC on gene expression in subcutaneous AT of finishing pigs. RAC was fed for 12, 28, or 42d and expression of adipogenic and lipogenic TFs was determined by Northern blot analysis. They determined that RAC supplementation caused reduction in lipogenic genes including PPAR-g, SREBP-1, and FAS after 42 days. These data indicate that RAC mechanism of action in AT has to do with attenuation of the processes of adipogenesis and lipid filling in this tissue although the specific mechanisms remain undefined. While it is likely that prolonged administration of RAC causes down regulation of the BAR, the anti-adipogenic effects observed at 42d indicate that alternative mechanisms may be responsible for the effects of RAC on SC AT.

Reiter et al. (2007) used divergently selected lean and obese pigs to examine the role of genetic background on metabolic pathways associated with anabolic and catabolic lipid metabolism in liver, skeletal muscle, and AT in animals supplemented with RAC for 52 days. While these animals showed differential expression of lipogenic and adipogenic genes and TFs between genotypes, treatment with RAC resulted in depressed anabolic and increased catabolic lipid-metabolism related gene expression irrespective of genetic background. This is similar to results by Mimbs et al. (2005) who reported enhanced growth performance in finishing lean and obese pigs fed RAC regardless of their degree of adiposity at the initiation of the trial.

In heifers RAC has been shown to improve average daily gain (ADG), while either decreasing or having no effect on dry matter feed intake (Gruber et al., 2007; Walker et al., 2006). Consumption of less feed with equivalent lean growth results in fewer days to market and improved feed and production efficiency making RAC a promising agent to improve the performance of feedlot cattle.

Winterholler et al. (2007) conducted a study to determine the effects of RAC in finishing heifers. They showed a 4.6% increase in ADG and increased hot carcass weight without any effects on marbling score or feed intake when RAC was fed at 200mg/hd/d. Winterholler et al. (2007) reported ADG, G:F, and DMI decreased and HCW, USDA Quality Grade, and Yield Grade increased as DOF increased from 150 to 192d. Winterholler et al. (2007) observed that RAC fed at a rate of 200 mg/hd/d for 28d increased ADG by 4.6% and HCW by 8 kg in heifers. Schroder and others (2004) determined Optaflexx® improved steer ADG by an average of 25.7% when compared to control steers. They also reported a 20.4% improvement in ADG was observed in heifers. No effects in feed intake were observed indicating improved feed efficiency in both groups across three treatment levels of Optaflexx (100, 200, and 300 mg/hd/d) and two treatment durations of 28 and 42d.

Zilpaterol HCl (ZH; Zilmax, Intervet Schering Plough Animal Health, DeSoto, KS) was recently approved for oral administration in beef cattle by the United States Food and Drug Administration (FDA) in 2006. Like RAC, this BAA is approved for use during the finishing phase and label uses include improved FE and increased carcass leanness in cattle when fed during the final 20-40 DOF. Treatment with ZH requires a minimum of a three day withdrawal period. Approval of ZH use in the US was preceded

by approval in South Africa, Mexico, and more recently Canada (Delmore et al., 2010). One negative impact of ZH is the perceived increase in Warner-Bratzler Shear Force (WBSF) in steaks from animals receiving ZH during the finishing period.

Vasconcelos et al. (2008) fed Zilpaterol HCl (ZH) to finishing steers for the final twenty days with a three day withdrawal period. They observed an increase in ADG and decreased DMI. They showed that feeding steers for more than twenty days did not give further advantage to ADG. They also observed a 9kg increase in BW and a 15kg increase in hot carcass weight resulting in changes in dressing percentage. They hypothesized that treatment with ZH resulted in partitioning of nutrients away from non-carcass components and towards valuable carcass depots (Elam et al., 2009). In this instance the BAA improved both the FCR as well as the yield of useable product thereby enhancing the overall efficiency of production.

Lopez-Carlos et al. (2011) recently conducted a study feeding RAC or ZH to finishing lambs in order to directly compare the effects of these two BAA. 112 crossbred lambs were treated with either RAC or ZH for the final 14, 28, or 42 DOF. While there was similar growth performance between lambs receiving ZH or RAC, lambs receiving either BAA had 9.6% higher final BW and 24% higher ADG compared to controls. Carcass characteristics were also improved including a 3.9% and 3.8% greater HCW and dressing percentage, respectively. Most notably perhaps was the 20% decrease they observed in fat thickness in animals fed BAA indicating that these compounds were effective at partitioning energy metabolism away from AT and towards SM resulting in observable changes in carcass composition.

Duration of Effects

The intensity or maximal response of a receptor to an agonist can diminish over time despite the continued presence of the agonists. This inherent regulatory mechanism serves to mediate the amplitude, frequency and duration of the ligand-induced response (Sun et al., 2003). One such mechanism is known as receptor desensitization and is a phenomenon that occurs with the BAR under chronic BAA administration (Lefkowitz, 2007). In the classical model of receptor desensitization G-proteins mediated signaling is subject to receptor desensitization and internalization in a signaling pathway involving arrestins and GPCR-related kinases (GRks, Clark et al., 1999). This occurs because receptor activation also induces regulatory processes that attenuate the duration of signaling via ligand-induced receptor internalization or ligand-induced desensitization of the receptor (Clark et al., 1999). Whether or not these classical paradigms apply to BAA-induced desensitization of the BAR in animals fed RAC is yet to be determined.

Smith (1989) determined that chronic administration of BAA can lead to temporal changes, down regulation, or desensitization of BAR. Kim et al. (1992) reported decreased BAR density in muscles from rats fed cimaterol. Likewise, Mills et al. (1990) found that porcine adipocytes isolated from pigs fed RAC were less sensitive to RAC-induced lipolysis in vitro. Similarly, Spurlock et al. (1994) looked at the effect of RAC on BAR density and affinity in porcine SM and AT. While RAC treatment did not affect the affinity of the BAR for RAC, and did not affect the maximal binding (Bmax) in SM tissue, there was a reduction in the Bmax in AT which may account for the diminished lipolytic response and plateau effect of chronic BAA administration (Spurlock et al., 1994). Be it receptor desensitization, down-regulation, or a yet undetermined

phenomenon, long-term diminishing effects have precluded effectiveness of these compounds over extended amounts of time or after previous exposure.

The use of RAC and other repartitioning agents is generally limited to the finishing phase. Optaflexx is approved for use in beef cattle for 28 to 42d prior to harvest and is to be administered at a rate of 70 to 430 mg/hd/d. Strategic timing of Optaflexx administration may be a valuable tool for managing and marketing of finished cattle. For example, it may be possible to group cattle according to expected end weights to increase effectiveness and efficiency of Optaflexx administration in finisher rations. With the high cost of feed, the number of days on feed can significantly affect the profitability of the enterprise and the relative value of administering BAA or other metabolic modifiers making it imperative to identify molecular cues that are indicative of optimal windows for treatment with these compounds.

Future of Beta-Adrenergic Agonists in Livestock

Historically, research with these compounds has been focused on optimizing dosage, duration, and timing of treatment to maximize the tangible outcomes. Less research has focused on understanding the molecular intricacies that are responsible for the action of these compounds. Besides an overall all effect on lean deposition/protein synthesis and lipid accretion, lipogenesis and lipolysis (Bergen, 2001), a clearer understanding of the molecular events responsible for the specific mechanism of action in finishing heifers is lacking and such understanding will contribute to optimizing management strategies for finishing cattle under SE conditions. With the advent of genomic platforms tailored for livestock production, the molecular underpinnings of energy partitioning and feed efficiency appear on the verge of elucidation. This will form

the basis for future technologies and management strategies toward effective implementation of these and other metabolic modifiers in beef cattle production.

Adipose Tissue

Adipose tissue (AT) is present to some extent in all mammals, and other organisms from *C. elegans* to Drosophila have also developed similar ways to store excess energy in the form of fat (Pond, 1992; Ashrafi, 2007; Gesta et al., 2007). In terms of energy metabolism the role of AT is to store energy in the form of triglycerides during periods of nutritional excess and to release this currency when nutrient availability is low or energy demand is high. In addition to its role in energy storage, AT is a dynamic organ with major impact on the organism's metabolic disposition (Waki and Totonez, 2007). Once thought of as simply a storage vat for excess dietary energy with lesser roles as a cushion and insulator in the body, we now know that AT participates in endocrine, paracrine, and autocrine signaling networks and that it plays critical roles in energy homeostasis, immune function and in reproductive processes (Kim and Moustaid-Moussa, 2001). AT also serves as the primary site for de novo lipogenesis (DNL) in livestock species such as pigs, and ruminant species; which is not the case for humans and rodents (Bergen and Mersmann, 2005). The role of AT in thermoregulation has also been defined with white AT serving as an insulator to maintain body temperature and brown AT being involved in thermogenesis via uncoupling of mitochondrial respiration. Novel physiological roles for AT are constantly being identified and the contribution of AT to the whole-body energy metabolism and cell-signaling in humans and animals has begun to garner much more appreciation.

Economic Value of Adipose Tissue in Beef Production

Aside from its physiological roles, AT is an economically important tissue when it comes to beef cattle production. AT physiology has been of interest to animal scientists and livestock producers for years due to the direct impact of this tissue on economic and quality aspects of meat production. A longstanding goal of animal scientists has been to minimize wasteful AT accretion because it represents a waste of dietary energy, making over-fattening of livestock an undesirable and inefficient process (Hausman et al., 2008; Dodson et al., 2009). While excessive trim fat decreases carcass value and production efficiency, the beef industry paradoxically rewards producers with premium prices for carcasses with high IMF or marbling (Schroeder et al., 2002). The issue of excess fat cover is not a trivial one because genetic selection for carcass leanness can come at the expense of IMF and thus decrease carcass quality and value. Therefore, understanding molecular intricacies of AT biology in general, as well as the regional peculiarities between specific AT depots are both imperative to understanding the contribution of AT to metabolic and production efficiency (Hausman et al., 2009).

Cellular Composition of Adipose Tissue

At the histological level AT can be described as an innervated loose connective tissue composed primarily but not exclusively of adipocytes, which originate from the mesenchymal stem cell lineage. The pluripotent cells that give rise to the population of preadipocytes present in AT also give rise to myocytes and chondrocytes among other cells (Gesta et al., 2007). AT itself is a heterogeneous tissue composed of preadipocytes, endothelial cells, macrophages and other cell types. Mature adipocytes capable of storing and releasing lipids make up about one-third of the cellular population. The heterogeneity

of the cells that comprise AT imparts a dynamic and depot-specific microenvironment within the tissue and thus AT exhibits a high degree of responsiveness and plasticity. In addition, AT is highly innervated and vascularized all of which are indicative of the dynamic role of AT in whole body metabolism (Hausman and Richardson, 2004; Jacobi et al., 2006).

Adipocytes

While they are not the exclusive residents in the AT depot, adipocytes are the primary functional cellular components of AT. Mature adipocytes are responsible for energy cycling (energy storage and release) and they serve as the primary source of the various factors synthesized and secreted by AT (Mohamed-Ali, 1998; Kim and Moustaid-Moussa, 2000). In addition, these adipocytes are responsive to local and distant metabolic cues allowing them to have an impact on whole body metabolism and contribute to energy homeostasis (Flier and Maratos-Flier, 1998).

Mammalian adipocytes can be classified into two types: those that give rise to white adipose tissue (WAT) and those that give rise to brown adipose tissue (BAT; Spiegelman and Flier, 2001). These cell types can be distinguished morphologically and functionally. WAT contain a large unilocular lipid droplet, which displaces the nucleus of the cell towards the periphery. BAT has multilocular lipid droplets and an increased proportion of mitochondria, which are responsible of the brown appearance of these cells. In terms of energy homeostasis, white adipocytes are responsible primarily for triglyceride synthesis and energy storage while brown adipocytes contain the cellular machinery for fatty acid metabolism and thermogenesis, and participate in energy dissipation via uncoupling of mitochondrial electron transport from ATP production.

Because these cell types act as counter actors in energy metabolism, understanding the molecular components that distinguish BAT and WAT has become increasingly important to understanding AT biology and overall energy homeostasis. While BAT has been thoroughly investigated in rodents and more recently humans, the evidence and role of BAT in livestock species has been scarcely studied (Martin et al., 1999; Smith et al., 2004). The balance of BAT and WAT can contribute to systemic energy balance (Spiegelman and Flier, 2001), and as such may warrant future investigation in livestock production efficiency research. Unless otherwise stated, the following discussion will deal exclusively with WAT and the adipocytes that comprise this tissue.

Adipogenesis

Adipogenesis is an inclusive term that describes the cellular events responsible for the commitment of pluripotent stem cells to the adipocyte lineage to form pre-adipocytes, and the proliferation and differentiation of these pre-adipocytes into mature adipocytes capable of assimilating and storing lipids and responding to various external stimuli (Rosen et al., 2000; Hausman et al., 2009). The process of adipogenesis begins in mid to late gestation in ruminant animals (Feve, 2005; Gnanalingham et al., 2005; Muhlhausler et al., 2006) and this process continues into adult life in accordance with normal cell turnover, as well as the expansion of AT depots to accommodate caloric excess. De novo AT development occurs in multiple distinct sites including between the muscle and dermis (SC AT), and around the kidneys and heart. These events are accompanied by functional and morphological changes that occur at the cellular level and lead to the formation and accretion of distinct AT depots in vivo (Hausman et al., 2001; Novakofski,

2004). Postnatal growth of AT can be attributed to adipogenic processes that lead to hyperplastic and hypertrophic expansion of the depot during the course of growth and development. The timing of this process differs between species and breeds, within species, as well as between depots in individual animals.

Numerous detailed studies on the stages and indices of adipogenesis have been conducted due to the agricultural and biomedical ramifications of AT biology and pathology, particularly with respect to excessive AT accretion. Much of our understanding of these events has been established using in-vitro techniques and various cell-lines (Reviewed in Novakofski, 2004; Poulos et al., 2010), which form the basis for hypothesis driven in vivo experimentation. The advent of various molecular techniques and platforms has allowed substantial progress in understanding how cellular heterogeneity and local micro-environment can impact the capacity and extent to which adipogenesis occurs in an ordered and depot-specific manner.

Regulation of Adipogenesis

The process of adipogenesis occurs as a series of temporally regulated events that are responsible for the development and expansion of AT in specific depots. Several studies have helped to determine that this process is under the control of specific transcription factors (TFs) that respond to environmental and developmental cues to establish and maintain adipogenic gene-expression paradigms (Christy et al., 1989; Totonoz et al., 1993; Yeh et al., 1994; MacDougald and Lane, 1995; Farmer, 2006). The gene products targeted by these TFs include metabolic proteins, lipid transport proteins, and those that impart hormone responsiveness on the adipocyte (Kim and Moustaid-Moussa, 2000; Fruhbeck et al., 2001). The expression of these TFs and the genes that

they regulate follows a specific pattern that allows one to track the progression of the differentiation process and may provide molecular targets to enhance or depress the development of specific AT depots in beef cattle.

The ultimate fate of adipocyte precursor cells is determined by several factors including the immediate endocrine/paracrine environment, adhesion to the extracellular matrix, the specific milieu of transcription and regulatory factors, and nutrient availability (Poulos et al., 2010; Hausman et al., 2009). The balance between pro- and anti-adipogenic factors present in the local environment dictate the commitment to, and progression through the adipogenic cascade. A discussion of some of the well-characterized adipogenic TFs and other markers of differentiation follows below.

Peroxisome Proliferator Activated Receptors (PPARs)

PPARs belong to a family of ligand-dependent nuclear receptor transcription factors and the role of these TFs as master regulators of adipogenesis is supported by both in vivo and in vitro studies. Members of the PPAR family included PPAR-alpha, PPAR-beta/delta, and PPAR-gamma (PPAR-γ).

In terms of adipogenesis, the regulatory transcription cascade is centered on the expression of PPARγ (Wu et al., 1996; Chinetti et al., 2000; Rosen et al., 2000; Rosen and Speigleman, 2001). PPARγ is a lipid activated nuclear receptor that is considered to be both necessary and sufficient to initiate the entire program of adipogenesis (Fernyhough et al., 2007; Rosen et al., 2000; Rosen and Speigleman, 2001; Wu et al., 1999). PPARγ is expressed as two isoforms designated PPARγ1 and PPARγ2, which are derived from alternative promoter regions in the same gene. PPAR□2 is identical to PPARγ1 with the exception of an additional 30 amino acid segment at the N-terminus of

the peptide (Meirhaeghe et al., 2003). While the PPARγ1 isoform is expressed in various tissues, PPARγ2 expression appears to be restricted to AT.

Thiazolidinediones are a class of pharmacological compounds that have insulin-sensitizing effects and have been shown to be ligands for PPAR-□ (Weng et al., 2006; Komers et al., 1998; Chinetti et al., 2000). These compounds are effective at improving insulin sensitivity in part by the PPAR-γ induced changes in expression of lipid metabolism and adipogenesis related genes including glucose transporters, FABPs, and lipoprotein lipase among other PPAR-γ targets (Weng et al., 2006). TZD's have been shown to effectively induce adipogenesis in several in vitro systems including rodent, human, bovine, and porcine adipose tissue stromal-vascular cell cultures (Reviewed in Hausman et al., 2007) While these compounds are widely used in treatment of type-2 diabetes, their usefulness in livestock species has garnered recent attention and warrants further consideration.

CCAAT-Enhancer-Binding Proteins (C/EBPs)

CCAAT-Enhancer-Binding Proteins (C/EBPs) are a family of TFs, which consists of six members (C/EBPα to C/EBPε). The expression of each of the C/EBP proteins occurs at specific time points during adipogenesis, indicative of their distinct roles in the process. CEBP/β and CEBP/δ are expressed early in adipogenesis and are responsible for initiating the differentiation of preadipocytes through eventual activation of PPARγ. Tang et al. (2003b) provided evidence for the role of CEBP/β in the adipogenic process. Using 3T3-L1 cells and murine embryonic fibroblasts (MEFs) they determined that CEBP/β is involved in regulating mitotic clonal expansion of preadipocytes which is

required for adipogenesis to occur, and precedes the expression of the TFs that give rise to the mature adipocyte phenotype (Tang et al., 2003a).

C/EBPα is also known as master regulator of adipogenesis and cooperates with PPARγ to establish and maintain the adipogenic program. In cell culture, expression of C/EBPα occurs relatively late during adipogenesis after the induction of PPARγ (Wu et al., 1998; Salma et al., 2006). Rosen et al. (2002) determined that while important, C/EBPα is not required to drive adipogenesis in the same manner that PPARγ does. This suggests that PPARγ and C/EBPα induce adipogenesis using a single pathway with PPARγ playing the predominant role and C/EBPα acting in a feed-forward loop to drive PPARγ expression. Terminal adipogenesis appears to be dependent on C/EBPα because failure to express C/EBPα results in insulin resistance and failure to accumulate WAT in vivo suggesting that C/EBPα is required to maintain PPARγ expression which in turn contributes to insulin sensitivity and AT accretion (Wu et al., 1999).

Preadipocyte Factor 1/DLK-1

The differentiation of adipocyte progenitor cells to adipocytes is subject to induction by transcriptional activators and suppression by transcriptional inhibitors (Rosen and Speigleman, 2000). The balance of inhibitors and activators is responsible for the rate and extent of adipogenesis among a population of progenitor cells. Preadipocyte factor 1 (Pref-1) also known as delta-like kinase homolog 1 (DLK-1) is a member of the epidermal growth factor-like protein family that was identified during differential screening of cDNAs expressed in differentiating murine 3T3-L1 adipocytes (Smas and Sul, 1993). Pref-1 expression is high in preadipocytes and is down-regulated during the

conversion of preadipocytes to adipocytes. Smas and Sul (1993) reported that Pref-1 was important in maintaining the preadipocyte state and that overexpression of Pref-1 or addition of the soluble ectodomain of Pref-1 to 3T3-L1-cell culture was sufficient to prevent adipose conversion in these cells. Pref-1 also has an inhibitory effect on expression of PPAR-γ and CEBP/α. Pref-1 mRNA and protein levels are high in 3T3-L1 preadipocytes, but Pref-1 expression decreases during adipocyte differentiation and is absent in mature adipocytes. The role of Pref-1 as an anti-adipogenic TF has since been characterized in beef cattle and other livestock species with the bovine PREF1 gene being mapped to chromosome 21q24 (Minoshima et al., 2001) and its importance in adipogenesis particularly in the development of intramuscular fat has recently gained attention.

Multipotent mesenchymal cells commit and differentiate into various cell lineages including myocytes, adipocytes, osteoblasts, and chondrocytes (Gesta et al., 2007). Pref-1 is capable of regulating both the commitment of multipotent MSCs to a particular cell lineage and the maintenance of these committed cells in an immature state (Wang and Sul, 2009). Using Pref-1 knockout and transgenic murine embryonic fibroblasts, Wang and Sul (2009) were able to elucidate some details on Pref-1 mechanism of action. They showed that Pref-1 inhibits the differentiation of adipocytes by upregulating the expression of Sox9 which is expressed in progenitor cells that give rise to osteocytes and chondrocytes and promotes chondrogenic commitment. Cells that eventually give rise to chondrocytes and adipocytes come from a common source of MSCs; Sox9 inhibits adipogenesis while promoting chondrogenesis in this pool of progenitor cells. In light of these findings they concluded that down regulation of Sox9 is required for adipocyte

differentiation and that Pref-1 inhibits adipocyte differentiation through upregulation of Sox9 expression. Sox9 directly binds C/EBPβ/δ promoters and inhibits their expression thereby preventing adipocyte differentiation. Additionally they showed that Pref-1 promotes chondrogenic determination of MSCs using a combination of Pref-1 null and overexpression mouse models. This provides in vitro evidence that Pref-1 directly targets and promotes Sox9 expression and that Pref-1 is capable of inhibiting MSC differentiation into adipocytes and directing them to chondrogenic lineage.

Zinc-finger Protein 423

Zinc finger protein 423 (Zfp423) is another recently identified regulator adipogenesis and preadipocyte determination that, unlike Pref-1, appears to promote the adipogenic events. Zfp423 is enriched in preadipogenic fibroblasts relative to non-adipogenic fibroblasts and its expression occurs upstream of PPARγ (Gupta et al., 2010). Ablation of Zfp243 expression via short-hairpin RNA reduces PPARγ expression and impairs differentiation in 3T3-l1 preadipocytes. In undifferentiated cells, over-expression of Zfp423 results in PPAR-γ induction and adipogenic differentiation of these cells (under appropriate differentiation conditions). Zfp423 has also been implicated as a regulator of neurologic development; therefore other specifying factors are likely to be involved in obtaining adipogenic competency in non-committed precursor cells. Taken together these data indicate a permissive role of Zfp243 in commitment of precursor cells to the adipogenic fate, and because Zfp243 is not expressed in non-adipogenic fibroblasts (Gupta et al., 2010) it may serve as a potential molecular marker to identify and define committed preadipocyte populations in vivo.

Carbohydrate Response Element Binding Protein

Carbohydrate response element binding protein (ChREBP) is a regulatory transcription factor that enhances the expression of lipogenic enzymes in response to cellular carbohydrate metabolite levels. Studies have shown that targeted disruption of ChREBP results in aberrant lipid metabolism in vitro and in vivo. Lizuka et al. (2004) reported that mRNA levels for all of the major lipogenic enzyme genes, as well as for hepatic pyruvate kinase, were significantly lower in ChREBP-null mice fed a high-starch diet compared to those in WT mice. In addition these mice showed decreased liver TAG and total body lipogenesis providing compelling evidence for the role of the ChREBP TF in glucose regulation of lipid metabolism in vivo. Ishii et al. (2004) also provided evidence for the direct role of ChREBP in lipogenic gene expression. Using various reporter-constructs containing ACC, FAS, or LPK gene promoters they showed ChREBPs were responsive to glucose when transfected into WT but not ChREBP null hepatocytes. In addition glucose transactivation of the constructs in ChREBP null hepatocytes was restored by co-transfection with a functional ChREBP expression plasmid. Any role of ChREBP is acetate driven lipogenesis (as in ruminants) is not clear.

Stearoyl-CoA Desaturase

Stearoyl-CoA desaturase (SCD) catalyzes the delta-9 desaturation of saturated fatty acids to form monounsaturated fatty acids (MUFAs). It has been studied extensively in the biomedical community as it pertains to metabolic syndrome and other disorders (Biddinger et al., 2005). In mouse 3T3-L1 adipocytes, SCD mRNA expression is regulated by various factors including sterol regulatory element binding protein 1 which is considered a main regulator lipid metabolism (Le Lay et al., 2002; Horton et al., 2002). Ntambi et al. (1988) reported that SCD gene expression of 3T3-L1 preadipocyte is

increased in the early stage of adipocyte differentiation, suggesting an important role for SCD in adipogenesis and subsequent lipid metabolism. Mouse SCD1 and human SCD show conserved binding sites for the TFs SREBP1 and C/EBP-α in their promoter regions (Bene et al., 2001). In addition, these elements in the promoter region of bovine SCD are highly homologous to that of mice and humans (Keating et al., 2005), suggesting that SREBP1 and C/EBP-α are also important transcriptional factor of bovine SCD. In addition, Taniguchi et al. (2004) reported that SCD genotype is associated with the fatty acid composition in Japanese Black cattle. SCD is therefore related not only to biomedical issues but also to quality aspects of beef production.

Fatty Acid Binding Proteins

Fatty acid binding proteins (FABPs) are intracellular lipid-binding proteins involved in cell signaling and metabolism. These are small proteins that reversibly bind hydrophobic ligands including saturated and unsaturated fatty acids, eicosanoids, and other lipids. They are abundantly expressed in a tissue specific manner and found across all species from Drosophila melanogaster to humans and livestock demonstrating strong evolutionary conservation and apparent importance of these proteins in cell physiology (Zimmerman and Veerkamp, 2002). Adipocyte fatty acid binding protein (aFABP) also known as aP2 or FABP4 is an adipocyte specific FABP that has been used as reliable indicator of terminal differentiation and lipid metabolism. This is a predominant cytosolic protein that makes up about 5% of the total cellular protein content in mature adipocytes and is indicative of active lipid metabolism within the cell (Boord et al., 2002). aFABP contains a PPAR response element in its promoter region and has been shown to be a target of PPAR-g in the adipogenic cascade (Shin et al., 2009). aFABP has also been

implicated in the development of Metabolic Syndrome (MetS) and related maladies and mice deficient in A-FABP are protected from development of diet-induced and genetic obesity and he associated hyperinsulinemia, hyperglycemia, and insulin resistance (Uysal et al., 2000; Hotamisligil et al., 1996).

Sterol Regulatory Element Binding Protein

Sterol Regulatory Element Binding Proteins (SREBPs) are helix-loop-helix TFs involved in adipogenesis and lipid metabolism (Horton et al., 2002; Shimano et al., 2001; Tontonoz et al., 1993). Tontonoz et al. (1993) described what was known as adipocyte determination and differentiation factor-1 (ADD1) as a basic helix loop helix domain containing protein which was expressed in brown adipose tissue and showed increased expression during differentiation of adipocytes in cell culture. They also showed that ADD1 could bind and effect expression of the Fatty acid synthase (FAS) gene, but was incapable of binding to other bHLH domain containing genes indicating the sequence specificity of this TF.

Adipogenesis in Beef Cattle

The process of adipogenesis in beef cattle has been well studied by animal researchers seeking to improve production efficiency and product quality. In cattle early adipogenesis occurs during fetal development and during the early postnatal period (Du et al., 2010). Adipocytes are recruited from pluripotent mesenchymal stem cells (MSCs) that are induced to commit to the adipocyte lineage. Skeletal muscle has an abundant supply of these MSCs the majority of which are destined to become myocytes; however, a variable portion of these cells are committed to become intramuscular adipocytes and

give rise to IMF. Cell culture studies have provided information on the specific chronology of TF expression. As discussed previously, this process occurs as the result of several coordinated events and is characterized by differentiation (basically by the well know canonical mechanism) of preadipocytes to mature adipocytes capable of storing and metabolizing lipids, as well as secretion of various adipocyte specific proteins that affect whole body energy homeostasis.

Adipose Tissue Depots

AT is widely distributed throughout the body in mammals and can be separated into anatomically and physiologically distinct depots. Unlike the liver or other morphologically defined organs, AT is rather amorphous which can lead to an underestimation of the anatomical and functional organization of the tissue. In terms of anatomical location, at least 16 distinct AT depots have been identified in mammals. These depots have differential responses to hormonal, metabolic, and environmental cues (Pond, 1992). In the simplest of classification schemes, the two major anatomic subdivisions are the visceral AT (VAT) and the subcutaneous AT (SC) depots. These subdivisions can be further divided into subcategories. The VAT can be divided into intraperitoneal and retroperitoneal, the latter of which can be again divided into the omental and mesenteric AT. SC AT can be divided into the superficial SC and deep SC, which is considered to be more metabolically active. In addition to the SC and VAT depots, there are also fat depots that surround the kidney, pelvis and heart (KPH), genitalia, and within and between muscle bundles (intramuscular and intermuscular fat, respectively). Several studies have identified differential metabolic and physiological characteristics between various AT depots and these depot-specific differences provide

opportunities to manipulate AT depots independently of one-another to manipulate beef carcass quality and provide consumers with a consistent and desirable product in an economically efficient manner.

Depot Specific Metabolism of Adipose Tissue

It is now generally accepted that not all AT depots are created equal with respect to their impact on energy partitioning, production efficiency, and meat-quality. AT depots do not always exhibit uniform patterns of metabolism or response to external stimuli (Goodpaster et al., 2005; Kissebah and Krakower, 1994; Mersmann, 1998). Moreover, depot-specific differences in the presence and number of fat cell precursors may contribute to the differential rates of AT growth between depots, which can have economic implications. Temporal and developmental changes in AT metabolism have been recognized for some time now; however, spatial differences that result in marked physiological disparities between depots are less clearly defined. Because early studies of AT cellularity and physiology were conducted with rodent models and more specifically with the epididymal fat pad in these animals, regional differences in AT metabolism are only recently being understood and appreciated.

In humans and rodents it has been shown that visceral adipose tissue (VAT) is a primary contributor to complications from diabetes and metabolic syndrome (MetS), while the subcutaneous adipose tissue (SAT) appears to play a neutral or protective role (Kisseba and Krakower, 1994; Giorgino, 2005). In addition several TFs and regulatory genes are differentially expressed in a depot specific manner, including C/EBP and carnitine palmitoyl transferase-1b (CPT-1b). These data indicate that the location and not the amount of fat per se is the causative agent resulting in the various maladies associated

with obesity and MetS. It has been shown that preadipocytes derived from subcutaneous AT show more accumulation of TAG compared to those derived from visceral AT.

Like humans and rodents, AT depots in meat animals possess different cellular, metabolic, and developmental signatures, all of which have an impact on the amount and distribution of fat in meat products. The AT depots of beef cattle are likely to exhibit differential expression of regulatory factors in a depot-specific and adipogenic state-specific manner which varies as the processes of growth and development occur. Targeting these factors is a potentially viable approach to independently manipulate AT depots to reduce overall carcass adiposity and simultaneously enhance IMF percentage in an economically efficient manner.

In vitro evidence for depot-specific metabolism also exists. Chen et al. (2010) conducted a molecular comparison of adipocytes derived from porcine visceral and IMF AT using cell culture techniques. Mature adipocytes were isolated and purified from the respective depots and allowed to undergo dedifferentiation and redifferentiation in vitro. They observed that early in the differentiation process, both visceral and IMF adipocytes demonstrated similar abilities to accumulate lipid, however by day 10 post-redifferentiation, the cells derived from IMF accumulated lipid at a much higher rate compared to visceral AT. The increased accumulation and metabolism of lipid was also supported by increased expression of lipogenic TFs including CEBP/α and PPAR-γ. These data support the paradigm of depot specific metabolism and may have biomedical and agricultural implications in terms of therapeutic interventions and management strategies, respectively.

Torii et al. (1998) conducted a study to determine the role of PPAR-γ in adipocytes within the longissimus muscle of beef cattle. Fibroblast-like cells were isolated from the longissimus muscle and cultured with known activators of murine PPAR-γ. Using Oil Red O staining the authors observed dose dependent induction of differentiation using T-174, a thiazolidinedione compound that is a specific activator of PPAR-γ. This study showed that bovine SM has adipose precursor cells that are responsive to PPAR-γ ligands and can be induced to differentiate into mature adipocytes in vitro.

Ohyama et al. (1998) reported increased differentiation of perirenal and IMF derived S-V cells isolated from Japanese Black cattle. Similarly, Wu et al. (2000) determined that treatment with PPAR-γ agonists caused induction of differentiation although it was to a greater extent in omental derived S-V cells compared with those derived from SC AT once again highlighting differential depot-specific responses to the local hormonal and regulatory milieu.

Pickworth et al. (2010) investigated differentiation state-specific gene expression as it related to carcass adiposity in steers selected for four levels of IMF. Their data indicated that differentiation state specific gene expression was not affected by the level of carcass adiposity; however, they observed differences between the SC and IMF depots indicating that adipogenesis in these distinct depots occurs independently. The expression of Pref-1 was higher in IMF versus SQ AT while PPAR-γ, FAS, LPL, and FABP4 were higher in SC versus IMF.

Grant et al. (2008) conducted a study to determine the differentiation capacity of stromo-vascular (S-V) cells isolated from bovine IMF and SC AT in response to

dexamethasone (DEX, a glucocorticoid analog) and troglitazone (TRO, a PPAR-γ agonist). Cells were cultured in differentiation media with added TRO, DEX, or both. Control cells had no added TRO or DEX. The addition of TRO and/or DEX caused enhanced differentiation of S-V cells derived from both SC and IMF compared to control cells. Morphological assessment indicated nearly a two-fold increase in the proportion of adipogenic colonies. Interestingly, they identified inherent differences in the overall capacity to differentiate between the cell types with the percentage of differentiated cells being 6.4 fold higher in SC versus IMF-derived cells.

Because AT metabolism is inextricably tied to whole-body energy metabolism, the dynamics of depot-specific adipose tissue metabolism are essential components to understanding metabolic efficiency and how it relates to overall production efficiency in beef cattle. The current understanding of depot specific adipose tissue metabolism in beef cattle is scarce although the advent of several molecular and genomic tools is expediting the rate at which information is acquired and analyzed.

Adipose Tissue Distribution in Beef Cattle

AT in beef cattle is separated into discreet depots that have varying economic impact on the value of the carcass (Dodson et al., 2010a; Dodson et al., 2010b). These include subcutaneous AT (SC which is located beneath the skin), visceral AT which is located around internal viscera (VAT), intermuscular fat which is located between muscles, and IMF located within the perimysium of an individual muscle. In terms of developmental order, VAT is generally quoted as developing first followed by SC, intermuscular and intramuscular fat. While the presence of at least some AT in various depots is obligatory for normal physiology, excessive partitioning of dietary energy into

expendable AT depots represents inefficient use of feedstuffs and has a negative impact on production efficiency. Strategies to selectively enhance the marbling depot will depend on using contemporary and classic tools to develop an improved understanding of depot-specific physiology and metabolism, as well as a more comprehensive understanding of the region-specific differences in adipocyte and preadipocyte biology (Dodson et al., 2010; Basu et al., 2009; Hausman et al., 2009).

Intramuscular Fat

Intramuscular fat (IMF) develops within the perimysial connective tissue that surrounds the myofibrillar bundles in SM tissue (Moody and Cassens, 1968). As mentioned before, this is distinct from intermuscular fat, which is the fat that is located between individual muscles of the same cut (Hoquette et al., 2009). The presence of intramuscular fat is responsible for important meat quality traits that have implications on beef cattle profitability and consumer preference. A longstanding goal of animal scientists has been to understand the processes that lead to IMF accumulation in attempts to manipulate these factors to improve product quality. The accumulation of intramuscular fat gives rise to the commercial trait known as "marbling", which has a direct impact on carcass value under the most meat quality grading systems.

One of the confounding issues that have increased total fat content of meat products is the drive to increase IMF as a result of both consumer preference and the emphasis of IMF in the USDA quality grading system. While development of the IMF depot occurs in pre-natal and early post-natal life, accumulation of fat in the IMF depot occurs after other AT depots have reached a plateau. During this "finishing phase" the

IMF depot expands and the animal attains a higher quality grade according to the USDA scale. The concomitant expansion of the earlier maturing subcutaneous and intermuscular fat depots prior to and during this finishing phase results in an undesirable increase in carcass adiposity and inefficient use of dietary energy. This has spearheaded efforts to enhance IMF development independently of other AT depots to improve production efficiency while producing a desirable and valuable product (Dodson et al., 2010; Pickworth et al., 2010; Smith et al., 2008; Sweeten et al., 1990).

Intramuscular Fat is a Distinct Adipose Tissue Depot

Regulation of IMF adipogenesis occurs at the cellular level and is under the control of the various TF and regulatory factors that govern metabolism in other AT depots (Hausman et al., 2009). Various studies have sought to understand the molecular components of marbling development in vitro and in vivo. While IMF is histologically a *bona fide* AT depot exhibiting the signature markers, extracellular matrix, and metabolic machinery present in other AT, it represents a distinct depot with specific differences in metabolism and responsiveness to external cues (Smith and Crouse, 1984; Hausman et al, 2009).

About 80% of the TAG in muscles is stored within the resident adipocytes that make up the IMF depot (Gondret et al., 1998). Intramyocellular lipid makes up the remaining portion of muscle TAG and contributes to the total fat content within the muscle; however, this is not discernible macroscopically and does not contribute to the quality grading to the extent that IMF AT does. Rates of fatty acid (FA) synthesis in marbling are generally depressed relative to subcutaneous AT (SC; Hood and Allen, 1978). In addition glucose appears also to be an important contributor to FA synthesis in

IMF compared to SC AT, which uses acetate as the primary carbon source (Smith and Crouse, 1984). Indeed feeding ruminant animals diets high in carbohydrates that escape rumen fermentation has been shown to increase visual marbling fat (Pethick et al., 1997).

Adipocyte differentiation in skeletal muscle initiates from multipotent mesenchymal stem cells (MSCs), which are abundant in skeletal muscle at early developmental stages. In addition to providing the source of progenitor cells for myogenesis, a portion of this same population of MSCs gives rise to intramuscular adipocytes, which serve as the sites for the accumulation of IMF (Tong et al., 2009). While adipogenesis occurs during fetal and early post-natal life, accumulation of muscle lipid as a result of energy partitioning occurs later in life in beef cattle and other species (Du et al., 2010).

Taniguchi et al. (2007) demonstrated that several genes are switched on and off during events associated with early adipogenesis and the authors suggest that a bovine specific gene network (distinct from that observed from cell lines derived from other species) was responsible for the adipogenic program in bovine IMF adipocytes. They showed that over 100 genes were differentially expressed and that these genes were involved in metabolic and cell signaling pathways. Of particular interest was the fact that typical adipogenic genes were upregulated during early differentiation of the preadipocytes.

In a subsequent study the same group (Taniguchi et al., 2008) conducted experiments using non-transformed adipofibroblasts that were derived from de-differentiation of mature adipocytes isolated from perimuscular fat tissue. These adipocytes were cultured with the appropriate media to induce differentiation and were

harvested daily for RNA isolation and gene expression analysis. They determined that the expression of PPAR-γ and SREBP-1 along with the downstream targets of these TFs was co-expressed at day 2 post-differentiation. Among the genes co expressed were SCD, ELOVL6, FABP4, LDLR, and FAS.

While it is generally accepted that adipocyte hyperplasia and hypertrophy is responsible for depot expansion, one unanswered question is the source of the progenitor cells that undergo adipogenesis. A recently discovered source of adipocytes that has been identified in vitro arise as a result of de-differentiation of mature adipocytes to form proliferation competent daughter cells capable of population expansion. It is estimated that 1 in 100 adipocytes is capable of undergoing de-differentiation, proliferation, and then redifferentiation (Fernyhough et al., 2004) making this phenomenon a potentially viable target for inducing independent expansion of the IMF depot (Dodson et al., 2010).

Marbling

Marbling is a cattle industry term that refers to the appearance of white flecks of fat between myofibrillar bundles. Economically, marbling is a major determinant of carcass value in the United States (USDA, 1996), and consumers are often willing to pay a premium price for guaranteed quality beef (Hocquette et al., 2009). Carcasses receive a marbling score based on the subjective evaluation of the amount and distribution of IMF at the cut surface of the rib eye between the 12th and 13th rib. High marbling is appreciated by the consumer because of the perceived influence it has on sensory qualities such as tenderness and juiciness of meat products (Platter et al., 2005). The high value placed on marbling scores provides incentive and motive to manipulate the IMF depot to maximize profitability. At the cellular level, marbling can be enhanced through

the enlargement of existing adipocytes (hypertrophy) and through an increase in the number of adipocytes (hyperplasia) in the IMF depot (Du et al., 2010). These are physiological processes affected by genetics, nutrition, and environmental cues therefore elucidating the molecular regulatory network specific to the IMF depot is essential to improving efficiency of beef production (Harper and Pethick, 2004).

There are several factors that affect IMF accumulation, which impacts the marbling scores and carcass value. The extent of marbling development varies between sexes and between different breeds of cattle. Some breeds have the genetic propensity to develop significant amounts of marbling and their carcasses are highly valued in markets around the world. Selection of sires and breeds of cattle that produce offspring with the genetic potential to direct energy into intramuscular adipocytes early in the growth period is a promising management strategy (Mir et al., 1999). Japanese Black cattle (JB) have the genetic potential to accumulate large amounts of IMF comprised of a high proportion of mono-unsaturated fatty acids (MUFA). These animals can accumulate IMF up to 40 months of age with the typical finishing period beginning around 10 months and continuing for another 20 months. These cattle also deposit fat at a rate that exceeds that of less marbled breeds such as the Japanese Brown and Holstein breeds (Zembayashi et al., 1995). The high MUFA content meat cuts from these animals a characteristic marbling composed of so called "soft-fat," due to the lower melting point of these fatty acids and this property is considered desirable to many consumers.

Oshaki et al. (2007) isolated stromal-vascular cells from perirenal adipose tissues of Japanese Black and Holstein steers to investigate gene expression profile during bovine preadipocyte differentiation. They reported a 10.8 and 6.3-fold increase in the

level of SCD mRNA in Japanese Black and Holstein, respectively, on day 1 of the cell culture. Differential SCD expression between the two breeds may reflect the phenotypic differences in the fat development and fatty acid profile between the breeds. SREBP1 and C/EBP-alpha are supposed to regulate SCD expression; however, in their study expression of these two factors was not completely consistent with that of SCD expression.

While it is known that cattle breeds have a differential propensity to develop regional adiposity, particularly IMF, the molecular cues responsible for this phenotypic variation is only recently being investigated. The high IMF in JB cattle appears to be due to an increase in the number of intramuscular adipocytes relative to other breeds; however an increase in adipocyte size was also reported (Gotoh et al., 2009). Wang et al. (2005) conducted microarray studies to compare the expression of genes in the longissimus muscle (LM) of JB and Holstein (HOL) cattle over an extended intensive feeding period. Using a consecutive biopsy approach they identified 335 genes that were differentially expressed between the breeds, which have differential propensities for adipose accretion. Genes that were upregulated in the LM of JB cattle included Adiponectin, SCD, FABP4, and LPL all of which are involved in unsaturated FA synthesis, adipogenesis and thyroid hormone pathways, while in HOL cattle, the genes involved in connective tissue and SM development were differentially expressed. The authors suggest that these data support the notion that at any given developmental time-point, JB cattle have more active IMF development than HOL cattle on the same diet. Jurie et al. (2007) reported significant correlations between expression of A-FABP and TAG content in steers of different genotype that showed high or low levels of marbling.

Studies of this nature give important clues to the molecular basis for the unique intramuscular adiposity of the Japanese Black breed and shed light on important phenomena including the onset and rate of adipose tissue development, metabolic differences between breeds, and signaling pathways involved in lipid metabolism in beef cattle.

Marbling development can also be altered by nutrition and management practices. Dietary roughage level has been shown to affect carcass adiposity in beef cattle. Yamada et al. (2009) subjected Wagyu steers to high, medium, and low roughage diets (HR, MR, and LR, respectively). They reported that dietary roughage levels induced differential expression of putative adipogenic TFs in mesenteric and intramuscular fat depots, while there were no effects of treatment on the SC depot. In mesenteric adipose tissue, the expression of C/EBPα in the LR and MR groups was significantly higher than that in the HR group. Adipocytes in the LR and MR group had intramuscular adipocytes that were significantly smaller than in the HR group. These results indicate that nutritional management can affect the expression of key adipogenic genes and the cellularity of AT in a depot specific manner in beef cattle.

In another study, Graugnard et al. (2009) looked at adipogenic gene networks in the longissimus lumborum muscle of Angus and Angus x Simmental cattle fed high- or low starch diets. They determined that various TFs and markers of mature adipose tissue differentiation were present during the early growing phase in these cattle. The genes identified appear to be part of a transcriptional network driven by the putative regulator of adipogenesis, PPAR-γ. They concluded that this network is responsible for coordinating IMF metabolism and lipid filling during the early growth phase.

Understanding these and other gene networks that contribute to IMF maturation will provide strategies tailored to maximize the marbling trait and avert excess carcass adiposity (Hausman et al., 2009).

Fat in Meat Products

In addition to the direct effects on efficiency and the economic implications, there are concerns about the impact of animal fats including those contained in meat products, on consumer health (Kouba and Mourot, 2011; Colmenero, 2000; Wood et al. 1999; Colmenero, 1996**;** Bergen and Merkel, 1991). While red meat has been shown to be a valuable source of dietary protein and other essential nutrients including B-complex vitamins, iron and zinc, the fat content of diets containing red meat have been linked to the development of obesity and heart disease (Kontogianni et al., 2008; Kelemen et al., 2005). Chronic overconsumption of dietary energy, particularly in the form of animal fat has been implicated in the onset of various metabolic diseases although it cannot be said definitively that the meat itself is the causative agent for this association (McAfee et al., 2010). In response to these increasing pressures to eliminate animal products as a source of excess dietary fat, the amount and distribution of AT in meat animals has become a concern to animal researchers, industry stakeholders, and end product consumers. The burgeoning obesity epidemic has prompted studies to develop a more comprehensive understanding the molecular aspects of AT biology both in animals to minimize excess fat content in meat products, and in humans in order to identify biological markers and potential targets for therapeutic intervention (Hausman et al., 2009; Vernon et al., 1999).

Post-Genomic Era Livestock Production

Livestock production in the post-genomic era is poised to benefit from innovative application of novel technologies originally designed for the sequencing of the human genome, which was completed in 2001 (Lander et al., 2001; Venter et al., 2001). The pre-existing infrastructure and advanced stage of the available reagents will speed the rate at which livestock genomic data is obtained and made useful (Rothschild and Plastow, 2007). With the DNA sequences of many livestock species available, discoveries in the laboratory are rapidly making their way to farms and commercial operations. The Bovine Sequencing and Analysis Consortium were responsible for the publication of the sequence, annotation, and comparative analysis of the cattle genome in 2009.

The already rapid adoption and innovation of these available platforms and technologies into livestock production is a testament to their effectiveness and usefulness in these systems. Indeed, several dedicated technologies for cattle, pigs, and other species have been developed to further improve economically relevant traits in these animals (Tsai et al., 2006; AffyMetrix, Santa Clara CA). In addition to the direct advantages to agriculture, another driving force for the improved technologies is the usefulness of livestock species as appropriate models suited for biomedical research (Ireland et al., 2008; Gibbs et al., 2004).

Zhao et al. (2010) looked at proteome differences associated with fat deposition in bovine SC AT. Using crossbred steers with different back fat thickness they sought to identify protein markers associated with fattening in beef cattle. Their result indicated that annexin 1 a protein was highly expressed in tissues of animals with increased back fat thickness identifying this protein as a potential marker for assessing this trait in beef cattle at various stages.

Barendse et al. (2007) conducted a study on 1472 cattle from various breeds to determine the association between single nucleotide polymorphisms in the bovine genome and phenotypic measures of and feed efficiency. Using this whole genome association approach they determined that DNA variants in or near proteins associated with energy usage were an order of magnitude more common compared to those affecting body mass and appetite. The largest group of polymorphisms consisted of genomic regions containing promoter regions, micro-RNA motifs and other non-coding elements. This suggests that a substantial portion of the phenotypic variation in feed efficiency is due to differences in the regulatory aspects of gene expression.

The use of genomic data to understand the molecular components of metabolic regulation will help identify targets to improve biological and production efficiency. Economically relevant traits such as lean muscle deposition and lipid accumulation are complex biological traits under the control of interacting gene networks (Wu and Lin, 2006). These networks act coordinately to control and integrate energy metabolism, adipocyte differentiation, and protein turnover. Transcriptional regulation of key metabolic enzymes is under the control of various transcription factors which themselves belong to coordinately regulated gene networks. Metabolic pathways in higher organisms require transcriptional regulation as a long-term mechanism to control the levels of regulatory genes and enzymes (Desvergne et al., 2006). It has been shown that there is a high correlation between mRNA expression of target genes and the recruitment of transcription factors, nuclear receptors, and their co-regulatory factors. This suggests that gene expression analysis is suitable for inferring transcriptional activity in various tissues.

With respect to coding genes, phenotypic changes or observations are attributable to gene expression (mRNA abundance) in so far as the changes in gene expression translate to changes in functional protein products. Efficiency of translation machinery can affect the rate at which proteins are translated and proteins are also subject to post-translational modifications and proteolysis by various regulatory and housekeeping mechanisms adding an additional layer of complexity when trying to extrapolate from gene expression to functional protein expression. Therefore, it is better to corroborate all three-genomic, transcriptomic and proteomic data in order to make a comprehensive assessment and deduction, although this is not always feasible, be it economically or otherwise. Taken all together, gene expression analysis has become a viable technique for understanding the dynamics of molecular regulation of metabolic pathways and for generating the basis for hypothesis driven and proof of function research.

Summary

It is evident that achieving maximum production efficiency while producing a desirable and valuable phenotype or product is paramount to the success of any beef cattle operation. There are several approaches, which can be taken ranging from selective breeding to nutritional management and administration of metabolic modifiers, all of which are aimed at minimizing production costs and increasing the yield of desirable product. The advent of genomics and the emergence of the post-genomic era have provided a viable avenue towards improving beef cattle production by allowing us to identify molecular components that are responsive to the various management and selection strategies being employed, and understanding those that are responsible for the fruition of profitable phenotypes. Elucidating and targeting these regulatory factors can

achieve rapid improvement in efficiency and profitability ensuring the long-term success and viability of the beef cattle industry.

Changes in gene networks that control metabolic pathways such as adipogenesis and energy metabolism are controlled by regulatory factors that can be used to manipulate AT depots. Transcriptional networks coordinate adipocyte differentiation and energy metabolism in rodents and other species (Desvergene et al., 2005). The recruitment of lipogenic transcription factors and nuclear receptors to promoter regions is highly correlated with the mRNA expression of the genes they target (Bennet et al., 2008). This suggests that gene expression (i.e. mRNA abundance) is useful for inferring transcriptional activity in vivo. Skeletal muscle tissue is a heterogeneous tissue composed of varying cell types (myocytes, non-differentiated stem cells, satellite cells, pre-adipocytes, adipocytes, and components of the extracellular matrix). Messenger RNA (mRNA) expression is but one of many regulatory factors to be considered when studying the complex molecular networks working simultaneously in a heterogeneous tissue such as SM. While care must be taken to avoid over simplification of the various mechanisms, gene expression profiling provides valid information to aid in future study design and identification of potential biomarkers. In addition cell specific makers may be used to elucidate the individual contribution of the constituent cells to the functionality of the network and the physiology of the tissue.

Chapter 3. Relationship between Residual Feed Intake and the Transcriptomic Signature of Performance Efficiency in Finishing Beef Cattle

Introduction

Feed represents the single largest input cost in most animal production systems and comprises about 70% of the input costs for commercial beef cattle operations (Herd at al., 1998; Liu et al., 2000; Arthur et al., 2001; Basarab et al., 2002). With the cost of feed continuing to rise, improvement in the efficiency of animal production remains one the highest priorities of animal science research and a necessity to ensure the viability of the beef cattle industry. Gibbs and McAllister (1999) reported that increasing FE by 5% would have an economic impact four times greater than a 5% enhancement in average daily gain (ADG). Swine and poultry producers have dramatically reduced the time and resources necessary to produce marketable animals through intense genetic selection for more efficient animals, and improvement in nutrition and management practices (Havenstein et al., 2003). During this time cattle have also experienced substantial improvement in overall efficiency although much of this has come as a result of grain feeding and other nutritional regimens, as well as reproductive and pharmaceutical technologies (Elam and Preston, 2004). While there is considerable variation for FE in beef cattle and this trait appears to be moderately heritable, there has been little improvement in FE or maintenance energy requirements during the past 50 years (Archer et al., 1999).

Residual Feed Intake (RFI) is a measure of FE that is independent of growth and bodyweight, factors, which are confounding to other measures of feed efficiency making RFI a more reliable and attractive measure than traditional feed conversion ratios. Koch

et al. (1963) suggested that the feed requirements could be adjusted for body weight (BW) and weight gain by partitioning feed intake into an expected component which is the amount of food that an animal would be expected to eat based on the estimated requirements, and a residual component which is the actual amount of feed intake above or below the expected intake levels. RFI partitions the drivers of feed intake into two categories: the feed expected for a given level of performance, and a residual component. RFI can then be defined as the difference between the actual and expected feed intake for a given level of performance with more efficient animals having a lower (more negative) RFI compared to less efficient ones. Considerable variation exists for RFI within animal breeds and genetic strains, and the trait has been shown to be moderately heritable (Fan et al., 1995). There are several biological factors responsible for variation in RFI of which protein turnover and tissue metabolism have been implicated as major contributors (Richardson and Herd, 2004; Figure 3.1), although the specifics of how these pathways are involved are less understood.

The need for improved FE along with the confounding factors for other measures of this parameter have driven interest and research in RFI into prominence (Hill and Azain, 2009). RFI is however difficult to measure due to the large number of animals required and the cost and labor involved in measuring individual animal weight and feed intake over a long period of time. These factors have led to a lack of adoption of RFI as a FE measure despite its advantages over other FE parameters. Predictive molecular markers for RFI have recently gained interest as an alternative to direct measurement, which would aid in the more practical implementation of RFI in management practice. Identification of predictive biomarkers for RFI will circumvent the need for individual

animal measurements and facilitate the use of RFI in breeding and management systems in order to improve the overall efficiency of animal production.

Objective

The objective of this study was to examine the expression of a panel of surrogate marker genes involved in lipid metabolism, energy balance, protein turnover and mitochondrial function and associate them with a measure of feed efficiency in finishing cattle. The selected cattle were the initial progeny of a RFI selection program expected to produce divergent phenotypes with respect of RFI. Therefore, we sought to determine the relationship between the selected GE profiles and the individual RFI of these animals in an effort to identify specific pathways or genes that can be used as indicators of RFI and/or targeted to improved feed efficiency. These efforts would reduce the costs and labor associated with RFI measurement and will lead to expedited improvement in terms of selecting for efficient animals.

Materials and Methods

Animals:

All procedures were conducted in accordance with the Auburn University Institutional Animal Care and Use Committee (IACUC No. 2004-0783). A total of 15 male cattle (n = 7 Bulls and 8 Steers) were selected from the initial progeny of a RFI selection program being established at the Alabama Black Belt Research and Experimentation Station. All cattle were Angus sired and their dams were Angus x Simmental crosses. Calves were individually fed twice daily for 84 days and refusals were collected once daily (See **Table 3.1** for diet composition). Animals were housed in a slatted floor barn and were allowed nose to nose contact for socialization. Feed intake

(FI) data was collected for each animal and used to determine residual feed intake (RFI) according to methods outlined by Okine et al. (2004). In addition for FI data, animals were weighed every two weeks for the duration of the experiment. Average daily gain and total weight gain were determined.

Sample Collection:

Adipose tissue and skeletal muscle biopsy samples were collected from each of the steers and bulls in the study. The cattle were restrained in a cattle chute and a 12 cm x 12 cm area centered over the loin area just caudal to the 13th rib was shaved and scrubbed in preparation for aseptic excision of skeletal muscle biopsy samples. Lidocaine hydrochloride 2% (15ml) was infused with a sterile 20 gauge x 1 ½ inch needle along the cranial and dorsal edges of the surgically prepared area to achieve local anesthesia. The incision was extended ventromedially into the longissimus dorsi muscle. A 1g sample of the dorso-lateral aspect of the longissimus dorsi muscle was removed by sharp dissection. The skin incision was closed with #3 braided coated nylon sutures (POLYWEB, Webster Veterinary Supply, Sterling, MA) in a cruciate pattern. For AT sample collection, a skin incision was made in the tail head and approximately 1g of subcutaneous adipose tissue was surgically excised from the area. Again, the skin incision was closed with #3 braided coated nylon sutures. Samples were placed in labeled aluminum foil wrappers and immediately snap-frozen in liquid nitrogen until they could be transferred into a -80 °C freezer for long-term storage and gene expression analysis.

RNA Isolation:

Total RNA was isolated from the SM an AT biopsy samples using TriZol reagent

(Invitrogen Corp., Carlsbad, CA) following the manufacturers recommended protocol. The samples were removed from the -80 °C freezer and placed into a container filled with liquid nitrogen. For each tissue sample, approximately 500mg of tissue was removed from the labeled foil wrapper and placed into a freezing cold metal mortar and pestle. The sample was then crushed hitting the pestle with a hammer and then the crushed tissue was placed into a tube containing 2ml of TriZol. The sample was then homogenized in this tube using a mechanical homogenizer (Polytron). Chloroform was then added to the 50ml tube; the tube was shaken for 15 seconds and then allowed to sit for 10 minutes. The contents were then transferred into a glass centrifuge tube which was then capped and placed into the chilled centrifuge maintained at 4 °C. The samples were spun at 10,000x g for 10 minutes and then the upper aqueous supernatant was removed and placed into another sterile 50ml tube. The remaining portions placed into a labeled hazardous material disposal jar until it could be appropriately discarded.

A second extraction was then performed on the layer by adding 1ml of TriZol and 1ml of Chloroform to the sterile tube containing the upper aqueous extract. Once again, samples were shaken for 15 seconds, allowed to sit for 10 minutes and then centrifuged again at 10,000 x g for 10 minutes. The supernatant was removed, placed into a sterile glass centrifuge tube and 1 volume of isopropanol was added. The samples were inverted to remove any precipitate that formed at the top of the tube and the samples were then centrifuged at 10,000 x g for 8 minutes. The isopropanol was gently discarded being careful not to disturb the nucleic acid precipitate which had formed on the side and bottom of the glass tube. Five ml of ice cold 70% ethanol was then added to the tube before a final spin at 10,000 x g. The ethanol was discarded and the RNA precipitate was

then re-suspended in 75-100µl of nuclease free water and placed into labeled, 2ml micro-centrifuge tubes for storage and subsequent use.

DeoxyRiboNuclease (DNAse) Treatment

Total RNA was subjected to deoxyribonuclease (DNAse) treatment using Turbo DNA Free (Kit No. AM1907M; Applied Biosystems/Ambion, Austin TX.). 20µl of the RNA isolate was transferred into a nuclease free tube and treated with 2µl of the supplied 10X reaction buffer and 1µg of DNAase (2 Units/µl). Samples were incubated at 37 °C for 30 minutes and then the DNAase reaction was stopped using 0.1 volumes (approximately 2.2µl) of DNAse inactivation reagent. Samples were allowed to sit for 2 minutes at room temperature and then centrifuged to pellet the inactivation reagent and DNAase enzyme. The RNA supernatant was collected via pipette and placed into a new, labeled, micro-centrifuge tube. DNAse treated samples were then purified using RNeasy mini RNA purification kits (Kit No. 74102; Qiagen, Vencia, CA). RNA quality was assessed using the 260nm/280nm absorbance determination observed via NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Willmington, DE). Acceptable quality RNA displaying a 260nm/280nm ratio of between 1.8 and 2.0 was then used for subsequent downstream procedures.

cDNA Synthesis:

Complimentary DNA (cDNA) was synthesized from the purified RNA isolate using high capacity cDNA Reverse Transcription kits (Kit # 4368814, Applied Biosystems, Foster City, CA). One microgram of DNAse treated total RNA was subjected to cDNA synthesis according to the manufacturer’s protocol and as follows.

cDNA Synthesis Master Mix

The cDNA synthesis kit is supplied by the manufacturer as individual components that are mixed to create a 1x 20µl reaction (see table 3.2). These components were allowed to thaw on ice and then the appropriate volumes were mixed for the RT reaction. A single reaction calls for adding 2µl of 10x RT buffer to a sterile micro-centrifuge tube followed by 4.2µl nuclease free water, 0.8ul of 25x deoxy nucleoside tri-phopsphates (dNTP) mixture, 2.0µl of random RT primers and finally 1.0µl of multiscribe reverse transcriptase (added last to prevent premature or non-specific polymerization). For consistency, a sufficient amount of master-mix containing reaction buffer, dNTP's, random oligonucleotide primers, and reverse transcriptase (RT) was prepared for each set of tissue samples. Once the master mix was created it was stored on ice.

Diluting RNA

For each tissue/RNA sample, one-microgram of RNA was diluted to 10µl using certified nuclease-free water. This 10µl RNA solution was the transferred into a labeled, 0.2ml PCR tube and 10µl of the previously prepared master-mix was added to make for a 20µl reaction. The reaction was gently mixed by pipetting and then centrifuged to remove air bubbles and bring all reaction components to the bottom of the tube. The tubes were then placed back into a PCR cooler to maintain the temperature while the other tubes were centrifuged.

cDNA Synthesis

The tubes containing the 20µl reaction were then placed in the PCR thermocycler and subjected to the 3-step thermocycling protocol recommended by the manufacturer

(Table 3.3). The 1st step is an initial primer annealing step (25 °C for 10 Minutes). This primer incubation step is necessary to maximize primer-RNA template binding when using oligo d (T) primers for first-strand cDNA synthesis. This step was then followed by an elongation step (37 °C for 120min) and finally an enzyme deactivation step (85 °C for 5 Min). Upon completion of these steps, samples were held at 4 °C in the thermocycler until they were transferred to a -20 °C freezer for storage. The resulting cDNA (20ng/µl) was used for quantitative Real-Time PCR (qRT-PCR) to determine the expression of specific target genes.

Gene Expression Assays

Assay Design

Custom TaqMan gene expression assays (Applied Biosystems, Foster City, CA) were designed for each of the genes of interest (see table 3.4) using the proprietary Applied Biosystems Custom TaqMan® Assay Design Tool (Applied Biosystems, Foster City, CA). Available sequence data was obtained from the National Center for Biotechnology Information database (NCBI; http://www.ncbi.nlm.nih.gov/nucleotide) for each gene and the online tool utilized the genomic input information, specifically mRNA sequence data, to generate custom primers and a sequence-specific fluorescent probe for real-time gene expression analysis (see Table 3.4).

TaqMan Gene Expression Assay

Gene expression analysis was conducted using TaqMan® Gene Expression assay chemistry (Figure 3.2), which uses a pair of custom, unlabeled PCR primers to target the gene of interest. In addition, a sequence-specific custom TaqMan® probe with a 6-

carboxyfluorescein (FAM®) fluorescent-dye label and minor groove binding (MGB) moiety on the 5' end, and 6-carboxy-tetramethyl-rhodamine (TAMRA) non-fluorescent quencher (NFQ) dye on the 3' end is used to monitor the accumulation of amplified product as the PCR cycles progress. This probe makes use of a NFQ and the principles of fluorescence resonance energy transfer (FRET) to suppress the fluorescence emitted by the FAM reporter dye while they are held in close proximity at the ends of the intact probe. After primer and probe annealing, polymerization occurs and the intrinsic 5' exonuclease activity of the Taq polymerase causes cleavage of the 5' reporter dye from the probe resulting in a fluorescent signal due to displacement from the non-fluorescent quencher. The Real-Time machine then detects and records the fluorescent signal once during each PCR cycle. The use of this custom fluorescent probe enables the specific detection of the target gene product as it accumulates during the polymerase reaction because the fluorescent signal increases as the exponential amplification of the target gene occurs.

Threshold Cycle (Ct)

The cycle at which the fluorescent signal surpasses a pre-determined level is known as the threshold cycle (Ct). The Ct is recorded and used in the quantification calculations to determine relative expression between samples and tissues. The fluorescence detected at each cycle, including the Ct, is directly proportional to the amount of fluorophore released during amplification and is indicative of the amount of cDNA template present at any given cycle. With that said the Ct value should be proportional to the initial amount of target transcript present in the sample and reflect the relative mRNA abundance when compared to the Ct from other samples.

Graphically, the threshold cycle for a given sample is defined as the point where the threshold line intersects the amplification plot which is readily observable using the real-time monitoring platform. The threshold, which can be manually adjusted, should be set above the baseline of the plot which includes the early cycles in which exponential amplification is not occurring. It should also fall within the exponential region of the curve in which the target sequence is being amplified exponentially. The SDS Software (v2.0.3 Applied Biosystems, Foster City, CA) automatically calculates the baseline and threshold values for a detector based on the assumption that the data exhibit the "typical" amplification curve with optimal amplification efficiency. In each experiment, amplification curves were observed and it was determined whether or not the automatic threshold value was occurred with the exponential portion of the curve. If not the threshold was manually adjusted to ensure proper quantification.

Relative Quantification Protocol

Relative quantification (RQ) determines the change in expression of a nucleic acid sequence(target) in a test sample relative to the same sequence in a calibrator sample by comparing the Ct values between these samples and adjusting for the initial cDNA loading concentrations using an internal control gene (Pfaffl, 2003). The calibrator sample can be a pooled control sample, an untreated sample, a sample at zero time-point etc. (Pfaffl, 2003; Livak and Schmittgen, 2001). The calibrator has its expression set to 1 or 100% and each of the other samples are quantified and expressed as a function of the calibrator sample. In the current study a pooled cDNA sample, representative of all treatments groups, was used as the calibrator sample for each qPCR run.

qPCR Reaction Set-up

For each 20µl multiplexed reaction the reaction components and PCR cycling conditions were as indicated in table 3.5 and Table 3.6, respectively. For each reaction 1µl (50ng) of cDNA synthesis reaction product was diluted to 8µl in a 2ml nuclease-free micro-centrifuge tube and placed on ice. A sufficient amount of master mix containing enough TaqMan gene expression master mix (Part No. 4369016; Applied Biosystems Foster City, CA), 18S probe, and the custom gene expression assay was prepared for the tissue/gene of interest and then placed on ice. For each sample 12µl of master mix was added to the tube containing the 8ul cDNA solution. The reactants were gently mixed and then the 20µl reaction was pipetted into individual tubes in 0.2ml MicroAmp® Optical 8-Tube Strips (Part No. 4316567; Applied Biosystems, Foster City, CA) that were resting in a chilled PCR cooler. The strips were then capped with MicroAmp® Optical 8-Cap Strip (Part No. 4323032 Applied Biosystems, Foster City CA) and centrifuged gently to remove air bubbles and ensure all components of the reaction were at the bottom of the tube. For each tissue sample, the multiplexed assay was run in duplicate and this process was repeated for each gene/tissue combination.

Quantitative Real-Time PCR

Quantitative Real-Time PCR was conducted to determine relative mRNA abundance for each of the genes of interest using an ABI7500 thermocycler and the relative quantification protocol of the SDS software package. The individual 8-tube strips containing the multiplexed reaction were placed into the sliding tray of the PCR machine and then sample location and identification were entered into the protocol. The samples were then subjected to qRT-PCR using the thermocyling conditions outlined in Table 3.6.

Quantitative Real-Time PCR Efficiency Determination

The RQ method of quantification used by the SDS software system assumes optimal reaction efficiency or doubling of template cDNA during each reaction cycle. The manufacturer purports 100% reaction efficiency from properly designed TaqMan gene expression assays, however efficiency can be affected by various factors including the presence of PCR inhibitors or other contaminants thus the PCR efficiency was determined for each assay using comparative Ct analysis for serial dilutions from a pooled control cDNA sample. For each assay, cDNA dilutions of 10μg, 5μg; 1μg, and, 0.1μg were prepared from a pooled sample of cDNA. Real-time analysis was then conducted and Ct values determined for each of the dilutions. A plot of the log cDNA input versus the Ct value was generated and the slope of the line determined. Assuming 100% efficiency, the value of this slope should be -3.32. This indicates that it takes 3.32 cycles to increase the Ct value by a factor of 10. This stands to reason because assuming that the amount of template doubles each cycle the relative amount of template can be determined using the equation: **X*2^Y=n** where X=initial cDNA input and n=amount of template at cycle Y. In the case of optimal efficiency as determined by dilution 2^3.32 is equal to 10. In other words, for each 10-fold change in cDNA input, there is a 3.32 change in Ct value. The actual efficiency (E) of the reaction can be determined using the equation $E= 10^{(-1/slope)}-1$. All assays showing efficiencies of equal to or greater than 1.9 were deemed acceptable for data analysis.

Data Normalization

In order to ensure accurate quantification of mRNA abundance, data must be normalized to an internal control gene to account for variation in initial cDNA input amounts. TaqMan chemistry allows for multiplexed PCR reactions when probes are designed using different reporter dyes. In the current experiment 18s ribosomal RNA was used as the endogenous control gene in a multiplexed real-time reaction. The 18s rRNA assay was designed with a separate, sequence-specific VIC®-labeled fluorescent probe and the cDNA's for the target gene and the 18s rRNA were co-amplified for each sample. The Ct values were recorded for simultaneously for both the 18s and target gene amplification and the ABI 7500 sequence detection system software (SDS; v2.0.3, Applied Biosystems, Foster City, CA) software calculates the relative expression value based on the normalized expression data from the multiplexed reaction.

RFI Calculations:

RFI values for this study were estimated as outlined by Okine et al., (2004) and modified by Anderson et al (Auburn University;Personal Communication). RFI for bulls and steers were calculated for each sex, respectively using the regression equation of:

Equation 1: $\textbf{Daily DMI} = \mathbf{X_0 + X_1\ (ADG) + X_2(MidWT^{0.75})\ +RFI}$,

where daily DMI is the average daily feed intake, X_0 is the regression intercept, X_1 is the partial regression coefficient of daily intake on ADG and X_2 is the partial regression coefficient of daily intake on body weight. ADG and $MidWt^{0.75}$ were used as regressors on daily DMI (SAS Inst., Inc., Cary, NC, 2003). RFI is the error term of the model.

Statistical Analysis:

The data were analyzed using the GLM Procedure (SAS Inst. Inc., Cary, NC) as a completely randomized design, with animal as the experimental unit.The mean relative gene expression (RQ) for each gene was compared with RFI each animal. For simple means comparisons of animal performance, gene expression, and RFI the PROC GLM procedure of SAS was used (SAS Inst. Inc., Cary, NC). The Proc CORR procedure of SAS was used to determine correlation coefficients between performance traits and average relative expression for each gene. All Differences were considered significant at $P < 0.05$.

Results and Discussion

Animal Performance and Residual Feed Intake:

The animals used in the current experiment were the initial progeny of a breeding experiment designed to select for RFI under production conditions in the SE US. Determination of RFI provides a measure of how animals perform relative to their contemporaries under the same circumstances and may be a more accurate measure of feed efficiency compared to other determinations. It has been shown that castration has an effect on animal feed efficiency and markedly increases IMF in LM of beef cattle (Park et al., 2002). Sixteen male progeny of the initial calf crop were used, one half of them castrated (Steers) and the rest intact (Bulls). Animals were fed a corn and soy based ration (Table 3.1) and daily feed intake and refusals were determined. Residual feed intake was calculated for each of the animals in the trial using equation 1. As expected using the regression equation, the mean RFI for the animals within the contemporary group was equal to zero (Table 3. 7) with some animals having lower or higher RFIs.

Our hypothesis was that bulls would have lower (more efficient) RFI compared to steers however there was no segregation by reproductive status. The range of RFI values varied within and between groups (Figure 3.7) with both having some high and low RFI animals indicating varying efficiency between animals regardless of reproductive status. We did not have a wide range of segregation of RFI values between animals (Figure 3.7). An arbitrary classification scheme was utilized to categorize animals into low, medium or high RFI based on their deviation from the mean (Table 3.7). Most cattle (9 out of 15) had RFI values that fell within one standard deviation of the mean (Table 3.7; standard deviation = 0.51). Based on this classification scheme, 9 animals were classified as average RFI. One bull and two steers were designated low RFI indicating greater efficiency in these animals compared to their average and high RFI contemporaries. Three of the animals were classified as high RFI (inefficient). Once again this was an arbitrary classification scheme in order to further mine our data based on biological efficiency of the individual animals.

One possible explanation for the lack of segregation in RFI is the small amount of animal numbers used in this preliminary experiment. In addition, as mentioned in the methods, these cattle were the 1st generation progeny of an RFI selection program intended to generate a divergent population of cattle with respect to RFI. In such a system, subsequent generations of progeny would be expected to achieve more divergence in RFI and may have resulted in a more segregated population of animals with respect to this efficiency parameter. Between, and within contemporary groups, there appeared to be a large amount of inter-animal variation indicating variable performance with respect to RFI. There was no effect of sex on RFI therefore we considered all

animals as one group when doing correlation analysis. We found that when taken on whole, the expression of some target genes was correlated with RFI and as such may warrant further research to determine exactly which components of their corresponding pathways are potentially related to RFI. These data are discussed below.

Gene Expression

Richardson and Herd (2004) suggested that up to 37% of the variation in RFI observed in beef cattle was due to tissue metabolism, protein turnover, and stress (Figure 3.1). The panels of selected genes in the current experiment represent regulatory sentinels responsible for governing the flux of nutrients to and from economically relevant tissues and orchestrating energy metabolism within these tissues. These are putative and novel factors that have been implicated in the dynamics of AT accretion, SM metabolism, mitochondrial function, and energy partitioning which are important processes that affect metabolic efficiency and carcass characteristics and production efficiency. We expected that RFI would be related to the expression patterns of these regulatory factors and that these expression profiles would be informative in terms of the molecular mechanisms that contribute to variation in RFI.

Adipose Tissue

In Adipose tissue, the expression of FAS, leptin and PPARγ2 was determined. These genes are involved in AT metabolism and indicative of metabolic activity in the tissue. Correlation analysis indicated that these genes were unrelated to RFI (Figure 3.8; $P > 0.05$). There were no differences in expression of FAS (Figure 3.13) or PPARγ2 (Figure 3.15) between bulls and steers and this was not correlated with RFI. Interestingly,

AT Leptin GE was higher (P < 0.05) in bulls compared to steers (Figure 3.14). This may be the result of increased AT deposition in bulls as leptin serves as an indicator of AT abundance. Considering the effect of castration on carcass adiposity, this is an interesting observation given that bulls generally produce leaner carcasses than steers and leptin is an indicator of fat cell size and fat cover in cattle (Delavaud et al., 2002). In many species including cattle, circulating levels of leptin and expression of the leptin (*ob*) gene are indicative of adipocyte size and volume (Delavaud et al., 2007; Chilliard et al., 2005; Delevaud et al., 2002; Soukas et al., 2000). There are several short and long term regulatory mechanisms that impact leptin expression which may have played a role in the current experiment. Of particular interest is body fatness which regulates leptin and its response to other factors such as feeding level and physiological status. Because of infrastructure limitations we did not look at carcass adiposity in this study; however, this would have been very valuable in order to relate our leptin expression data to a known factor that regulates leptin expression.

Skeletal Muscle

In SM, UCP-2 and E2 were positively correlated with RFI (Table 3.7; $P < 0.05$). These genes are involved in energy oxidation and protein turnover, respectively. Mitochondria serve as the primary site for cellular energy production accounting for upwards of 90% of cellular ATP production. Because of the predominant role of mitochondria in energy balance, it has been hypothesized that differences in mitochondrial function may be responsible for disparities in FE amongst groups and breeds of animals. Differences in mitochondrial function have been reported pigs, broilers, and steers divergently selected for RFI (Grubbs et al., 2013; Kolath et al., 2006a;

Lancaster et al., 2007). In the current study, expression of UCP-2 was positively correlated with RFI (Table 3.7; P = 0.02) and with the expression of poly-ubiquitin (P = 0.006) and E2 Conjugase indicating that more efficient animals had lower expression of the genes involved in skeletal muscle protein turnover and mitochondrial uncoupling.

In 2006 Kolath et al., conducted a study to determine the relationship between RFI and mitochondrial function in finishing Angus steers. In their study they conducted a feeding trial to identify low and high RFI steers and used only these animals in subsequent analysis. There were no differences in expression of UCP2 and 3 in high and low RFI steers and it was determined that the expression (mRNA abundance) of these proteins was not related to RFI in finishing steers. It is worth noting that in the Kolath study the author's collected LM samples post-slaughter as opposed to the biopsy samples that were collected from live animals in the current study.

Kelly et al. (2011) conducted a study to determine the effect of phenotypic ranking for RFI on the expression of genes involved in the mitochondrial respiratory chain and TFs responsible for mitochondrial biogenesis. Beef heifers were subjected to contrasting feeding regimens (High forage vs. High Concentrate) and RFI was determined. Similar to the Kolath study, these authors selected animals that were either feed inefficient (High RFI, low FE; n=10) or feed efficient (Low RFI, High FE, n=10) to represent phenotypic extremes. They determined that UCP3 expression tended to be up-regulated (2.2-fold, $P = 0.06$) for in high-RFI versus low-RFI animals. PGC-1α which is a master regulator of mitochondrial biogenesis was 1.7-fold higher ($P = 0.01$) in low compared with high-RFI animals. These data along with the data from the current study suggest an important role for mitochondria in improving feed efficiency.

Much of skeletal muscle metabolism is devoted to the dynamics of protein synthesis and degradation. In meat producing animals, protein metabolism is important because the net differences between protein synthesis and protein degradation is the determining factor in the amount of marketable product (Bergen, 2008). Additionally, the process of protein synthesis requires dietary energy therefore the rates of synthesis and degradation affect the efficiency of animal production. Ubiquitin (Ub) is a regulatory protein that is located in nearly all-eukaryotic cells as its name implies. Ub can exist as a monomer or in the form of Polyubiqutin (UB), which is a homo-polymer of ubiquitin molecules. Among its various signaling functions, ubiquitin is best recognized for its role in targeting proteins to the UPS for degradation and recycling. (Pickart and Fushman, 2004). Ubiquitin Conjugating Enzymes (E2) performs the second step in the ubiquitination reaction that targets proteins for degradation via the ubiquitin-proteasome system (UPS). While UB expression was not correlated with RFI (Table 3.7) it was highly correlated with E2 GE. This is to be expected, as these are two components of the ubiquitin proteasome protein degradation system and would be expected to be auto-correlated. An autocorrelation of this nature makes physiological sense and may help to validate the technical soundness of the approach despite large amounts inter-animal variation within and between bulls and steers.

Energetic homeostasis is determined by the balance between energy consumed and stored versus energy requirements and oxidation in an organism. Energy oxidation, particularly FA oxidation is an important component of the energy equation and the balance between storage and oxidation can determine the net accretion of AT in beef cattle. To determine the energetic disposition of the finishing animals we looked at

oxidative gene expression as indicators of oxidative metabolism. CPT-1b is the rate-limiting enzyme controlling the oxidation of FA in skeletal muscle mitochondria. There were no differences between the expression of CPT-1b in bulls and steers (Figure 3.12) and there was no significant correlation with RFI (Table 3.7). Similarly, PPARα another indicator of cellular energy oxidation was not correlated with RFI (Table 3.7). Because energy expenditure is a major component of the energy balance equation, it was interesting to note that there were no differences in the energy oxidation genes between bulls and steers in the current experiment. Again, variable performance between groups may be responsible for the lack of a clear expression pattern with respect to these genes, but we expected that these genes might be associated with RFI which was not the case. While these genes may be involved in the overall energy equation, the lack of segregation in performance efficiency that we observed may have diluted the relationship of these genes with RFI.

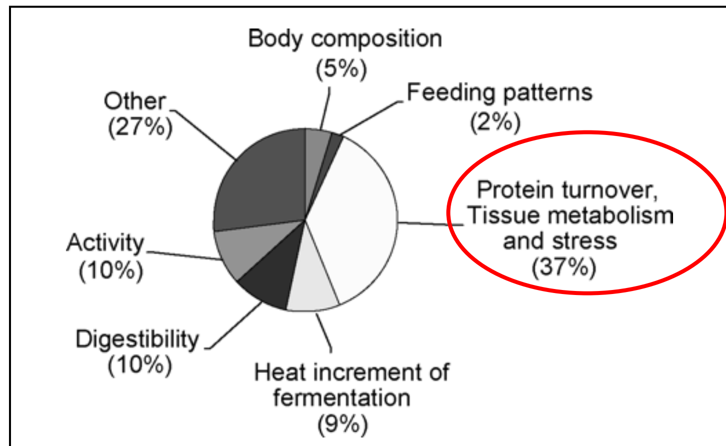
Adipose tissue genes and genes for fat deposition appeared to be minimally related to RFI and FE (Table 3.7). It is possible that the range of RFI values in bulls and steers was too narrow to ascertain a relationship between fattening and feed efficiency. Our results do suggest that feed efficiency, measured as RFI is related to the expression of genes involved in SM metabolism as well as weakly correlated with other metabolic genes (Table 3.7). The current project utilized only 15 animals and future work will be focused on identifying other genes related to RFI in an effort to find predictive biomarkers that can be used to determine RFI without the need for intense and laborious data collection. Such studies should include a divergently selected (High vs. Low RFI) population of animals so as to have a more robust model of RFI phenotypes.

Identification of putative molecular markers of RFI will also make the cost of determining RFI more reasonable which can lead to this efficiency parameter being more readily adopted as a selection tool.

Serial tissue sampling may also be warranted to garner a longitudinal perspective of the transcriptomic signature as the animal progresses through the finishing process. This would be informative in terms of determining how “highly-efficient (low RFI) animals” profile compared to their “less-efficient (high RFI)” counterparts. Furthermore, this may elucidate early developmental time points at which low- vs. high- RFI animals can be identified, which would be the ultimate practical use of this methodology. The goal of this work will be to provide an improved method of more rapidly identifying efficient animals and to maximize the potential for RFI to reduce the overall cost and environmental impact of beef cattle production.

Chapter 3 Figures

Figure 3.1 Contributions of biological mechanisms to variation in residual feed intake as determined from experiments using divergently selected cattle[1]



[1]Circled area represents pathways controlled by modulations in gene expression and other factors; Determined from experiments using divergently selected cattle; Modified from Richardson, E. C., and R. M. Herd. 2004.

Table 3.1 Experimental diet composition[1]

Ingredient	Percentage
Cracked Corn	28%
Cotton Seed Meal	7%
Soyhull pellets	60%
Syrup	3%
Bovitec Minerals	2%

[1]Experimental Diet composition only; Proximate analysis data not presented; Diet was fed ad libitum with refusals collected daily

Table 3.2 High-capacity cDNA synthesis reaction components[1]

Component	Volume/Reaction (μl)
10x RT Buffer	2
25x dNTP Mix (100mM)	0.8
10X Random RT Primers	2
MultiScribe Reverse Transcriptase	1
Nuclease Free Water	4.2
Total Reaction Volume	10

[1]Individual Reaction components for complementary DNA synthesis from total RNA isolate. For each tissue a master mix containing sufficient quantities of the individual reaction components was created and cDNA synthesis was conducted for each RNA sample; RT- Reverse Transcription; dNTP- deoxynucleoside triphosphates.

Table 3.3 cDNA synthesis reaction conditions[1]

	Step 1	**Step 2**	**Step 3**	**Step 4**
Temperature (°C)	25°C	37°C	85°C	4°C
Time	10min	120min	5min	Hold

[1]Reaction conditions for the 4 step cDNA synthesis reaction; Temperatures were controlled in PCR thermocycler; upon completion, reactions were held at 4°C until samples were removed.

Table 3.4 TaqMan gene-specific primer and TaqMan probe sequences for the genes and tissues of interest

Gene of interest	Accession No.	Forward Primer	Reverse Primer	TaqMan Probe	Tissue
FAS	NM_00102669	CCTGGCCTTCGTGAGCAT	AGCACGGCGTAGCCA	CAGCAGCCATGCCCTT	AT
Leptin	NM_173928	GCCTTCCAGAAATGTGGTCCAAATA	CGGCCAGCAGGTGGA	CCTCCGGGACCTTC	AT
PPAR-gamma	Y12420	CCGCTGACCAAAGCAAAGG	AGTTCATGTCATAGATAACAAACGGTGAT	TTTCCCGTCAAGATCG	AT
Poly-Ubiquitin	NM_174133	ACCTGGTCCTCCGTCTGA	CCTCCAGGGTGATGGTCTTG	CCGGTCAGGGTCTTCA	LM
Ubiquitin E2	LOC100138178	AGTTCATGCATAGATA	GCCTTCCAGAAATGTGGTCCA		LM
CPT-1b	NM_001034349	GACTGGCAGCCCTCACT	CTTGTTCTTGCCAGAGCTGAAG	CCCACTCCACTCTTCC	LM
PPAR-alpha	AF229356	TTGCCGGGAAGACCAACA	CCATACACAGCGTCTCCATGTC	ACAACCCCGCCTTTCT	LM
UCP-2	AF127029	GGACTCTGGAAAGGGACATCTC	CCAGCTCAGCACAGTTGACA	TCGCTCGCAATGCCAT	LM

[1]Accession No. = NCBI database accession number (http://www.ncbi.nlm.nih.gov/); FAS- Fatty Acid Synthase; PPAR-gamma - Peroxisome proliferator activator protein gamma; Ubiquitin E2 – E2 Ligase; CPT-1b - Carnitine Palmitoyl Transferase 1b; PPAR-alpha - Peroxisome proliferator activator protein alpha; UCP-2 –Uncoupling protein 2; AT- Tail Head Adipose Tissue Biopsies; LM- *Longissimus dorsi* Muscle Tissue Biopsies.

Table 3.5 Reaction components for Real-Time PCR gene expression analysis

Component	**Volume**
TaqMan Gene Expression Master Mix	8µl
Vic-Labeled 18S rRNA Probe[1]	1µl
Custom TaqMAn Gene Expression Assay	1µl
cDNA[2] + H20	10µl
Total Reaction Volume	20µl

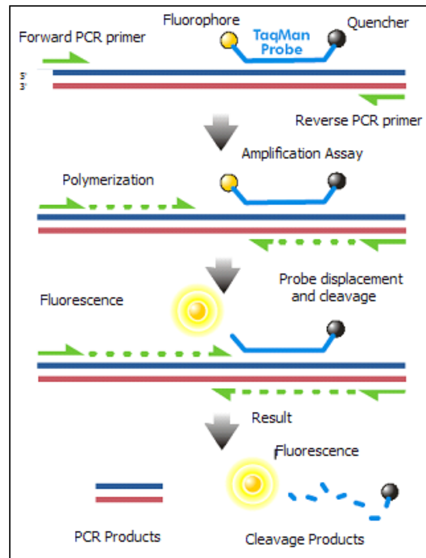
[1]18S Vic Labeled 18S ribosomal RNA probe for data normalization in multiplexed quantitative Real-Time PCR reaction; cDNA = Complementary DNA.

Table 3.6 Real-Time PCR thermocycler reaction conditions

Step	Description	Time	Temperature
1	AMPerase UNG Activation	2 min	50°C
2	Taq Activation/UNG Inactivation	10 min	95°C
3	Polymerization	15 sec	95°C
4	Primer Annealing/Detection	1 min	60°C
5	Repeat Steps 3-4 for 40 cycles	NA	NA

[1]UNG = AMPerase Uracil N Glycosylase- Prevents Carryover Contamination; Taq = Thermostable Taq DNA Polymerase; Fluorescence Detected step 3.

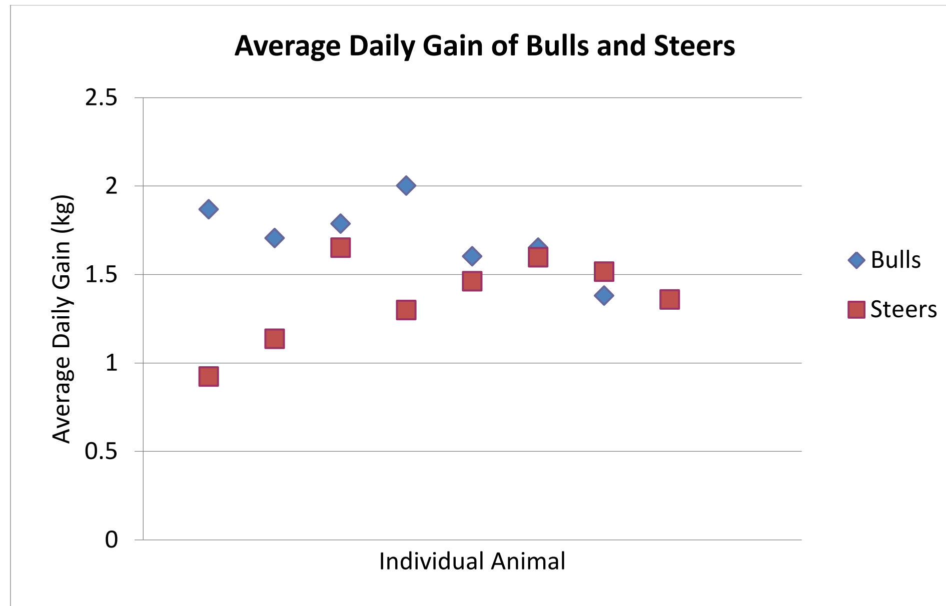
Figure 3.2 Overview of TaqMan® gene expression chemistry[1]



[1]**Description:** Unlabeled PCR primers anneal to template DNA. In addition a fluorophore-labeled probe containing a quencher moiety also anneals to the target sequence. Polymerization and exonuclease activity of Taq Polymerase results in displacement of the reporter from the probe causing a detectable fluorescent signal proportional to the amount of amplified DNA present. (Adapted from Applied Biosystems.com)

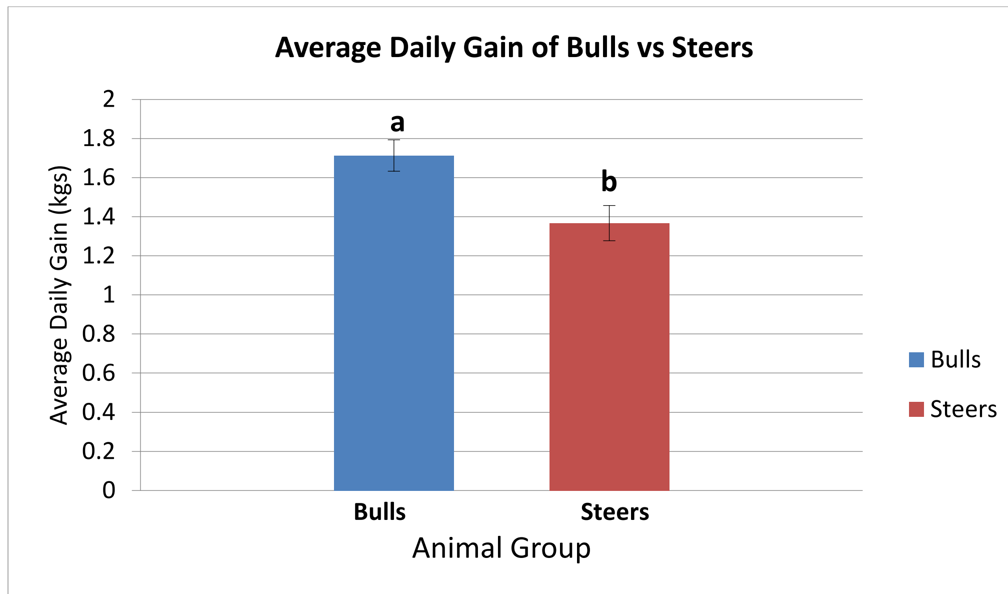
Results

Figure 3.3 Average daily gains of individual bulls and steers on feed for 84 days[1-3]



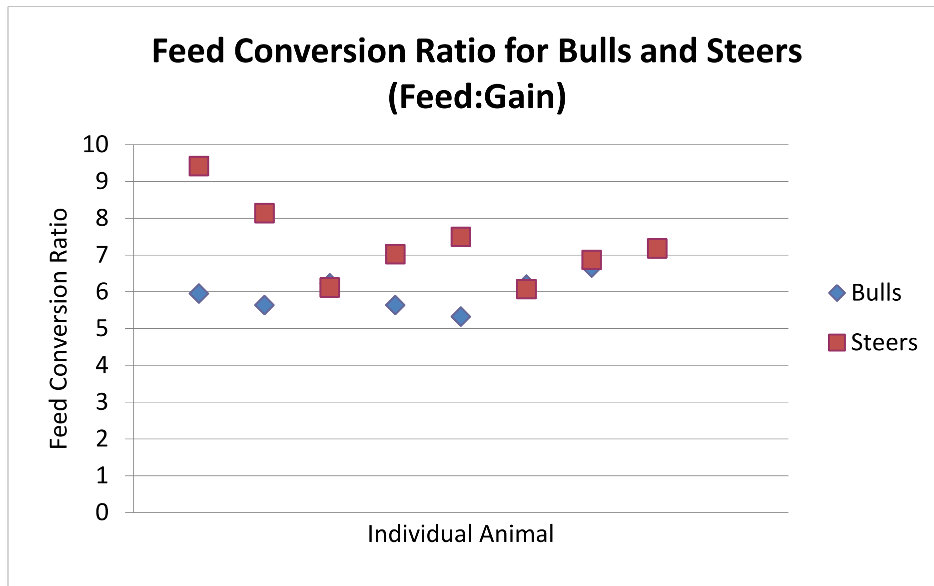
[1]Range of Average Daily Gains for Individual bulls and steers in the feeding trial.
[2]Bulls are represented by diamonds and steers represented by squares
[3]Average Daily Gain (kg) calculated for 84 days on feed.

Figure 3.4 Comparison of average daily gains of bulls vs. steers on feed for 84 days[1]



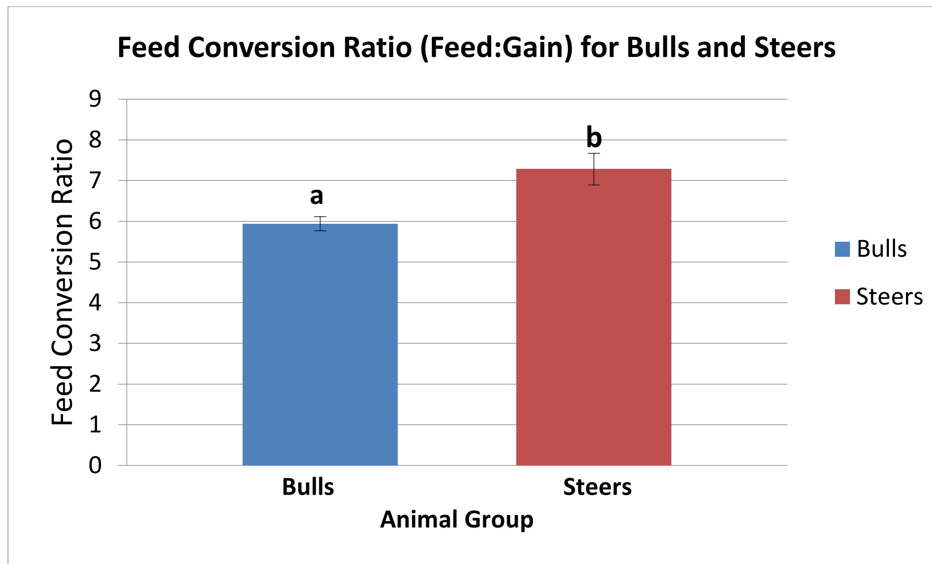
[1]Animal Group = Bulls vs. Steers; Average daily gain determined for 84 days on feed; data are presented as Mean +/- SEM; [a,b]Means with different superscripts differ ($P<0.05$).

Figure 3.5 Dry matter feed conversion for individual bulls and steers on feed for 84 days[1]



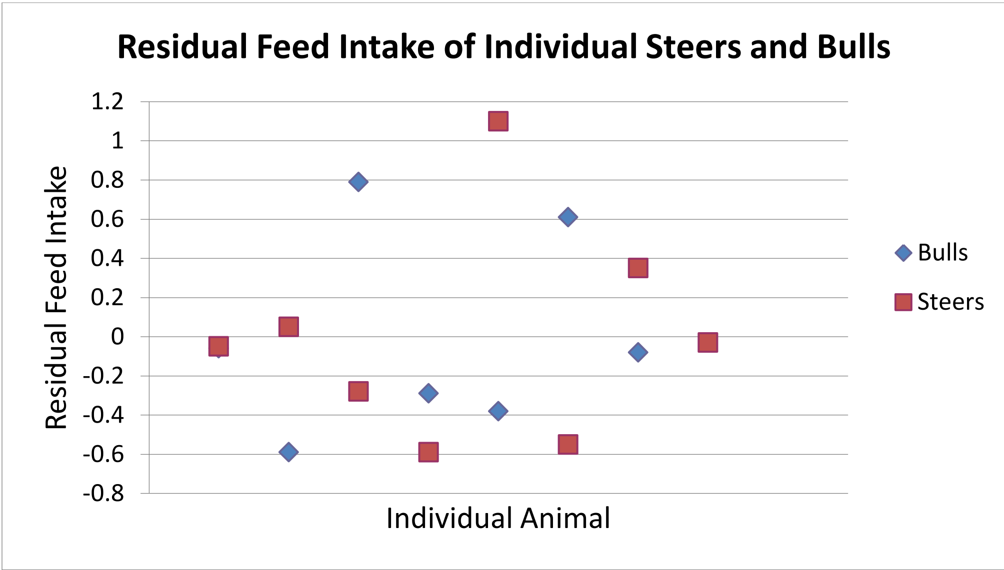
[1]Dry matter feed conversion for individual animals on feed for 84 days; Individual bulls are represented by diamonds and steers are represented by squares.

Figure 3.6 Dry matter feed conversion for individual bulls and steers on feed for 84 days[1]



[1]Animal Group = Bulls vs. Steers; Dry matter feed conversion (kg fed: kg weight gained) determined for 84 days on feed; data are presented as Mean +/- SEM; [a,b]Means with different superscripts differ (P<0.05).

Figure 3.7 Calculated residual feed intakes for individual bulls and steers on feed for 84 days[1]



[1]Residual Feed Intake in individual animals on feed for 84 days; Individual bulls are represented by diamonds and steers are represented by Squares.

Table 3.7 Arbitrary classification of cattle as high, average, or low RFI based on individual animals' deviation from the mean RFI for the population[1]

Sex	Individual RFI	Classification based on STDV from the mean
Bull	-0.06	Average
Bull	-0.59	Low
Bull	0.79	High
Bull	-0.29	Average
Bull	-0.38	Average
Bull	0.61	High
Bull	-0.08	Average
Steer	-0.05	Average
Steer	0.05	Average
Steer	-0.28	Average
Steer	-0.59	Low
Steer	1.1	High
Steer	-0.55	Low
Steer	0.35	Average
Steer	-0.03	Average
Mean RFI	0	
STDV	0.51	

[1]Arbitrary Classification Scheme based on Calculated Individual Residual Feed Intake; Low RFI- More efficient; Individual RFI is more than one standard deviation lower than the mean RFI; Average RFI- Average Efficiency compared to contemporaries; Individual RFI is within one standard deviation of the mean RFI; High RFI- Less efficient; Individual RFI is more than one standard deviation higher than the mean RFI; Mean RFI = Average RFI for the Entire Group of Animals (Bulls and Steers); STDV = Standard Deviation.

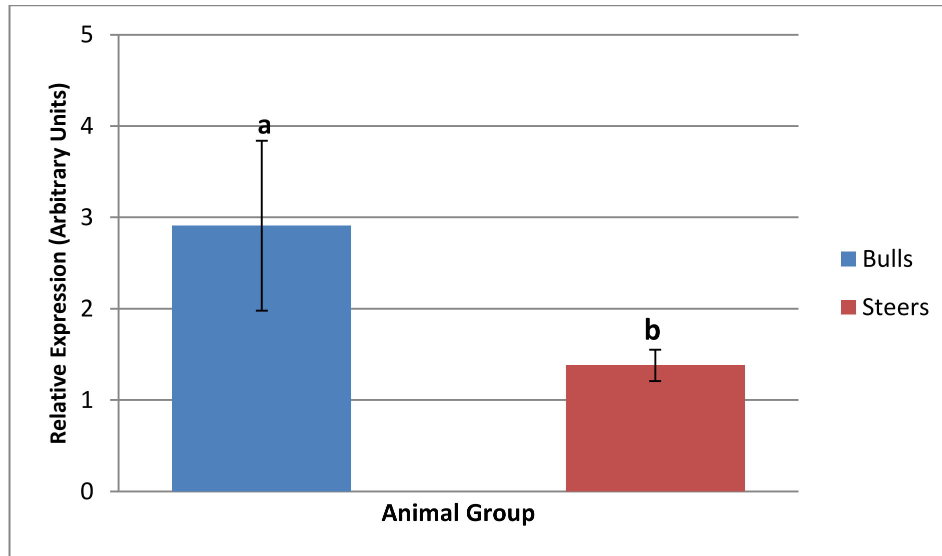
Table 3.8 Pearson correlation coefficients relating the relative expression of each gene with estimated RFI for steers on feed for 84 days[1]

Gene	Pearson Corr. Coefficient	*p*-value
PPARγ2	-0.18	0.58
FAS	-0.23	0.52
Leptin	0.1	0.76
PPARα	-0.2	0.44
CPT-1b	-0.48	0.15
Ubiq-E2	0.51	0.05
Polyubiquitin	0.18	0.53
UCP-2	0.59	0.02

[1]PPAR-γ2 = Peroxisome Proliferator Activator Protein Gamma 2; FAS = Fatty acid Synthase; PPARα = Peroxisome Proliferator Activator Protein; CPT-1b = Carnitine Palmitoyl Transferase- 1b; Ubiq-E2 = Ubiquitin E2 Ligase; UCP-2 = Uncoupling Protein 2.

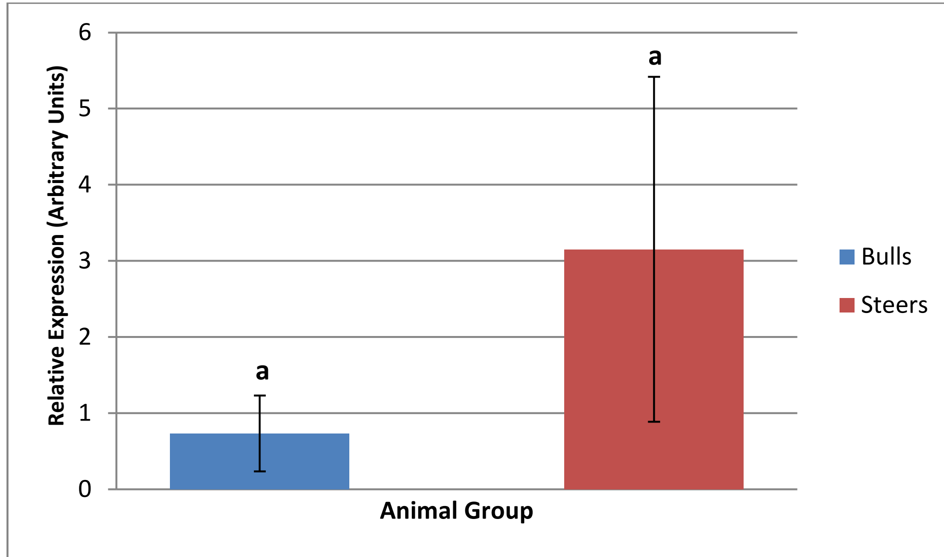
Gene Expression

Figure 3.8 Relative expression of PPAR-α in SM biopsies of finishing bulls vs. steers on feed for 84 days



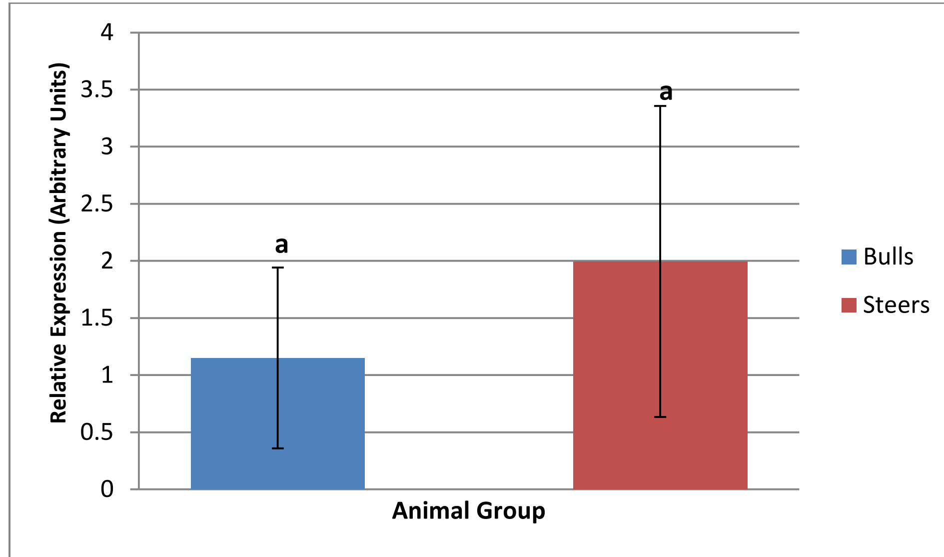
[a,b] Mean +/- SEM; Means not sharing a common superscript differ ($P < 0.05$); Animal Group = Bulls (Black) vs. Steers (Grey); SM = Skeletal Muscle; PPAR-α = Peroxisome Proliferator Activator Receptor-alpha.

Figure 3.9 Relative expression of UCP-2 in SM biopsies of finishing bulls vs. steers on feed for 84 days



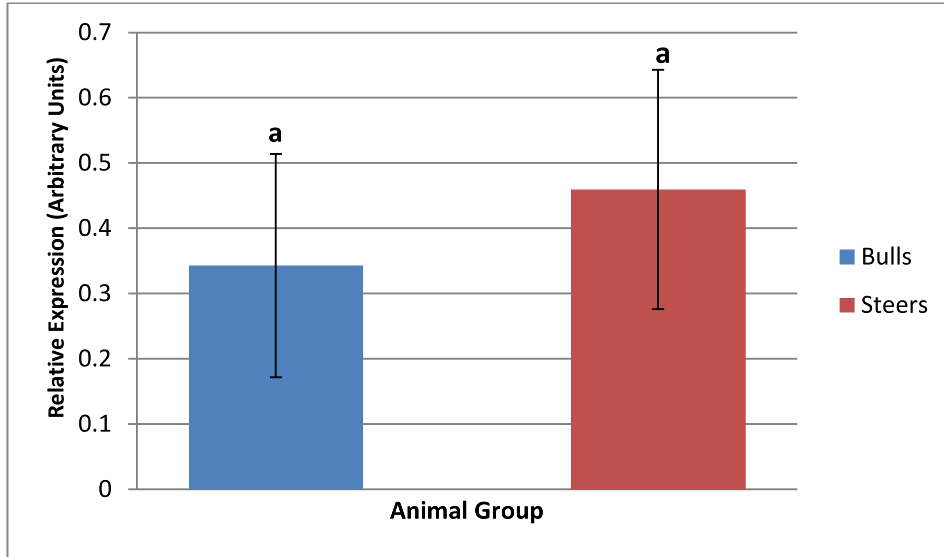
[a,b]Mean +/- SEM; Means not sharing a common superscript differ ($P < 0.05$); Animal Group = Bulls (Black) vs. Steers (Grey); SM = Skeletal Muscle; UCP-2 = Uncoupling Protein 2.

Figure 3.10 Relative expression of Ub-E2 in SM biopsies of finishing bulls vs. steers on feed for 84 days



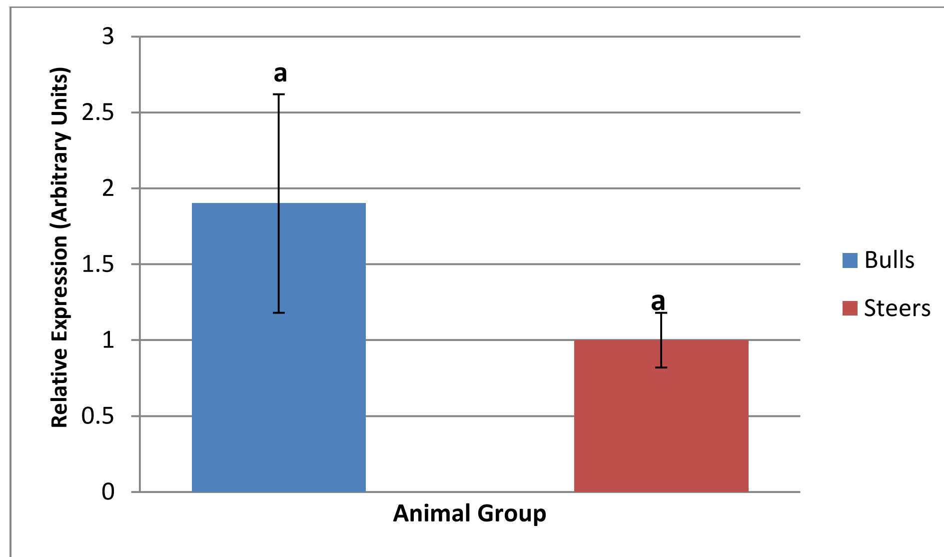
[a,b] Mean +/- SEM; Means not sharing a common superscript differ ($P < 0.05$); Animal Group = Bulls vs. Steers; SM = Skeletal Muscle; UB-E2 Ubiquitin E2 Ligase.

Figure 3.11 Relative expression of poly-ubiquitin in SM biopsies of finishing bulls vs. steers on feed for 84 days



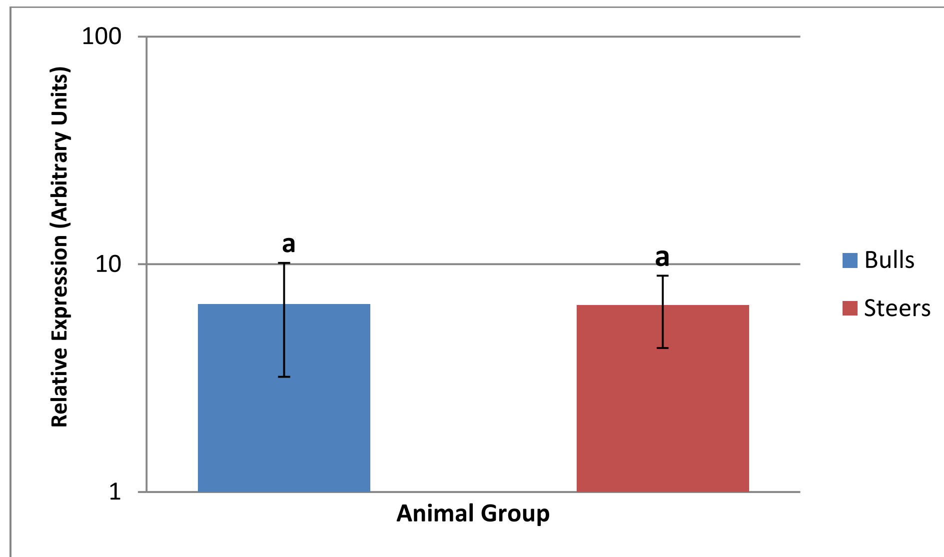
[a,b] Mean +/- SEM; Means not sharing a common superscript differ ($P < 0.05$); Animal Group = Bulls vs. Steers; SM = Skeletal Muscle.

Figure 3.12 Relative expression of CPT-1b in SM of finishing bulls vs. steers on feed for 84 days[1-4]



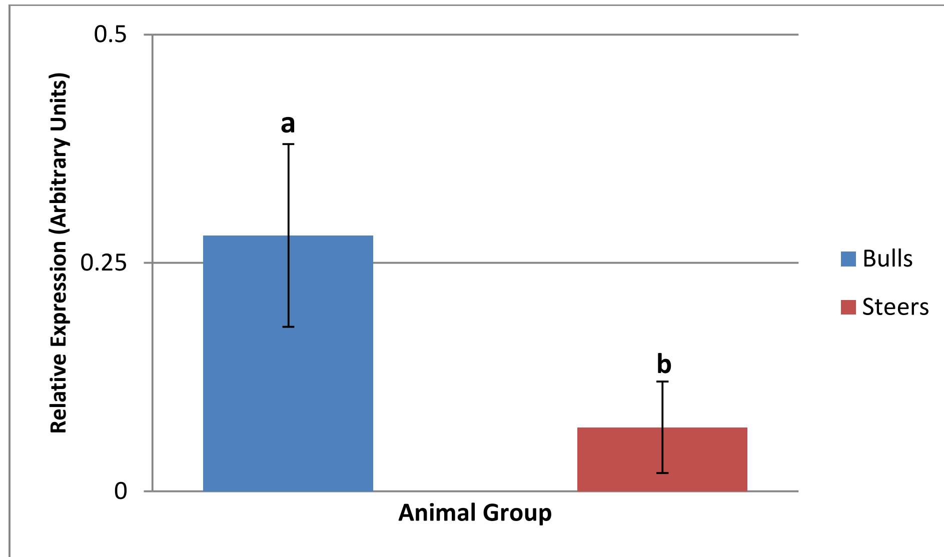
[a,b] Mean +/- SEM; Means not sharing a common superscript differ ($P < 0.05$); Animal Group = Bulls vs. Steers; SM = Skeletal Muscle; CPT1-b = Carnitine Palmitoyl Transferase 1-b.

Figure 3.13 Relative expression of FAS in AT of finishing bulls vs. steers on feed for 84 days



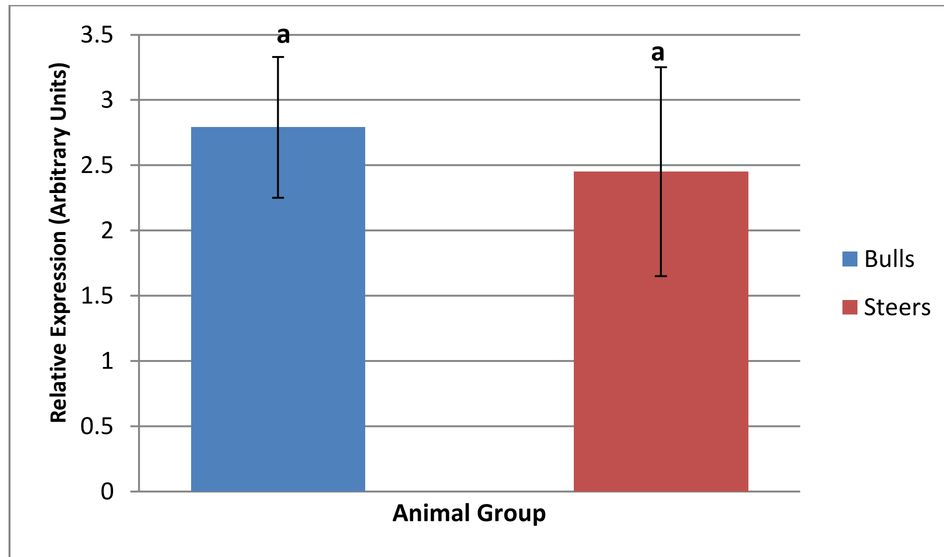
[a,b] Mean +/- SEM; Means not sharing a common superscript differ ($P < 0.05$); Animal Group = Bulls vs. Steers; AT = Adipose Tissue; FAS = Fatty acid synthase.

Figure 3.14 Relative expression of Leptin in AT of finishing bulls vs. steers on feed for 84 days



[a,b] Mean +/- SEM; Means not sharing a common superscript differ ($P < 0.05$); Animal Group = Bulls vs. Steers; AT = Adipose Tissue.

Figure 3.15 Relative expression of PPAR-γ in AT of finishing bulls vs. steers on feed for 84 days



[a]Mean +/- SEM; Means not sharing a common superscript differ ($P < 0.05$); Animal Group = Bulls vs. Steers; AT = Adipose Tissue; PPAR-γ – Peroxisome Proliferator Activator Receptor- gamma.

Chapter 4. The Effect of Days on Feed and Beta-Agonist Administration on the Expression of Regulatory Genes in Skeletal Muscle and Adipose Tissue of Finishing Heifers

Introduction

Rising feed costs and less than optimal production efficiency are major factors that have a marked impact on the bottom line of any beef cattle operation. These factors also affect the overall economic and environmental sustainability of the beef cattle industry in the Southeastern United States (SE). Feed represents 60 to 70% of total input costs for commercial beef production (Basarab et al., 2002; Arthur et al., 2001; Herd et al., 1998; Ritchie et al., 1992) and beef cattle are amongst the least efficient of livestock species (Rosegrant et al., 1999). Heifers are even less efficient than their steer counterparts making them less desirable in a finishing situation and highlighting the need for continuing research to improve beef production efficiency. Heifers comprise about one-third of the annual beef harvest in the US (USDA, 2010) and generally have poorer feed efficiencies, lower hot carcass weights, and produce less tender beef than steers (Tatum, 2007; Choat et al., 2006). These problems have been a driving force for researchers and producers to develop and apply various management and selection strategies to improve production efficiency and product quality in finishing heifers (Beerman, 2002; Mills, 2002). Advances in genetics and animal nutrition have made substantial improvements in production efficiency, but considerable variation still exists with regard to maintenance energy requirements (Archer et al., 1999) which dictates the relative efficiency individual animals and has a marked impact on the efficiency of the operation.

In addition to genetic selection and nutritional management, several exogenous agents have been developed and proven safe and effective for improving meat animal productivity and product quality (Dikeman, 2007; Beerman et al., 2005). Ractopamine HCl (RAC) is a member of

the beta-adrenergic agonist (BAA) group of growth modifying repartitioning agents, which are chemically and pharmacologically similar to the endogenous catecholamine's epinephrine and norepinephrine (Figure 4.1, Figure 4.2; Smith, 1998). Historically BAAs have been used therapeutically in human medicine due to their effects on smooth muscle, their potential as anti-obesity agents, and other medicinal uses (Tattersfield, 2006). Interest in livestock production is due to their effectiveness in repartitioning dietary energy away from peripheral fat synthesis and increasing the rate and efficiency of protein deposition in lean muscle tissue (Dikeman, 2007; Beerman et al., 2002; Mersmann, 1998). This results in increased muscle mass and lean muscle percentage (Beerman, 2002; Mills 2002) without increasing feed intake making these compounds effective repartitioning agents and valuable tools for improving production efficiency. In 1999, the USDA approved the use of RAC under the trade name "Paylean (Ractopamine HCl; Elanco Animal Health, Deerfield IN) for finishing swine and subsequently in 2003 approved Optaflexx (Ractopamine HCl; Elanco Animal Health, Deerfield IN) as a formulation of RAC for use in finishing cattle. These compounds have since been incorporated to varying degrees in the swine and cattle industries. Currently RAC is approved for use for the final 28-42 days on feed at a dosage of 70-430mg/hd/d in finishing cattle.

The phenotypic effects of RAC in livestock species have been well documented and the proposed benefits include increased feed efficiency, leaner carcasses, and decreased wasteful AT accumulation (Walker et al., 2006; Beerman et al., 2002; Mersmann, 1998). The mechanisms that accomplish these phenotypic changes are less clearly understood. While some evidence have pointed to indirect actions resulting in changes in concentration of circulating hormones and target cell sensitivity (Beerman, 2002; Mersmann, 1998), it is generally accepted that direct, receptor mediated changes are responsible for BAA action in vivo (Figure 4.3; Winterholler et

al., 2007; Beerman, 2002). The presence of BAR subtypes on cells in AT and SM provide evidence for the direct effect of RAC on these tissues and binding of BAA to these receptors has been shown to induce metabolic responses in cell lines and animal models (Lefkowitz, 2007; Bergen, 2001; Peterla and Scanes, 1990). Many of the studies that have reported on the effect of RAC on metabolite concentrations are confounded by the inclusion of various steroidal implants (Winterholler et al., 2008; Sissom et al., 2007) which are common in the beef cattle industry, but can affect the metabolic response to RAC or other agents making the independent mechanism of BAA action difficult to determine in production scenarios.

RAC is purported to increase hot carcass weight, rib eye area, and dressing percentage and decrease carcass fat (Winterholler et al., 2008; Schroeder et al., 2005). The basis for these effects lies the metabolic activity of AT and SM which are of economic importance to producers and consumers alike. Of particular interest is the ability of these compounds to produce leaner carcasses and decrease waste fat accretion. In pigs fed BAA increased rates of lipolysis and decreased lipogenic enzyme activity has been reported (Perterla and Scanes, 1990; Merkel et al., 1987). In various animal and cell models modulation of lipolytic enzyme activity has been observed in response to BAA administration (Hausman et al., 2009; Halsey et al., 2011; Reiter et al., 2007; Leftkowiz 2007; Bergen 2001; Mersmann, 1998; Peterla and Scanes, 1990) and lipolytic/lipogenic enzyme activity is known to be regulated by a myriad of transcription factors (Desvergne et al., 2006; Griffin and Sul, 2004; Sul and Wang, 1998). Halsey et al. (2011) and Reiter et al. (2007), each reported decreased lipogenic gene expression in SC AT of pigs treated with RAC however similar studies in cattle are limited.

In AT the response to RAC is transient and may be affected by down-regulation of BAR during chronic administration of the BAA (Liang and Mills, 2002; Liu et al., 1994). Spurlock et

al. (1994) showed that the density of the BAR is decreased in SC AT in pigs administered RAC which may account for the diminished lipolytic response to chronic RAC dosages. This fleeting effect makes it essential to determine the optimal timing of administration to maximize the benefit of using these compounds with respect to distribution of carcass fat. Additionally, because AT development is regulated in a temporal fashion and in particular the IMF depot is late maturing, it is necessary to determine the regulatory mechanisms affected by RAC administration in order to avoid any detrimental impact on the IMF depot for the sake of increasing lean tissue. The response of AT to RAC in has been fairly well characterized using a variety of biochemical and molecular studies in pig models (Halsey et al., 2011; Reiter et al., 2007; Liang and Mills, 2002; Dunshea et al., 1993, 1998; Liu et al., 1994), however to date, these studies have not been widely conducted in beef cattle.

RAC administration leads to muscle cell hypertrophy, increased muscle mass and more lean tissue for meat products. Several studies have shown increase in individual muscle weights with administration of various BAA in sheep, cattle, and poultry (See et al., 2004; Rehfeldt et al., 1997; Bergen et al., 1989; Wheeler and Koohmarie 1992; Claeys et al., 1989) The mechanism of muscle hypertrophy induced by RAC is related to its ability to cause a shift in the metabolic activity of muscle fibers and induce a switch from type-I to type-II muscle fibers (Gonzalez et al., 2007, 2008). RAC also affects the dynamics of protein metabolism via increased rates of protein synthesis, decreased rates of protein degradation or a combination of both contributing to SM hypertrophy (Grant et al., 1993; Bergen et al., 1989; Mersmann, 1998; Sainz et al., 1993). Also, it has been shown that administration of BAA results in increased net uptake of AA in SM, along with lowered muscle fractional degradation rates and increased fractional accretion rates (Wheeler and Koohmaraie, 1992). These basic metabolic changes are governed by regulatory

signals which must be responsive to BAR activation by RAC, but their identity and mechanism of action are not clearly understood.

The effects of BAA on muscle protein metabolism are also transient and mediated by direct binding of the BAA to specific isoforms of the BAR, but the downstream target and effectors of the pathway are less clear. Moreover BAAs have been shown to be less efficacious in younger beef cattle with the maximum benefits occurring in the finishing phase of production although the physiological mechanisms for this phenomenon have not been identified. These are likely tied to temporal growth patterns and protein dynamics in young versus older animals (Beerman, 2002; Mills, 2002). These processes are governed by genes regulating energy metabolism and protein turnover and thus the identification of regulatory factors implicated in these pathways may provide molecular targets for improving beef cattle production (Wang et al, 2009). Understanding the molecular mechanisms will allow for more tailored management strategies to optimize the timing of administration and maximize the animal response without the potential deleterious effects of these compounds.

Increased dry matter feed efficiency is another proposed benefit of RAC. Schroeder et al., (2004) reported increases of 25 and 20% in steers and heifers fed RAC, respectively, without an increase in daily feed intake. Similarly, Gruber et al. (2007) and Walker et al. (2006) reported increased ADG without an increase in feed intake indicating increase efficiency with RAC administration. Avendano-Reyes et al. (2006) reported decreased dry matter feed intake in heifers treated with RAC compared to control animals. By increasing protein deposition and decreasing fat deposition, RAC increases efficiency of production by changing body composition in an economically favorable manner. Accomplishing this repartitioning at without affecting feed intake levels further increases efficiency due to the decreased resources necessary to achieve a

marketable phenotype (Williams et al., 1994) but it is not clear how these compounds are apparently able to manipulate energy flux in a manner that improves production efficiency.

Other than the improvement in feedlot performance and feed efficiency, there are modest and variable changes in carcass composition in cattle compared to that noticed in pigs. This may be due to the distribution of BR subtypes on target tissues between the two species. Winterholler et al., 2007 reported heaver hot carcass weights but noticed no difference in rib eye area, yield grade, and indicators carcass adiposity in finishing heifers. Similar results have been reported for heifers treated with RAC. Still, others have reported no differences in HCW or loin area in heifers treated with RAC (Walker et al., 2006) highlighting a disparate response to RAC in fed cattle under various conditions. Furthermore Gonzalez et al. (2008) reported a differential response by different muscles to RAC administration which adds another layer of complexity because various muscles may respond differently to RAC treatment. These disparities must be addressed and considered in order to effectively incorporate RAC into beef cattle management plans and garner consistent and reproducible results.

While metabolic modifiers have been used in livestock production for the last half century, recent interest and our understanding of these compounds has been aided by advances in livestock genomics and the advent of innovative molecular strategies (Etherton et al., 2003). These advances have allowed researchers to track the signaling cascades induced by various exogenous agents and to identify putative and novel mediators of biological efficiency as determined by the pathways they effect (Riedmaier et al., 2011; Bionaz and Loor, 2007; Reiter et al., 2007). The goal of this project was to determine the effect of days on feed and ractopamine administration on the expression of genes involved in regulation of energy and protein metabolism and to relate the expression of these genes to performance and carcass data for

finishing heifers under SE conditions. This is in an effort to monitor the expression patterns of these regulatory genes and determine how they are affected by duration of the finishing phase and BAA supplementation. These data may provide a framework for optimal inclusion of BAA in management strategies based on molecular events and determinants of energy metabolism and partitioning. Optimal administration timing can be developed to coordinate with the temporal patterns of growth and development of valuable tissues and will help to mitigate unexpected and undesired effects of these compounds. In addition, identification of downstream targets for the BAA will lead to potential target genes for improving production efficiency and product quality. This would augment the economic and environmental benefits of these exogenous agents and improve the efficiency and sustainability of beef cattle production in the SE and elsewhere.

Materials and Methods

Animal Care and Use

All procedures were approved of by the Auburn University Institutional Animal Care and Use Committee for the use of live animals in experiments (IACUC Approval Number: 2007-1273).

Animal Trial

Seventy-one crossbred heifers were purchased via tele-auction in 2007. These heifers were co-mingled after purchase and were backgrounded for 66 days on summer a perennial Bermuda grass and Bahia grass pasture mix supplemented with soy hull pellets (3.2kg/hd/d). The heifers were then transported to the Auburn University Beef Cattle Evaluation Center where they were housed for the duration of the feeding trial. Cattle were assigned to one of six pens (12 cattle per pen) and were stratified according to height and weight to minimize social dominance.

Cattle had continuous access to automatic water troughs and each pen contained 12 Calan Gates® (American Calan, Northwood, NH) to allow for individual feed provision and intake determination. A 21d warm-up period was used to train the heifers to use the Calan Gates®. During this period the heifers received a corn based diet (Table 4.1) at 2% of BW, and had access to hay ad libitum. After the warm-up period, hay was removed and the heifers had ad libitum access to the corn-based diet. Orts were recorded daily, and MGA (0.5 mg/hd/d) was added to the diets to suppress estrus.

Treatment groups consisted of days on feed (DOF) with the following assignments: 79 (n=16), 100 (n=16), 121 (n=16), and 142 (n=16) DOF. Furthermore, for each DOF group, half (n=8) of the animals were treated with ractopamine hydrochloride (RAC; Optaflexx®; Elanco Animal Health, Greenfield IN) at 300mg/hd/d for the final 35d prior to harvest. The other half (n=8 per DOF group) did not receive treatment and served as controls for the experiment (CON).

Sample Collection

Heifers were harvested at the Auburn University Lambert-Powell meats laboratory under USDA inspector supervision. Heifers were transported to the meats laboratory 24 hours prior to harvest and were humanely euthanized beginning at 8:00 am on each harvest day. During the normal slaughter and dressing process, skeletal muscle tissue samples were collected from the gastrocnemius and longissimus muscle (13th Rib for consistency) immediately upon hide removal. Subcutaneous AT was collected from the rump area and a deep SC AT sample was also collected from the site of LM collection. All tissue samples were approximately 2-5 grams and care was taken to ensure consistent collection sites between animals. Upon collection, tissues were placed in labeled aluminum foil wrappers and immediately snap frozen in a portable liquid

nitrogen container before being transported and stored in a minus 80 degree C freezer until subsequent use.

RNA Isolation

Total RNA was extracted from frozen tissue samples using TRIzol reagent (Invitrogen, Carlsbad, CA). Approximately 0.5 grams of tissue was removed from the minus 80 degree freezer and placed in a portable liquid nitrogen container. The frozen tissue was then crushed using a metal mortar, and the crushed tissue was placed in 5ml of TRIzol. The sample was then homogenized using a polytron® (PT 1200, Kinematica; Bohemia, NY) tissue homogenizer and then the extraction carried out as per the manufacturers protocol (Invitrogen, Carlsbad, CA). The total RNA isolate was subjected to RNA purification using RNeasy mini elute columns (Kit 74102; Qiagen, Vencia, CA). RNA quality was assessed electrophoretically under denaturing conditions. Samples were deemed accessible if they showed a prominent 28s band and a lesser 18s band along with minimal evidence of degradation as previously described in our laboratory (Reiter et al., 2007). RNA concentration was determined using a Nano drop (ND-1000) spectrophotometer (Thermo Fisher Scientific; Wilmington, DE).

Complimentary DNA synthesis

Complimentary DNA (cDNA) was synthesized from the purified RNA isolate using high capacity cDNA Reverse Transcription kits (Kit # 4368814, Applied Biosystems; Foster City, CA). For each reaction, one microgram of DNAse treated total RNA was subjected to cDNA synthesis according to the manufacturer's protocol. Table X and Table X show the cDNA reaction components and thermocycling conditions for cDNA synthesis, respectively. The

resulting cDNA (50ng/μl) was used for quantitative Real-Time PCR (qRT-PCR) to determine the expression of specific target genes.

Selected Genes of Interest

The panel of selected genes for this study included genes involved in lipogenesis (FAS, CEBP/a, and PPAR-g), **Lipid Oxidation** (CPT-1b, PPAR-a), **mitochondrial function** (PGC-1a, UCP2), **Protein turnover** (PolyUb, UbE2, and PSDM 11), and **energy balance** (leptin). For this study genes expression was assayed in loin muscle (LM) and gastrocnemius muscle (GM) and in two subcutaneous AT depots (Rump AT and Deep SC AT). Expression was measured across 3 DOF groups (79, 121 and 142) and two treatment groups (CON vs. RAC). Table 4.6 shows the genes that were assayed for each tissue and treatment.

Gene Expression Assays

Assay Design

Custom TaqMan gene expression assays (Applied Biosystems; Foster City, CA) were designed for each of the genes of interest using the proprietary Applied Biosystems Custom TaqMan® Assay Design Tool (www.appliedbiosystems.com; Applied Biosystems; Foster City, CA). Available sequence data was obtained from the NCBI database (www.ncbi.nih.nlm.gov) for each gene and the online assay design tool utilized the genomic input information, specifically mRNA sequence data, to generate custom primers and a sequence-specific fluorescent probe for real-time gene expression analysis. For some genes of interest, there were available made-to-order kits which utilize existing/available sequence to design proprietary assays for commonly studied and/or thoroughly vetted genes.

qPCR Reaction Set-up

For each 20ul multiplexed qPCR sample the reaction components and PCR cycling conditions were as indicated in Table 4.4 and Table 4.5, respectively. For each reaction 1ul (50ng) of cDNA synthesis reaction product was diluted to 8ul in a 2ml nuclease-free micro centrifuge tube and placed on ice. A sufficient amount of master mix containing enough TaqMan gene expression master mix (Part No. 4369016; Applied Biosystems; Foster City, CA), 18S probe, and the custom gene expression assay was prepared for the tissue/gene of interest and then placed on ice. For each sample 12ul of master mix was added to the tube containing the 8ul cDNA solution. The reactants were gently mixed and then the 20ul reaction was pipetted into individual tubes in 0.2ml MicroAmp® Optical 8-Tube Strips (Part No. 4316567; Applied Biosystems; Foster City, CA) that were resting in a chilled PCR cooler. The strips were then capped with MicroAmp® Optical 8-Cap Strip (Part No. 432303; Applied Biosystems; Foster City, CA) and centrifuged gently to remove air bubbles and ensure all components of the reaction were at the bottom of the tube. For each tissue sample, the multiplexed assay was run in duplicate and this process was repeated for each gene/tissue combination.

Data Normalization

Gene expression data was normalized to an internal control gene to account for variation in initial cDNA input amounts. 18s ribosomal RNA was used as the endogenous loading control in the multiplexed real-time reactions. The 18s rRNA assay was designed with a separate, sequence-specific VIC®-labeled fluorescent probe and the cDNA's for the target gene and the 18s rRNA were co-amplified in a single, multiplexed reaction for each sample. Relative quantification (RQ) was determined using the ABI 7500 sequence detection system software (SDS; v2.0.3, Applied Biosystems, Foster City, CA) using the normalized expression data from the multiplexed reaction.

Statistical Analysis

Statistical analysis was done using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The least square means statement was used to compare average relative gene expression. Differences in least square means were considered significant at $P < 0.05$. Tissue (LM, GM, Rump Fat, LM Fat) Treatment (RAC vs. Control), Days on feed (DOF) and tissue*treatment*DOF interactions were analyzed. The main effects of tissue, treatment, and harvest date were considered in the model. The interaction of these effects was also considered.

Tissue Collection and Gene Expression Analysis

In the current project we profiled the expression of individual regulatory genes involved in protein turnover, AT development, and energy metabolism as heifers progressed through the finishing process. Additionally, the effect of Optaflexx®, a member of the BAA class of metabolic modifiers, on expression of these genes was determined. Skeletal muscle and adipose tissue samples were collected immediately after animals were harvested and were used for gene expression analysis. Heifers were transported to the USDA- inspected Auburn University Lambert-Powell meats laboratory and fasted for 24 hours prior to harvest. In retrospect, it may have been more prudent to collect biopsy samples prior to transport or fasting the heifers because these processes may affect gene expression patterns over the course of the 24-hour period. Changes in gene expression occur over the course of hours to days, and this should be considered with respect to our sampling process. Robust changes in expression could be diluted by molecular changes to accommodate the stress of transport and the shifts in the metabolic paradigm bought about by the 24hr fast. Of course these changes and stresses due to the sampling process cannot be completely eliminated even with the use of biopsy sampling etc., but steps can be taken to mitigate confounding factors particularly when metabolic genes are of

interest. In future studies this should be taken into consideration to obtain a more accurate snapshot of tissue-specific GE expression profile while the animal is on feed. With that said, there were still some notable changes in expression with respect to tissue, treatment, and DOF that will be discussed individually below.

SM Energy and Lipid Metabolism

Skeletal muscle growth and metabolism are essential to the efficient production of meat products from beef cattle. The longissimus muscle (LM) is a supportive muscle located along the spinal cord and is responsible for some of the more valuable retail cuts of beef including rib eye, top loin and strip loin. LM area is an important parameter used in selection programs as an indicator of muscling potential in breeding animals (Wilson et al., 1995) as well as an estimate of carcass composition in finishing animals. Additionally, LM area is used as an adjusting coefficient for USDA yield grading (USDA, 1996). There is a substantial amount of variation for IMF content between different muscles in the same animal (Brackebush et al 1991; Johnson et al., 1973), however there is a high correlation between the IMF content of the LM and other muscles over a range of IMF content (Brackebush et al., 1991) thus the LM has become the customary location for IMF assessment in beef cattle. Ultrasound techniques have been developed to predict marbling scores based on the IMF content of the LM (Newcome et al., 2002; Hassen et al., 2001; Wilson et al., 2001; Hassen et al., 1999; Amin et al., 1997). The LM therefore contributes to both the quality and yield grades according to the USDA system (USDA, 1996) and AT and protein metabolism within this muscle is important to production efficiency and profit margins.

Gastrocnemius muscle (GM) was selected because it provided a readily accessible muscle that differed physiologically and anatomically from the LM. The GM is a located in the

lower leg and is responsible for the bulge shape in the calf muscle. This locomotive muscle is involved in standing, running, walking, and jumping and does not have the retail value of the LM. Gonzalez at al., (2008) determined that there is a differential response to RAC between SM of different location and function and that RAC may be able to improve the value of certain low value cuts (Gonzalez et al., 2008). This also shows that results cannot simply be extrapolated from muscle to muscle.

Skeletal muscle is one most metabolically demanding tissues in the body. The goal of meat production is to maximize SM growth and optimize the nutrient content to produce valuable meat products. These objectives are governed by tightly regulated metabolic pathways. Physiologically these pathways are responsible for nutrient partitioning, protein turnover, energy homeostasis etc., but from a production standpoint these pathways determine the growth and composition of SM tissue and affect profit margins. Given the two functionally, anatomically, and economically distinct muscles (LM and GM), we profiled the expression of genes involved in protein turnover and adipogenesis/lipid metabolism to gain a perspective into how days on feed and RAC affects metabolism in these tissues.

Skeletal Muscle Gene Expression

Lipid Metabolism: Triglycerides (TG) are a primary storage form of neutral lipids in adipocytes, and in the SM serve as an important source of energy for SM contraction and function. Increase in IMF is generally due to increased accumulation of TG (Gao and Zhao et al., 2009) making TG metabolism an attractive target for biomarkers of IMF accumulation (Gondret et al., 2008). In meat animals TG accumulation in the SC depot results in the wasteful deposition of costly feed energy, and accumulation in the IMF results in expansion of the valuable marbling depot. Thus depending on the tissue of interest, TG accumulation can represent a paradoxical

phenomenon. We profiled the expression of genes related to adipogenesis and lipid metabolism in skeletal muscle tissue from the finishing heifers in the current project the current project as these factors likely regulate the extent to which lipid accumulation occurs in response to days on feed and a BAA repartitioning agent.

PPARg expression did not differ between control and RAC treated heifers ($P > 0.05$; Figure 4.6). BAAs enhance production efficiency by decreasing AT accumulation and directing nutrients to SM for protein synthesis. It is possible that the adipocytes in the IMF are not as responsive to RAC as in other depots. It has been shown that the IMF depot shows differential response to nutritional and hormonal stimuli compared to the SC and other AT depots (Hausman et al., 2009; Dodson et al., 2010). These metabolic distinctions could lead to a lack of response of IMF to the BAA compounds.

Interestingly, relative expression of PPARg decreased from 70 to 142 DOF in the SM of control heifers ($P < 0.05$; Figure 4.7). PPARg drives the adipogenic program and we expected increased PPAR gamma expression in SM tissue as DOF increases as this would be indicative of enhanced lipid metabolism and AT accumulation during this timeframe. It is not clear why expression would decrease in SM of control heifers as DOF progressed. PPARg was increased in RAC heifers at 142 DOF compared to 72 DOF ($P < 0.05$; Figure 4.8). This may indicate that RAC was more effective at limiting adipogenesis in heifers harvested at the earlier portions of the finishing period as opposed to the latter phases. Because of the effects of age and maturity on fattening, the temporal aspects of RAC on AT development warrant further investigation. Additionally, because IMF is a metabolically distinct depot that is inherently late developing, the optimal timing of BAA administration must be determined to mitigate potential interference with the critical time window for marbling development.

Pref-1 expression was highest in the SM of control animals at 79 DOF compared to RAC treated heifers ($P < 0.05$; Figure 4.12). There were no differences between the two treatment groups at 142 DOF ($P > 0.05$ Figure 4.12). RAC treated heifers had lower Pref-1 expression compared to control heifers ($P < 0.05$; Figure 4.9), and GM showed higher expression levels compared to LM ($P < 0.05$ Figure 4.11). For Pref-1 there was a significant interaction effect of treatment and SM tissue ($P < 0.05$; LM vs. GM). Expression of Pref-1 was much higher in the GM of control animals compared to the LM of these same animals and compared to LM and GM of RAC treated heifers ($P < 0.05$). We found no difference between LM and GM in RAC treated animals in the current study (Figure 4.13; $P > 0.05$).

Glycerol-3-phasphate acyl transferase (GPAT) is involved in esterification of acyl groups to glycerol-3-phosphate in the process of TG biosynthesis, which is a metabolic characteristic of mature adipocytes actively synthesizing and depositing lipid. Tissue and treatment did not have an effect on GPAT expression (Figure 4.14 and 4.15; $P > 0.05$).We found that GPAT expression was nearly 3 times higher at 142d compared to 72d (Figure 4.16; $P < 0.05$). This may indicate that deposition of lipid increased as the IMF depot matures which is consistent with the general school of thought regarding marbling maturation (Du et al., 2010.) Jeong et al., 2012 determined the expression of lipid deposition and lipolytic genes in LM of Korean cattle and then developed a predictive model to determine IMF based on gene expression. Among 9 fat deposition genes they determined that GPAT showed the highest correlation with IMF ($r = 0.74$; $P < 0.001$). They also showed that adipose tissue triglyceride lipase (ATGL), a lipolytic gene, was negatively correlated with IMF in these cattle ($r = -0.68$; $P < 0.001$). These data show that metabolic gene expression is important in determining the IMF phenotype and that expression profiling may be a viable tool in predicting IMF content. The

cattle in the current experiment did not show substantial differences in marbling scores between treatment groups (Grubbs et al., 2009) which is also reflected in our gene expression analysis for GPAT.

Carnitine palmitoyl transferase 1b (CPT1b) is rate-limiting for the oxidation of long chain fatty acids via mitochondrial beta oxidation in SM. The function of CPT-1b is to facilitate the transfer of LCFA from the cytoplasm across the mitochondrial membrane to access the machinery for β-oxidation. For CPT-1b expression only the effect of harvest date was significant ($P < 0.05$; Figure 4.19). Loin muscle tended to have higher expression of CPT-1b but this was not significant ($P = 0.12$; Figure 4.18). RAC and control heifers did not differ ($P > 0.05$; Figure 4.17). From a metabolic standpoint it was interesting to note that while GPAT expression was up at 142 DOF, CPT-1b expression was lower. This could possibly indicate a metabolic shift towards lipid accumulation and away from oxidation as days on feed increased. CPT-1b expression was more than 3 times higher in muscle tissue samples taken at 79d compared to 142d ($P < 0.05$; Figure 4.19). Given its role in energy oxidation, CPT-1b expression may be used to infer the metabolic disposition in a given tissue. Finishing animals are expected to deposit lean muscle and as a result of dietary energy provision also deposit fat in specific depots. High CPT-1b expression at the earlier harvest date in the IMF depot may indicate the use of IMF as an energy source for the surrounding SM as protein deposition occurs. Later in the finishing phase when relative rates of protein deposition decline and IMF deposition occurs (Du et al., 2010; Hausman et al., 2009;), decreased CPT-1b expression could allow for accumulation of the IMF responsible for the all-important marbling trait in beef cattle. It would be interesting to profile the histological and biochemical activities of the IMF depot and relate it to gene expression data to understand the metabolic progression of this depot during the finishing period.

While the IMF depot is generally cited as the last major AT depot to mature**,** Pethick et al. (2004) argue that IMF is not a late maturing AT depot and that it develops at a similar rate to other AT depots. They believe this common misconception arises because as an animal matures the rate of fat deposition will increase relative to lean deposition, and the visual appearance of IMF increases. Hence, the commercial trait "marbling" is indeed late maturing; however this does not necessarily reflect IMF development (adipogenesis and lipid filling) which occurs at a time and rate comparable to other depots. They also contend that an important determinant of final levels of IMF after finishing is the initial amounts of IMF (i.e. number and size of preadipocytes/adipocytes present in the depot) before entering the feedlot. With the heifers in the current study being relatively advanced composition-wise, it may have been difficult to observe more concrete differences in expression patterns due to the metabolic shifts that occur as the animal matures. Aoki et al. (2001) reported that IMF does not increase beyond a "maximum value" point due to decreased feed intake and less changes in carcass composition as animals reach maturity.

Our data support the notion of a temporal pattern of AT metabolism with changes in GE for key genes however due to limited time points and sample size did not reveal any discernible mechanism for lipid metabolism with respect to treatment and DOF. A more complete temporal landscape representing animals at different ages and compositional phenotypes is necessary to make comprehensive assessments of GE profiles and relate them the patterns of growth and development. This is true both with respect to natural maturation patterns as well as with the use of BAA which have been shown to have differential effects on younger versus older animals. Also, because there are metabolic distinctions between AT depots, it can also be assumed that the specificity and proportion of BARs would also vary between depots. It would be interesting

to focus on isolated intramuscular adipocytes and determine their complement of β-receptors. This may provide some insight into the molecular basis of the RAC response on a depot specific basis with the ultimate goal of being able to target and manipulate these depots independently of one another.

Skeletal muscle is a heterogeneous tissue that has a complex assortment of cell types (including adipocytes) all contributing to the anatomy and physiology of the tissue. mRNA abundance is one of many ways to assess the metabolic disposition of the tissue however like most techniques it does not stand alone as a comprehensive tool. Still, this approach of molecular profiling in the IMF depot is novel and will prove to be valuable in ascertaining the regulatory factors that govern this depots expansion. In a complex tissue such as SM, metabolic control depends on the activity of transcription factors which modulate nutrient flux through various metabolic pathways (Desvergne et al, 2001) and the mRNA abundance for these transcription factors may be useful in determining their activity *in vivo* (Bennett et al., 2008). The presence of the IMF depot within the larger SM tissue both necessitates and facilitates specific methods for inferring metabolic activity and adipogenesis within this tissue. The molecular tools for this assessment in beef cattle are becoming more widely available and, while their potential for commercial application must be thoroughly evaluated, their possibility of depot specific intervention is highly coveted (Dodson et al., 2010a; Dodson et al., 2010b; Hausman et al., 2009).

Mitochondrial Function: Mitochondria are responsible for 80-90% of cellular ATP production (Szewczyk and Wojtczak, 2002) therefore we also profiled mitochondrial genes that have roles in energy oxidation and metabolism. Mitochondrial uncoupling protein 2 (UCP-2) is a member of the larger UCP family which function to dissipate the mitochondrial proton gradient

and separate mitochondrial oxidation from ATP production. UCP-2, which is expressed in bovine adipocytes and skeletal muscle, has been shown to be involved in feed efficiency in poultry (Raimbault et al., 2001), however Kolath et al. (2006) did not see differences in UCP expression in loin muscle from high versus low RFI cattle. In skeletal muscle, there were no effects of treatment tissue, or harvest date on UCP2 expression ($P > 0.05$; Figures 4.20, 4.21, and 4.22).

Protein Turnover: Protein turnover is the sum of protein synthesis and degradation and the balance of these processes determines net protein accumulation within a cell or tissue. Because these processes are energy dependent, they have a direct impact on the efficiency and profitability of livestock production (Bergen, 2008). Additionally, the efficiency of muscle growth is inextricably tied to meat production and it is critical to understand the dynamics of protein turnover to maximize beef cattle productivity (Bergen, 2008). This is important not only to SM protein metabolism as it pertains to lean tissue accretion, but also because protein metabolism is closely tied to energy metabolism. Peptide bond formation is estimated to require 4 ATP equivalents and degradation is estimated to require one. Given this energy requirement, increased rates of protein turnover can prove to be energetically demanding and detrimental to production efficiency and whenever repeated rounds of protein turnover can be avoided, there is a net savings on metabolic energy (Bergen, 2008; Bergen and Merkel, 1991). Metabolic redundancies or inefficiencies identified in this process are likely to contribute to variations in production efficiency in cattle and other species (Bergen 2008; Bergen and Merkel, 1991). BAAs have been shown to increase lean protein deposition by modulating the balance of protein degradation and synthesis in favor of net accretion of protein.

Protein degradation has traditionally been determined by differences between measurements of fractional protein accretion and synthesis rates, by measurement of urinary 3-methyl histidine excretion, or by measurement of the activity of known proteases within the SM tissue (Goll et al., 1998). Skeletal muscle contains three primary systems for proteolysis. These include the ATP-dependent ubiquitin proteasome system, a lysosomal system, and a calcium dependent (calpain/calpastatin) system. It should be stated that more diverse functions for ubiquitin in cell signaling have recently been identified including protein trafficking and signal transduction (Welchman et al., 2005) but it is generally accepted that the much of SM proteolysis is accomplished via the UPS system (Attaix et al., 1998; Solomon and Goldberg, 1996). Changes in the activity of components of the UPS is primarily responsible for protein turnover dynamics under different physiological conditions including periods of disease induced proteolysis, autophagy and sepsis (Goll et al., 2008; Reinstein and Ciechanover, 2006; Minnard et al., 2005; Hasselgren et al., 1997); however the dynamics of this system in growing, healthy animals are less clearly understood (Bergen, 2008).

The 76 amino acid protein ubiquitin is regulatory protein that targets proteins for degradation via the UPS. There were no differences in polyubiquitin expression between control and RAC treated animals ($P > 0.05$; Figure 4.26). We observed a 20% decrease in polyubiquitin expression from 79 to 142 DOF in SM tissue ($P < 0.05$; Figure 4.27). There was substantial variation in both treatment groups however the average relative expression between the groups was very similar. There was no significant interaction between kill date and treatment for polyubiquitin expression ($P = 0.15$). There was no difference in ubiquitin expression between control and RAC heifers at 142 DOF ($P > 0.05$; Figure 4.28).

Ubiquitin E2 enzymes (UbE2) also known as ubiquitin conjugating enzymes are responsible for the second step in the covalent attachment of ubiquitin to target proteins destined for proteolytic degradation by the UPS. Ubiquitinated proteins are then recognized and hydrolyzed by the regulatory and core particles of the 26S proteasome, respectively. There were no differences in expression UbE2 between harvest dates (Figure 4.29 $P > 0.05$) and treatment did not affect UbE2 expression in SM samples (Figure 4.30 $P > 0.05$).

While ubiquitin and E2 are responsible for targeting proteins to the proteasome, PSDM11 is a non-ATPase regulatory subunit of the 26S proteasome itself. Ubiquitinated proteins are targeted to and recognized by the regulatory subunits of the proteasomes which are found in the cytosol and nucleus of various cells. As a regulatory unit, the expression of PSDM11 can be used to monitor the activity of the UPS system. Ractopamine did not affect the expression of PSMD11 (Figure 4.32; $P > 0.05$) however expression tended to be higher in LM vs. GM (Figure 4.33 $P < 0.10$). As mentioned earlier, these muscles represent physiologically distinct muscle groups which have different fiber type composition and different metabolic profiles. The functional demands of these different muscles may be responsible for differential protein metabolism between these two tissues. At 142 DOF, expression of PSDM11 was increased by nearly 3 fold compared to at 79 DOF ($P < 0.05$; Figure 4.34). Increased protein turnover can potentially be detrimental to muscle growth if rates of protein synthesis are not comparably increased. Additionally, individual animal variation in PSMD11 may contribute to variable efficiency and response to BAA administration. Lower levels of protein turnover at earlier time points may be indicative of rapid net protein accretion in these growing/finishing animals earlier in the finishing period. Unfortunately we did not assay for parameters of protein synthesis which would

have provided a complementary data set and a more comprehensive analysis of SM protein metabolism in these finishing heifers.

There are several examples of UPS modulation in response to physiological status in farm animals. During lactation induced SM proteolysis, transcriptional upregulation of UPS components has been observed in dairy cattle (Clowes et al., 2005). UPS expression has also been monitored in growing chickens (Harper et al., 1999); however it is unclear how these relate to growth rate and efficiency. Given the known effects of RAC on lean tissue accretion and the purported effects on protein turnover we expected to see marked differences between control and treatment animals with respect to UPS marker genes. The effects of RAC and DOF on protein metabolism were not evident with the panel of marker genes we selected and may occur through other mechanisms affecting protein turnover. There were no differences in expression of ubiquitin E2 conjugase between treatment groups or across days on feed. These results are mainly consistent with polyubiquitin expression which was not different between treatment groups or DOF with the exception of the 142DOF control animals which appeared to have slightly lower expression of polyubiquitin compared to other groups. This would seem to indicate increased protein turnover by way of the ubiquitin proteasome system, however we did not observe significant increases in other components of the UPS that me measured. It is unclear the extent to which this modest decrease in expression effected protein turnover in these tissues and more extensive biochemical analyses should be done to further investigate this process.

We did not observe marked down regulation of the components of the UPS that we tested however and it may be necessary to look at specific ubiquitin ligases such as those mentioned above before making an affirmative conclusion on the involvement of the UPS in the RAC response in finishing heifers.

The exact mechanism by which BAAs increase net protein deposition in cattle is not clearly understood. We looked primarily at genes involved in the UPS to compare rates of protein turnover (in the form of degradation); however this is only one component of the protein turnover equation. We did not measure protein synthesis in the current experiment, but it should be mentioned because despite not observing marked changes in proteolytic machinery, it cannot be conclusively stated that the overall dynamics of protein turnover where not shifted in response to DOF and/or treatment group. In addition, as mentioned previously, there are 2 other major proteolytic systems responsible for protein degradation in the cell. Aspects of these systems could be contributors to the apparent enhanced protein accretion observed with RAC administration. For example Pascual et al. (1993), observed increased cathespin A activity with administration of a mixed, non-selective BAA in SM of rats treated with. Cathespin A is an indicator of lysosomal protein degradation and upregulation of this system may be responsible for the effects of RAC however we did not measure expression of genes involved in lysosomal proteolysis. Wheeler and Koohmarie (1991) treated cattle with $L_{644,939}$ and they attributed the hypertrophic effects of this BAA to increased activity of calpastatin which is the inhibitory component of the calpain/calpastatin system of protein degradation. They observed improved feed efficiency, reduced urinary nitrogen excretion, and increased calpastatin activity in BAA treated animals. This increased calpastatin activity would decrease the activity of this proteolytic system. While our current study used RAC, it could be possible that components of the calpain/calpastatin system were involved with the effect of RAC on protein turnover although one must be careful in generalizing results between different BAAs.

BAAs have been shown to increase cross sectional area of muscle fibers contributing to the hypertrophy of SM tissue (Gonzalez et al., 2008; Aalhus et al., 1991). Several studies have

looked at expression of myofibrillar genes to explain the increased protein synthesis in SM in response to BAA administration. α-actin mRNA abundance increased in pigs (Bergen et al., 1989) and sheep (Koohmaraie et al; 1991) treated with BAA. These effects have not been directly linked to elevated cAMP in response to BAR activation, however they point to increased capacity for protein synthesis in these models. Smith et al. (1989) conducted a study in which they used Northern blot analysis to assay the expression of a putative myosin heavy chain (MHC) cDNA clone and determined that RAC either increased expression of this MHC gene or increased the stability of the mRNA either of which could lead to increases in SM protein synthesis. Anderson et al. (1990) incubated cultured rat myotubes with RAC to determine the effect on protein synthesis. They showed that RAC stimulated protein synthesis and increased the apparent rate of MHC synthesis. Direct measurements of protein synthesis were not taken in the current study but the lack of differences between control and RAC treated heifers suggests that other dynamics of protein turnover are likely in play with respect to the mechanism of RAC action on SM tissue in beef cattle. We did not look at MHC expression or muscle fiber morphometrics however these are important considerations when making conclusions about muscle protein metabolism.

In farm animal species there is a wide range of efficiency with respect to protein synthesis (Bergen, 2008). Theoretically the efficiency of protein synthesis is projected to be around 80%, however much lower efficiencies have been estimated (Bergen, 2008; Bergen and Merkel, 1991). Increased protein turnover can potentially be detrimental to muscle growth and profits if rates of protein synthesis are not comparably increased. While metabolic processes such as protein synthesis and degradation have fixed thermodynamic efficiencies, the aggregate impact of these processes is likely amenable to genetic selection and therapeutic intervention

once the molecular basis of these processes are more thoroughly understood. It would be interesting to compare our gene expression results with measures of protein synthesis (i.e. RNA/Protein ratio, or other translational machinery) to gain a comprehensive perspective on the rates and extent of protein synthesis as days on feed progressed and as affected by RAC. Emerging techniques in proteomics and other molecular innovations will allow for more sophisticated assessments of protein turnover in efforts to regulate this process and enhance the efficiency of muscle growth in meat producing animals (Bergen, 2008).

There are currently very few reports regarding direct measurements of protein turnover in cattle receiving RAC. These data would be valuable both in ascertaining the direct mechanism of RAC action in cattle, but also in corroborating molecular data on the regulation of the process in specific target tissues. Walker et al. (2011) conducted a study to determine the effect of gender and RAC administration on protein turnover in steers and heifers. They determined that there was a differential effect of RAC on biceps femoris and LM muscle and that RAC decreased protein degradation rates in LM but did not affect rates in biceps femoris. They also reported that RAC did not affect protein synthesis rates isolating its effects to protein degradation. Their study, not unlike many preceding it, utilized various measures of protein synthesis/degradation to determine rates of protein turnover. We utilized a targeted gene expression approach which is relatively novel and does not calculate direct rate of synthesis and/or breakdown but utilizes molecular information to infer the activity of the governing pathways. This data should be reconciled with biochemical protein turnover assays to determine and optimize the efficacy of this approach. Identification of sentinel genes that can report on protein turnover will provide an innovative tool to determine the efficiency of protein metabolism which directly impacts muscle growth and the production efficiency in the beef cattle industry.

Adipose Tissue Metabolism: RAC treated heifers showed PPARg expression levels that were twice that of the control animals ($P < 0.05$). This profile is contrary to what would be expected given the purported mechanism of BAA action on adipose tissue which includes increased lipolysis and decreased AT accumulation in treated animals. PPARg increased, although not significantly from 70 to 142 DOF ($P < 0.10$). This trend although not significant, is expected as animals would increase fattening as DOF increase. Additionally we saw an opposite pattern in FABP4 expression which is peculiar because FABP4 is a target of PPARg (Hausman et al., 2009) and expression is increased as adipocytes reach terminal differentiation. Therefore PPARg should coordinately regulate FABP4 expression as demonstrated by Graugnard et al. (2009). In their study Graugnard et al. (2009) fed cattle high or low starch diets to produce divergent metabolic profiles. They showed that PPARg regulates a host of target genes that are involved in adipogenesis and lipid accumulation. One possible reason for the expression patterns observed in the current study may be due to the physiological stage of the heifers compared to the young calves used in the Graugnard study which would likely influence the dynamics of lipid metabolism. The steers used in the Graugnard study were at a much younger compositional and chronological age and this may be responsible for the more robust expression patterns observed in their study with respect to days on feed.

RAC treated heifers had showed PPARg expression levels that were double that of the control animals. This profile is contrary to what would be expected given the purported mechanism of BAA action on adipose tissue which includes increased lipolysis and decreased AT accumulation in treated animals.

RAC heifers had lower expression of FABP4 compared to control heifers ($P < 0.05$; Figure 4.40). The decrease in FABP4 could be a result of decreased lipid accumulation in the

subcutaneous AT depot. These expression data should be compared to carcass data to determine if the phenotypes substantiate the expression profiles. There were no effects of tissue or harvest date on FABP4 Expression ($P > 0.05$; Figure 4.40, 4.41, and 4.43). There was substantial variation in FABP4 expression within and between treatment groups. This may have contributed to the lack of significant differences in FABP4 expression patterns.

Expression of UCP-2 was affected by treatment and by harvest date.There was substantial variation within each harvest date but expression at 79 DOF was low relative to 142 DOF which was nearly two-fold higher (Figure 4.43; $P < 0.05$). Animals treated with RAC had higher expression of UCP2 compared to control animals (Figure 4.43; $P < 0.05$). This result is interesting because increased UCP-2 activity in AT could lead to oxidation of FA and reduced AT accumulation although it is difficult to reconcile this with increased feed efficiency. SC AT location (Deep SC vs. Rump) did not affect UCP2 expression (Figure 4.44, $P > 0.05$).

Summary and Conclusions

Much of the role of energy substrates and how they provide fuel for SM have been investigated in human and rodent species and driven by interests in exercise physiology and more recently the impacts of ectopic lipid accumulation on risk factors for diabetes etc. (Watt et al., 2004; Watt et al., 2002). In meat animals, interest in energy metabolism is driven by the desire to manipulate energy partitioning towards valuable tissues and away from less valuable ones. This longstanding objective has been assisted with the application of metabolic modifiers and repartitioning agents; however there is still a dearth of information on the specific mechanisms of action and effects of these compounds on meat quality. The molecular processes involved have been scarcely evaluated in cattle but interest in these mechanisms as tools to improve beef production is emerging. The current experiment did not identify specific

mechanisms that are responsible for the repartitioning effect of the BAA Optaflexx but we did observe differential expression of some of the target genes in response to DOF and RAC treatment as well as distinct expression between depots and tissues.

Fattening is affected by nutrition, gender, and age in cattle (Hausman et al., 2009). The heifers in this study were relatively advanced on their growth and compositional curve (Grubbs et al., 2008) which may have impacted the gene expression profiles observed both with respect to days on feed and response to Ractopamine treatment. Marbling is considered to be a late developing trait and some believe adipogenesis in this depot occurs later than in the SC depot (Hausman et al., 2009) so profiling during the latter phases of the finishing process are certainly informative in terms of the molecular regulation of the expansion of the IMF depot. However, populations of progenitor cells in the IMF and other AT depots are determined earlier in the developmental process (Du et al., 2010) which provides yet another time frame for which a molecular profile needs to be determined. Perhaps more importantly, this may provide an earlier time frame in which superior animals can be identified and nutritional regimens can be modified to optimize production. Much of the fattening that occurs during the finishing phase is a result of adipocyte hypertrophy as oppose to increased adipocyte number (Hausman et al., 2009). The populations of progenitor cells that undergo this hypertrophy is established during prenatal, early postnatal, and adolescence in beef cattle (Du et al., 2010). Therefore much of the dietary energy provided during the finishing process is used to reach a desired level of fatness with the ultimate goal of driving some of those nutrients to the IMF depot which has the lowest priority in terms of adipogenesis and nutrient partitioning compared to the SC and visceral AT depot (Du and Dodson 2011; Hausman et al., 2009). This makes future approaches two-fold. Understand how these resident populations are established in various depots so that populations of progenitor

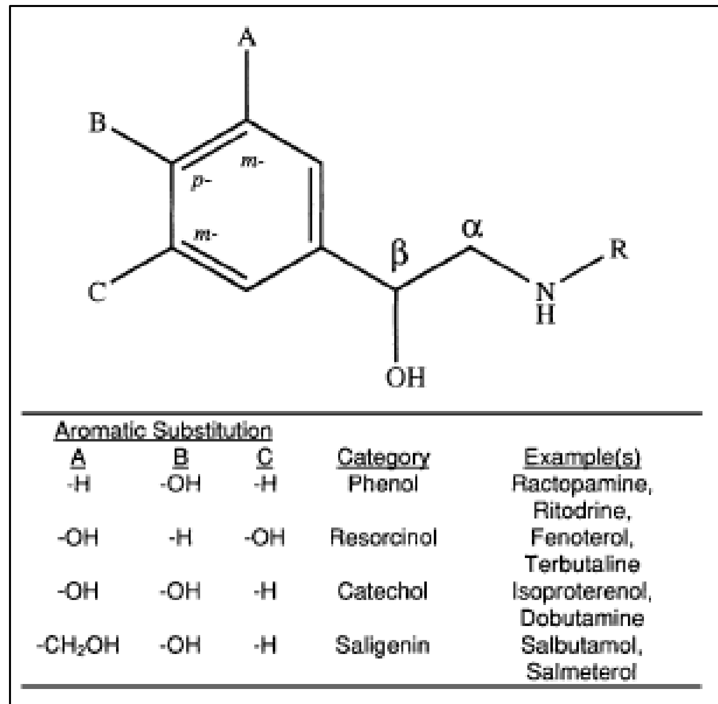
cells in low commercial value depots can be minimized thereby limiting their capacity to expand and the nutrient demand needed to support this expansion; and to understand how depot specific regulation coordinates and prioritizes AT depots for nutrient partitioning and adipogenic expansion.

Future work should focus on relating gene expression data with performance data and histological analysis of target tissues to understand how molecular regulation governs tissue structure and function and gives rise to desirable phenotypes that support a profitable industry. The limited availability of resources for the current study may have hampered our ability to garner a comprehensive perspective of the GE profiles across the entire feeding period, but for the selected harvest dates we did observe some notable changes in expression between treatment groups and across DOF assignments. Future studies utilizing all of the sampling dates will augment the current data set and provide GE snapshots of cataloging the temporal changes in expression that give rise to specific beef cattle phenotypes. The ability to identify and monitor the expression of these regulatory factors has only recently been reported (many since the inception of this project) and has been met with variable success. Advances in sampling techniques, tissue and cell manipulation, and increase availability of genomic information will lead to more innovated approaches to monitor and modulate metabolic pathways via genetic selection or direct manipulation in order to improve the efficiency of beef cattle production. An improved understanding of how and when to administer BAA will maximize their efficacy and knowledge of their specific molecular targets will allow for advanced compounds that capitalize on their benefits and minimize the detrimental and/or unwanted side-effects of these metabolic modifiers. Moreover, it will provide mechanistic perspective on how nutrient partitioning and

tissue priority is established and manipulated providing a valuable approach to efficiently manipulate carcass composition in beef cattle.

Chapter 4 Figures

Figure 4.1 General structure of beta-adrenergic agonists including common aromatic substitutions[1]

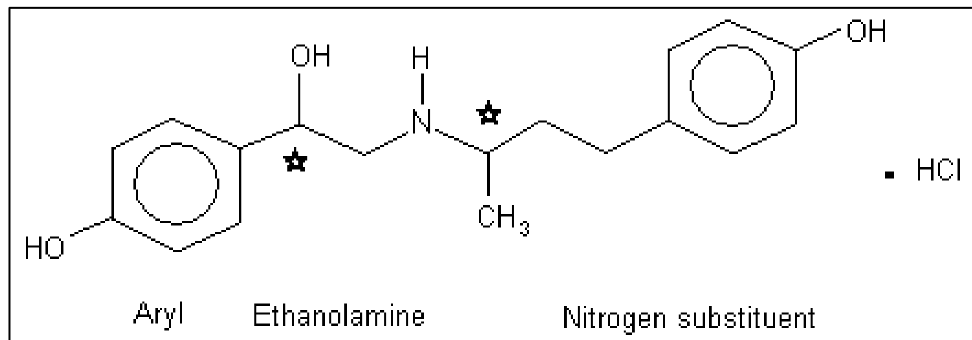


Aromatic Substitution

A	B	C	Category	Example(s)
-H	-OH	-H	Phenol	Ractopamine, Ritodrine,
-OH	-H	-OH	Resorcinol	Fenoterol, Terbutaline
-OH	-OH	-H	Catechol	Isoproterenol, Dobutamine
$-CH_2OH$	-OH	-H	Saligenin	Salbutamol, Salmeterol

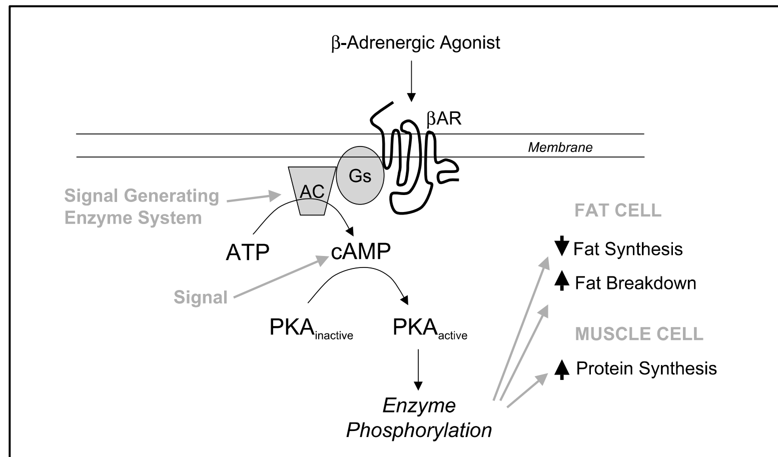
[1]Substitutions about the aromatic ring impart distinct chemical structure and function on the family of molecules; The R- group is generally a bulky chemical group such as a t-butyl or isopropyl group; *m-* and *p-* designated the meta- and para- positions relative to the □-carbon of the phenolethanolamine molecule, respectively; From Smith D.J. 1998. J. Anim. Sci. 76:173-194.

Figure 4.2 Chemical structure of Optaflexx® (Ractopamine HCl)[1]



[1]Stars indicate Chiral Centers on the Ractopamine Molecule; Specific stereochemistry is not indicated on this figure; Optaflexx is a registered trademark of Elanco Animal Health (Greenfield, IN).

Figure 4.3 Putative β-adrenergic agonist mechanism of action via β-adrenergic receptor[1]



[1]βAR = β−Adrenergic Receptor; PKA = Protein Kinase A; cAMP = Cyclic Adenosine Mono Phosphate; AC = Adenylate Cyclase; Gs = G-Stimulatory Protein; Binding of the BAA to the BAR results in activation of adenylate cyclase causing an increase in intracellular cAMP. This activates protein kinase A (PKA) which initiates a phosphorylation cascade resulting in modulation of specific cellular processes in adipose tissue and skeletal muscle including those noted in the diagram; Adapted from Hancock et al., 2006.

Table 4.1 Composition of diets fed to animals during the feeding trial period[1]

Ingredient	Percentage
Corn	38.5
Corn Gluten Pellets	17.5
Cottonseed Hull Pellets	10
Dried Distillers Grain	9.5
Wheat Midds	6.5
Soyhulls	6.5
Cottonseed Hulls	5
Molasses	2.5
Limestone	1.25
Bicarb	1
Fat	1
Salt	0.5
Vitamins A,D,E	0.1
Trace Minerals	0.1
Rumensin 80	0.019

[1]As Fed Basis. Dry Matter= 90%, CP=13.7%, NEgain00.78Mcal/Kg, NDF=32.2%, ADF= 15.7%; Adapted from Grubbs et al., 2008

Table 4.2 High-capacity cDNA synthesis reaction components[1]

Component	Volume/Reaction (μl)
10x RT Buffer	2
25x dNTP Mix (100mM)	0.8
10X Random RT Primers	2
MultiScribe Reverse Transcriptase	1
Nuclease Free Water	4.2
Total Reaction Volume	10

[1]Individual Reaction components for complementary DNA synthesis from total RNA isolate. For each tissue a master-mix containing sufficient quantities of the individual reaction components was created and cDNA synthesis was conducted for each RNA sample; RT- Reverse Transcription; dNTP- deoxynucleoside triphosphates

Table 4.3 cDNA synthesis reaction thermocycling conditions[1]

	Step 1	Step 2	Step 3	Step 4
Temperature (°C)	25°C	37°C	85°C	4°C
Time	10min	120min	5min	Hold[3]

[1]Reaction conditions for the 4 step cDNA synthesis reaction; Temperatures were controlled in PCR thermocycler; upon completion reactions were held at 4°C until samples were stored for further use.

Table 4.4 Individual Reaction Components for Real-Time PCR Gene Expression Analysis[1]

Component	Volume
TaqMan Gene Expression Master Mix	8ul
Vic-Labeled 18S rRNA Probe[2]	1ul
Custom TaqMAn Gene Expression Assay	1ul
cDNA + H20	10ul
Total Reaction Volume	20ul

[1]Volumes shown are for individual Real-Time PCR reactions. For each gene a sufficient amount of master-mix with the appropriate amount of each component was generated to ensure consistency of the mix for all samples; 18S Vic Labeled 18S rRNA probe for Data Normalization in Multiplexed Reaction; cDNA = Complementary DNA.

Table 4.5 Real-Time PCR thermocycler reaction conditions[1]

Step	Description	Time	Temperature
1	AMPerase UNG[1] Activation	2 min	50°C
2	Taq[2] Activation/UNG Inactivation	10 min	95°C
3	Polymerization	15 sec	95°C
4	Primer Annealing/Detection[3]	1 min	60°C
5	Repeat Steps 3-4 for 40 cycles	NA	NA

[1]UNG = AMPerase Uracil N Glycosylase- Prevents Carryover Contamination; Taq = Thermostable Taq DNA polymerase; Fluorescence Detected at Step 4

Table 4.6 Gene-Specific primer and TaqMan® probe sequences for the genes and tissues of interest[1]

Target Gene	Accession Number[1]	Forward Primer	Reverse Primer	MGB-Probe
PPAR-α	AF229356	TTGCCGGGAAGACCAACA	CCATACACAGCGTCTCCATGTC	ACAACCCCGCCTTTCT
Poly-ubiquitin	NM_174133	ACCTGGTCCTCCGTCTGA	CCTCCAGGGTGATGGTCTTG	CCGGTCAGGGTCTTCA
UCP2	AF127029	GGACTCTGGAAAGGGACATCTC	CCAGCTCAGCACAGTTGACA	TCGCTCGCAATGCCAT
FAS	NM_00102669	CCTGGCCTTCGTGAGCAT	AGCACGGCGTAGCCA	CAGCAGCCATGCCCTT
PGC-1 α	AB10607	CGAGAATGAGGCTAGTCCTTCCT	GCTTCTTAAGTAGAGACGGCTCTTC	CTGACGGCACCCCTC
PPAR-γ	Y12420	CCGCTGACCAAAGCAAAGG	AGTTCATGTCATAGATAACAAACGGTGAT	TTTCCCGTCAAGATCG
Cpt1-b	NM_001034349	GACTGGCAGCCCTCACT	CTTGTTCTTGCCAGAGCTGAAG	CCCACTCCACTCTTCC
Pref-1/Dlk1	NM_174037.2	CGGAGGCAGCTGCGT	GCAGAAGTTGCCCGAGAAG	CCCCACGCTGTCTGC
Leptin	NM_173928	GCCTTCCAGAAATGTGGTCCAAATA	CGGCCAGCAGGTGGA	CCTCCGGGACCTTC
GPAT	NM_001012282	GGACTGACCTCTCTGGACTCT	CGGAGCAGCAGCTCTTAGTG	CCCGCCTGTGACTTC
Ubiquitin E2 Ligase	NM_001099372.1	AGTTCATGCATAGATA	GCCTTCCAGAAATGTGGTCCA	

[1]Accession Number = NCBI Accession Number; MGB-Probe = FAM-labeled TaqMAN minor groove binding probe; CPT-1b = Carnitine Palmitoyl Transferase 1b; FAS = Fatty acid synthetase; GPAT = Glycerol phosphate acyl transferase; PPAR-α = Peroxisome Proliferator Activator Protein-α; PGC-1α = Peroxisome Proliferator Activator Protein Coactivator-1α; PPAR-g = Peroxisome Proliferator Activator Protein-γ; Pref-1 = Preadipocyte factor 1 (aka Dlk-1); UCP-2 = Uncoupling Protein 2.

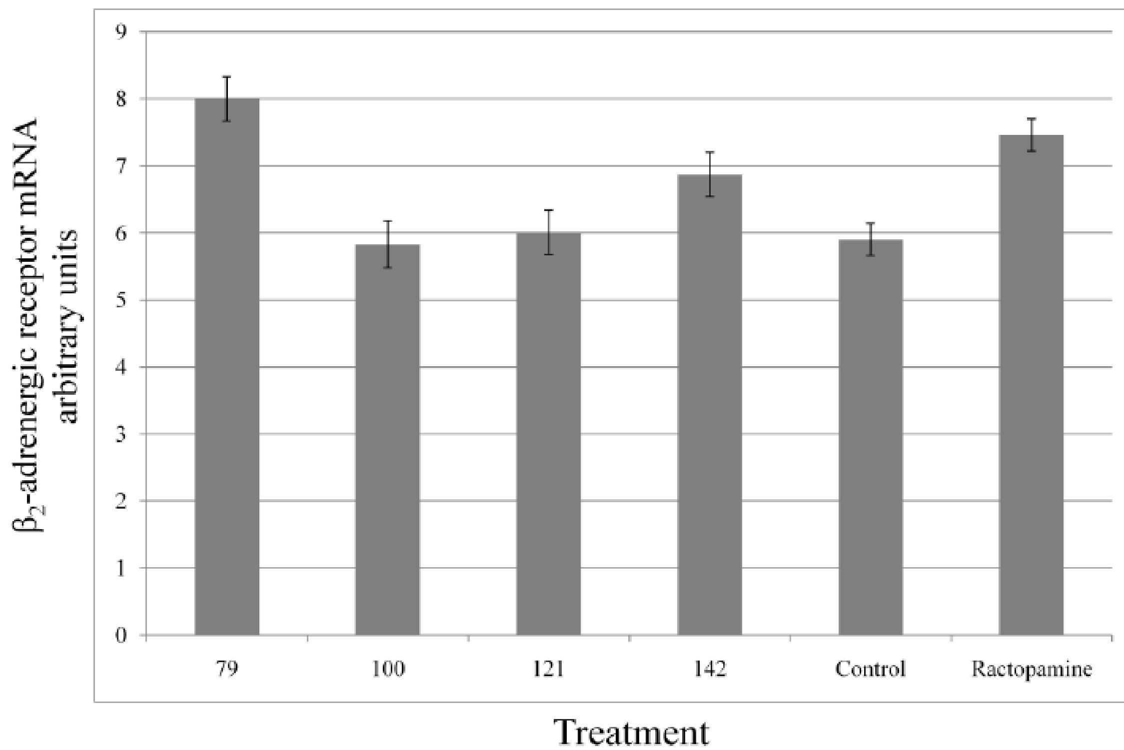
Animal Performance (from Grubbs et al., 2009)

Table 4.7 Animal performance during the treatment period (final 35 DOF for each group)[1]

Performance Measure	Treatment		SEM	P- Value
	Control	RAC		
Trt ADG (kg/d)	1.1	1.24	0.04	0.02
Trt Gain (kg)	38.6	43.7	1.5	0.02
Trt DMI (kg/d)	329	322	5.5	0.4
Trt DMFE (feed:gain)	9.86	7.57	0.69	0.02

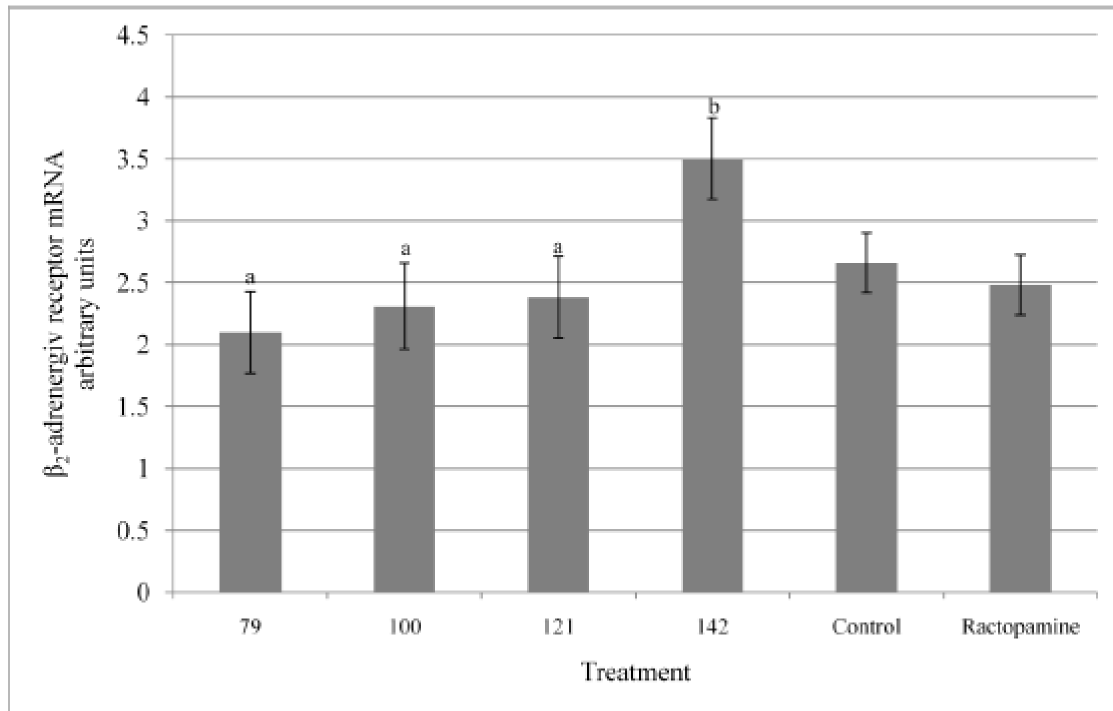
[1]Least Square Means (+/-) SEM, Differences are significant at $P<0.05$; RAC = Ractopamine HCL 300mg/h/d; Trt ADG = average daily gain during treatment period; Trt Gain = Total gain during treatment period; Trt DMI = Total DMI during treatment period; Trt DMFE = Dry matter feed efficiency during treatment period; Modified from Grubbs et al., 2009

Figure 4.4 β2-adrenergic receptor mRNA expression in rump adipose tissue from finishing heifers[1-4]



[a-b] For days on feed 79, 100, 121 and 142 bars lacking common superscript differ by ($P < 0.05$); Control and Ractopamine heifers did not differ in β2-adrenergic receptor mRNA expression ($P > 0.05$); 79 (79 d on feed), 100 (100 d on feed), 121 (121 d on feed), 142 (142 d on feed); Control (heifers not fed Ractopamine); Ractopamine (rRctopamine administered at 300 mg/hd/d 35 d prior to harvest; Modified From Grubbs et al., 2009.

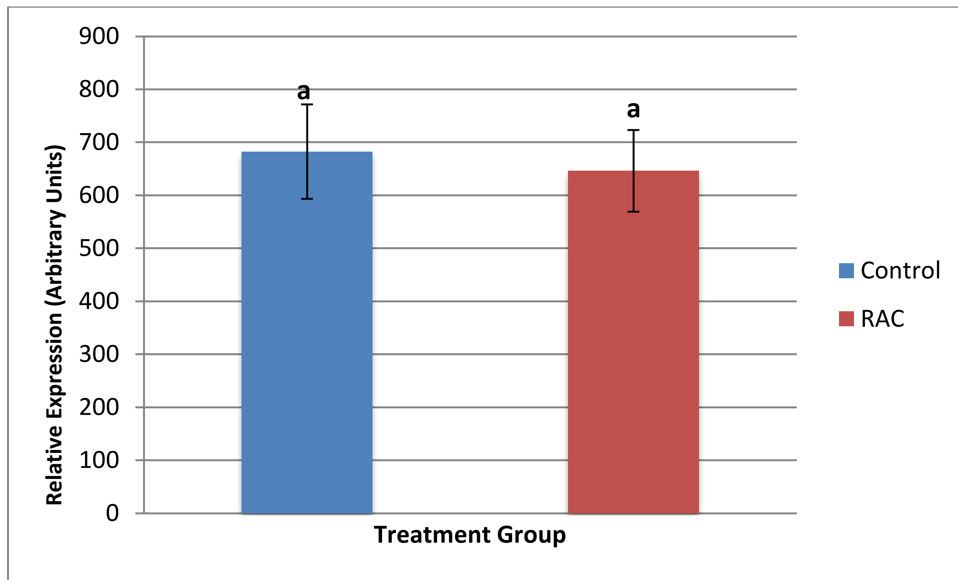
Figure 4.5 β2-adrenergic receptor mRNA expression in gastrocenimus muscle from finishing heifers[1-4]



[1]79 (79 d on feed), 100 (100 d on feed), 121 (121 d on feed), 142 (142 d on feed), Control = (heifers not fed Ractopamine); Ractopamine (Ractopamine administered at 300 mg/hd/d 35 d prior to harvest; [a]For days on feed 79, 100, 121 and 142 bars lacking common superscript differ by ($P < 0.05$); Control and Ractopamine heifers did not differ in β2-adrenergic receptor mRNA expression ($P > 0.05$); Modified From Grubbs et al., 2009

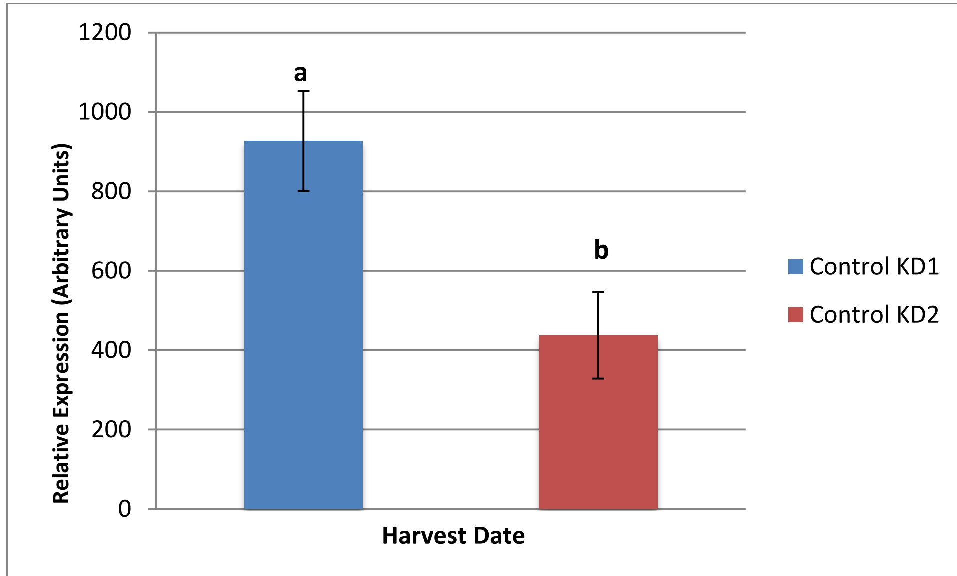
Gene Expression

Figure 4.6 Relative PPAR gamma Expression in SM of Control vs. Ractopamine treated heifers[1]



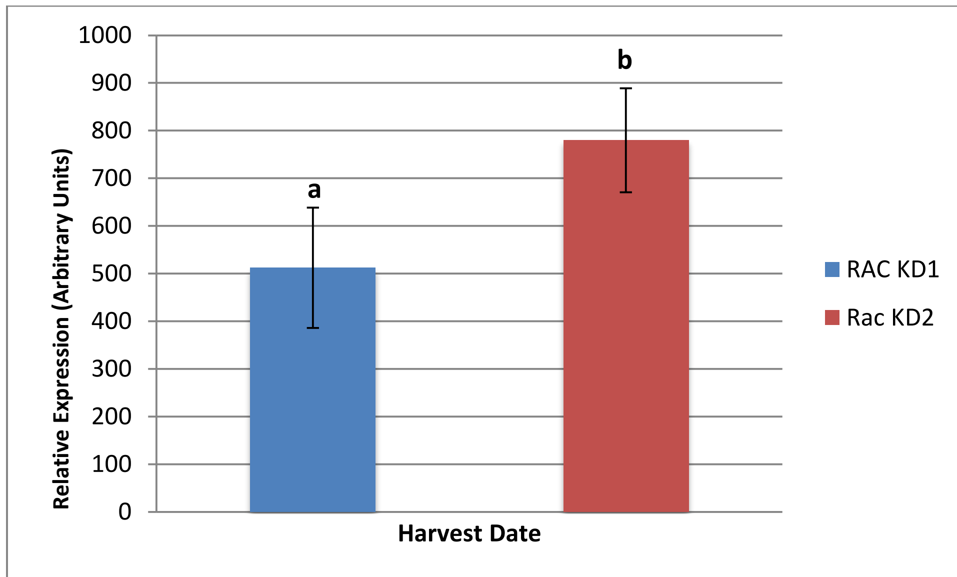
[1]LSM +/- SEM. Means not sharing a common superscript differ (P < 0.05); PPAR-g = Peroxisome Proliferator Activator Protein-gamma; Control= Control Heifers received no ractopamine for final 35 days on feed; RAC= Received ractopamine HCL (300mg/h/d) for final 35 days on feed.

Figure 4.7 Relative PPAR gamma expression in SM of control heifers harvested at 79 and 142 days[1]



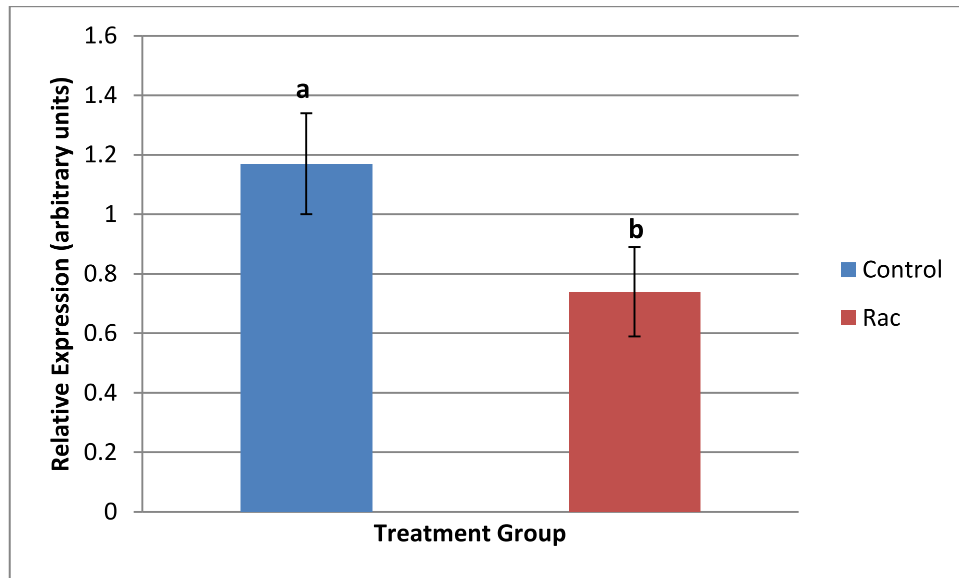
[1]LSM +/- SEM. Means not sharing a common superscript differ (P < 0.05); PPAR-g = Peroxisome Proliferator Activator Protein-gamma; KD1= Control Heifers harvested at KD1 (79 Days on Feed); KD2= Control Heifers harvested at the final harvest date (142 Days on Feed)

Figure 4.8 Relative PPAR gamma Expression in SM of RAC heifers harvested at 79 and 142 days[1]



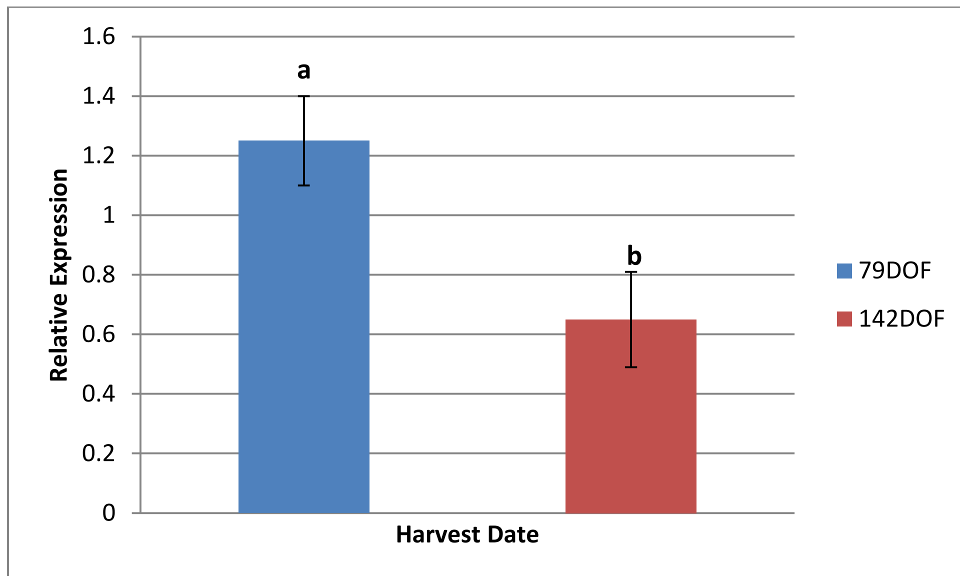
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); PPAR-g = Peroxisome Proliferator Activator Protein-gamma; RAC KD1= RAC Heifers harvested at KD1 (79 Days on Feed); RAC KD2= RAC Heifers harvested at KD2 (142 Days on Feed)

Figure 4.9 Relative PREF-1 expression in skeletal muscle tissue harvested from Control and Ractopamine treated finishing heifers[1]



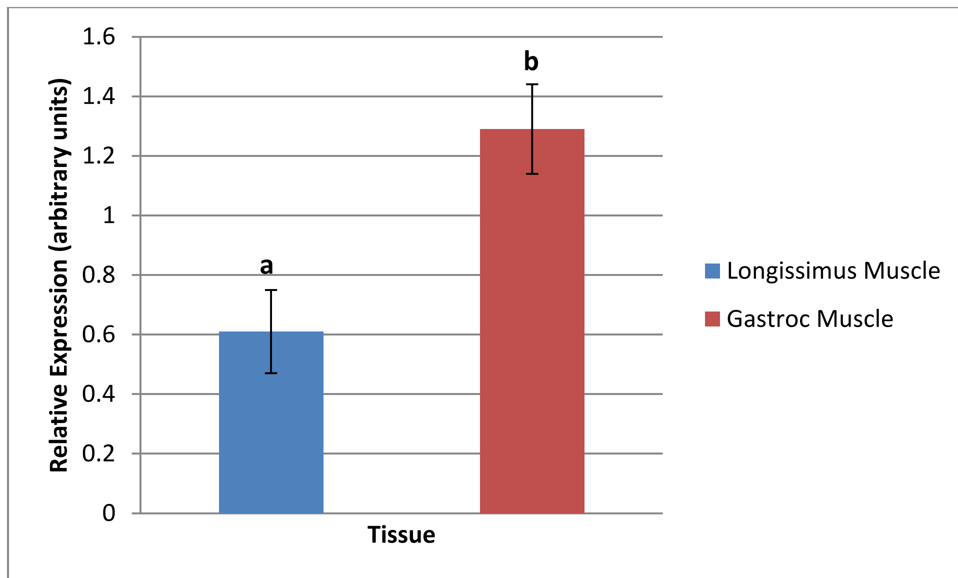
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); Pref-1 = Preadipocyte factor 1/Dlk1; Control= Control Heifers received no ractopamine for final 35 days on feed; RAC= Received ractopamine HCL (300mg/h/d) for final 35 days on feed.

Figure 4.10 Relative Pref-1expression in skeletal muscle tissue of finishing heifers harvested at 79 and 142 days[1]



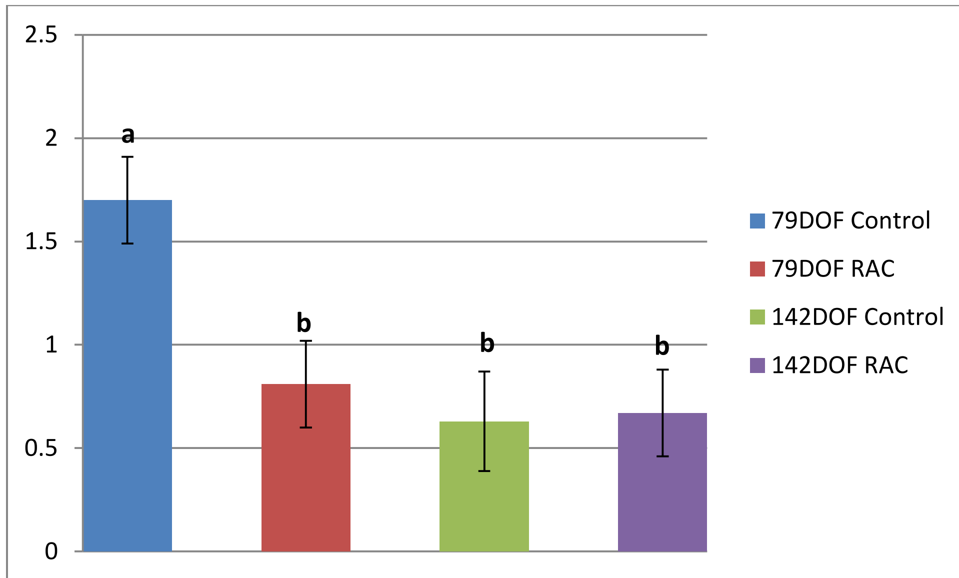
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); Pref-1 = Preadipocyte factor 1; DOF = Days on feed.

Figure 4.11 Relative Expression of PREF-1 in *Longissimus dorsi* and gastrocnemius muscle tissue harvested from finishing heifers[1]



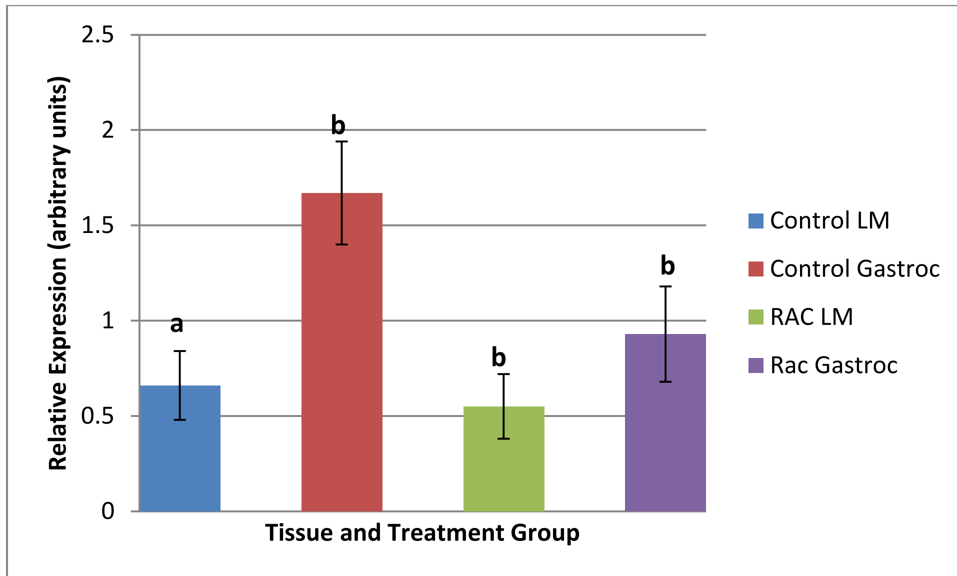
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); Gastroc = Gastrocnemius muscle; Pref-1 = Preadipocyte factor-1/Dlk-1.

Figure 4.12 Relative Expression of PREF-1 in skeletal muscle tissue harvested from Control and Ractopamine treated heifers at 79 and 142 days on feed[1]



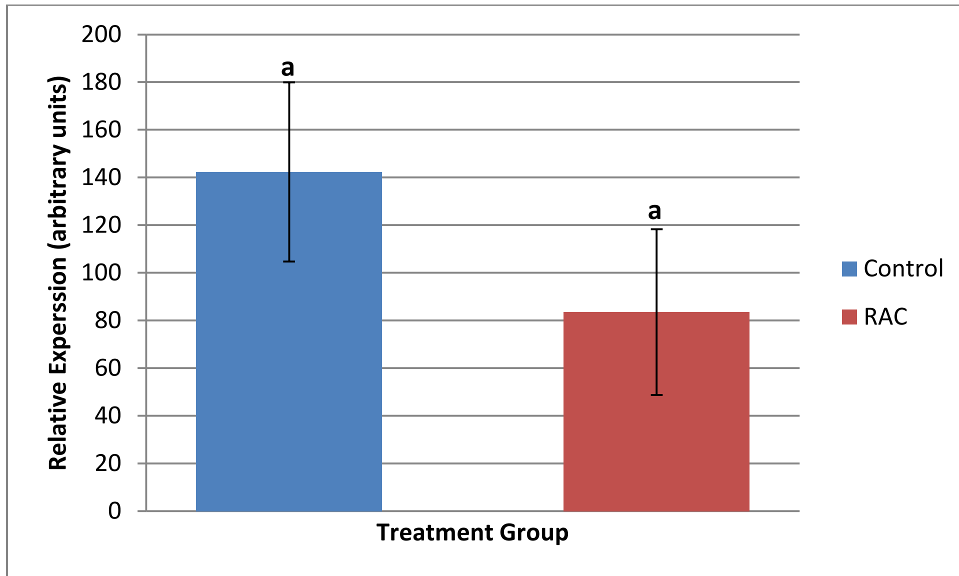
Least square means +/- SEM; [a,b]Columns not bearing a common superscript differ (P < 0.05); 79 DOF = heifers Harvested at 79 days on feed; 142 DOF = Heifers harvested at 142 days on feed; Control = Heifers not treated with Optaflexx; RAC= Heifers treated with Optaflexx at 300mg/hd/d for the final 35 days prior to harvest.

Figure 4.13 Relative Expression of PREF-1 in gastrocnemius and longissimus muscle samples harvested from Control and Ractopamine treated heifers[1]



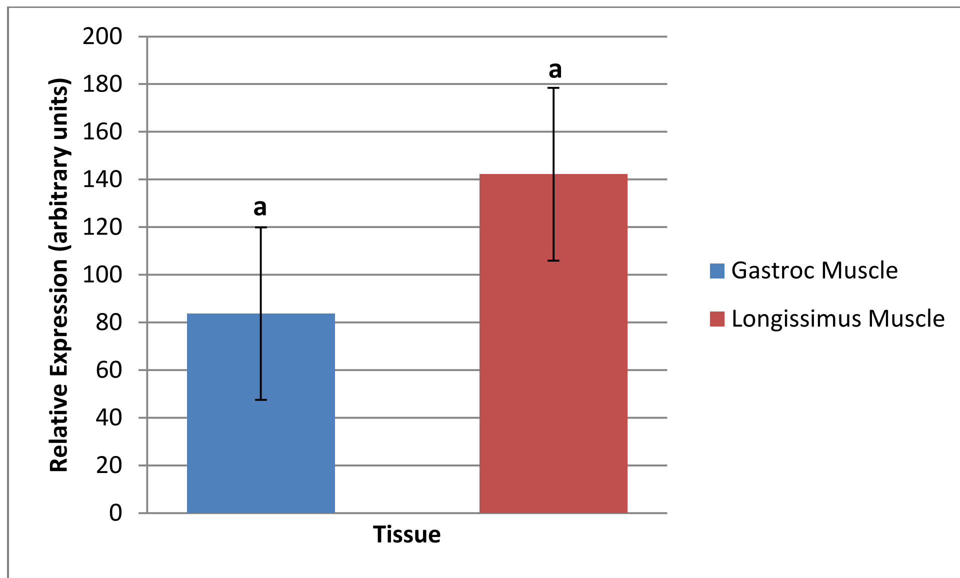
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); Pref-1 = Preadipocyte factor 1; Control= Control Heifers received no ractopamine for final 35 days on feed; RAC= Received ractopamine HCL (300mg/h/d) for final 35 days on feed; LM = Longissimus dorsi muscle; Gastroc = Gastrocnemius muscle.

Figure 4.14 Relative expression of glycerol-phosphate acyl transferase in skeletal muscle tissue collected from heifers in Control vs. Ractopamine treated finishing heifers[1]



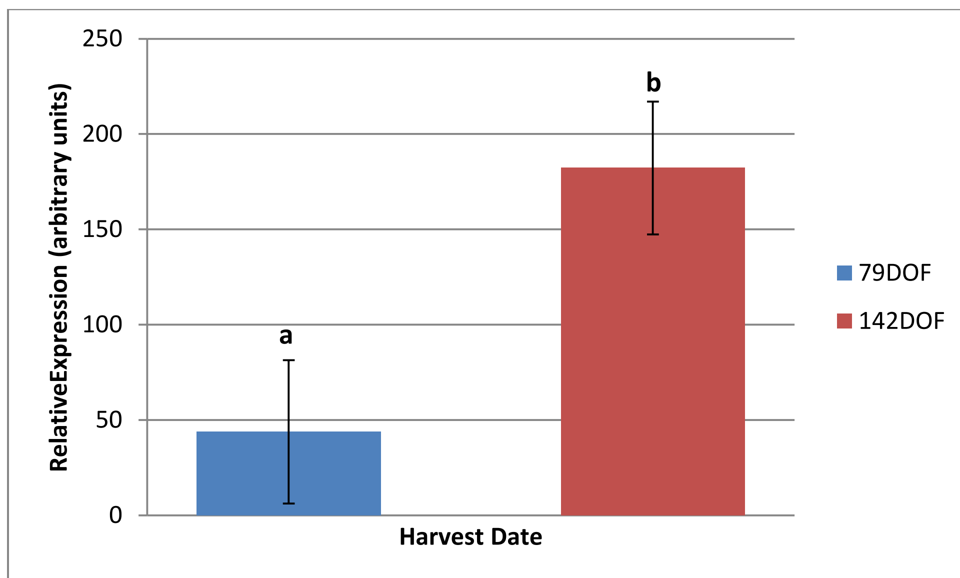
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); GPAT = glycerol-3-phosphate acyl transferase; Control = received no ractopamine for the final 35 days on feed; RAC= received Optaflexx at 300mg/hd/d for the final 35 days on feed.

Figure 4.15 Relative expression of glycerol phosphate acyl transferase in gastrocnemius vs. longissimus muscle collected from finishing heifers[1]



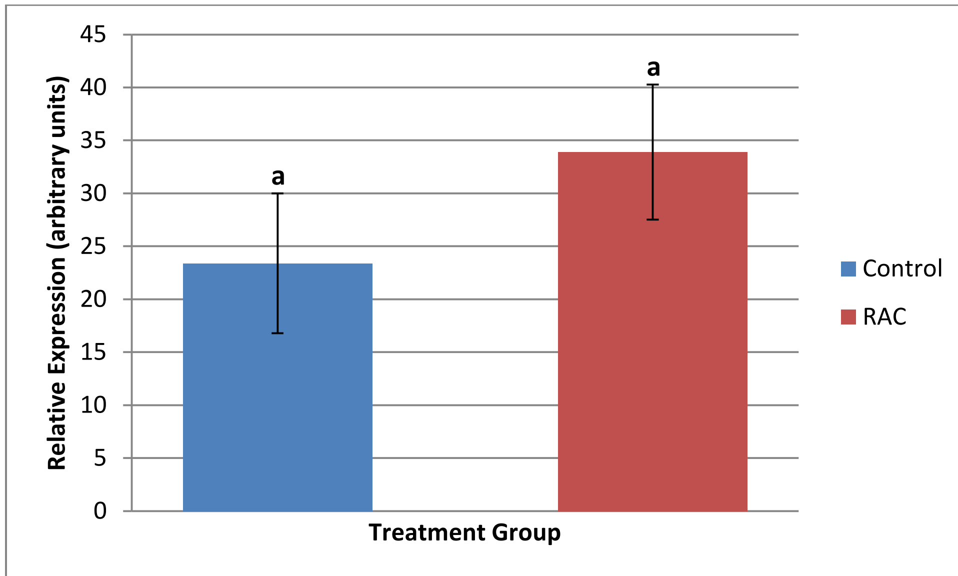
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); GPAT = glycerol-3-phosphate acyl transferase.

Figure 4.16 Relative expression of glycerol-phosphate acyl transferase in skeletal muscle tissue collected from finishing heifers harvested at 79 and 142 days[1]



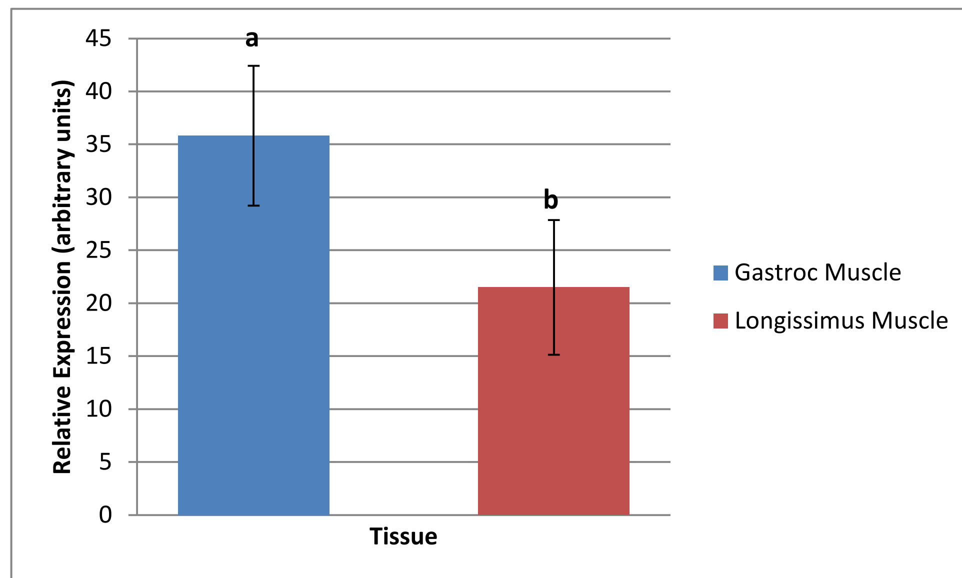
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); GPAT = glycerol-3-phosphate acyl transferase; DOF = Days on feed

Figure 4.17 Relative expression of CPT-1b in skeletal muscle tissue collected from control vs. ractopamine treated finishing heifers[1]



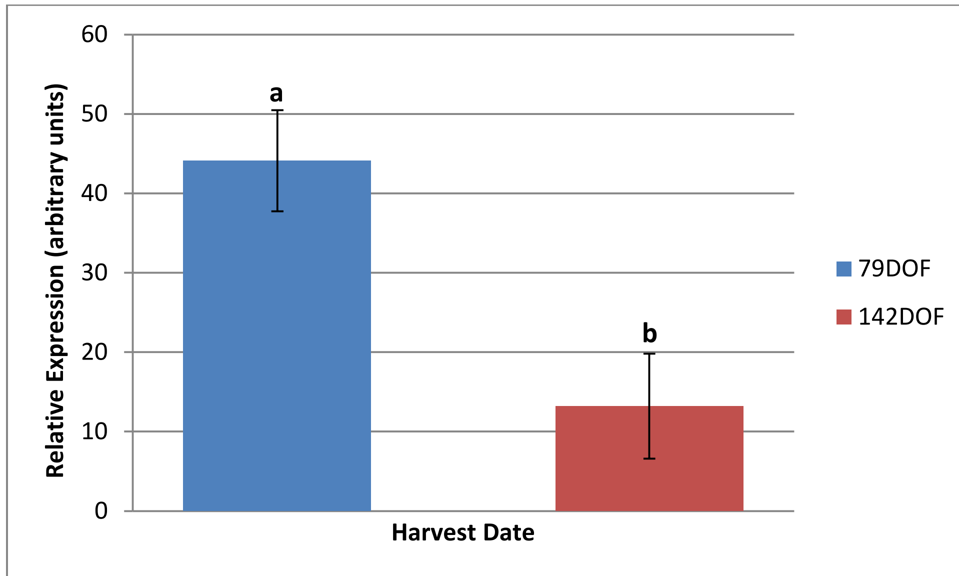
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$; CPT1-b = Carnitine palmitoyl acyl transferase 1b; Control = received no ractopamine for the final 35 days on feed; RAC= received Optaflexx at 300mg/hd/d for the final 35 days on feed.

Figure 4.18 Relative expression of CPT-1b in gastrocnemius and longissimus muscle collected from finishing heifers[1]



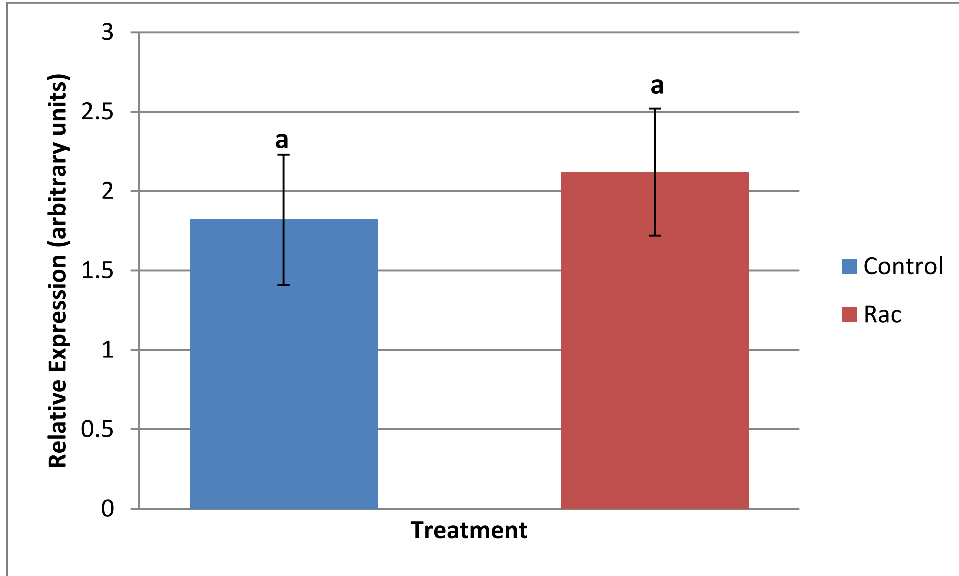
[1]LSM +/- SEM. Means not sharing a common superscript differ (P < 0.05; CPT1-b = Carnitine palmitoyl acyl transferase 1b

Figure 4.19 Relative expression of CPT1b in skeletal muscle tissue collected from finishing heifers harvested at 79 and 142 days on feed[1]



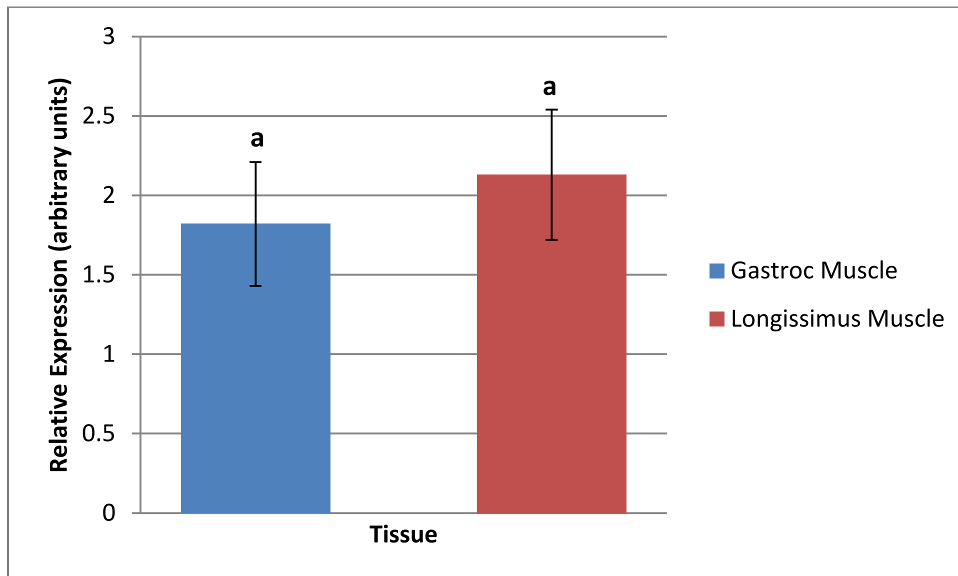
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$; CPT1-b = Carnitine palmitoyl acyl transferase 1b; DOF = Days on feed.

Figure 4.20 Relative expression of UCP2 in skeletal muscle tissue collected from control vs. ractopamine treated heifers[1]



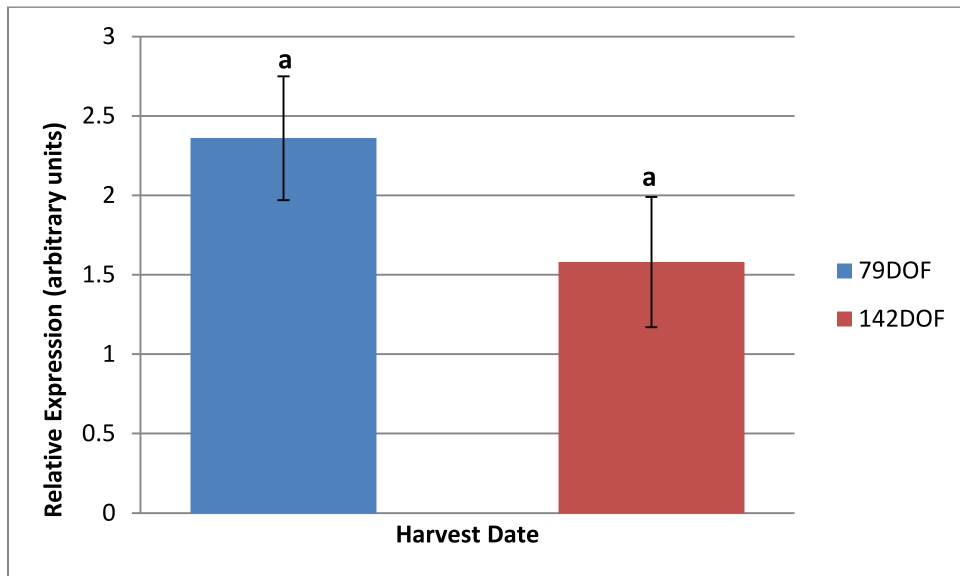
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$; UCP2 = Uncoupling protein 2; Control = received no ractopamine for the final 35 days on feed; RAC= received Optaflexx at 300mg/hd/d for the final 35 days on feed.

Figure 4.21 Relative expression Of UCP2 in gastrocnemius and longissimus muscle collected from finishing heifers[1]



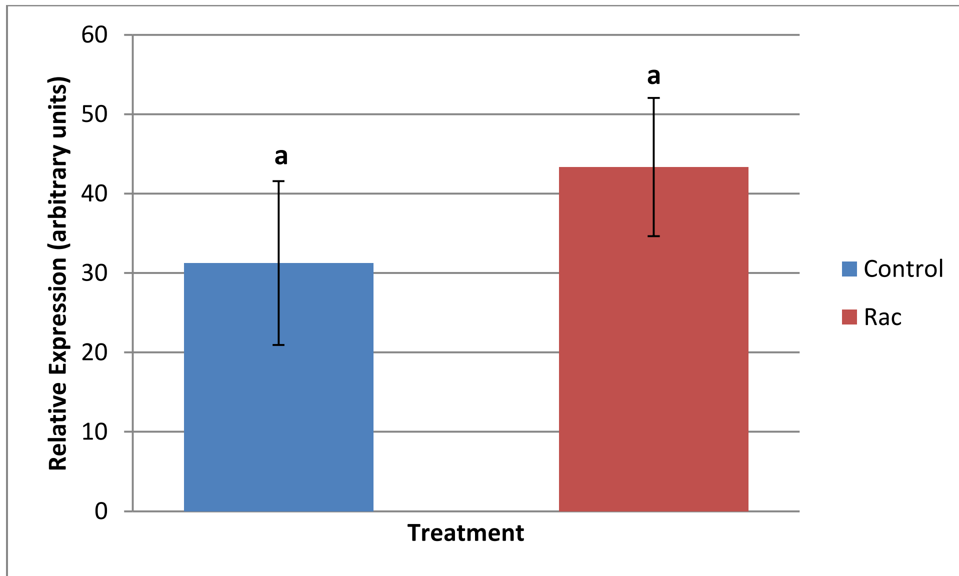
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); CPT1-b = Carnitine palmitoyl acyl transferase 1b.

Figure 4.22 Relative expression Of UCP2 in skeletal muscle tissue collected from finishing heifers harvested at 79 and 142 days[1]



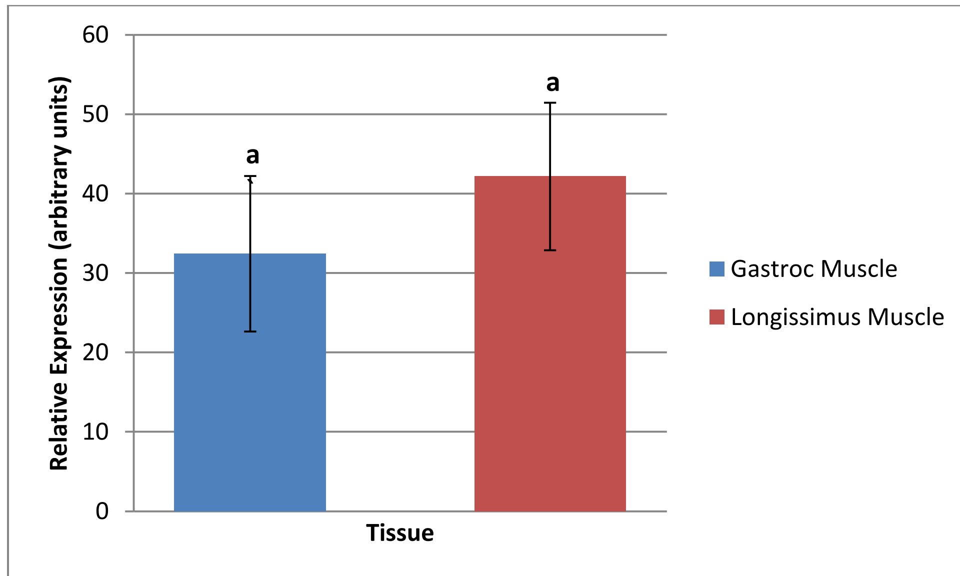
[1]LSM +/- SEM. Means not sharing a common superscript differ (*P* < 0.05; CPT1-b = Carnitine palmitoyl acyl transferase 1b; DOF = Days on feed.

Figure 4.23 Relative expression of PPAR-α in skeletal muscle tissue collected from control vs. ractopamine treated finishing heifers[1]



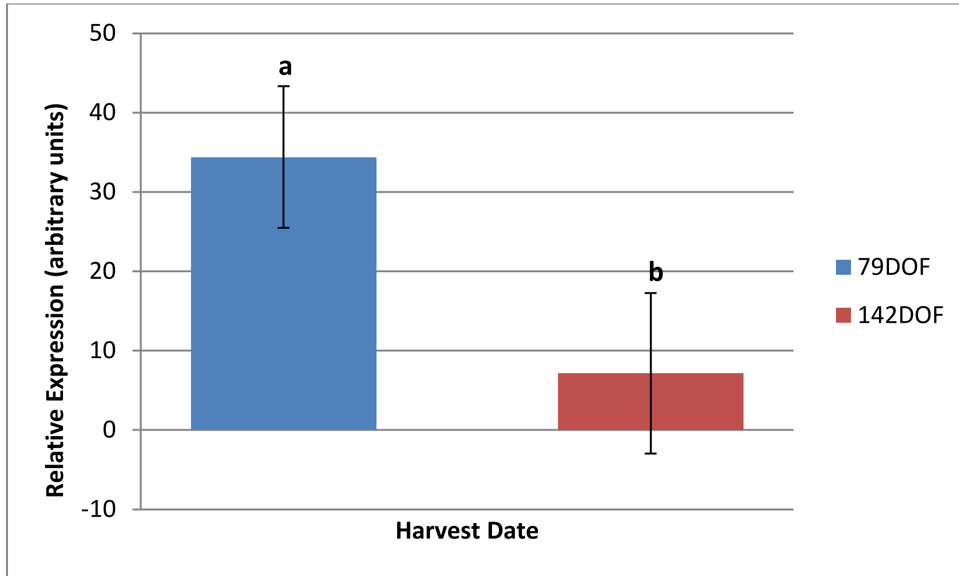
[1]LSM +/- SEM. Means not sharing a common superscript differ (P < 0.05; PPAR-a = peroxisome proliferator activator receptor alpha; Control = received no ractopamine for the final 35 days on feed; RAC= received Optaflexx at 300mg/hd/d for the final 35 days on feed.

Figure 4.24 Relative expression of PPAR-α in gastrocnemius and longissimus muscle tissue collected from finishing heifers[1]



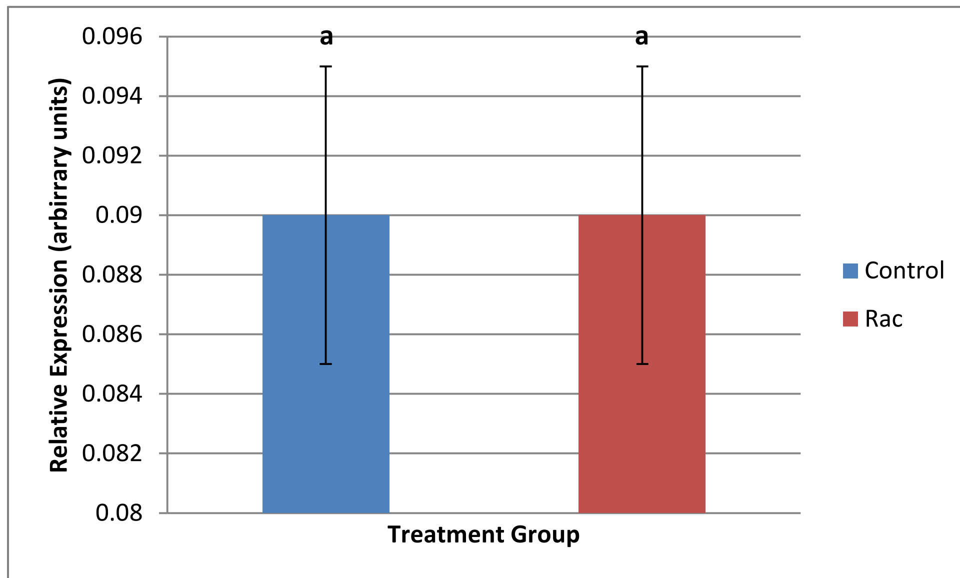
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$; PPAR-a = peroxisome proliferator activator receptor alpha.

Figure 4.25 Relative Expression of PPAR−α in skeletal muscle tissue collected from finishing heifers harvested at 79 and 142 days[1]



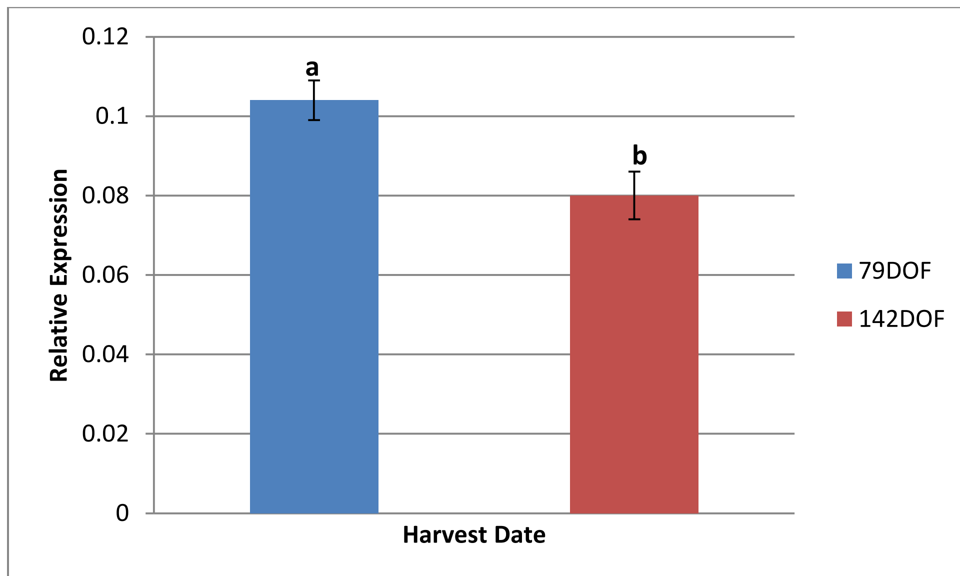
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$; PPAR-α = peroxisome proliferator activator receptor alpha. DOF = Days on feed.

Figure 4.26 Relative expression of polyubiquitin in SM of control and ractopamine treated finishing heifers[1]



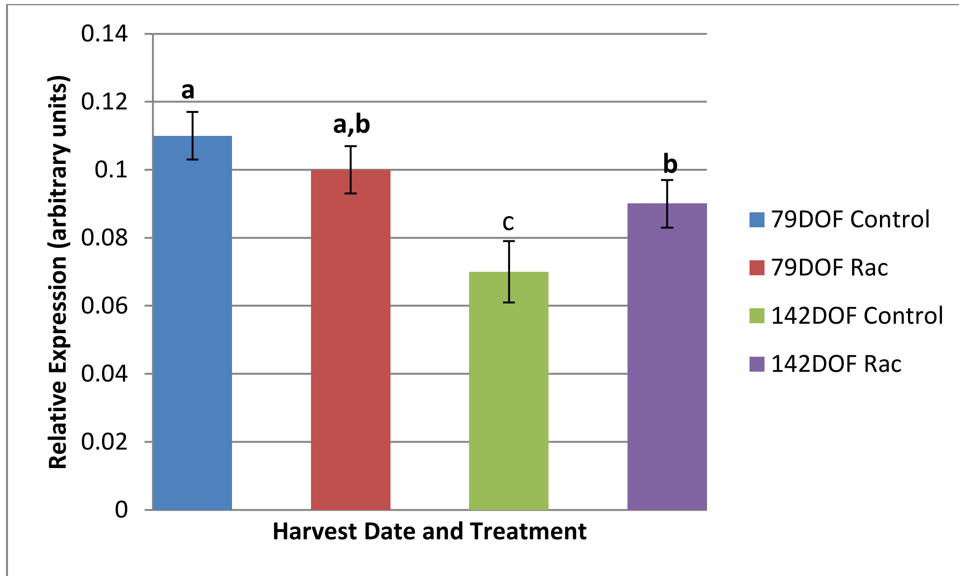
[1]LSM +/- SEM. Means not sharing a common superscript differ (P < 0.05); Control = received no ractopamine for the final 35 days on feed; RAC= received Optaflexx at 300mg/hd/d for the final 35 days on feed.

Figure 4.27 Relative expression of polyubiquitin in skeletal muscle of finishing heifers harvested at 79 and 142 days[1]



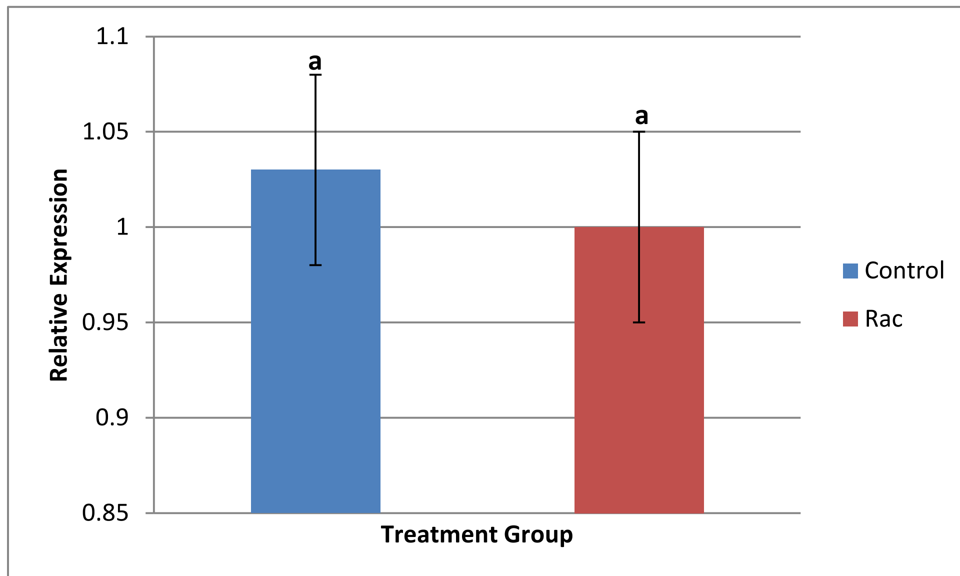
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); 79DOF = 79 days on feed; 142DOF = 142 days on feed.

Figure 4.28 Relative expression of polyubiquitin in gastrocnemius and longissimus dorsi muscle samples harvested from control and ractopamine treated heifers at 79 and 142 days[1]



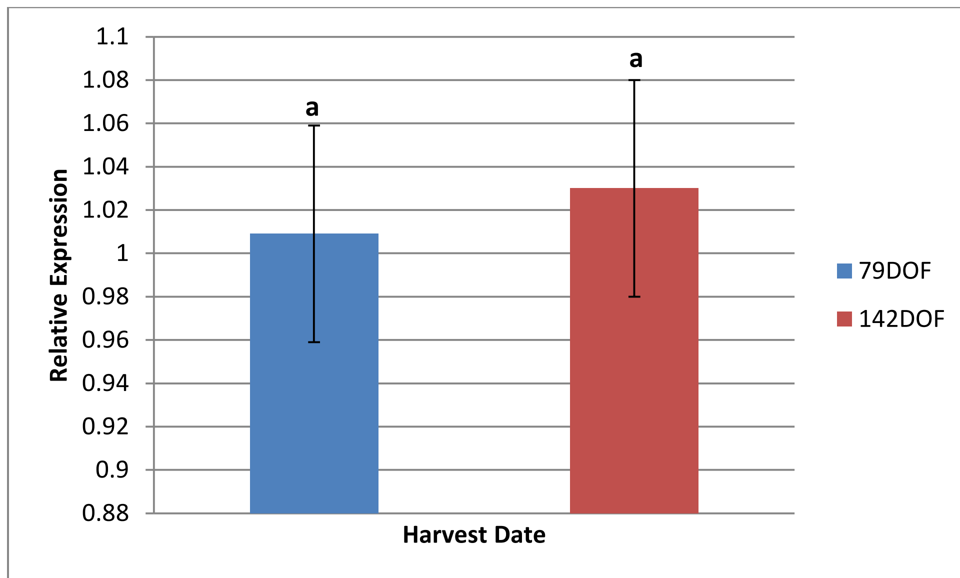
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); Control = received no ractopamine for the final 35 days on feed; RAC= received Optaflexx at 300mg/hd/d for the final 35 days on feed. Harvest date 1 = 79 days on feed; Harvest date 2 = 142 days on feed.

Figure 4.29 Relative expression of ubiquitin E2 in skeletal muscle of control and ractopamine treated finishing heifers[1]



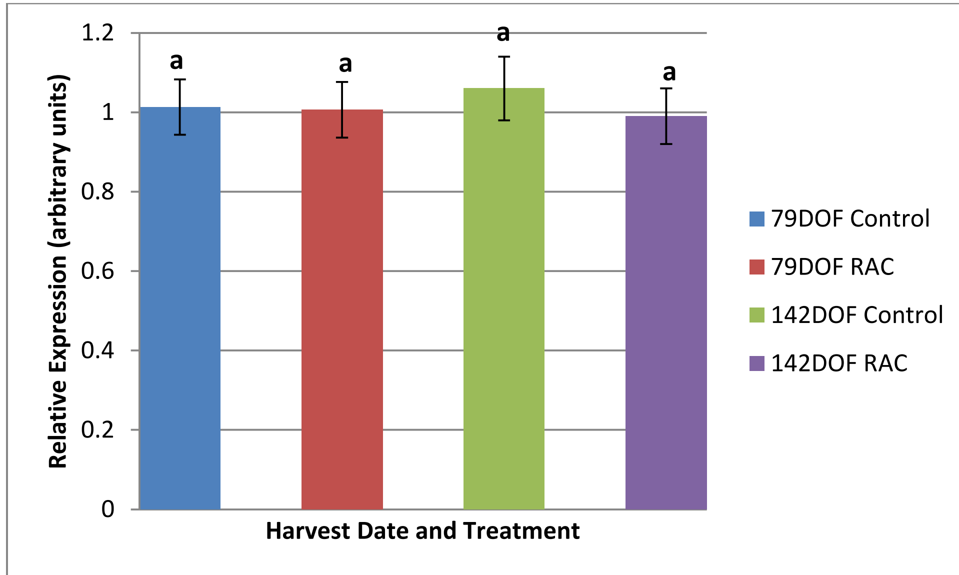
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); Control = received no ractopamine for the final 35 days on feed; RAC= received Optaflexx at 300mg/hd/d for the final 35 days on feed.

Figure 4.30 Relative expression of ubiquitin E2 in skeletal muscle tissue of finishing heifers harvested at 79 and 142 days[1]



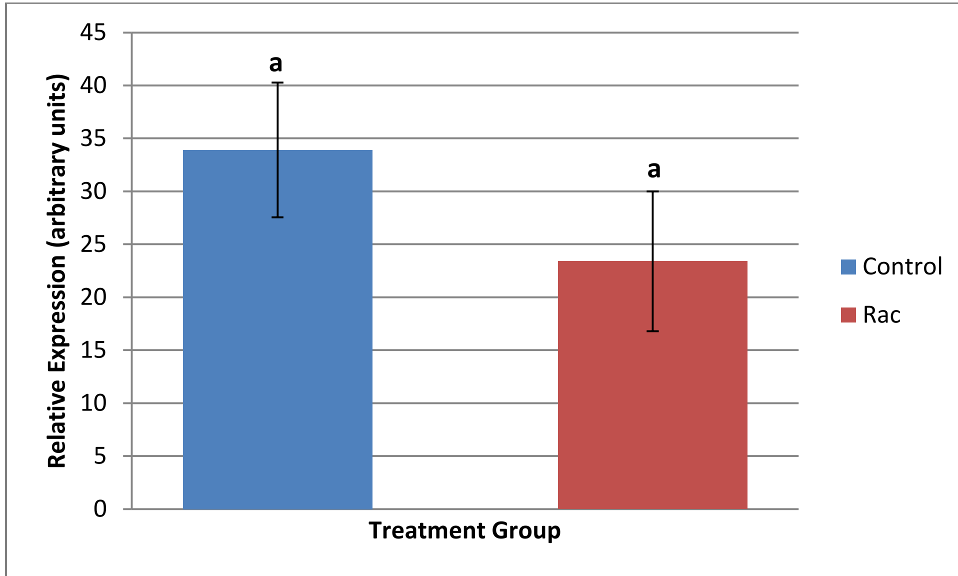
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); Harvest date 1 = 79 days on feed; Harvest date 2 = 142 days on feed.

Figure 4.31 Relative expression of ubiquitin E2 in gastrocnemius and longissimus dorsi muscle samples from control and ractopamine treated heifers harvested at 79 and 142 days[1]



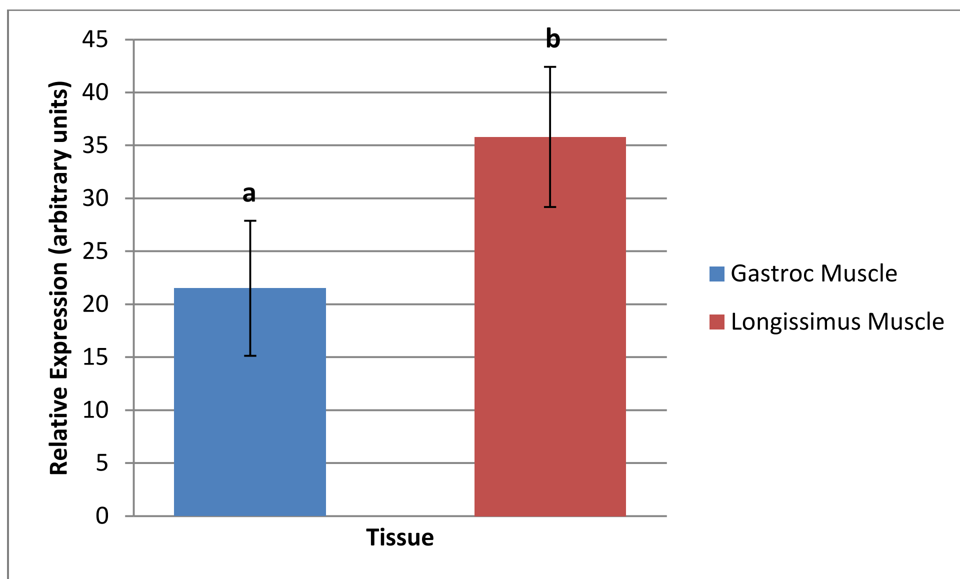
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); Control = received no ractopamine for the final 35 days on feed; RAC= received Optaflexx at 300mg/hd/d for the final 35 days on feed. Harvest date 1 = 79 days on feed; Harvest date 2 = 142 days on feed.

Figure 4.32 Relative expression of PSMD11 in skeletal muscle tissue collected from control vs. ractopamine treated finishing heifers[1]



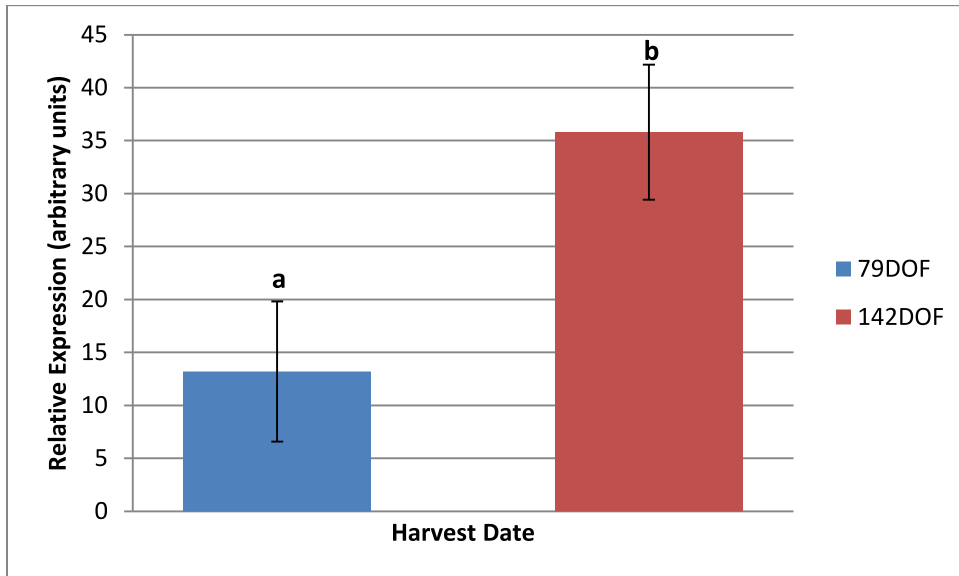
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); PSMD11 = proteasome (prosome, macropain) 26S subunit, non-ATPase 11; Control = received no ractopamine for the final 35 days on feed; RAC= received Optaflexx at 300mg/hd/d for the final 35 days on feed.

Figure 4.33 Relative expression of PSDM11 in gastrocnemius and longissimus muscle collected from finishing heifers[1]



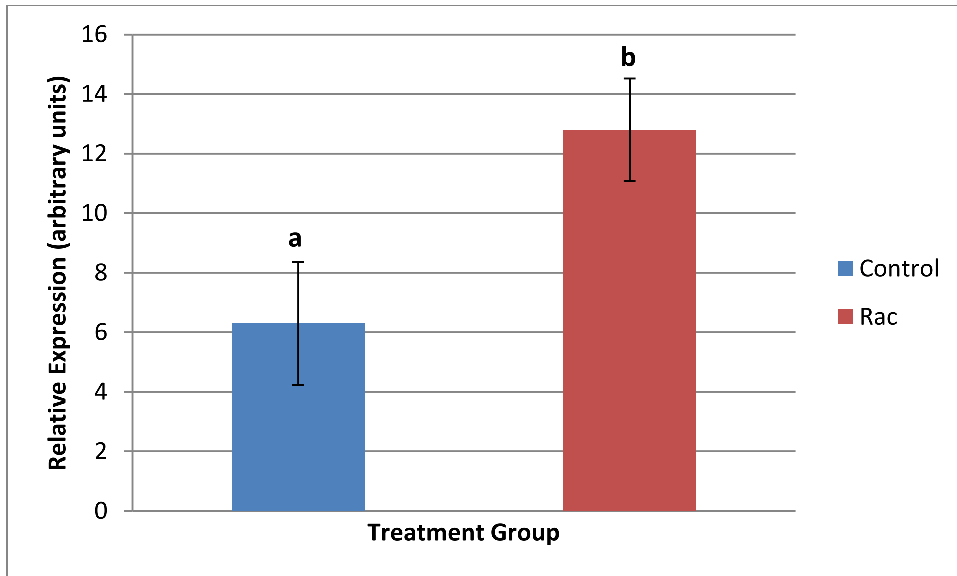
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); PSMD11 = proteasome (prosome, macropain) 26S subunit, non-ATPase 11.

Figure 4.34 Relative expression of PSDM11 in skeletal muscle tissue collected from finishing heifers harvested at 79 and 142 days[1]



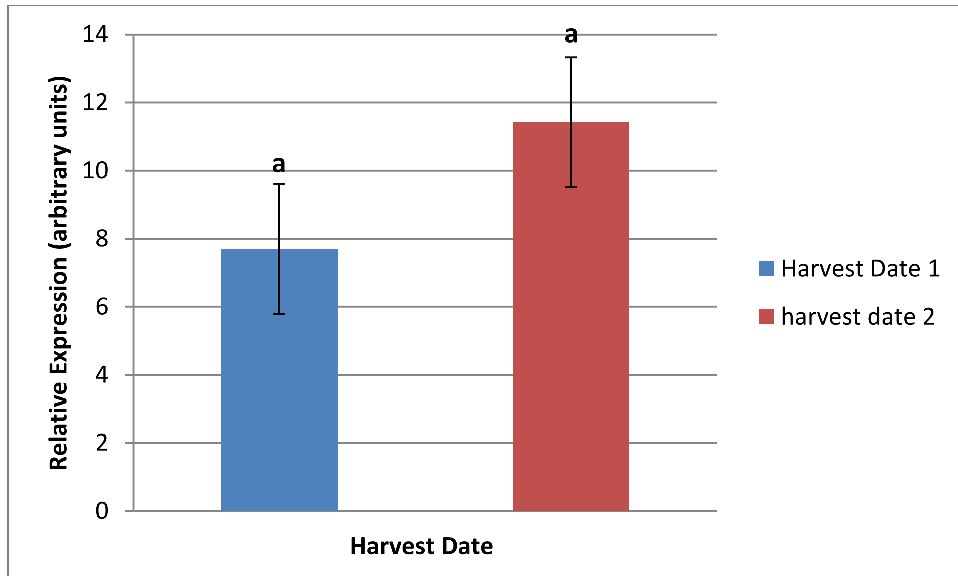
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); PSMD11 = proteasome (prosome, macropain) 26S subunit, non-ATPase 11; Harvest date 1 = 79 days on feed; Harvest date 2 = 142 days on feed.

Figure 4.35 Relative expression of PPAR-gamma in adipose tissue of control vs. ractopamine treated finishing heifers[1]



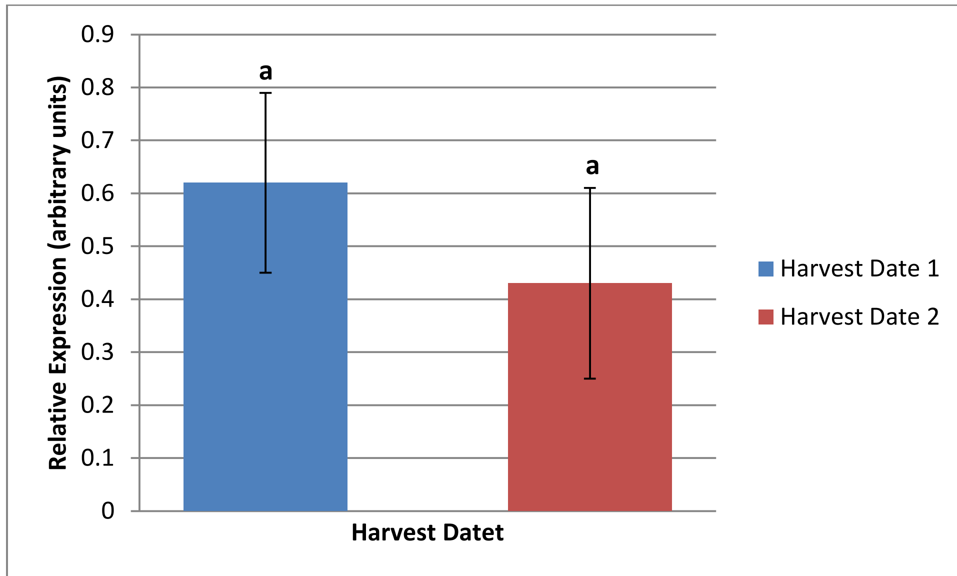
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); PPAR-g= peroxisome proliferator activator receptor–gamma; Control = received no ractopamine for the final 35 days on feed; RAC= received Optaflexx at 300mg/hd/d for the final 35 days on feed.

Figure 4.36 Relative expression of PPAR-gamma in adipose tissue of finishing heifers harvested at 79 and 142 days



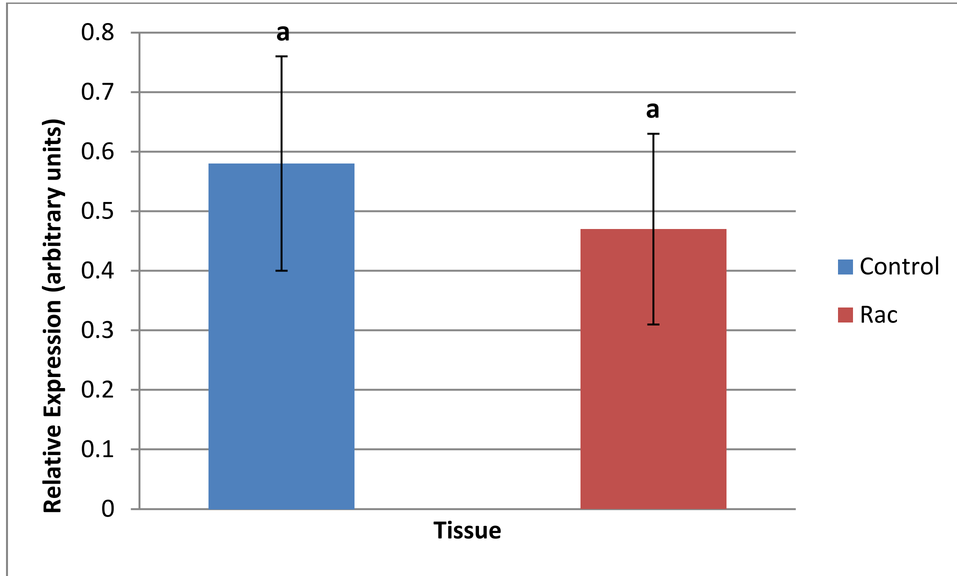
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); PPAR-g= peroxisome proliferator activator receptor–gamma; Harvest date 1 = 79 days on feed; Harvest date 2 = 142 days on feed.

Figure 4.37 Relative expression of Pref-1 in SC AT of finishing heifers at 79 and 142 days[1]



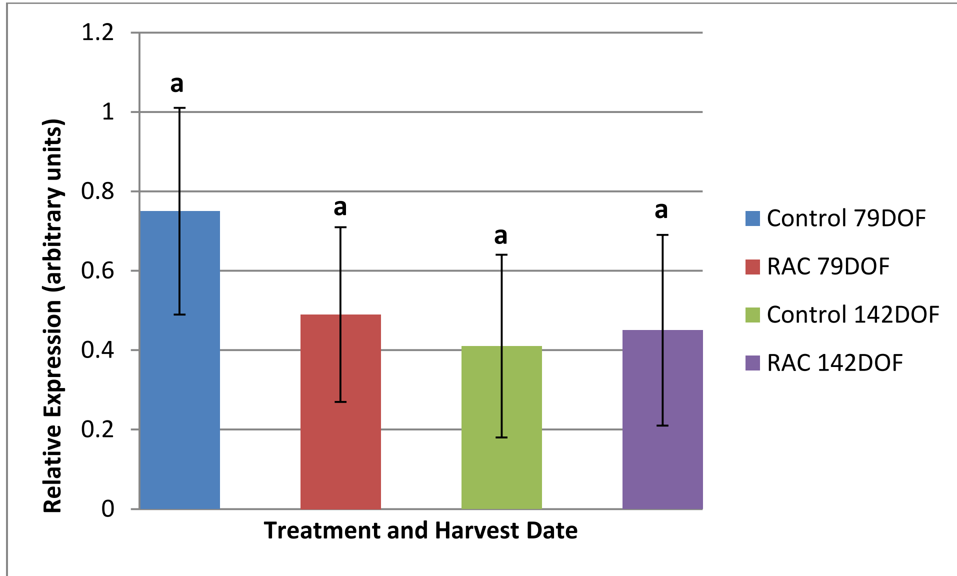
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); Pref-1 = preadipocytes factor 1; Harvest date 1 = 79 days on feed; Harvest date 2 = 142 days on feed.

Figure 4.38 Average Relative Expression of Pref-1 in SC AT of control and ractopamine-treated finishing heifers[1]



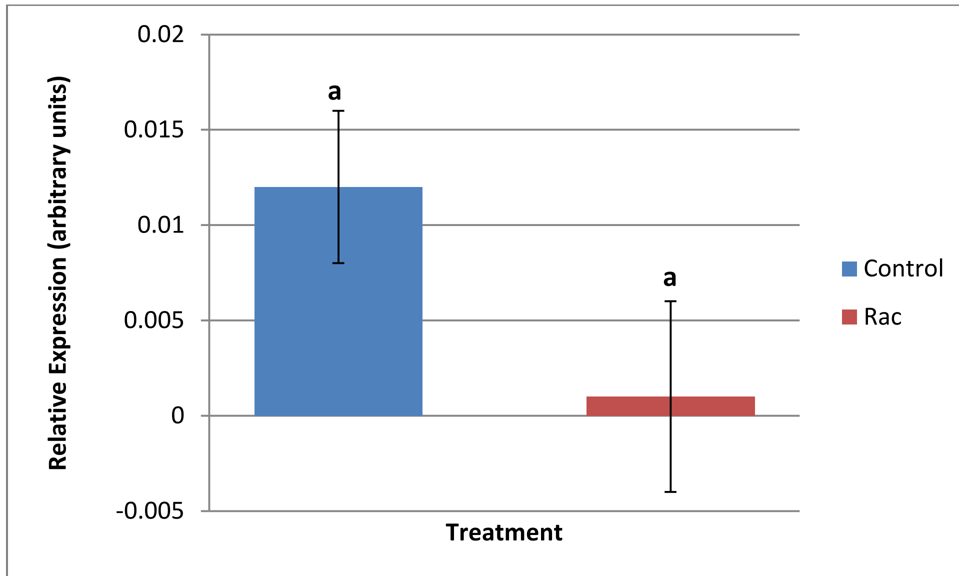
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); Pref-1 = preadipocytes factor 1. Control = received no ractopamine for the final 35 days on feed; RAC= received Optaflexx at 300mg/hd/d for the final 35 days on feed.

Figure 4.39 Relative Expression of Pref-1 in SC AT harvested from control and ractopamine-treated finishing heifers at 79 and 142 days[1]



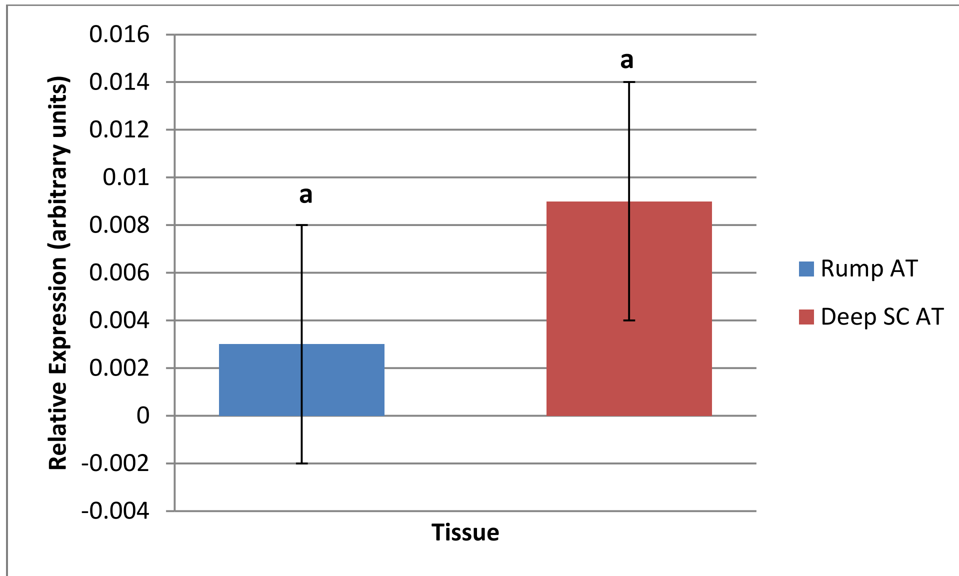
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); Control = received no ractopamine for the final 35 days on feed; RAC= received Optaflexx at 300mg/hd/d for the final 35 days on feed. 79DOF = 79 days on feed; 142DOF = 142 days on feed.

Figure 4.40 Relative Expression of FABP4 in adipose tissue collected from control vs. Ractopamine treated heifers[1]



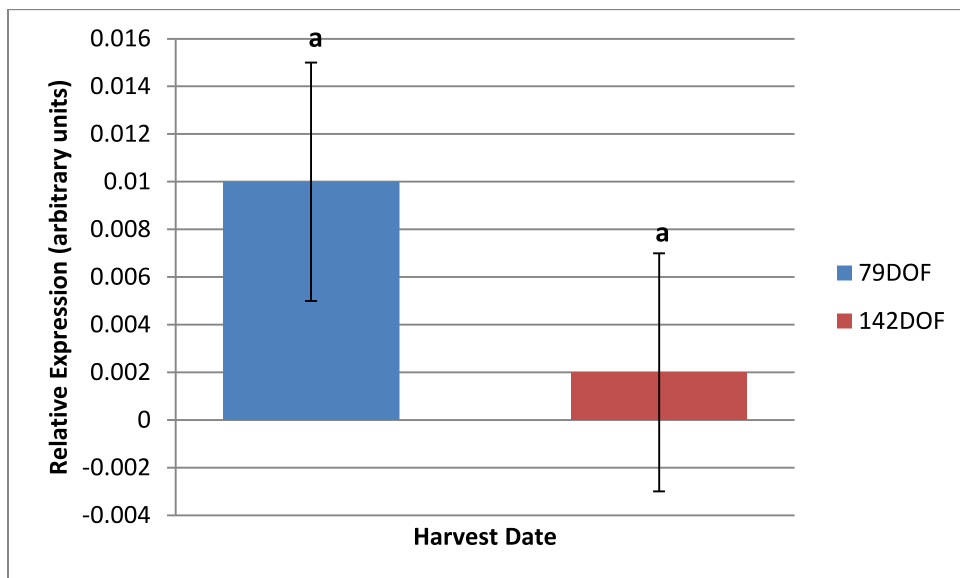
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); FABP4 = Fatty acid binding protein 4; Control = received no ractopamine for the final 35 days on feed; RAC= received Optaflexx at 300mg/hd/d for the final 35 days on feed.

Figure 4.41 Relative expression of FABP4 in rump and deep subcutaneous adipose tissue collected from finishing heifers[1]



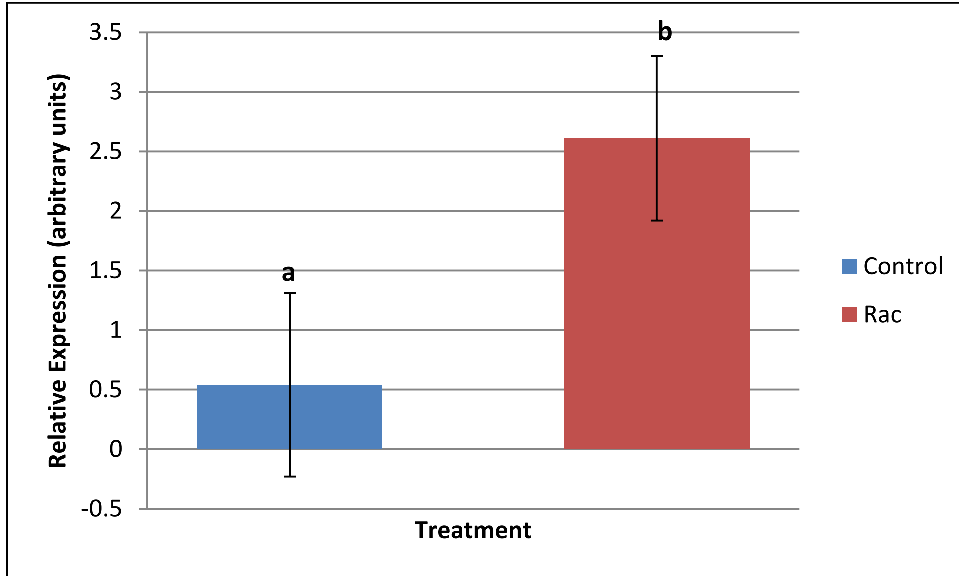
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); FABP4 = fatty acid binding protein 4; Deep SC adipose tissue = subcutaneous adipose tissue harvested from the 13th rib directly proximal to the loin muscle.

Figure 4.42 Relative expression of FABP4 in adipose tissue collected from finishing heifers harvested at 79 and 142 days[1]



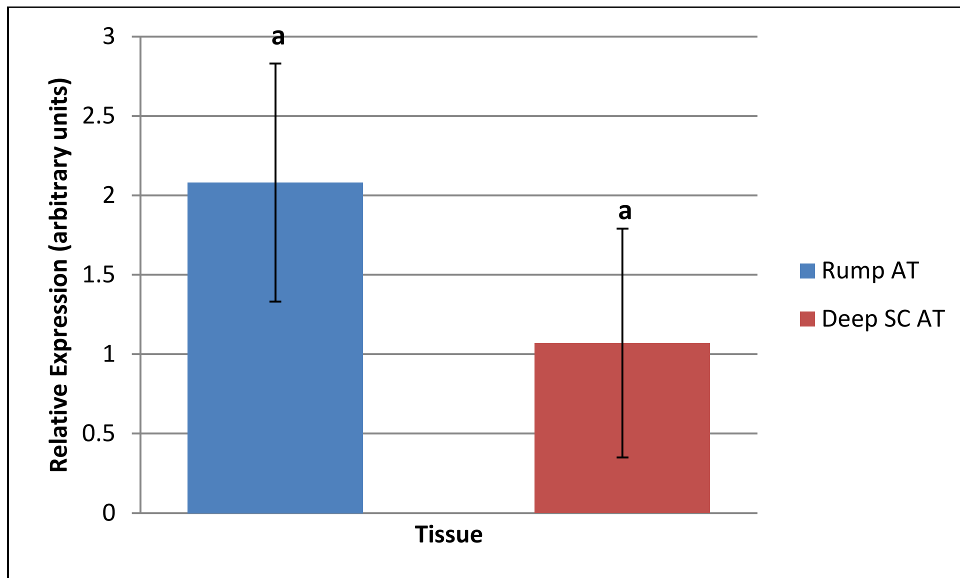
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); FABP4 = fatty acid binding protein 4; Harvest date 1 = 79 days on feed; Harvest date 2 = 142 days on feed.

Figure 4.43 Relative Expression of UCP-2 in adipose tissue of control vs. ractopamine treated finishing heifers[1]



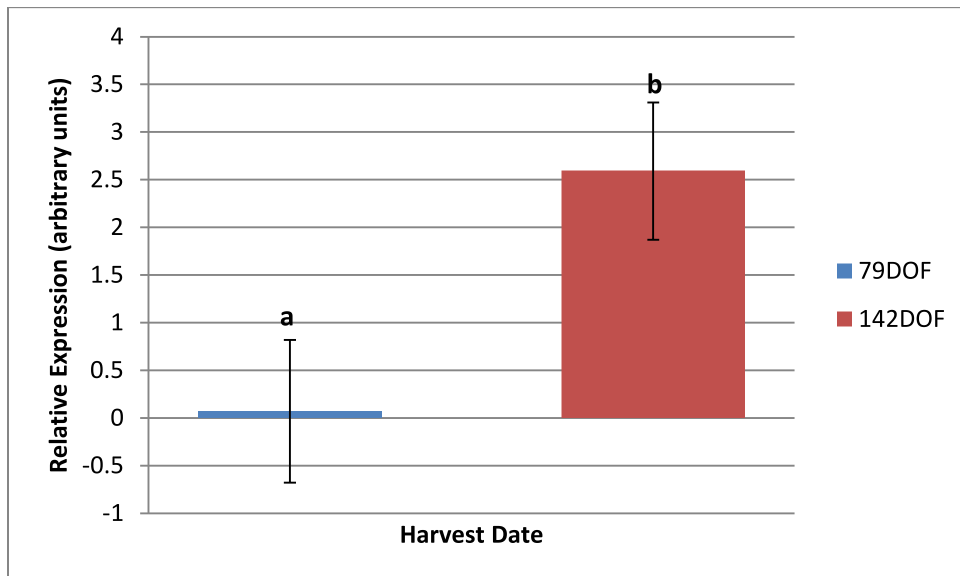
[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); PSMD11 = proteasome (prosome, macropain) 26S subunit, non-ATPase 11; Control = received no ractopamine for the final 35 days on feed; RAC= received Optaflexx at 300mg/hd/d for the final 35 days on feed.

Figure 4.44 Relative expression of UCP 2 in rump and deep deep subcutaneous adipose tissue of finishing heifers[1]



[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); UCP2 = Uncoupling protein 2; Deep SC adipose tissue = AT harvested from the 13th rib directly proximal to the loin muscle.

Figure 4.45 Relative expression of UCP 2 in adipose tissue collected from finishing heifers harvested at 79 and 142 days[1]



[1]LSM +/- SEM. Means not sharing a common superscript differ ($P < 0.05$); UCP2 = Uncoupling protein 2; Harvest date 1 = 79 days on feed; Harvest date 2 = 142 days on feed.

Chapter 5. The Effects of Feed Restriction and Re-feeding on Gene Expression Patterns in the Loin Muscle and Subcutaneous Adipose Tissue of Forage Fed Beef

Introduction

Various factors contribute to the economic success of a beef finishing operation. Utley et al. (1975) summarized these factors and reported that selecting an efficient, economical finishing system is driven by: market specifications, the type of feed available, and cost per unit gain. More recently consumer preference and expectation have emerged as driving factors for beef industry success. Feed can represent upwards of 60% of production costs in cow/calf feeding systems and over 70% in finishing systems (Anderson et al., 2005). With these, constant input cost, the efficiency of animal growth and nutrient utilization is of major concern and a deciding factor in determining profitability. Management strategies including specialized nutrition regimens and breeding programs have emerged in order to make maximum use of available resources and amplify animal productivity and efficiency. These practices have improved the overall volume and efficiency of livestock production. With an estimated seventy percent increase in current production levels needed to sustain the world population by 2030 (FAO, 2009); the industry will be required to make major advances in the face of dwindling land resources and increasing costs. Livestock genomics has begun to make notable contributions to our ability to identify, understand, and manipulate metabolic pathways to improve animal production (Bauman et al., 2011; Sellner et al., 2007; Dekkers, 2004).

Forage-Finished Beef

With proper pasture management, the ability to grow quality renewable forages year round is an advantage afforded to the beef industry in the south eastern US (SE), compared to less temperate climates in other regions of the country (Allen et al., 1996; McMillin et al., 1990).

Factors leading to increased adoption of a forage-based system have historically included increases in grain prices (Hoveland, 1975; Seideman et al., 1985), declining cattle prices, and increased concern about the environmental impact of beef production (Capper, 2011; Cook et al., 1984). Currently, with rising cost and competition for various grains, the use of forage-based finishing systems is becoming an increasingly popular option in areas that can support such a system.

The thought of returning to the forage finishing process seems ironic, considering the reasons for the widespread adoption of the feedlot process in the first place. Up until the middle of the last century cattle in the US were traditionally finished on pastures. This was the industry standard at the time and is still practiced in many countries around the world. Following World War II the United States witnessed a gastronomic revolution, which saw an increase in the per capita calorie intake (Unger and Scherer, 2010), and increased demand for beef and other high quality meat products (Gaylean et al., 2011). Increased consumption and the need for improved production efficiency led to intensive research and development regarding feedlot and grain-finishing processes. These practices have produced larger, higher quality cattle in a more efficient manner than previously experienced (McMurray, 2009; Ball and Cornett, 1996). Commercial feedlots have since become commonplace in the industry (MacDonald and McBride, 2009; Gaylean et al., 2011), and in the US consumers have become accustomed to the flavor and palatability (and cost), of grain-finished beef (Wood et al., 2003; Wood et al., 1999). Today, feedlots are larger, more highly specialized, and increasingly consolidated due to a rise in corporate ownership (Gaylean et al., 2011; MacDonald and McBride, 2009). Despite advances in feedlot production there is a “grass-roots” movement (no pun intended) to return to more pastoral

cattle rearing methods due to several tangible and purported advantages of forage-based finishing systems (Allen et al., 1996; Wilkins, 2008).

According to the USDA Agricultural Marketing Service (AMS, 2007), in order to qualify as grass-fed beef (GFB), cattle must have consumed grass and forage for their entire lifetime with the exception of milk consumed prior to weaning. Diets must not include grain or grain-byproducts of any sort (AMS, 2007). The notion that cattle can be reared on forages is by no means a novel concept, but with the cost of feed continuing to rise and profit margins decreasing, commercial pasture-based systems have become an attractive and viable option for beef producers in some geographic locations, and some consumers are willing are to pay a premium for GFB products (Gaylean, 2011; Cox et al., 2006).

Generally speaking cattle and other ruminants reared on forage-based systems are lighter, smaller, and leaner, and less tender compared to grain-finished animals (Neurnberg et al., 2005; Borton et al., 2005; McClure et al., 2000). Forage-finished animals also have slower growth rates compared to grain fed animals (Neurnberg et al., 2005; Mandell, 1998). Brown et al. (2005) showed that forage-fed cattle have smaller rib eye area (REA), which is an indicator of lean muscle growth. This trend is generally extended to most cattle on pasture vs. forage-based systems. Neel et al. (2007) found that in cattle fed to a similar age, the REA in feedlot cattle was significantly greater than that of forage-fed cattle. Supplementing animals on a pasture-based system with concentrates, such as soy-hull pellets or corn, can produce animals with greater REA and improve production efficiency compared to pasture alone (Baublits et al., 2004; Kerth et al., 2006). This practice disqualifies animals from being considered GFB (AMS, 2007). Smaller, leaner carcasses can lead to reduced dressing percentage, and less acceptable lean, fat, and quality grades (Faucitano et al., 2008; Berthiaume et al., 2006). These lower scores can

jeopardize the already volatile profitability of a beef cattle operation (Berthiaume et al., 2006; Bidner et al., 1986). Based on the estimated decreases in production per animal, Capper et al., (2011) suggested that forage-finishing systems alone are not currently capable of meeting domestic and international demands for beef.

The phenotypic differences observed in grass vs. grain-fed beef are the manifestations of differences in available energy in these feedstuffs and the amount of food consumed in these systems. There are several possible explanations for the "underperformance" of GFB vs. grain-fed animals in terms of carcass size and production efficiency. From a physiological standpoint energy metabolism and partitioning mechanisms are responsible for directing the utilization of feed energy and for producing the resulting carcass phenotypes. Depending on the plane of nutrition, all animals will undergo shifts in metabolic partitioning to accommodate maintenance 1st and then subsequent growth, meat production, lactation etc. The increased available energy in concentrates compared to forage supports more rapid growth and increased fattening in beef cattle (Muir et al., 1998; Fontenot et al., 1995; Byers, 1982). This has been substantiated with molecular evidence that profiles the induction of various regulatory factors involved in skeletal muscle (SM) and adipose tissue (AT) development in rapidly growing animals on a high plane of nutrition compared to restricted animals (Schmidt et al., 2011; Graugnard et al., 2010; Graugnard et al., 2009). Transcription factors and other regulatory players that control the accretion and composition of body tissues orchestrate these metabolic shifts (Graugnard et al., 2009). The exact mechanisms and identity of these regulatory factors particularly in GFB cattle are yet unknown.

GFB and Adipose Tissue

Regardless of the finishing system, beef is a nutrient dense food packed with a variety of high quality nutrients. While the economic impact of carcass size and quality are obvious, forage finishing can affect the composition of beef carcasses in a manner that alters the nutrient profile and may affect consumer health (Neurnberg et al., 2005; Daley et al., 2010; Alfaia et al., 2009; Desmet et al., 2004). Different finishing strategies i.e. grain, forage, or different combinations thereof, consistently produce distinct carcass phenotypes in beef cattle. This is true particularly with respect to adipose tissue depots (Roberts et al., 2009; Hausman et al., 2009; Alfaia et al., 2009; Faucitano et al., 2008; Kerth et al., 2006; Nuernberg et al., 2005; Realini et al., 2004), which has a direct impact on production efficiency as well as consumer preference and health (Kouba and Mourot, 2011; Scollan et al., 2001; Wood, 1999).

Grass fed beef is leaner than grain-finished beef (Alfaia, et al., 2009; Leheska, et al., 2008; Realini, et al., 2004), and grass feeding results in beef products with different fatty acid profiles, which affects organoleptic qualities and consumer acceptability (Daley et al., 2010, Faucitano et al., 2008; Kerth et al., 2006; Cox et al., 2006; French et la., 2000). GFB reportedly has increased proportions of conjugated linoleic acids (Noviandi et al., 2012; French et al., 2000), which have been purported to be beneficial to human health. Higher concentrations of anti-oxidant compounds have also been observed in GFB compared to grain-fed animals. While potentially beneficial to consumer health, changes in FA composition and antioxidant content can impart an "off" flavor and/or color to GFB that may be undesirable to most consumers (Daley et al., 2010; Wood et al., 2003). These distinctions are part of the consumer appeal for GFB for those that desire it but also represent concerns for less reluctant producers or consumers.

This distinct carcass composition in GFB is a function of nutrient content and availability in the forage. Changes in age or quality of forage result in differences in nutrient availability to

the animal (Rayburn et al., 2005; Drouillard and Kuhl, 1999; Preston, 1994), and ultimately affect carcass composition. Thus, proper management of forage for grazing is an important consideration for producing GFB (Funston et al., 2011; Rayburn et al., 2005). Additionally, it is also important to note that while GFB may have a higher proportion of beneficial FA, the absolute amounts due to the differences in animal size and total volume of AT depots between the two systems may dilute the beneficial effects of GFB. As such, it remains to be seen how these and other "improved" characteristics of GFB can translate into improved consumer health compared to beef from grain-fed animals. In order to improve upon this beneficial profile, specific metabolic pathways can be targeted to augment the proportions of favorable FA.

Various factors including age, genetics, and sex, influence the volume and composition of SM tissues and AT depots in beef cattle and other species (Hausman et al., 2009; Desmet et al., 2004; Utrera and Van Vleck, 2004; Owens et al., 1993). While these components are objective or predetermined, other production aspects, such as management strategies and nutrition are controllable factors and have a marked impact on carcass characteristics (Dalet et al., 2010; Owens et al., 1993; Crouse et al., 1984). There are quantitative and qualitative differences in AT volume and composition in grain-fed vs. GFB that occur in a depot-specific fashion. In particular, marbling is lower in GFB, a critical factor that must be addressed because IMF directly impacts meat quality scores and consumer acceptance of beef (Wood et al., 1999). Different AT depots (e.g. IMF, SC, Intermuscular Fat, etc.) are metabolically distinct entities and require individual attention with respect to their specific regulatory paradigm (Bergen and Burnett, 2011; Hausman et al., 2009; Rosen et al., 2000). Regulatory networks governing the processes of adipogenesis and AT metabolism have been described in various model systems (Poulous et al., 2010; Bergen and Mersmann, 2005). Putative players such as PPAR-γ and

CEBP/α (Sierebaek et al., 2011; Roesen et al., 2002; Christy et al., 1989;) have been identified and substantiated, while the identity and role novel factors are constantly being investigated (Romao et al., 2011; Stegar and Lezar, 2011; Wang et al., 2011). The ability to independently manipulate individual AT depots in a manner that improves carcass quality and production efficiency is a priority goal for beef producers and researchers and would be a major benefit to the GFB industry due to concerns with AT accretion and carcass quality.

Variability in the types and quality of forages in various seasons and regions of the country must be considered because it can affect the widespread adoption of forage-finishing systems (Preston, 2004; Drouillard and Kuhl, 1999). Different varieties of forages, geography, and climate affect the ability of different forages to thrive in various areas of the country (Preston, 2004; Drouillard and Kuhl, 1999). This threatens the ability of a commercial forage-feeding operation to produce carcasses of consistently high value due to the inherent differences in plant composition, nutrient availability, geography/climate, and management practices that can affect animal growth and development (Drouillard and Kuhl, 1999). Understanding the molecular components that regulate nutrient-gene interactions in economically relevant tissues can lead to strategies that can minimize fluctuations in carcass quality and increase consistency despite the inherent differences in forage composition and quality in different areas of the country.

Along with health awareness, consumers are becoming increasingly environmentally conscious making the perceived sustainability of beef production as important a factor as the quality of the product itself (Capper, 2011; Gaylean et al., 2011; Harper and Makatouni, 2002). While there is data to the contrary (Capper and Caddy, 2010), some of today's consumers view more pastoral agricultural practices as more "natural" and "environmentally friendly (Capper et

al., 2009)," which has been a driving force for the grass-fed beef revolution. The alleged renewability of a forage-based system lends itself to a long-term sustainability given proper land and animal management practices. In order to live up to these "greener" expectations; the production efficiency (FE, growth rate, REA etc.) must be improved in forage-based finishing systems (Capper, 2011; Capper et al., 2008). The advent of genomic technologies in beef cattle will aid in this progress (Hill, 2009; Sellner et al., 2007; Dekkers, 2004).

The wealth of evidence supporting disparities in growth rate, mature size, and to a less conclusive extent the nutrient profiles and production sustainability of grass-fed versus grain-fed beef have yet to be conclusively explained on a biological level. Of particular interest to our laboratory are the molecular distinctions that give rise to these divergent phenotypes and how they can be manipulated to deliver a consistently high-quality product in an efficient manner. These regulatory mechanisms have been scarcely investigated in beef cattle. Understanding these molecular distinctions will allow for specific interventions and manipulations to mitigate the undesirable aspects associated with grass-fed beef while capitalizing on the advantages of this sustainable finishing system. Livestock production in the post-genomic era will benefit from molecular platforms that will advance our understanding of how feeding systems and nutrient availability in these systems effects phenotypic outcome and the production efficiency associated with delivering a quality beef product. The application of these and other genomic technologies to GFB production can lead to improved consumer acceptance and demand, better production efficiency, and larger profit margins in the GFB industry.

Compensatory Growth

Another strategy that has been utilized to maximize production efficiency in beef cattle is the use strategic feed restriction/re-feeding regimens to capitalize on physiological phenomenon

known as compensatory gain. Compensatory gain is observed in animals and is characterized by a period of accelerated growth following a period of suppressed growth as a result of nutrient restriction (Gerrard and Grant, 2002; Wilson and Osbourne, 1960). As a result of this occurrence, animals on a lower plane of nutrition show a retarded growth trajectory but upon re-alimentation eventually match that of their non-restricted counterparts (Gerrard and Grant, 2002). From a production standpoint, this practice can be advantageous because if animals can be fed on a restricted level and eventually reach the same level of performance (meat, milk wool, etc.) as non-restricted animals, this will reduce the cost to produce a marketable product and have a positive impact profit margins. Evidence for compensatory growth has been reported in various animals including pigs, cattle, chickens, and other species (Austad 2010; Zhan et al., 2007; Lebret et al., 2007; Lin et al., 2004; Therkildsen et al., 2003; Chiba et al., 1999; Zubair and Leeson, 1996 Sainz et al., 1995 Mersmann et al., 1987).

The concept that caloric restriction can be beneficial to metabolic efficiency in animals has been investigated in various species ranging from roundworms (*C. elegans,*) to yeast (*S. cerevisiae*), rodents, and larger mammals (Lin et al., 2004; Austad, 2010; Higami et al., 2000). It has been observed that caloric restriction can prolong lifespan and improve metabolic efficiency in these various species (Ungarvi et al., 2008; Kritchevsky, 2002; Merry, 2002). Prolonging lifespan is usually not the objective of livestock production, but the effects on metabolic efficiency and nutrient partitioning are relevant to the comprehensive understanding of the molecular regulation of production efficiency. In a livestock production setting caloric restriction is generally not conducive to generating product, but the benefits of strategic caloric restriction followed by re-alimentation may increase profit margins and reduce environmental impact in the beef industry (Funston et al., 2011). It may also allow producers to tailor management strategies

to seasonal forage growth patterns and fluctuations in feed prices or availability, to make their overall operation more efficient. The effect of a properly managed restriction and re-feeding regimen can be so pronounced that animals that have been feed restricted prior to finishing can often attract a premium price in the feedlot due to the expected improved efficiency of gain relative to well-fed animals (McCurdy et al., 2010; NRC, 1996; Sainz et al., 1995). As such, this warrants further investigation to understand the molecular mechanisms responsible for this response.

Compensatory growth in livestock has been studied for years in livestock and other species (Fox et al., 1972; Osbourne and Mendel, 1916) but our understanding of the mechanistic basis of this phenomenon remains vague. Additionally, how the mechanisms of compensatory gain impact growth, carcass composition, and energy partitioning is still poorly understood (McCurdy et al., 2010; Sainz and Bently, 1997; Sainz et al., 1995). In terms of putative knowledge, it has been documented that during the re-alimentation period, animals undergoing compensatory growth are more efficient compared to their counterparts fed a more suitable and consistent plane of nutrition and that this improved efficiency is transient as the effect is diminished once the animals are re-alimented for extended periods (Sainz et al., 1995; Kabbali et al., 1992; Abdallah et al., 1988).

Adipose tissue and SM tissue protein, serve as repositories for cellular energy and building blocks, respectively (Havel, 2000). These are dynamic metabolic tissues that are tightly regulated in order to meet the energetic and metabolic demands of the organism based on the availability of energy and nutrients. In the case of nutritional challenges (in this case, restriction/re-feeding) these tissues adapt and remodel in an attempt to accommodate the metabolic demands of the organism and achieve relative homeostasis. The degree and duration of

caloric restriction dictates the metabolic response to this challenge and this response is orchestrated by molecular regulatory factors. Patterns of SM protein metabolism are important in terms of the lean tissue accretion in beef cattle production. Adipose tissue metabolism during feed restriction and re-feeding is also important due to the economic ramifications of AT accretion in specific depots. Because of the plasticity of these tissues, their central roles in energy and protein metabolism, as well as their economic value in terms of beef carcasses, AT and SM warrant special focus with respect to their contribution their metabolic response to feed restriction and repletion.

The involvement of protein metabolism during restriction and re-feeding have been documented in rodents, pigs (Whang et al., 2003; Therkildsen et al., 2002; Hornick et al., 2000; Jones et al., 1990; Millward et al., 1975). Whang et al. (2003) reported that pigs fed a protein-deficient diet exhibited compensatory growth once re-alimented and during the compensatory period the crude protein requirements are higher than that of non-restricted pigs. These authors argue that increased efficiency of protein utilization is responsible for the compensatory gain following restriction observed in pigs. This evidence substantiates the dynamics of protein turnover as a likely contributor to the compensatory response. The role of protein turnover, the interaction of various factors including increased feed intake, altered composition of gain, and energy partitioning during the compensatory response are vaguely understood and but it is well known that these processes are controlled by molecular and cellular alterations.

Endocrine and blood metabolite changes observed during the restriction and re-feeding periods have been studied in a variety of cattle species and production settings (Hersom et al., 2004; Hornick et al., 1998; Yamabayamba et al., 1996; Hayden et al., 1993; Hammond et al., 1990). The GH-IGF axis has often been implicated in the compensatory response in beef cattle

(Hayden et al., 1993). The somatotropic axis, which includes GH, Insulin-like Growth Factors, and their associated proteins, is responsible for growth and lactation in cattle and swine (Lucy, 2008; Renaville et al., 2002) and other species. GH secretion induces an anabolic paradigm in which protein accretion is enhanced and AT accretion is decreased. During fasting (or feed restriction) GH secretion spares protein breakdown and mobilizes energy from AT depots. This has been substantiated in several species (Lucy, 2008; Thirkilsden et al., 2004; Norrelund et al., 2001; Norrelund et al., 2000; Jones et al., 1990) and supports the observed increased body protein gains and increased efficiency of nutrient use in compensating cattle.

Yamabayamba et al. (1996) conducted a study in beef heifers fed *ad libitum* or restricted for 95 days followed by re-feeding. They measured several blood metabolites as well as resting metabolic rate before during and after restriction and concluded that the lower metabolic rate along with activation of the IGF-GH axis was responsible for the enhanced growth rates and efficiency observed in restricted cattle. While these and other metabolites have been identified the molecular signaling pathways responsible for, or responsive to these changes remain unclear.

Moderate caloric restriction results in a metabolic paradigm which attempts to conserve SM tissue protein and mobilizes AT energy by releasing FA. During re-alimentation, protein accretion continues transiently before protein synthesis decreases and AT accretion commences. The general pattern has been seen in cattle in studies that demonstrate that following feed restriction, the empty body weight of cattle is composed of increased proportion of protein and water compared to non-restricted cattle and that upon re-feeding the proportion of fat increases with declining proportions of protein and water (Hornick et al., 1998; Wright and Russell, 1991). Hornick (1998) conducted an experiment using Belgian blue bulls subjected to a low growth restriction period followed by a fattening re-alimentation period. They showed that the

compensatory effect lasted for a maximum of 2 months and that it resulted in increased FE in compensating animals. Additionally, compensating animals showed lower intramuscular fat and peripheral fat was higher compared to non-restricted animals. An interesting observation by the authors is that although the carcass quality grades suffered from increased peripheral AT, the fat content of the meat was actually lower than well-fed animals. These data show differences in peripheral and IMF metabolism that result in markedly different carcasses however the molecular components responsible have not been conclusively identified.

Mitochondrial efficiency and liver size have also been investigated in feed restricted and compensating animals (Connor et al, 2009; Pamplona and Barja, 2006; Merry, 2002). Connor et al. (2009) reported that during the re-alimentation period, feed-restricted cattle showed increased expression of genes involved in cellular metabolism, cholesterol synthesis, oxidative phosphorylation, glycolysis, and gluconeogenesis. They also reported reduced hepatic size, and based on these data concluded that liver size and mitochondrial function might be responsible for the compensatory response in these animals.

Understanding the ideal timing of compensatory process is an important economic consideration because the costs and rate of gain can vary depending on production phase, and with the use of different production inputs (Sainz et al., 1995; Coleman et al., 1993; Wright and Russel, 1991). Much of this is affected by, or coincides with molecular events that occur as a normal part of growth and development (Du et al., 2010) as well as those induced by environmental factors. For example, studies have shown that age of the animal affects the degree of the compensatory response and can also have an impact on composition of gain during this period (Boddicker et al., 2011; Choasap et al., 2011; Coleman et al., 1993; Wright and Russel, 1991). In order to implement and maximize the benefits of this practice in SE beef cattle

production, the optimal initiation and duration of the restriction period, particularly in a forage-based system, must be determined. An improved understanding of the molecular adaptations that are responsible for the accelerated growth and nutrient partitioning during the restriction and re-alimentation periods will provide critical pieces in the understanding of metabolic efficiency and provide the basis for management strategies for maximizing the economic and production benefits of this practice without compromising animal health and/or performance.

While the exact mechanisms for compensatory gain have not been elucidated, it is clear that metabolic shifts are responsible for the observed effects of restriction and re-feeding (Hornick et al., 2000; Hornick et al., 1998; Christensen et al., 1997). Energy conservation pathways are likely to be employed under periods of restriction and improved efficiency in the use of available nutrients may carryover for a transient time during the re-alimentation period leading to rapid compensatory growth. Mitochondrial function is also a likely player and this information must be identified. The identity of these mechanisms and pathways were the subject of the current inquiry because they may play a critical role in our understanding of the compensatory growth phenomenon and improve our ability to manipulate it towards more efficient and profitable beef production.

Rationale and Objective:

The initial thinking upon undertaking this project was to evaluate the effects of a forage-based finishing system using nutrient rich perennial ryegrass (*Lolium perenne*) pastures compared to dry lots on animal performance and gene expression patterns in finishing steers. Feeding forage is a common practice in the SE and when properly managed, can produce beef of desirable quality in an economically efficient and environmentally sustainable manner. It has been documented that molecular changes occur throughout the course of the feeding period that

result in changes to animal phenotype and meat quality (Graugnard et al., 2010; Du et al., 2010; Dayton and White, 2009; Hausman et al., 2008; Johnson and Chung, 2007). In addition, there is substantial evidence that caloric restriction followed by re-alimentation is capable of inducing a compensatory gain effect in cattle and other species, a phenomenon that may be useful in improving the efficiency of production in beef cattle. Unpublished data have shown a marked effect on growth trajectory when cattle subjected to ryegrass pastures after being on dormant pastures. The purpose of this project was to determine the effects of feed restriction and re-feeding in a pasture system, accomplished using differing periods of forage grazing (nutrient availability), on the expression of regulatory genes involved in lipid, protein, and energy metabolism in grazing steers.

Materials and Methods

Animals:

All procedures were conducted in accordance to the Auburn University Animal Care and Use Committee guidelines (IACUC Approval Number: 2008-1490). Animals were housed at the Auburn University E.V. Smith Beef Cattle Research Center. Animals were maintained on dormant and/or growing pastures (per experimental design) for the different time periods during the experiment.

Feeding Trial:

Animals were divided into one of four feeding groups to receive perennial Rye grass (*Lolium perenne),* or dormant pasture for different durations during the trial. **Figure 5.1** shows the grazing assignments for each treatment group during the feeding period. The first group (Group 126; n=12) received ryegrass for the full 126 days of the experiment. The second group

(Group 84; n=12) was on dormant pasture for the initial 42 days and then grazed the ryegrass pasture for the final 84 days. Group 42 cattle (n=12) were on dormant pasture for half of the trial and then received ryegrass for the final half. Group 0 cattle (n=12) were on dormant pasture for the entire duration of the experiment and did not receive Ryegrass. All Animals were weighed every 21 days and biopsy samples were collected on day 42, 84, and 126 of the trial.

Skeletal Muscle and Adipose Tissue Biopsies

For each of the treatment groups, half (n=6) of the animals were subjected to an excisional biopsy to collect subcutaneous adipose tissue (SC) and *Longissimus dorsi* muscle tissue (LM) in order to obtain samples for gene expression analysis. This procedure was conducted in the field during the collection of fecal samples and weight and height data. While the animal was restrained in a cattle chute, a 12 cm x 12 cm area centered over the loin area just caudal to the 13th rib was clipped and scrubbed for aseptic surgery. Lidocaine hydrochloride 2% (15 mL SQ) was infused with a sterile 20ga. x 1 ½ inch needle along the cranial and dorsal edges of the surgically prepared area to achieve local anesthesia. A 2cm vertical skin incision was made and approximately 1 gram of subcutaneous adipose tissue was surgically excised. The incision was extended ventromedially into the longissimus dorsi muscle. A 1-gram sample of the dorso-lateral aspect of the *Longissimus dorsi* muscle was removed by sharp dissection. The skin incision was closed with #3 braided coated nylon suture (POLYWEB, Webster Veterinary Supply, Sterling, MA) in a cruciate pattern. Skeletal muscle and adipose tissue samples were labeled and immediately snap frozen, in liquid nitrogen, until they could be taken back to the lab and stored in a freezer at minus 80 °C. Cattle were then sprayed with fly repellant and monitored for complications post procedure. Each sample was taken from the same approximate location at the 42, 84, and 126d collection points. In addition samples were taken at slaughter from the loin

muscle, gastrocnemius muscle and from the subcutaneous back fat depot. A 0d sample to provide a gene expression baseline for each animal was scheduled to be collected, but was not due to logistical complications. For the current experiment only biopsy samples collected at 84 and 126d were analyzed.

RNA Isolation

Total RNA was extracted from tissue samples using TRIzol reagent (Invitrogen, Carlsbad, CA). Approximately 0.5g of tissue was removed from the minus 80 °C freezer and placed in a portable liquid nitrogen container. The frozen tissue was then crushed using a metal mortar, and the crushed tissue was placed in 5ml of TRIzol. The sample was then homogenized using a polytron® tissue homogenizer and then the extraction carried out as per the manufacturers protocol (Invitrogen, Carlsbad, CA). The crude RNA extract was subjected to deoxyribonuclease (DNAse) treatment using Turbo DNA free kits (Kit No. AM1907M; Ambion Inc., Austin, TX) to remove residual genomic DNA that could interfere with downstream procedures. A 20ul aliquot of the crude RNA isolate was placed in a nuclease free micro-centrifuge tube and 2ul of Turbo DNAse (2U/µl) and 2ul of 10X DNAse reaction buffer were added to each sample. The tubes were then placed in an incubator at 37 °C and the DNAse reaction was allowed to continue for 30 minutes. After the 30 minute period the 2ul of the re-suspended DNAase inactivation reagent was gently mixed into the reaction and allowed to sit for 5 minutes before being centrifuged at 10,000 x g. The supernatant containing the RNA was then collected and placed into a new, labeled, nuclease-free tube. The total RNA isolate was then subjected to RNA purification using RNeasy mini elute columns (Kit 74102; Qiagen, Vencia, CA). RNA concentration was determined using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Willmington, DE).

Complimentary DNA synthesis

Complimentary DNA (cDNA) was synthesized from the purified RNA isolate using high capacity cDNA Reverse Transcription kits (Kit # 4368814, Applied Biosystems, Foster City, CA). One microgram of DNAse treated total RNA was subjected to cDNA synthesis according to the manufacturer's protocol. The resulting cDNA was then stored in the freezer until it was used for quantitative Real-Time PCR (qRT-PCR) to determine the expression of specific target genes.

Quantitative Real-Time PCR

Quantitative Real-Time PCR was used to determine the expression (mRNA abundance of a selected panel of target genes. Custom TaqMan gene expression assays (Applied Biosystems, Foster City, CA) were designed using available gene bank information for the genes of interest in this study. The assays in this category are indicated by an asterisk in table 1. Gene expression assays were conducted using an ABI 7500 Real-Time Thermocycler (Applied Biosystems, Foster City, CA). As described in the previous chapters, 18s ribosomal RNA was used as an endogenous control and relative mRNA abundance was determined using the ABI SDS software v2.0.3 (Applied Biosystems Foster City, CA).

Real-Time PCR Reactions

For each 20μl-multiplexed reaction the reaction components and PCR cycling conditions were as indicated in table 2 and table 3, respectively. For each reaction 1ul (50ng) of cDNA product was diluted to 8ul in a 2ml nuclease-free micro-centrifuge tube and placed on ice. A sufficient amount of master mix containing enough TaqMan gene expression master mix (Part No. 4369016; Applied Biosystems Foster City, CA), 18S probe, and the custom gene expression assay was prepared for the tissue/gene of interest and then placed on ice. For each sample 12ul of

master mix was added to the tube containing the 8ul cDNA solution. The reactants were gently mixed and then the 20ul reaction was pipetted into individual tubes in 0.2ml MicroAmp® Optical 8-Tube Strips (Part No. 4316567; Applied Biosystems, Foster City, CA) that were resting in a chilled PCR cooler. The strips were then capped with MicroAmp® Optical 8-Cap Strip (Part No. 4323032 Applied Biosystems, Foster City CA) and centrifuged gently to remove air bubbles and ensure all components of the reaction were thoroughly mixed at the bottom of the tube. For each tissue sample, the multiplexed assay was run in duplicate and this process was repeated for each gene/tissue combination.

Statistical Analysis

Statistical analysis was done using the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The GLM model statement included the main effects of biopsy date and treatment group as well as the interaction effect of biopsy date and treatment group. Treatment means were compared using the Least Square Means statement in SAS. Differences in least square means were considered significant at $P< 0.05$ and tendencies were considered at $P < 0.10$. The Proc CORR procedure of SAS was used to determine correlation coefficients between performance traits and average relative expression for each gene. Correlations were considered significant at $P < 0.05$ and tendencies were considered at $P < 0.10$.

Results and Discussion

For the current experiment, gene expression (relative mRNA abundance) and animal performance data were analyzed. Adipose tissue and skeletal muscle biopsy samples collected at 84d and 126d were used for GE analysis. Weights were collected every 21d. Total weight and

average daily gain were analyzed to correspond to the biopsy dates. Overall gain/ADG for the 126 day trial was also determined and related to gene expression data.

Animal Performance

Both overall gain and ADG for the duration of the trial showed significant differences between treatment groups (Figure 5.2). For the full 126 day trial, Group 126 had the highest total gain and ADG of any group (Figure 5.2). These Group 126 animals were allowed to graze growing ryegrass for the entire feeding period and gained an average of 205kg. Group 84 animals were heavier ($P < 0.05$) compared to Group 0 animals, which only gained around 68kg in 126d (Figure 5.2). The group 0 animals were relegated to the lowest plane of nutrition and were not allowed to graze growing rye grass at any point during the 126d trial. This impact of this low energy regimen is evidenced by their lack of substantial weight gain compared to other groups after 126d (Figure 5.2). Group 42 and 84 animals reported similar weight gains ($P > 0.05$) that were higher ($P < 0.05$) than group 0 but lower ($P < 0.05$) compared to Group 126 animals (Figure 5.2). As expected, similar patterns were observed for average daily gain. Also expected was the decreasing trend in weight total gain/ADG as days on ryegrass decreased.

Compensatory Effect

The differing periods of access to growing ryegrass served to alter the plane of nutrition of the finishing steers in the current study. During the trial period group 84 and 42 animals were allowed to graze growing ryegrass after being subjected to a dry lot and supplemented with hay for 42 and 84d, respectively. This was intended to impart caloric restriction on these animals for varying times in the finishing period. Upon re-alimentation (being released on ryegrass pasture) there were noticeable changes in growth trajectory indicating a potential compensatory effect in

these animals. From 63 to 84d, the group 84 animals showed similar total and average daily gains to group 126 (Figure 5.5; $P > 0.05$) which were higher compared to group 42 and group 0 animals which had not grazed ryegrass ($P < 0.05$). In contrast, group 42 animals were placed on ryegrass at 84d and recorded weight gains that were higher ($P < 0.05$) than all other groups between 84 and 105d despite groups 84 and 126 also consuming ryegrass during this period (Figure 5.8). These results indicate that a period of feed restriction followed by re-alimentation did indeed provoke a compensatory response in grass-fed cattle under these conditions. While there was an observable compensatory effect with immediate impact on growth trajectory upon re-alimentation on the ryegrass pasture, this effect was not substantial enough to accomplish the same total weight as animals receiving ryegrass for the entire 126d (Group 126; Figure 5.2).

While there were significant differences for overall gain and ADG for the length of the trial, there was substantial variation within and between treatment groups with respect to weight gain during the treatment phases. Group 126 appeared to level off with respect ADG and thus total gain during the final phase of the treatment period gaining an average of ADG of 0.4lkgs/d and averaging 13.3kgs total gain during the final 42d of the trial period (Figure 5.8, 5.10). Despite continuously grazing on the ryegrass, this group reported similar gains ($P > 0.05$) to the other treatment groups during this period (Figure 5.10). This was particularly interesting with respect to group 0 which only grazed the dry lot during the trial. One possible explanation for the tapering off of growth towards the end of the experiment may be the result of improper pasture management to sustain high levels of gain as animals grew and began to consume more. This would lead to a wash out of the high plane of nutrition and decrease overall growth during the later periods of the trial as evidenced by ADG and total gain data. Nonetheless, at this time point

there were no differences in gain between the treatment groups regardless of diet (Figure 5.10; $P > 0.05$).

When attempting to rear cattle on pasture, an important consideration is the carrying capacity and quality of the pasture. It is possible that the levels of growth under the SE climatic conditions during the trial period were not sufficient to support the consistent growth of these maturing steers during the latter phase of the trial thereby relegating the animals to a relatively restricted caloric regimen by default. The lack of differences in the late phases is likely due to the substantial individual animal variation with respect to gain during this treatment phase (Figure 5.7, and 5.12) which can possible be attributed to variation in individual animal feed efficiency. That is to say that some animals still performed well during this period while others recorded declining weight gains compared to previous periods (Figure 5.12) At 84d groups 126 and 84 showed similar weight gain (around 2.3kg, $P > 0.05$) which was higher than groups 42 and 0 ($P < 0.05$) which showed similar gains to each other (Figure 5.5; $P > 0.05$). Additionally, we noted that total Gain for the 126d trial was highly correlated with gain determined at 84d (R= 0.71; $P = 0.0004$).

Gene Expression Analysis

The performance of the animals in the current study was used to compare to expression of key metabolic genes that are responsible for differences production efficiency in finishing animals. While these factors have been investigated in other settings, the effect of a forage-based restriction/re-feeding paradigm under SE finishing conditions on the selected GE profile has not been investigated. Composition of gain was not determined, but this would have been very valuable in order to determine the net effect of the balance of metabolic networks determine carcass phenotype.

Serial Biopsy of Loin Muscle Tissue

Excisional biopsies of *longissimus dorsi* (LM) were collected from steers at three points during the feeding trial. The original intention for this experiment was to conduct a longitudinal grazing study in order to collect and analyze economically relevant SM and AT samples at important developmental time points to follow gene expression patterns. The use of skeletal muscle samples to monitor the expression of adipogenic genes involved in the development of the marbling depot has been previously established (Barnes et al., 2012; Graugnard et al., 2010; Graugnard et al., 2009; Wang et al., 2010; Lee et al., 2007). Graugnard et al., (2009) contend that alterations in gene expression networks are responsible for driving adipogenesis, lipid filling, and intracellular energy metabolism in the IMF depot. This provides an opportunity to evaluate long-term effects of nutrition and other management practices on adipogenic gene expression in SM of beef cattle as the IMF and other AT depots develop. In terms of sample collection strategy, the efficacy of the serial biopsy approach in understanding temporal and developmental aspects of adipogenesis in SM has also been documented in previous studies (Barnes, 2012; Graugnard et al., 2010; Wang et al., 2009; Lee et al., 2007; Lehnert et al., 2006). In the current study biopsy samples were collected at 84 and 126d for gene expression profiling. As mentioned before a 0d sample to provide a gene expression baseline for each animal was scheduled to be collected, but was not due to logistical complications. Additionally, based on the experimental design we only looked at the final 2 biopsy dates due to the fact that it was at this point where we truly had 4 treatment groups. Prior to this animals had received either ryegrass or dormant pasture with no combinations of the two (Group 126 on ryegrass for 41 d; Groups 84, 42, and 0 for 41d) on dormant pasture. Therefore it was hypothesized that these time points would be most informative in terms of developmental windows in response to dietary regimens.

Effect of Biopsy Date on Relative Gene Expression in Skeletal Muscle Biopsies

Energy and protein metabolism are dynamic processes that change throughout the course of growth and development. In addition, the process of adipose tissue deposition in cattle and other species is a developmental process that follows a temporal pattern and is affected by genetic, environmental, nutritional, and other factors (Du et al., 2010; Hausman et al., 2009). Many studies regarding the developmental process have been conducted in tissues from fetal and early postnatal cattle (Du et al., 2010a; Du et al., 2010b). To garner a perspective of this developmental time course in finishing cattle we collected biopsy samples at time points during the finishing process to understand how the expression of target genes might change with time and treatment and how these relate to animal performance.

In skeletal muscle, PPAR-γ, the putative master regulator of adipogenesis, showed increased expression in SM at biopsy date 126 compared to 84 (Figure 5.14; $P < 0.05$). This is consistent with results from Lee et al. (2007) who observed marked up-regulation of these PPAR-γ and CEBP/α in the latter fattening stage of finishing Hanwoo steers compared to earlier time points. It should be noted that their study lasted much longer and they collected biopsy samples at 12 and 27 months. The current study lasted only 126 days. Unlike the Lee study we did not observe a significant increase in CEBP-α expression in the later biopsy date (Figure 5.16; $P > 0.05$). In a study profiling gene expression patterns during IMF in Wagyu x Hereford and Piedmontese x Hereford cattle, Wang et al., (2009) determined that there were noticeable differences in the expression of adipogenic genes in a time-frame specific manner. They collected a series of 6 biopsy samples from 2 to 25 months and noticed that PPAR gamma expression was elevated at later time points compared to the earlier in animals that had the higher genetic propensity to have high IMF. Although we used animals from a similar genetic

background, the Wang et al., (2009) study highlights a temporal PPAR-γ gene expression pattern similar to that observed in the current study. These and other data support the notion that the marbling depot experiences a lag in maturation that is controlled by transcriptional regulators of adipose tissue metabolism.

PGC-1α expression was higher at 126d vs. 84d (Figure 5.20; $P < 0.05$). Increased PGC-1α expression at the latter biopsy date may indicate increased mitochondrial biogenesis/function as the animal's age or in response to changes in diet by the 126d time point. In a study Connor et al., (2009) fed 8-month old steers ad libitum (n=6) or restricted them to 60-70% of the intake of the control animals (n=6). The authors collected liver biopsies at -14, +1, and +14d relative to re-feeding. They determined that during re-alimentation that the restricted steers showed improved feed efficiency relative to their control counterparts. They also reported an increase in the expression of genes encoding mitochondrial complex proteins indicating a potential role for increased mitochondria in improving feed efficiency. While our study utilized specifically SM and AT tissues and not liver, the findings of Connor et al., (2009) are informative in terms of the integration of mechanisms responsible for improved feed efficiency experienced during compensatory gain. In addition to PGC-1α we looked at UCP-2 expression. Uncoupling proteins are capable of dissipating the proton gradient used to drive the synthesis of ATP in them mitochondria. UCP-2 expression in skeletal muscle was not affected by biopsy date (Figure 5.15; $P > 0.05$). Previous studies have reported a relationship between UCP expression and feed efficiency (Erlanson-Albertsson, 2003). While uncoupling proteins are involved in the dissipation of energy, the oxidation of FA serves as a major source of ATP for cellular energy purposes in ruminants and other species. CPT-1b expression is indicative of increase FA oxidation as this gene represents a major regulatory point governing the entrance of FA into the

mitochondria, the site of β-oxidation in the cell. CPT-1b expression was higher in SM biopsy samples collected at the 126d compared to d84 (Figure 5.13 $P < 0.05$).

Proteasome Macropain Subunit 11 expression was about four times higher at d126 compared to day 84 (Figure 5.18; $P < 0.05$) indicating higher rates of turnover at this time point. Increased protein turnover later in the feeding phase may be indicative of decreased efficiency. Protein turnover is an energetically expensive process given the ATP investment required to synthesize and breakdown proteins and efficiency of production can be increased by decreasing the rate of protein turnover (Bergen, 2008; Bergen and Merkel, 1991). Rate of weight gain decreased later in current trial (Figure 5.10 and Figure 5.11) and this could be due to increased protein turnover to supply energy for basic metabolic needs.

Effect of Treatment Groups on Relative Gene Expression in Skeletal Muscle Biopsies

In addition to the temporal aspect of growth and development, plane of nutrition and energy balance can impact the metabolic disposition and efficiency of growing animals. We sought to understand the effects of treatment group (duration of ryegrass vs. dry lot exposure) on the expression of regulatory genes involved in energy and protein metabolism. Despite observing significant changes in animal performance with respect to treatment groups, there were limited effects of treatment on the expression of genes in SM in these cattle.

UCP-2 expression was highest in treatment Group 126 (Figure 5.23 $P < 0.05$) compared to Groups 84, 42, and 0 which all showed similar expression ($P > 0.05$). FABP4 expression in SM showed substantial variation within treatment groups and there were no significant differences between treatments (Figure 5.24; $P > 0.05$). This could be indicative of differential marbling potential within animal groups. FABP4 is a putative marker of late adipocyte differentiation and would be expected to increase as the depot develops and adipocyte

metabolism and lipid storage ensues. Treatment group 42 showed increased expression of FABP4 relative to the other groups ($P < 0.05$). Pref-1 expression was higher in treatment group 42 compared to group 126 ($P < 0.05$), but was not different compared to group 84 and 0 (P > 0.05). PPAR-γ expression in SM was not affected by treatment ($P > 0.05$). CEBP-α expression was lower in group 42 compared to the group 126 (P < 0.05), but did not differ from group 84 and group 0 ($P > 0.05$). Groups 126, 84, and 0 all showed similar expression to each other (P > 0.05).

Pref-1 expression was higher at the second biopsy date compared to the d84 (Figure 5.19; $P < 0.05$). This is counter-intuitive given the putative school of thought on marbling development. It is generally accepted that the marbling depot is the late maturing therefore, as a negative regulator of adipogenesis, Pref-1 expression would be expected to decrease as the finishing phase progressed and the IMF depot expands. This has been demonstrated in a recent study by Key et al., (2013) in which cattle were subjected to grain or forage based diets for differing numbers of days on feed. The authors determined that Pref-1 mRNA in subcutaneous AT was decreased in response to DOF and grain finishing. The authors did not however measure Pref-1 expression in the IMF depot. It could be possible that the duration of the trial period was not long enough to truly monitor the Pref-1 profile over an adequate developmental window. Because Pref-1 is involved in muscle and adipose development which begins prenatally and continues throughout early postnatal and adult life, it would also be helpful to analyze earlier developmental time points to determine the overall effect of Pref-1 expression early and late in IMF development which has yet to be established.

PGC-1α drives mitochondrial biogenesis and as such is a major factor in energy metabolism. It has been shown that mitochondrial function is related to metabolic efficiency in

cattle and other species. Mitochondria are responsible for 90% of intracellular oxygen consumption and serve as the primary site for ATP synthesis. PGC-1α expression has been shown to be higher in elevated in low RFI (more efficient) animals compared to high RFI animals indicating that it may play a role in determining metabolic efficiency (Kelly et al., 2010). In the current study PGC-1α expression was highest in group 42 (Figure 5.27; P < 0.05) compared to the other groups which had similar levels of expression (P >0.05). Animal variation with respect to weight gain and performance could have contributed to the lack of differences in the current study. More robust treatments and animal numbers that generate larger performance disparities may be necessary to define the role of PGC-1α in metabolic efficiency of forage-finished steers.

As mentioned, the efficacy of the biopsy approach in assessing skeletal muscle gene expression has been documented in previous studies (Barnes, 2012; Graugnard et al., 2010; Wang et al., 2009; Lee et al., 2007; Lehnert et al., 2006). In the current study we observed fluctuations in gene expression that corresponded to the some of the variation we observed in animal performance. Larger animal numbers would likely help to mediate the amounts of variation we observed in performance and gene expression. It would be useful to correlate our expression data with specific parameters associated with the genes of interest such as marbling for adipogenic gene, skeletal muscle fiber types for oxidative and mitochondrial genes, and loin eye area for genes involved in protein metabolism. These carcass data were not made available for the current study.

Effect of Biopsy Date on Relative Expression of Genes in AT Biopsies

Adipose tissue is an important repository of cellular energy and also plays an active role in energy homeostasis, adaptations of physiological status and plane of nutrition. The selected

genes in AT for study here have been established as important regulators and indicators of AT accretion and fat metabolism. Understanding of these genes and the pathways they regulate will be critical to the understanding and ability to manipulate AT depots to the economic and health advantage of producers and consumers, respectively.

In AT Leptin expression decreased from biopsy date 84 to 126 ($P<0.05$). Leptin is an indicator of adiposity in various animals ranging from rodents to humans (Jequier 2006; Benoit et al., 2004). Serum leptin levels increase linearly with adiposity in sheep and cattle (Delavaud et al., 2007; Delavaud et al., 2002), and would be expected to increase as time on feed increased in finishing cattle as AT accretion occurs. The effects of feeding level on leptin expression can be long term, mid-term and short-term (Chilliard and Bonnet, 2005). Long term effects of feeding level are as a result of changes in adiposity in response to nutrition and development. Days on feed at a given plane of nutrition can also affect leptin levels as can size and frequency of meals. As evidenced by the decrease in weight gain towards the end of the feeding trial (Figure 5.10 and Figure 5.11), feed intake may not have been adequate to support substantial weight gain, much less fattening at this point in the trial. In addition to governing energy homeostasis, leptin serves an adaptive role for periods of under nutrition or caloric restriction. When feed is restricted, leptin concentrations decrease and the physiological effect of this is to stimulate re-feeding. In terms of metabolic adaptations this results in increased glucocorticoid secretion, decreased energy expenditure and decreased protein synthesis.

A similar pattern was observed for GPAT which was higher at 84 compared to 126d (Figure 5.28; $P < 0.05$). GPAT is an indicator of triglyceride synthesis and increased levels would be expected to correspond with increased fat deposition in AT. In ruminant animals, GPAT expression is highest in adipose tissue compared to non-ruminants where the liver is a

primary site of triglyceride synthesis and shows high levels of expression (Roy et al., 2006). GPAT expression has been shown to be highly correlated with IMF deposition in Korean steers ($r = 0.71$; $P < 0.001$) however studies investigating GPAT in subcutaneous AT are lacking. In the current study, molecular adaptations to plateauing nutrient availability and weight gains may have given rise to this expression pattern. Earlier in the trial when feed may have been substantial enough to support growth (84d) and fattening GPAT expression was higher versus later (126d) when animals were larger and required more nutrients for fattening and GPAT expression was lower. Biochemical assessments of tissue and circulating triglycerides could have been conducted to determine if GPAT expression influenced these parameters as well as carcass composition.

There were no differences in expressions of or ZFP423 or FABP4 across biopsy dates (Figure 5.29 and Figure 5.30, respectively; $P > 0.05$). These are indicators of adipogenesis and their steady expression across biopsy dates may be responsible for, or indicative of, the lack of substantial AT accumulation across treatment groups throughout the trial. Key et al., 2013 showed that transcription factors involved in adipogenesis and backfat thickness increased with days on feed and grain feeding in subcutaneous AT of finishing heifers. In the current study carcass data to support or refute these conclusions was not available.

UCP2 expression was also higher in the d84 compared to d126 (Figure 5.31 $P < 0.05$). One of the effects of reduced leptin in the face of caloric restriction is to reduce energy expenditure via direct and indirect mechanisms. While one must be careful not to over speculate, it is possible that the decreased leptin levels at 126d corresponded to decreased mitochondrial energy oxidation however biochemical assessment of mitochondrial function is necessary to support this conclusion. Regardless, increased UCP2 expression later in the trial may correspond

to decreased metabolic efficiency and thus decreased production efficiency as cattle grew and feed resources became scarcer.

Effect of Treatment on Relative Expression of Genes in AT Biopsies

Leptin expression was not impacted by treatment group other than Group 0 which showed lower expression compared to groups 126, 84, and 42 (Figure 5.37; $P < 0.05$). Group 0 did not graze ryegrass at all in the current experiment and thus were subjected to the lowest plane of nutrition. Animals in this group would be expected to have relatively less fat cover due to their relative feed restriction and this may be responsible for the lower leptin expression in this group. Groups 126, 84, and 42 did not differ (Figure 5.37 $P > 0.05$) in relative leptin expression. In addition, leptin levels are known to decrease with feed restriction and this may have contributed to lower leptin expression in Group 0 steers.

GPAT expression was similar across all treatment groups (Figure 5.36; $P > 0.05$). As mentioned before, GPAT is involved in to formation of glycerolipids and has been shown to be upregulated by nutritional and other factors. Up-regulation of this gene results in increased TAG synthesis. The relatively constant level of GPAT expression in subcutaneous adipose tissue across treatments is interesting given the differences in energy intakes expected in treatment diets. Variation in animal performance and lack of substantial forage growth throughout the trial may have led to the lack of significant differences. Individual animal carcass data would be useful to determine if GPAT levels can be related to backfat thickness or other adipose tissue carcass traits as has been previously described for IMF (Jeong et al., 2012).

UCP2 expression was higher in group 42 compared each of the other groups (Figure 5.35; $P < 0.05$) which all showed similar expression to each other ($P > 0.05$). Uncoupling proteins dissipate the proton-gradient in the mitochondrial respiration chain and energy is lost in

the form of heat instead of being used for ATP production. UCP2 expression can therefore be associated with metabolic efficiency because energy lost as heat, even in the necessity of thermoregulation, cannot be used for productive purposes. Increased expression of UCP2 in group 42 did not affect weight gain for the duration of the trial as group 42 had similar gains to group 84 (Figure 5.2; $P > 0.05$), and higher total gain than group 0 ($P < 0.05$).

Zfp423 is a novel metabolic regulator that has been shown to promote adipogenesis through its influence on preadipocyte determination (Gupta et al., 2010). In the current study Zfp423 expression was higher in group 42 AT biopsies compared to group 126 and 84 (Figure 5. 23; $P < 0.05$). Group 0 expression did not differ from any of the other groups ($P > 0.05$). Increased Zfp423 expression is expected to indicate increased adipogenic metabolism and as such should correspond to increased backfat thickness in cattle with high expression. The exact regulatory effect of Zfp423 in vivo remains unclear. Gupta et al., (2010) showed that ectopic expression of Zfp423 in non-adipogenic NIH-3T3 L1 fibroblasts induces PPAR-γ expression in undifferentiated cells. In the current study there was no discernible relationship between Zfp423 and PPAR-γ. While this mechanism has been studied in cell lines, little information exists regarding Zfp423 expression or involvement in adipogenesis in beef cattle particularly in response to differing planes of nutrition. In this regard more detailed studies are warranted to understanding the timing and significance of this contribution.

Correlations between Animal Performance and Gene Expression

When taken alone, performance data yielded relatively expected information as animals responded based on the plane of nutrition they were exposed to, and gene expression values varied between and within treatments in some cases. When performance and gene expression were taken together, a few interesting observations emerged.

Total ADG tended to be correlated with FABP4 expression at 84d (*P* = 0.08). A similar trend was observed with CEBP/α expression (*P* = 0.09). The expression of these two genes, both involved in AT differentiation and metabolism, were also highly correlated with each other ($P < 0.0001$) at 84d. CEBP/α expression was also correlated with PGC1α expression at 84d in LM samples (*P* = 0.03). The true significance of these correlations remains to be elucidated but it is interesting to note the transcriptomic picture that emerged and how this transcriptomic pattern could be related to observed animal performance.

Another interesting, but expected correlation was the expression of UBE2 and PSDM11 which was highly correlated at 126d (0.71; *P* = 0.01). These genes are involved in protein turnover pathways and would be expected to be auto-correlated based on their function in these pathways.

It may be prudent to look directly at indices of fat accumulation including back-fat thickness and marbling scores, as well as biochemical and histological assessments of IMF, as it would be interesting to relate these parameters with gene expression data. These are the types of associations that are likely to make practical sense of biological data as they can be readily compared or observed in terms of a measurable production parameter of economic importance. Not only that, stakeholders are likely to respond more favorably to genomics based platforms when they can be directly related to production parameters that impact their bottom line.

Summary and Conclusions

The objective of the current study was to determine the effects of a feed restriction and re-feeding paradigm in a pasture-based system on the expression of regulatory genes involved in lipid, protein, and energy metabolism in grazing steers subjected to differing levels of forage growth. We sought to determine the efficacy of a targeted transcriptomic approach in elucidating

the molecular determinants of energy and protein metabolism, and how these components are affected and employed during a pasture based restriction/re-feeding regimen. The results from this study indicate that there. Earlier sampling may be informative in terms of monitoring and relating developmental trajectory with gene expression profiles.

Unfortunately forage based research of this nature is dependent on adequate conditions to maximize forage growth and generate robust performance differences in treatment groups. This was not the case during the trial period as Alabama experienced formidable drought conditions resulting in retarded forage growth (especially toward the end of the trial) which may have confounded the design and results of the experiment. There were limited differences in expression across treatment groups particularly within the treatment phases. Again, the lack of differential performance amongst the treatment groups may have hampered the robustness of this expression profiling approach. The differences in expression observed in biopsy date indicate that metabolic paradigms appear to shift as growth and development occur, however the application of these specific paradigms may be limited to the conditions experienced during this pasture/feeding trial because of the lack of substantial forage growth that would likely not be experienced under more ideal production conditions.

Chapter 5 Figures

Figure 5.1 Grazing Assignments and Treatment Phases for Steers for the 126d Feeding Trial[1]

Group Number	**Feeding Trial Phase and Assignments**		
	0-41 Days	**42-83 Days**	**83-126 Days**
Group 126			
Group 84			
Group 42			
Group 0			
Ryegrass =		Dormant Pasture =	

[1]Ryegrass = Growing Perennial Ryegrass Pasture (*Lolium perenne*); Dormant Pasture = Dry lot supplemented with hay; Group 126, 84, 42, 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively.

Table 5.1 Reaction components for cDNA synthesis from total RNA from skeletal muscle and adipose tissue biopsies from grazing steers[1]

Component	Volume/Reaction (μl)
10x RT Buffer	2
25x dNTP Mix (100mM)	0.8
10X Random RT Primers	2
MultiScribe Reverse Transcriptase	1
Nuclease Free Water	4.2
Total Reaction Volume	10

[1]Individual Reaction components for complementary DNA synthesis from total RNA isolate. For each tissue a master mix containing sufficient quantities of the individual reaction components was created and cDNA synthesis was conducted for each RNA sample; RT = Reverse Transcription; dNTP = deoxynucleoside triphosphates.

Table 5.2 Thermocycling conditions for cDNA synthesis from total RNA extracted from skeletal muscle and adipose tissue biopsies from grazing steers.[1]

	Step 1	**Step 2**	**Step 3**	**Step 4**
Temperature (°C)	25°	37°	85°	4°
Time	10min	120min	5min	Hold

[1]Reaction conditions for the 4-step synthesis of cDNA from RNA; upon completion, reactions were held at 4°C until samples were removed.

Table 5.3 Reaction components for gene expression analysis using quantitative real-time PCR.[1]

Component	**Volume**
TaqMan Gene Expression Master Mix	8μl
Vic-Labeled 18S rRNA Probe[1]	1μl
Custom TaqMAn Gene Expression Assay	1μl
cDNA[2] + H20	10μl
Total Reaction Volume	20μl

[1]18S Vic Labeled 18S ribosomal RNA probe for data normalization in multiplexed quantitative Real-Time PCR reaction; PCR = Polymerase Chain Reaction; cDNA = Complementary DNA.

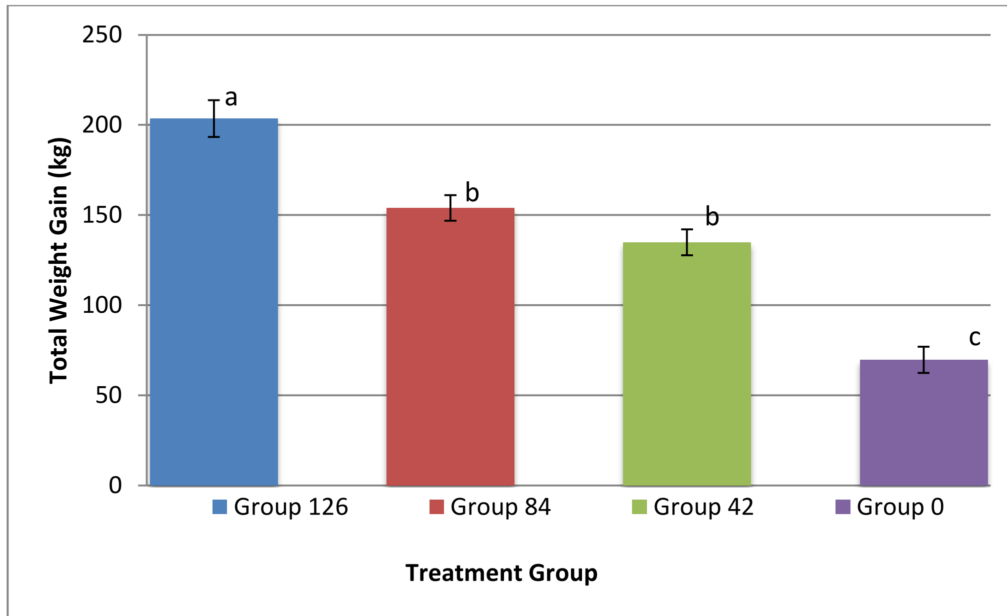
Table 5.4 Thermocycling conditions for quantitative Real-Time PCR analysis[1]

Step	Description	Time	Temperature
1	AMPerase UNG Activation	2 min	50°C
2	Taq Activation/UNG Inactivation	10 min	95°C
3	Polymerization	15 sec	95°C
4	Primer Annealing/Detection	1 min	60°C
5	Repeat Steps 3-4 for 40 cycles	NA	NA

[1]UNG = AMPerase Uracil N Glycosylase- Prevents Carryover Contamination; Taq = Thermostable Taq DNA Polymerase.

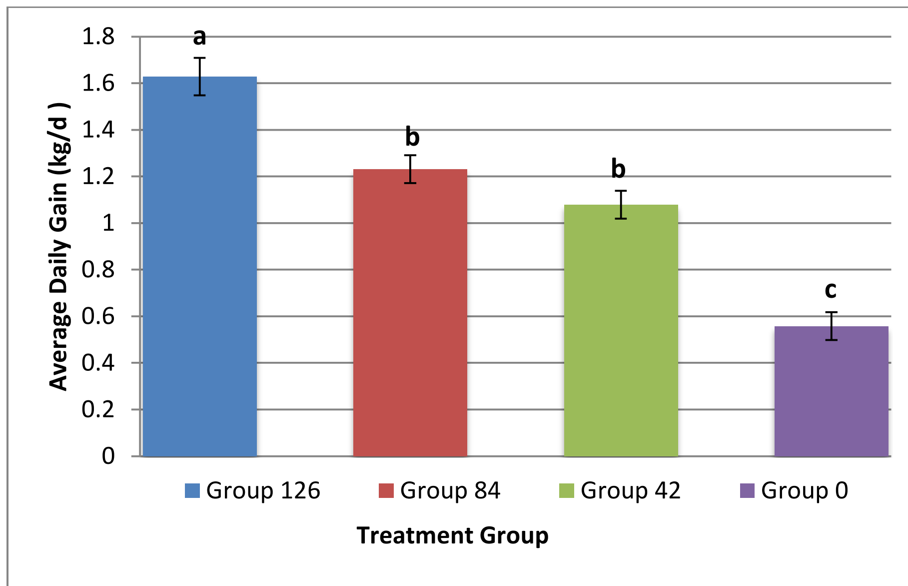
Animal Performance

Figure 5.2 Effect of treatment group on total weight gain in forage-fed steers for 126d trial period



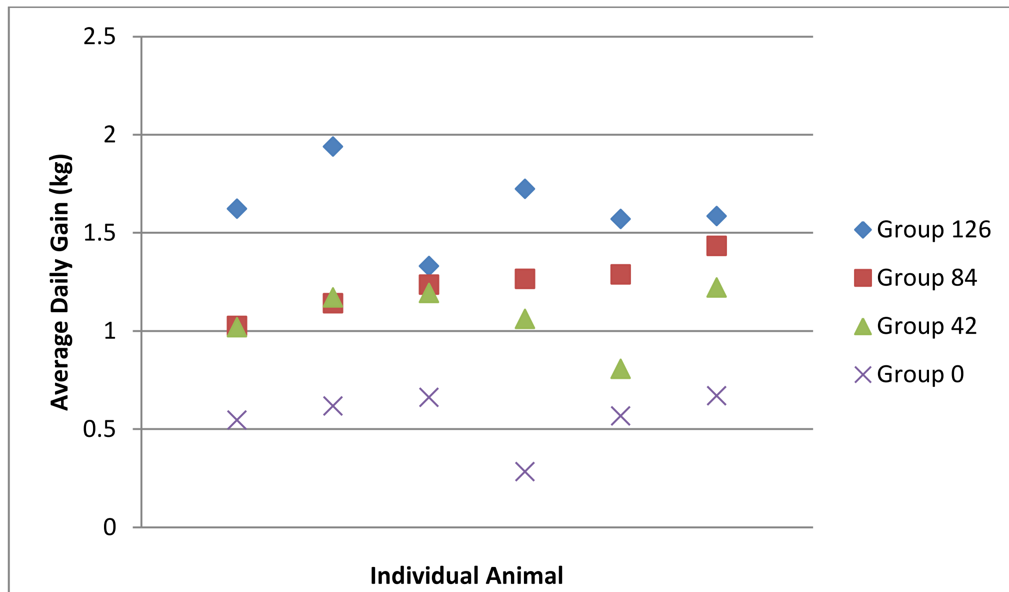
[a,b,c] Columns represent simple mean comparisons (+/- SEM) of average total weight gain in each treatment group for the duration of the 126d trial. Columns not sharing a common superscript differ ($P > 0.05$); Group 126, 84, 42, 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively.

Figure 5.3 Effect of treatment group on average daily gain in forage-fed steers for 126d trial period



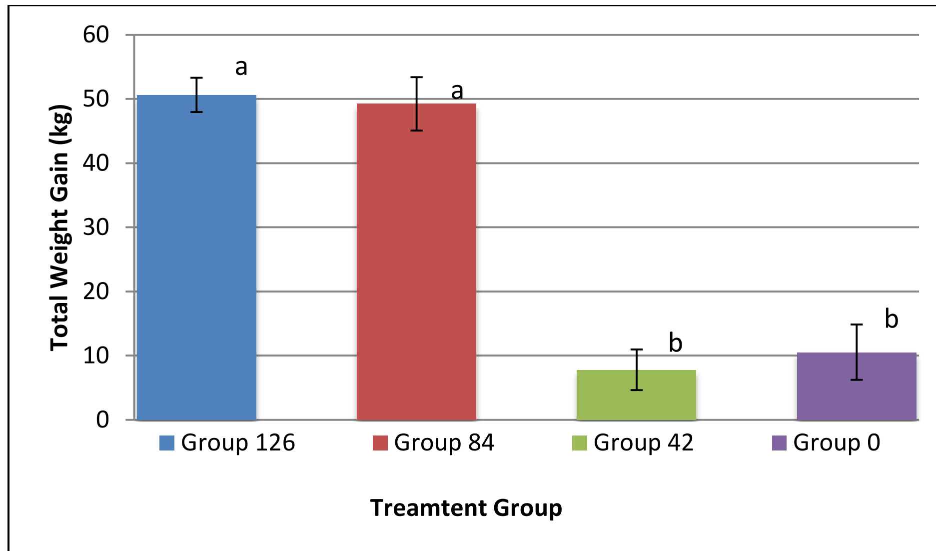
[a,b,c] Columns represent simple mean comparisons of average daily weight gain (+/- SEM) in each treatment group for the duration of the 126d trial. Group 126, 84, 42, 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively; Columns not sharing a common superscript differ (P > 0.05).

Figure 5.4 Effect of treatment on average daily gain of individual forage-fed steers for 126d trial period



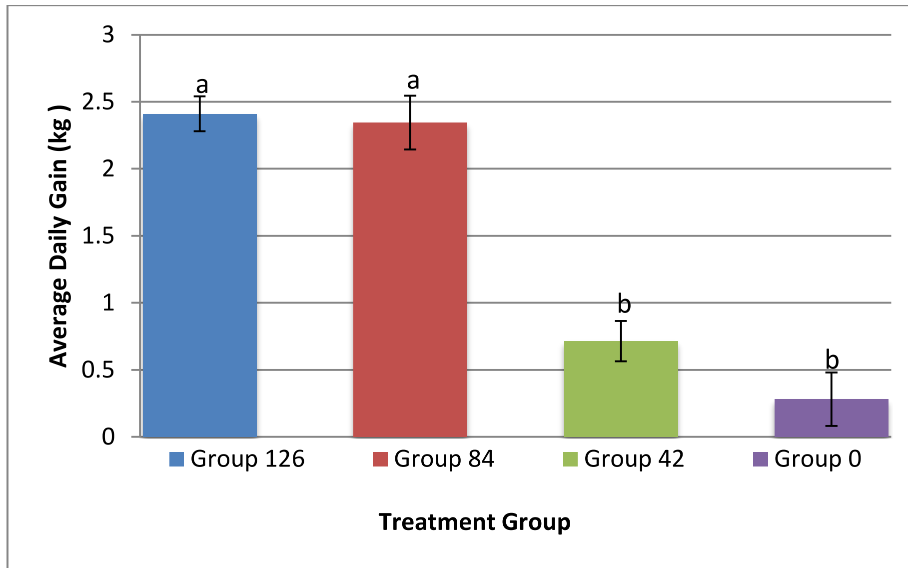
[1]Average daily weight gain for individual animals in each treatment group from 0 to 126d of the trial; Group 126, 84, 42, 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively; Average daily gain = kg of weight gained per day for duration of the 126d trial.

Figure 5.5 Effect of treatment group on total weight gain in forage-fed Steers from 63d to 84d of the trial period



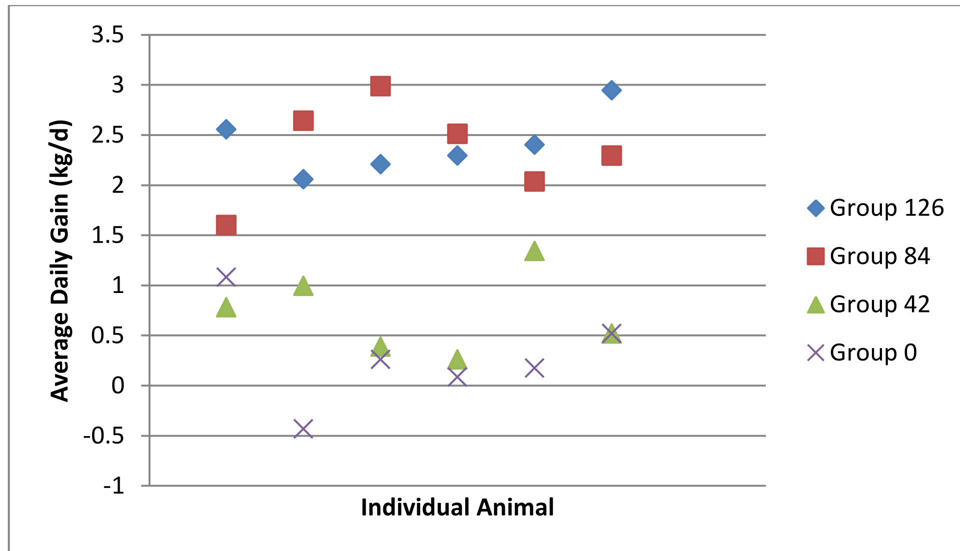
[a,b] Columns represent simple mean comparisons (+/- SEM) of average total weight gain in each treatment group from 63 to 84d of the trial. Columns not sharing a common superscript differ (P > 0.05); Group 126, 84, 42, 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively.

Figure 5.6 Effect of treatment group on average daily gain in forage-fed steers from 63d to 84d of the trial period



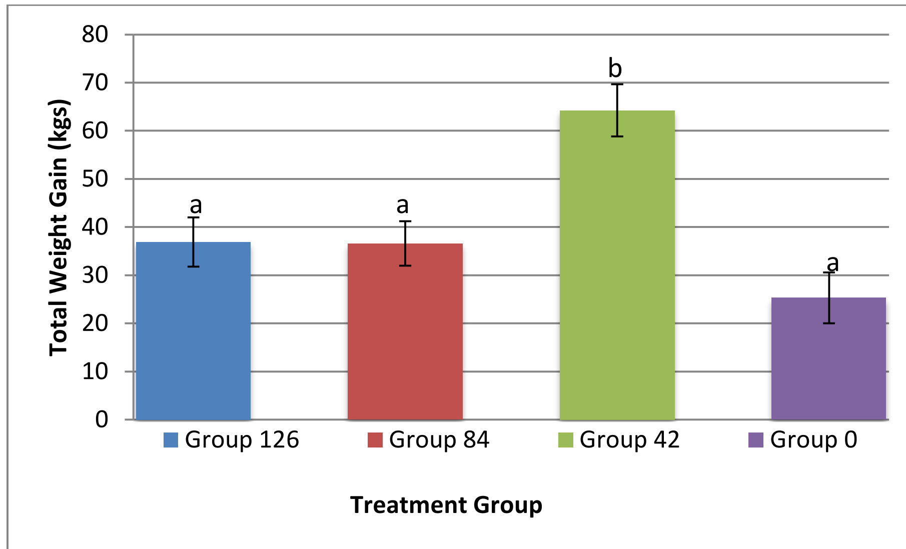
[a,b] Columns represent simple mean comparisons (+/- SEM) of average daily weight gain in each treatment group from 63d to 84d of the trial. Columns not sharing a common superscript differ ($P > 0.05$); Group 126, 84, 42, 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively.

Figure 5.7 Effect of treatment on average daily gain of individual forage-fed steers from 63d to 84d of the trial period[1]



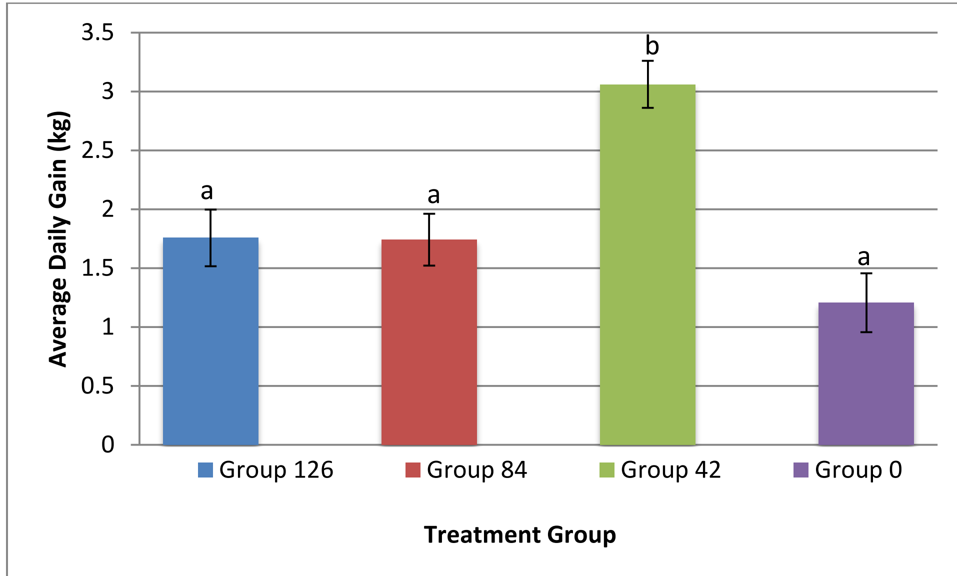
[1]Average daily weight gain for individual animals in each treatment group from 63d to 84d of the trial; Average daily gain = kg gained per day from 63 to 84d; Group 126, 84, 42, 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively.

Figure 5.8 Effect of treatment group on total weight gain in forage-fed steers from 84 to 105 of the trial period[1]



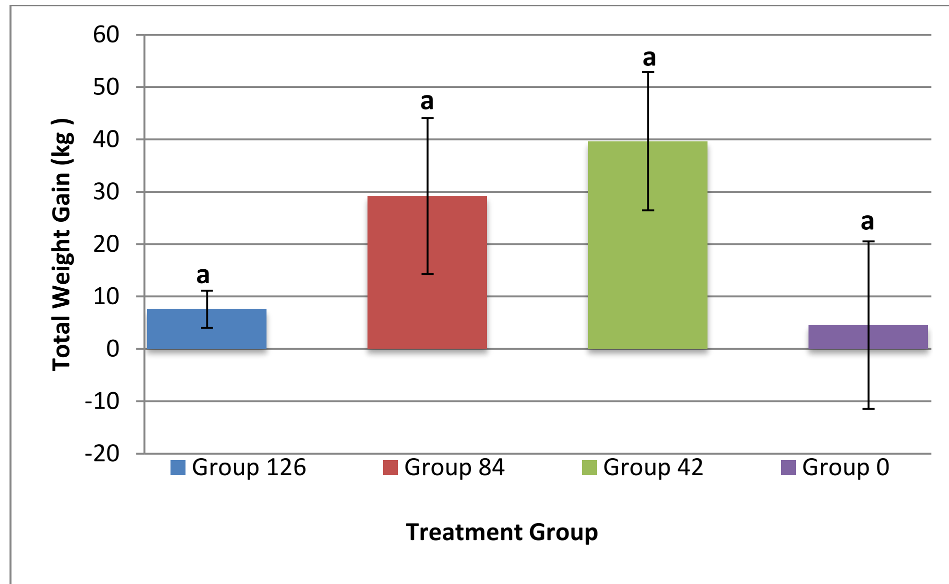
[a,b] Columns represent simple mean comparisons (+/- SEM) of average total weight gain in each treatment group from 84d to 105d of the trial. Columns not sharing a common superscript differ (P > 0.05).

Figure 5.9 Effect of treatment group on average daily gain in forage-fed steers from 84d to 105d of the trial period



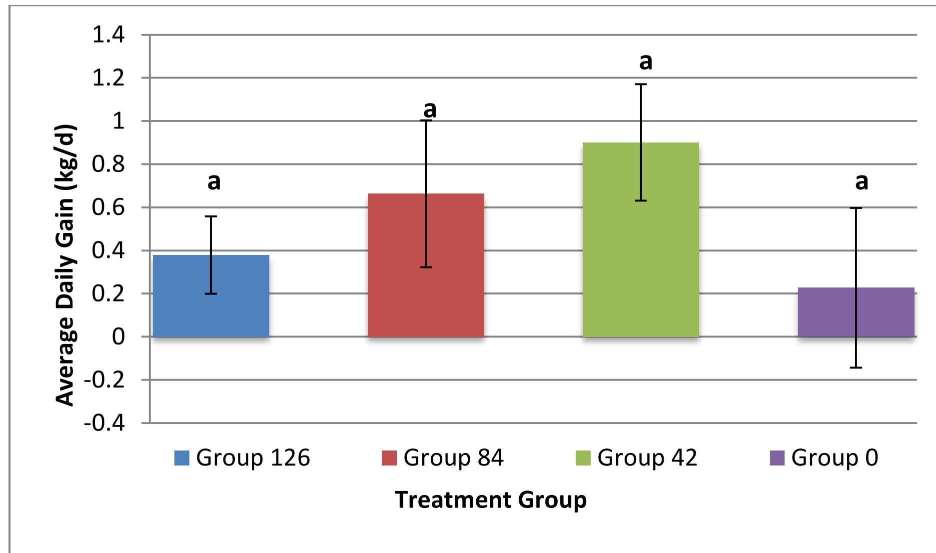
[a,b] Columns represent simple mean comparisons (+/- SEM) of average daily weight gain in each treatment group from 84d to 105d of the trial. Columns not sharing a common superscript differ ($P > 0.05$). Group 126, 84, 42, 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively.

Figure 5.10 Effect of treatment group on total gain in forage-fed steers from 105 to 126d of the trial period



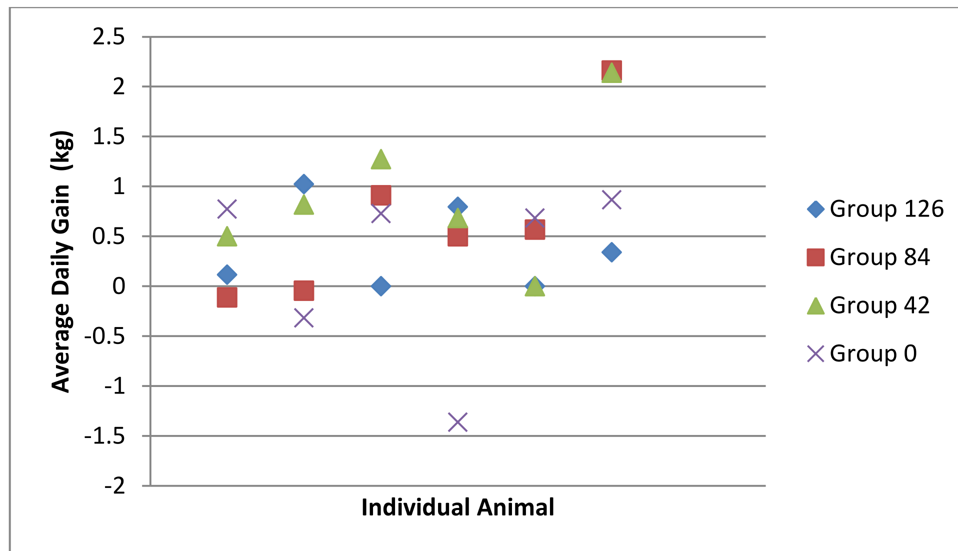
[a] Columns represent simple mean comparisons (+/- SEM) of average total weight gain in each treatment group from 105d to 126d of the trial. Columns not sharing a common superscript differ (P > 0.05). Group 126, 84, 42, 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively.

Figure 5.11 Effect of treatment group on average daily gain in forage-fed steers from 105 to 126d of the trial period



[a] Columns represent simple mean comparisons (+/- SEM) of average daily weight gain in each treatment group from 105d to 126d of the trial. Columns not sharing a common superscript differ (P > 0.05). Group 126, 84, 42, 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively.

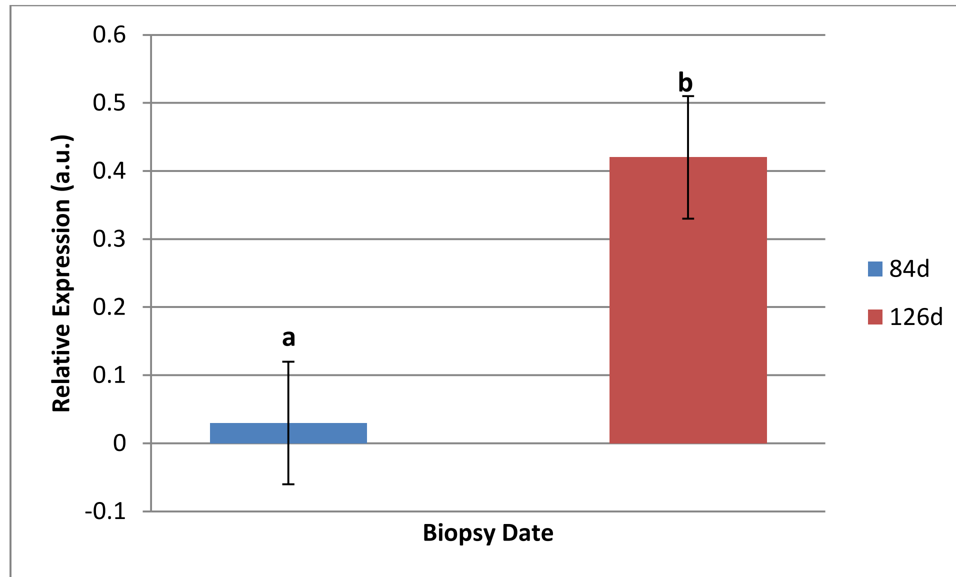
Figure 5.12 Effect of treatment on average daily gain of individual forage-fed steers from 105 to 126d of the trial period[1]



[1]Average daily weight gain for individual animals in each treatment group from 63d to 84d of the trial; Average daily gain = kg gained per day from 105-126d of trial; Group 126, 84, 42, 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively.

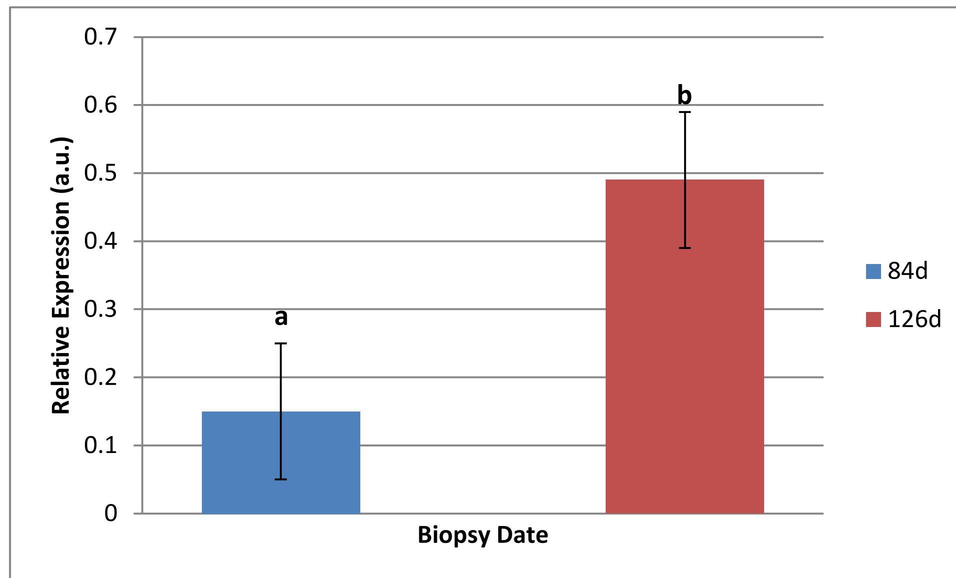
Gene Expression

Figure 5.13 Effect of biopsy date on relative expression of CPT-1b in steer skeletal muscle biopsies[1]



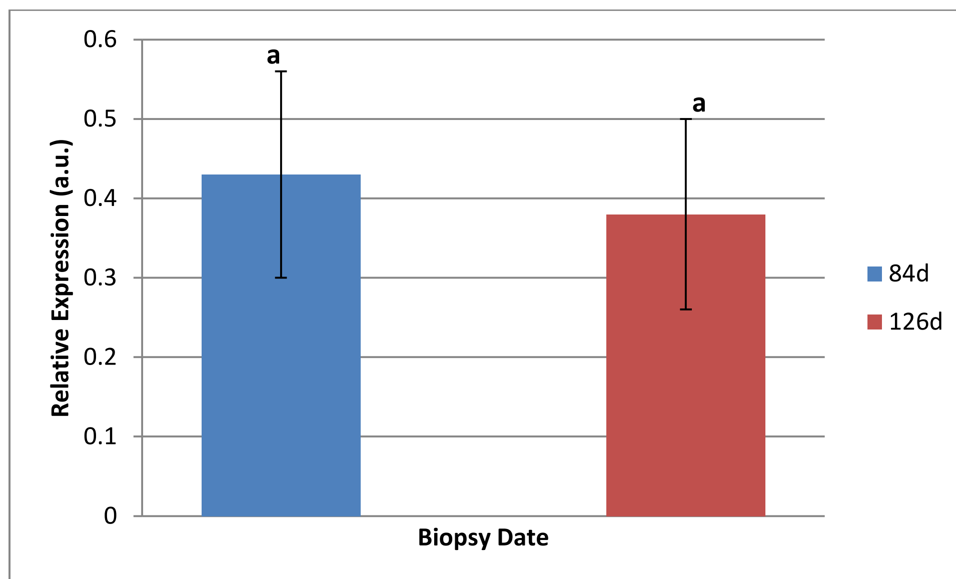
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); 84 and 126d = skeletal muscle biopsies taken at 84d and 126d;
CPT-1b = Carnitine Palmitoyl Transferase-1b.

Figure 5.14 Effect of biopsy date on relative expression of PPAR-γ in steer skeletal muscle biopsies[1]



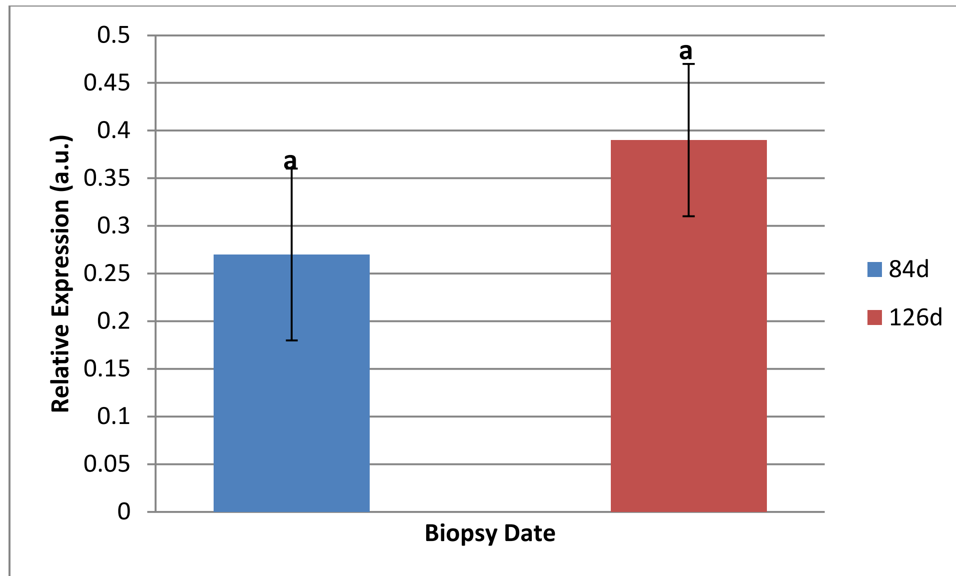
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); 84 and 126d = skeletal muscle biopsies taken at 84d and 126d;
PPAR-γ = Peroxisome Proliferator Activator Receptor-gamma.

Figure 5.15 Effect of biopsy date on relative expression of UCP2 in steer skeletal muscle biopsies[1]



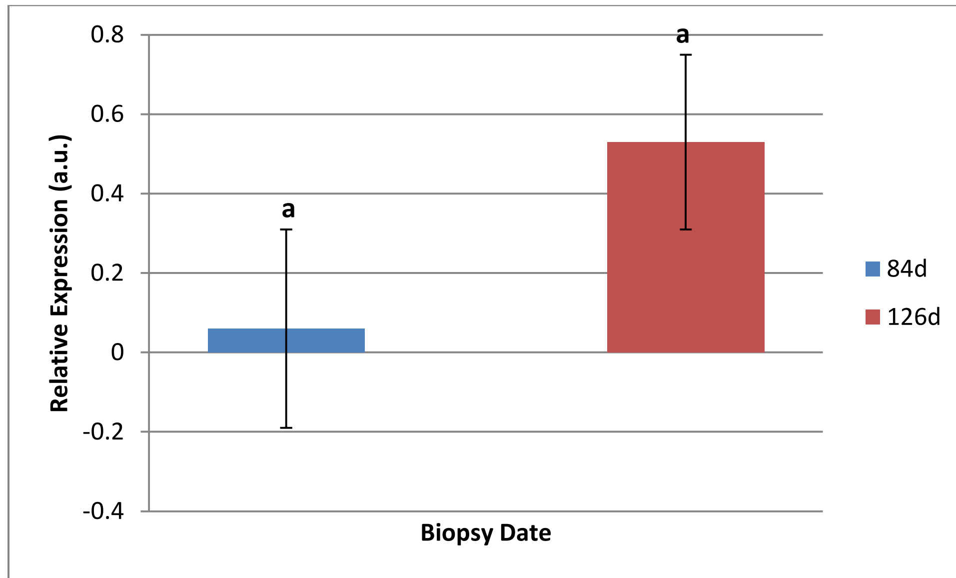
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); 84 and 126d = skeletal muscle biopsies taken at 84d and 126d;
UCP-2 = Uncoupling Protein-2.

Figure 5.16 Effect of biopsy date on relative expression of CEBP/α in steer skeletal muscle biopsies[1]



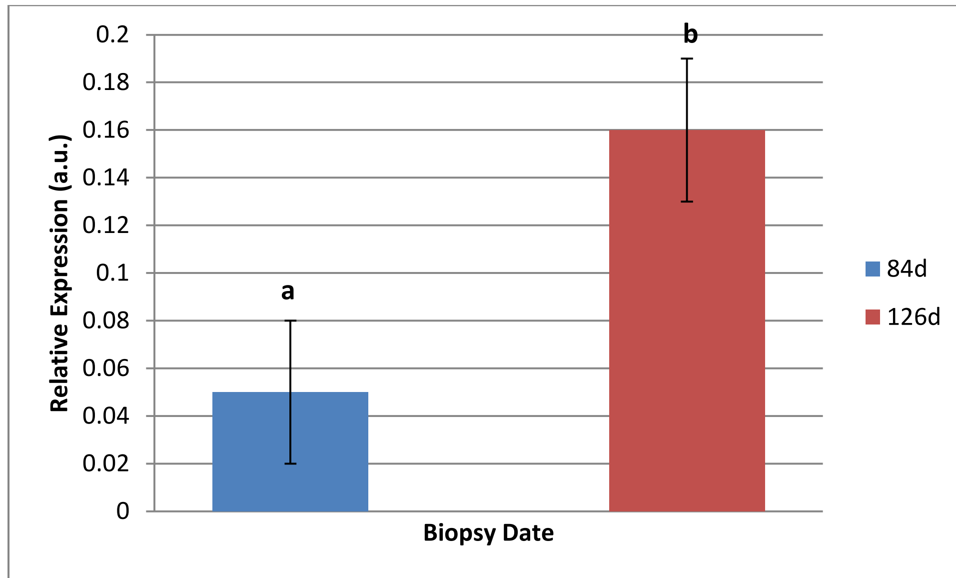
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); 84 and 126d = skeletal muscle biopsies taken at 84d and 126d;
CEBP/a = CAAT Enhancer Binding Protein/alpha.

Figure 5.17 Effect of biopsy date on relative expression of FABP4 in steer skeletal muscle biopsies[1]



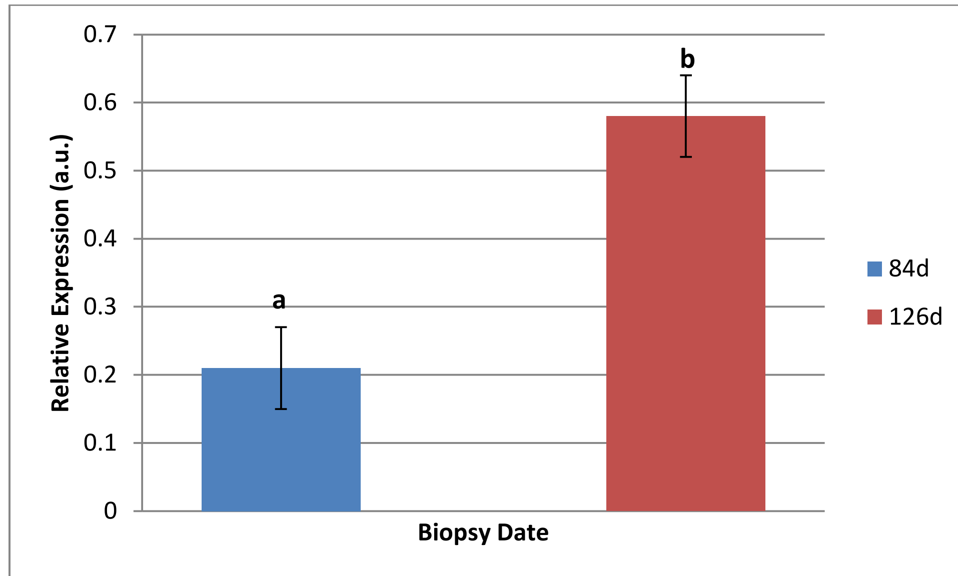
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); 84 and 126d = skeletal muscle biopsies taken at 84d and 126d;
FABP4 = Fatty Acid Binding Protein 4.

Figure 5.18 Effect of biopsy date on relative expression of PSDM11 in steer skeletal muscle biopsies[1]



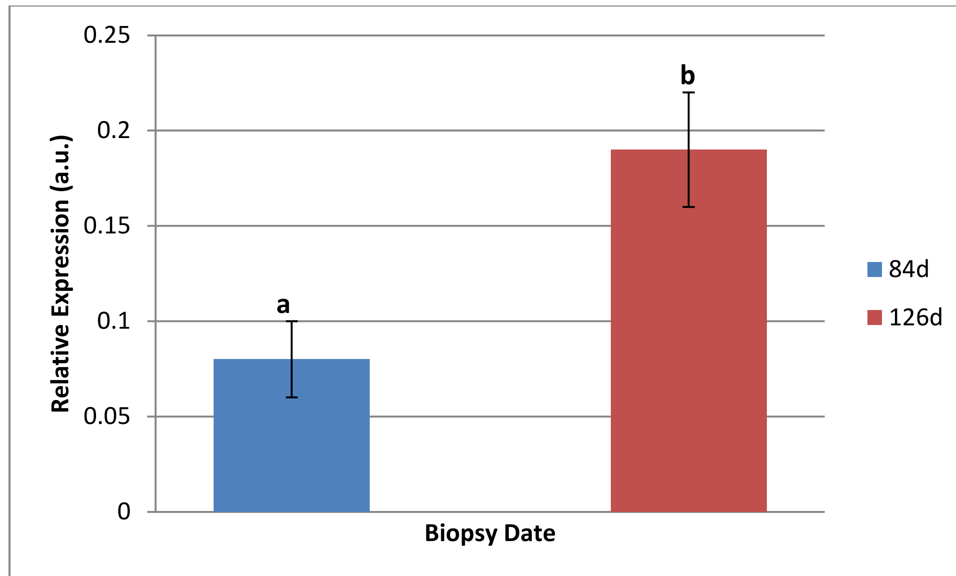
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); 84 and 126d = skeletal muscle biopsies taken at 84d and 126d; PSDM11 = Proteasome Macropain Subunit 11.

Figure 5.19 Effect of biopsy date on relative expression of Pref-1 in steer skeletal muscle biopsies



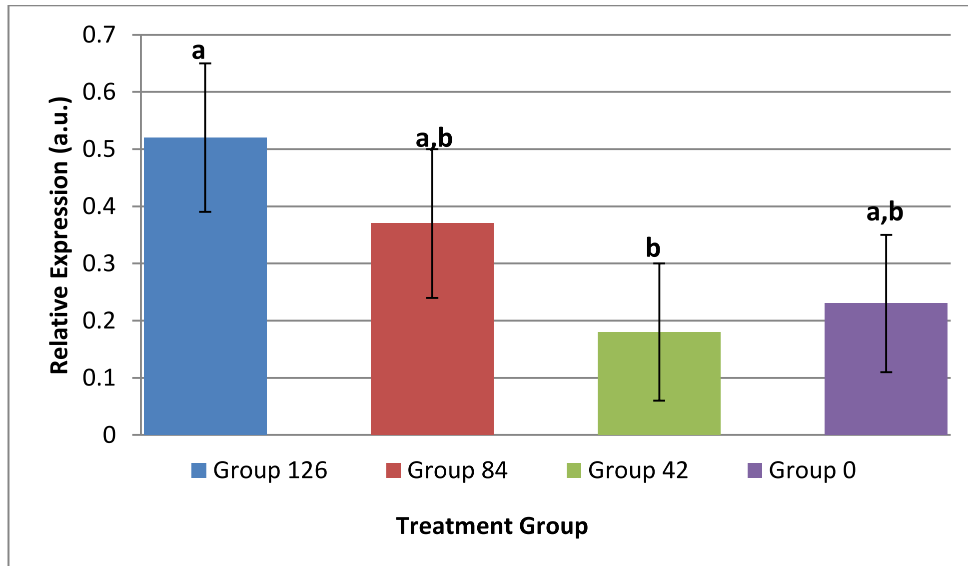
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); 84 and 126d = skeletal muscle biopsies taken at 84d and 126d;
Pref-1 = Preadipocyte Factor 1; Dlk1.

Figure 5.20 Effect of biopsy date on relative expression of PGC-1α in steer skeletal muscle biopsies[1]



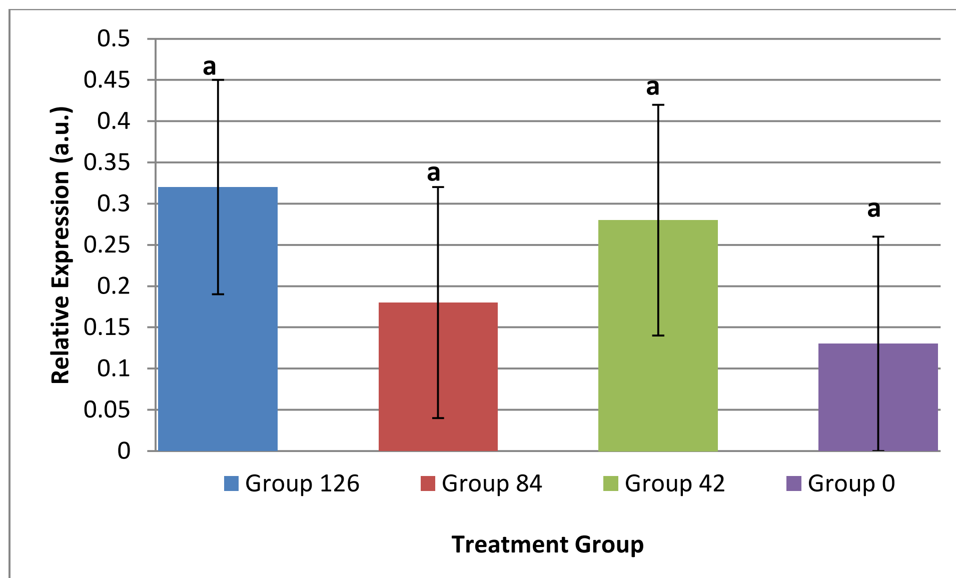
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ (P < 0.05); 84 and 126d = skeletal muscle biopsies taken at 84d and 126d;
PGC-1α = Peroxisome Proliferator Activator Protein Coactivator-1α.

Figure 5.21 Effect of treatment on relative expression of CEBP/α in steer skeletal muscle biopsies[1]



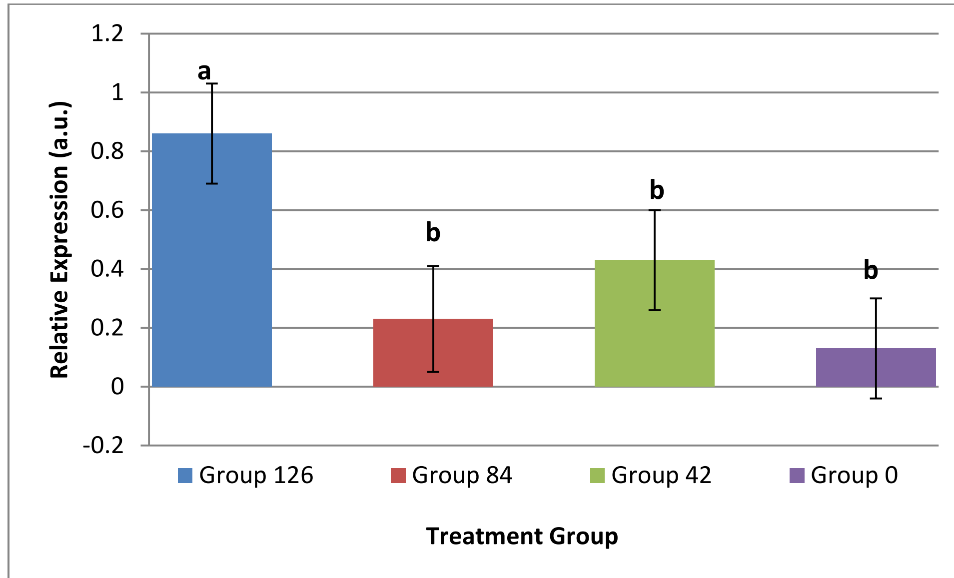
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P <$ 0.05); Group 126, 84, 42, and 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively; CEBP/α = CAAT Enhancer Binding Protein/alpha.

Figure 5.22 Effect of treatment on relative expression of CPT-1b in steer skeletal muscle biopsies[1]



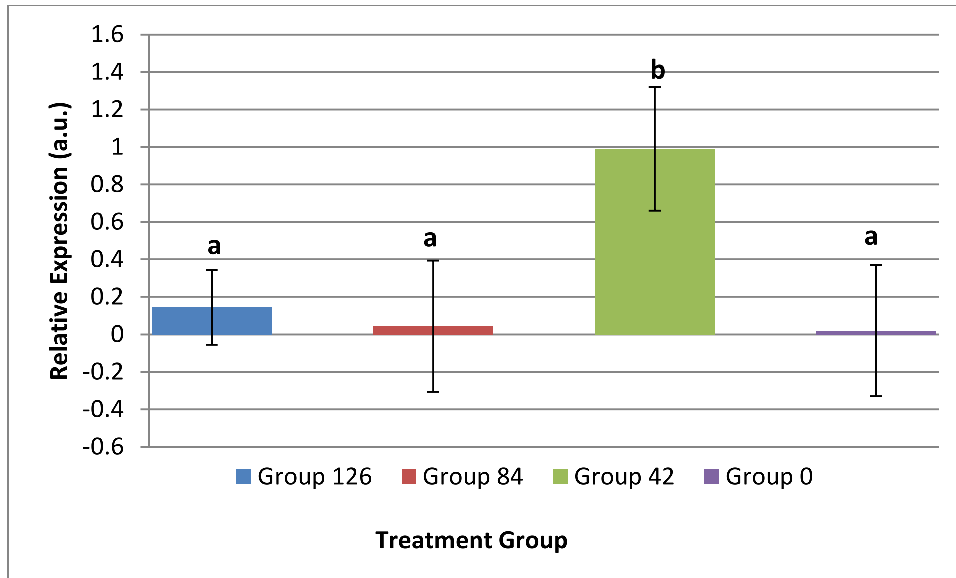
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); Group 126, 84, 42, and 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively; CPT1-b = Carnitine Palmitoyl Transferase 1-b.

Figure 5.23 Effect of treatment on relative expression of UCP-2 in steer skeletal muscle biopsies[1]



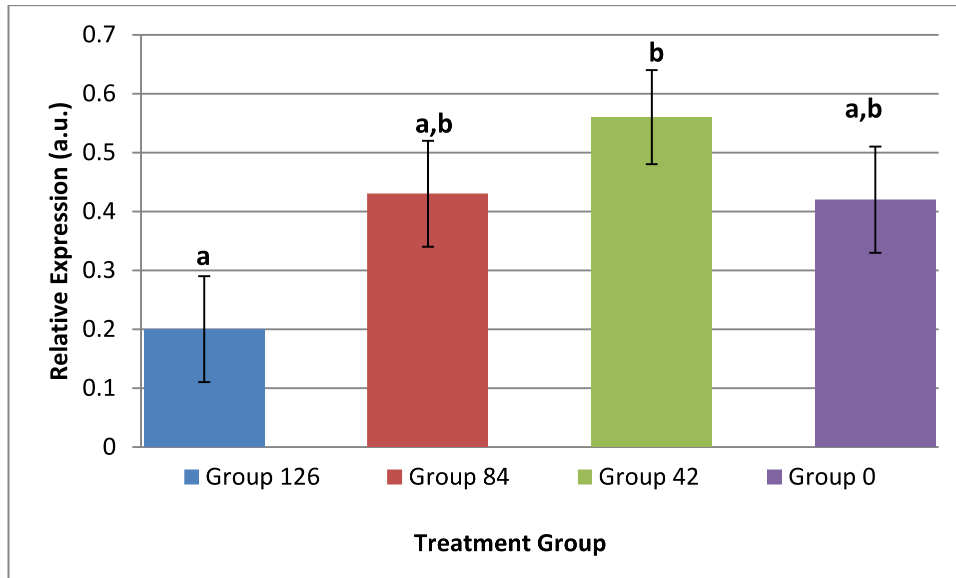
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); Group 126, 84, 42, and 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively; UCP-2 = Uncoupling Protein-2.

Figure 5.24 Effect of treatment on relative expression of FABP4 in steer skeletal muscle biopsies[1]



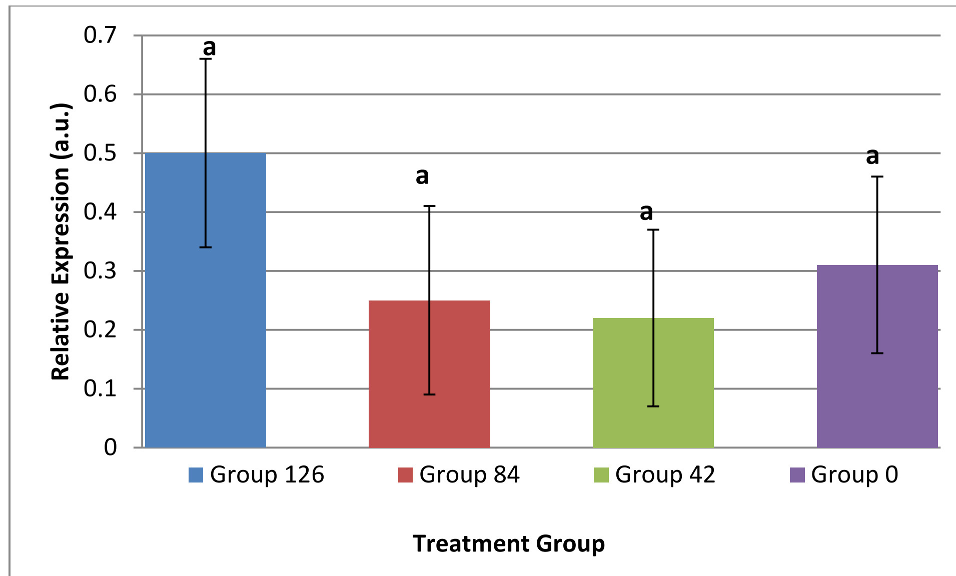
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); Group 126, 84, 42, and 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively; FABP4 = Fatty Acid Binding Protein 4.

Figure 5.25 Effect of treatment on relative expression of Pref-1 in steer skeletal muscle biopsies[1]



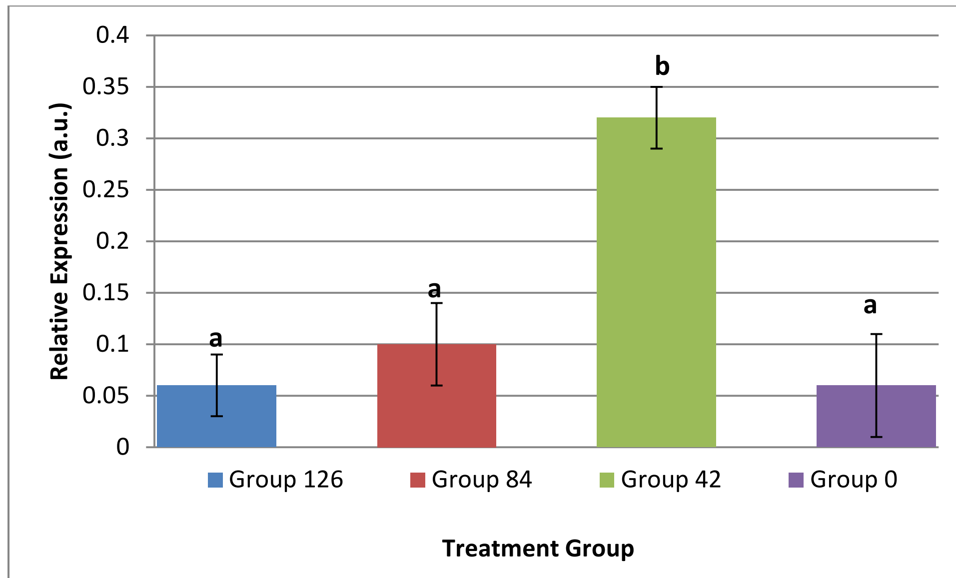
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); Group 126, 84, 42, and 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively; Pref-1 = Preadipocyte Factor 1; Dlk-1.

Figure 5.26 Effect of treatment on relative expression of PPAR-γ in steer skeletal muscle biopsies[1]



[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); Group 126, 84, 42, and 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively; PPAR- γ = Peroxisome Proliferator Activator Protein- gamma.

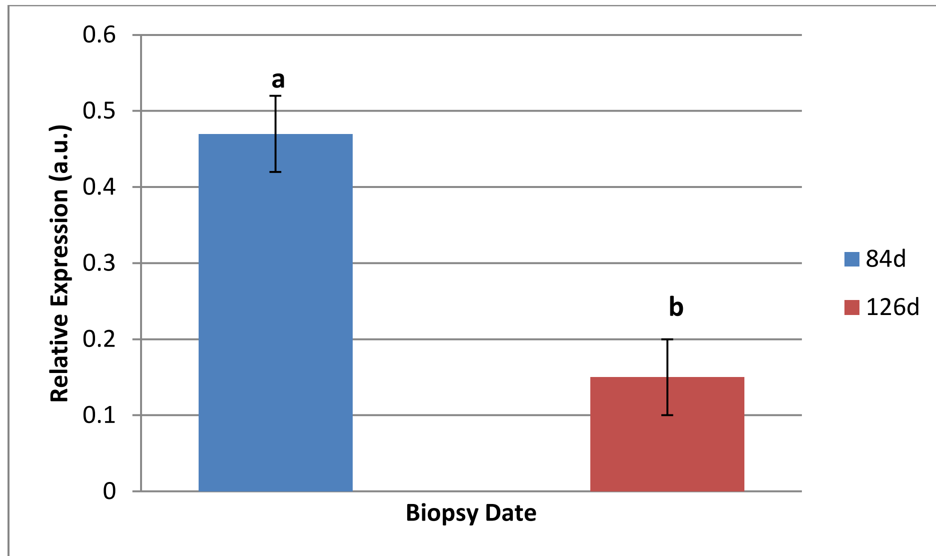
Figure 5.27 Effect of treatment on relative expression of PGC-1α in steer skeletal muscle biopsies for the 126d trial period[1]



[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); Group 126, 84, 42, and 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively; PGC-1α = Peroxisome Proliferator Activator Protein-1α.

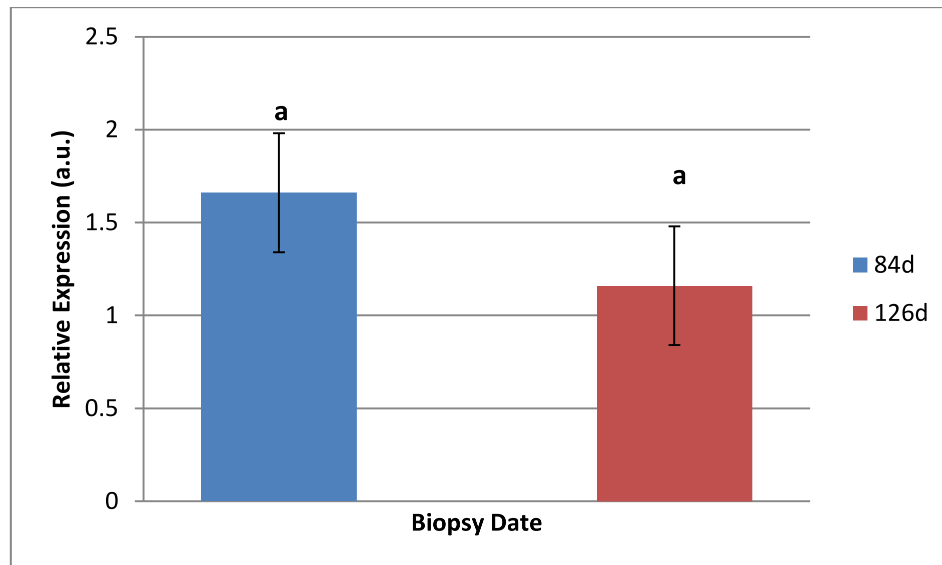
Adipose Tissue Biopsies

Figure 5.28 Effect of biopsy date on relative expression of GPAT in steer adipose tissue biopsies collected at 84 and 126d[1]



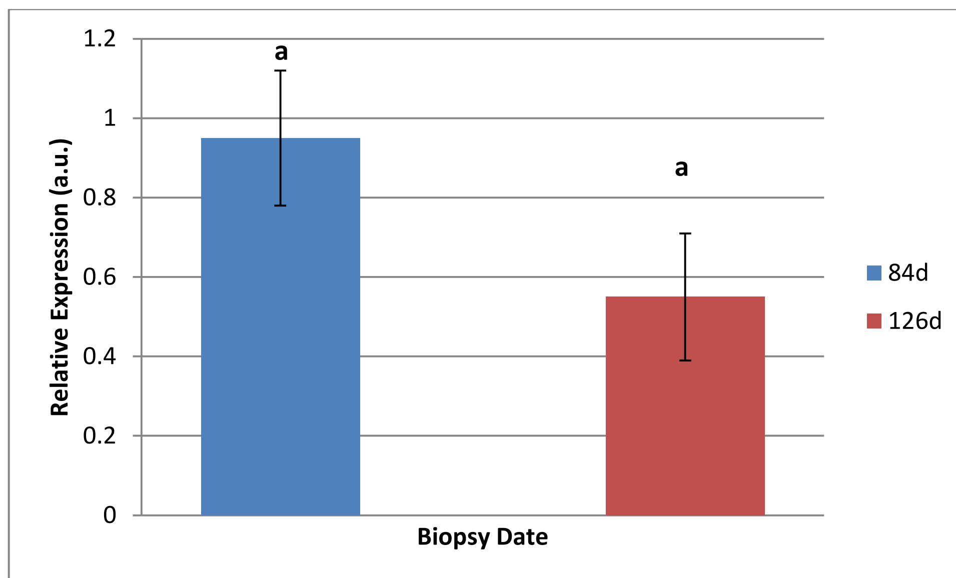
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); 84 and 126d =Adipose Tissue biopsies taken at 84d and 126d; GPAT = Glycerol Phosphate Acyl Transferase.

Figure 5.29 Effect of biopsy date on relative expression of ZFP423 in steer adipose tissue biopsies collected at 84 and 126d[1]



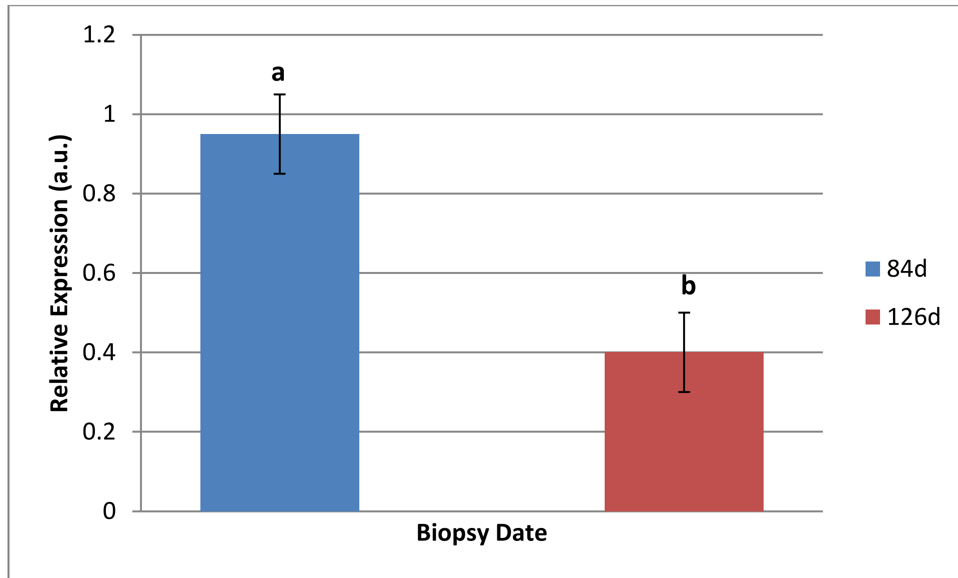
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); 84 and 126d =Adipose Tissue biopsies taken at 84d and 126d. ZFP423 = Zinc Finger protein 423.

Figure 5.30 Effect of biopsy date on relative expression of FABP4 in steer adipose tissue biopsies collected at 84 and 126d[1]



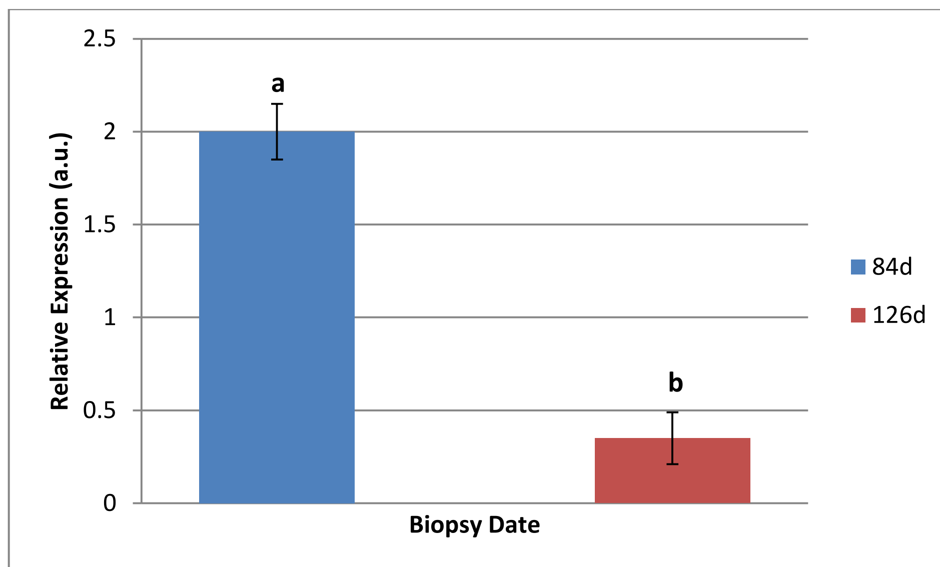
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); 84 and 126d =Adipose Tissue biopsies taken at 84d and 126d; FABP4 = Fatty Acid Binding Protein 4.

Figure 5.31 Effect of biopsy date on relative expression of UCP2 in steer adipose tissue biopsies collected at 84 and 126d[1]



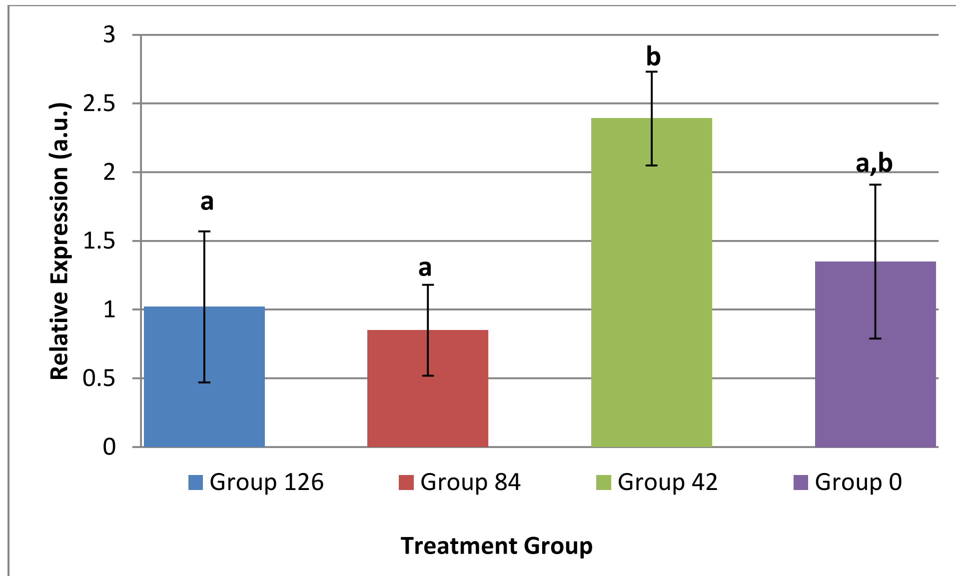
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); 84 and 126d =Adipose Tissue biopsies taken at 84d and 126d; UCP-2 = Uncoupling Protein 2.

Figure 5.32 Effect of biopsy date on relative expression of Leptin in steer adipose tissue biopsies[1]



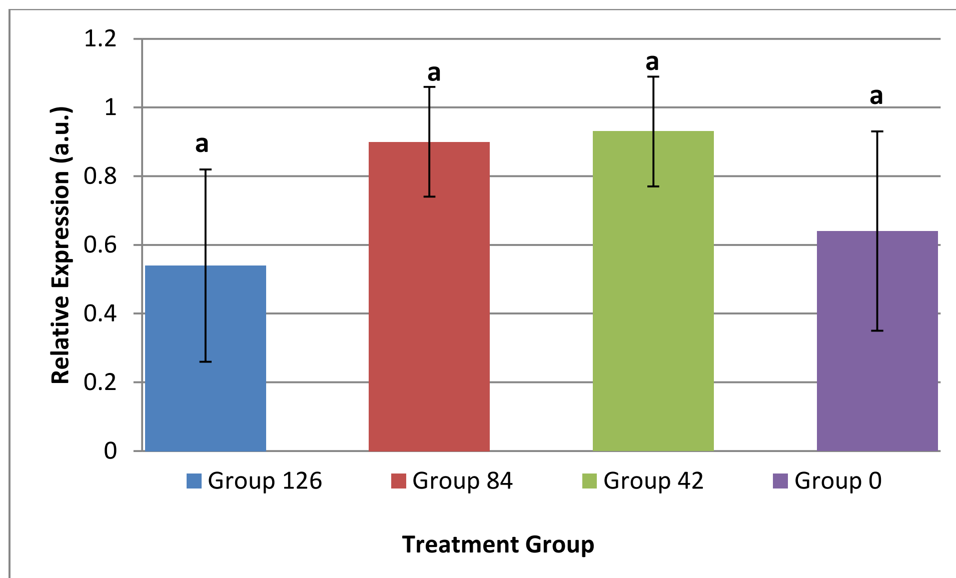
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ (*P* < 0.05); 84 and 126d =Adipose Tissue biopsies taken at 84d and 126d; LSM not sharing a common superscript differ (P > 0.05).

Figure 5.33 Effect of treatment on relative expression of ZFP423 in steer adipose tissue biopsies[1]



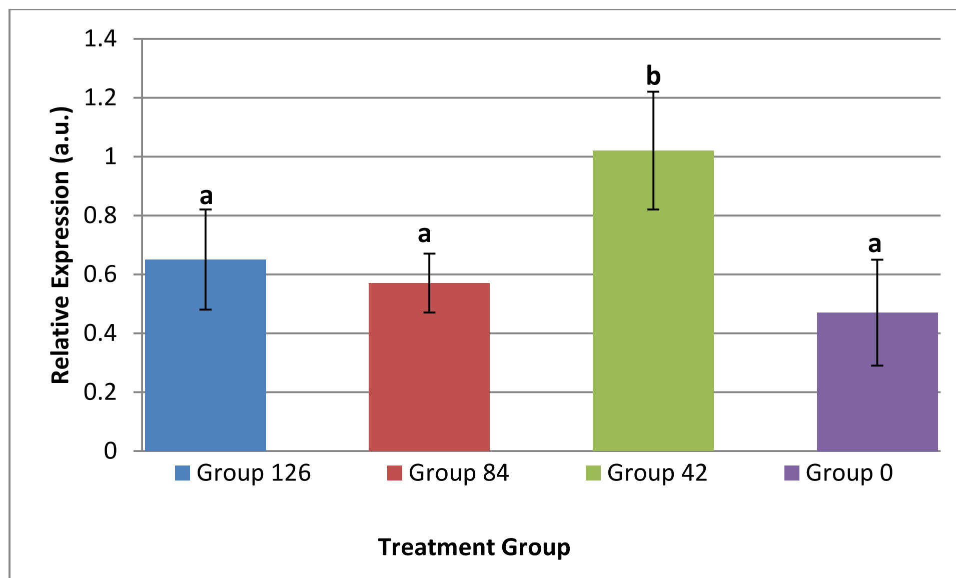
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); Group 126, 84, 42, and 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively; ZFP423 = Zinc Finger Protein 423.

Figure 5.34 Effect of treatment on relative expression of FABP4 in steer adipose tissue biopsies[1]



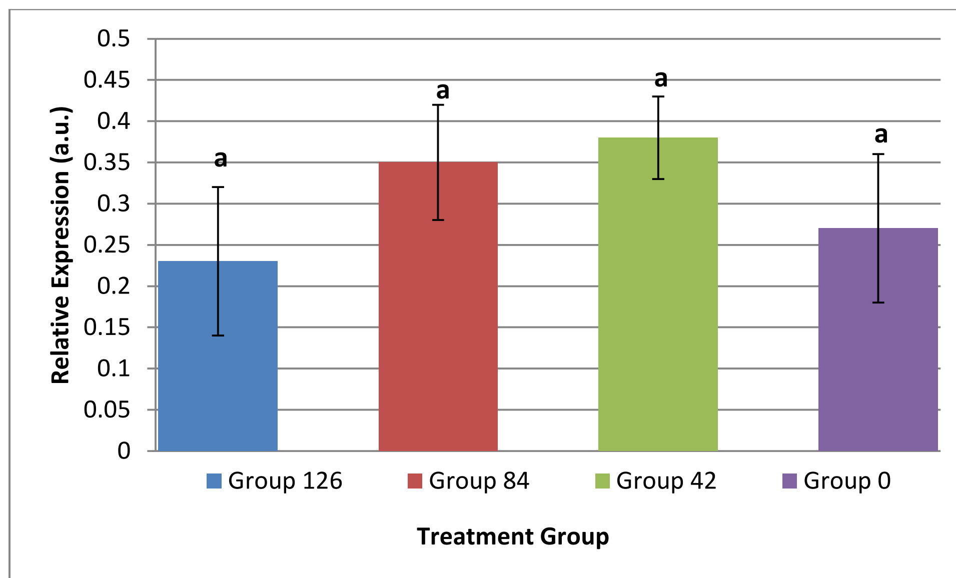
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); Group 126, 84, 42, and 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively; FABP4 = Fatty Acid Binding Protein 4.

Figure 5.35 Effect of treatment on relative expression of UCP-2 in steer adipose tissue biopsies[1]



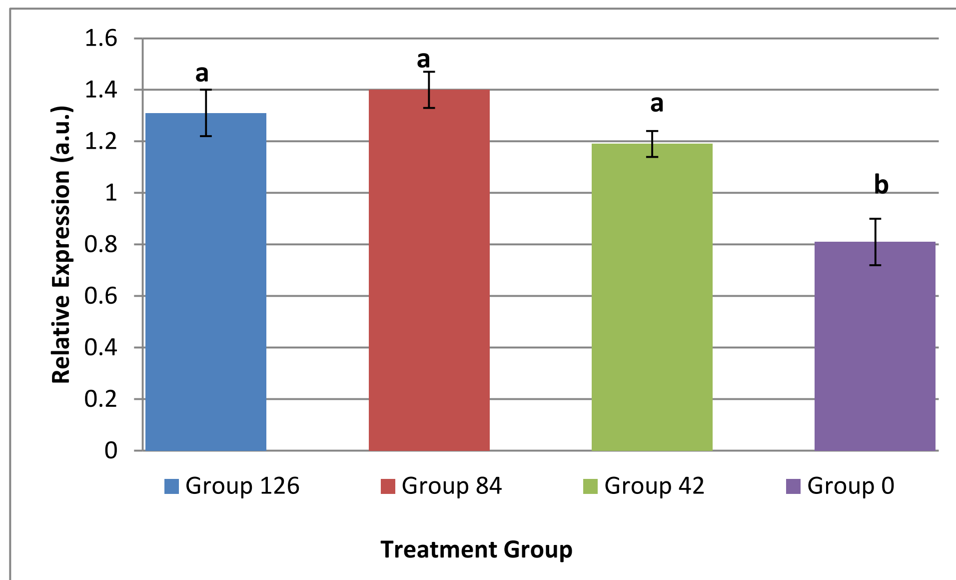
[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); Group 126, 84, 42, and 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively; UCP-2 = Uncoupling Protein 2.

Figure 5.36 Effect of treatment on relative expression of GPAT in steer adipose tissue biopsies[1]



[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); Group 126, 84, 42, and 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively; [1]GPAT = Glycerol Phosphate Acyl Transferase.

Figure 5.37 Effect of treatment group on relative expression of Leptin in steer adipose tissue biopsies[1]



[1]Least Square Means +/- SEM; Columns not bearing a common superscript differ ($P < 0.05$); Group 126, 84, 42, and 0 = 126, 84, 42, and 0 days grazing ryegrass, respectively.

Chapter 6. Summary of Experiments

Rising feed costs represent a constantly looming threat to profitability of beef cattle operations worldwide. These pressures come in the midst of a burgeoning global population with increasing demand for beef and other meat products. To compound the issue, environmental concerns have led to anxieties over the long-term sustainability of intense livestock production in general and the beef cattle industry in particular. These factors create the following dilemma for the industry: How to produce more product in the face of decreasing profit margins, waning resources, and increased regulatory pressures.

Terms like 'efficiency' and 'sustainability' can be vague and context dependent, and there are individual aspects (i.e. metabolic efficiency, production efficiency, economic efficiency, ecological efficiency, etc.) that cooperate to determine the overall efficiency and environmental impact of beef production. A longstanding goal of animal research has been to understand the specific factors that are responsible for variations in feed efficiency and nutrient partitioning because of their impact on production costs and sustainability. These objectives are complex but livestock operations in the post-genomic era are in a position to make use of existing and emerging molecular technologies to identify and consistently produce more efficient animals with the specific carcass characteristics that appeal to consumers.

Metabolic processes that have been hypothesized (and many cases substantiated) to effect performance efficiency include protein turnover, mitochondrial function, and AT tissue and lipid metabolism. Furthermore, research regarding metabolic regulation has identified several governing transcription factors that up or down regulate pathways involved in these pathways and can affect nutrient partitioning. While energetics and thermodynamics determine biological efficiency of individual nutrient use, the important consideration with respect to performance

efficiency is the effective use of feed resources to deliver a valuable phenotype. The ability to independently manipulate the metabolic disposition of individual tissues in favor of efficient nutrient use and an economically valuable phenotype is the ultimate objective of animal researchers in the post-genomic era. In this respect, three studies were conducted in order to determine molecular events associated with metabolic efficiency in specific SM and AT depots in finishing beef cattle.

Study 1: Relationship between Residual Feed Intake and the Transcriptomic Signature of Performance Efficiency in Finishing Beef Cattle

Residual feed intake is a feed efficiency parameter. High-cost and labor associated with determining RFI are limitations that have prevented wide-scale adoption as a FE parameter. The factors that contribute RFI variation within a contemporary group of animals are likely to be due metabolic processes that are governed by molecular-regulatory factors. If these particular regulatory pathways can be ascertained and articulated they can subsequently be targeted to identify more efficient animals. In addition, the comprehensive understanding of the pathway dynamics will allow for effective targeting using genetic and molecular tools in order to achieve a more desirable RFI phenotype. The results from our study indicate that RFI appears to be related to genes involved in protein turnover and to a lesser extent, other metabolic genes; however genes involved in AT metabolism did not appear to be related. It should be noted that the animals used in this study were the initial progeny of a breeding program designed to select for RFI, and these animals were only moderately segregated with respect to this parameter. A future study using subsequent generations that have been divergently selected (High vs. Low RFI) will be prudent to complete to aid our understanding of these relationships.

Study 2: Effect of Days on Feed and Beta-Agonist Administration on the Expression of Regulatory Genes in Skeletal Muscle and Adipose Tissue of Finishing Heifers

It is well known that production efficiency is one of the primary factors that contribute to the success and profitability of a beef cattle operation. Wasteful accumulation of AT in the SC depot is an economically inefficient process that has a negative impact on profit margins. Metabolic modifiers including beta-adrenergic agonists have been utilized to improve feed efficiency and repartition nutrients towards economically valuable tissues and away from AT. These compounds are not without controversy with respect to their effect on meat quality and the specific mechanism of action of these compounds is not completely understood. Identification of specific molecular targets of these compounds and optimal timing of administration with respect to the finishing period can maximize the effectiveness of these compounds and/or lead to strategies to mitigate undesirable effects. Our objective was to determine the effect of days on feed and Ractopamine administration on regulatory gene expression in skeletal muscle and adipose tissue of finishing beef heifers. We observed temporal changes in gene expression between days on feed but no discernable effects of Ractopamine on molecular regulation of metabolic pathways with respect to the genes we examined.

Study 3: The Effects of Feed Restriction and Re-feeding on Gene Expression Patterns in the Loin Muscle and Subcutaneous Adipose Tissue of Forage Fed Beef

Temperate climates and year-round forage growth provide the basis for niche forage-based finishing systems in the SE. Forage based systems eliminate the costs and labor associated with purchasing and transporting concentrates and other feeds. This system is dependent on proper land and animal management, and adequate forage growth in order to support a profitable and sustainable operation. In our final study we sought to determine the effect of a forage-based finishing system on gene expression patterns in SM and AT of finishing steers subjected to

differing levels of forage growth (nutrient availability) and to determine how these patterns reflected phenotype and animal performance. In terms of animal performance, treatment group did affect total and ADG for the duration of the trial. Animals on the highest plane of nutrition gained the most weight and those on the lowest gained the least weight. Likewise, the intermediate plane of nutrition supported intermediate levels of animal growth. The results from this study were variable and equivocal with respect to the gene expression analysis. Some genes showed differences with respect to treatment group. In terms of performance there was some variation within treatment group which may have led to the variation in GE patterns. As a result of normally variable rainfall and agronomic conditions, which are naturally occurring phenomena, raising cattle on grass in the SE is a potentially rewarding niche market that is not without its drawbacks. The genes selected in this study represent pathways that are putatively recognized as important in regulating key metabolic processes involved in SM and AT metabolism. These pathways contribute to the efficiency and quality of meat production and as such are of the utmost economic interest in beef cattle production. It appears that the transcriptomic changes were not robust enough for these current data to be very impactful in terms of identifying *bona fide* biomarkers. That is not to say that the genes selected are not critical in these processes, however under the conditions experienced over the course of the trial, the targeted gene expression based performance evaluation tools could not be verified as consistently effective for phenotypic evaluation in the current study.

Implications and Conclusions

The preceding research was conducted to determine if targeted transcriptomic profiling could be used to monitor the progression and metabolic activity within specific SM and AT

depots in finishing beef cattle. The results with respect to the overarching objectives were variable and equivocal, but taken en masse provides the informative basis for further research. Depot specific AT metabolism is likely to remain of intense research interest due to the economic and biomedical implications of AT accretion in agricultural species, and humans, respectively. In future related studies, advanced histology and imaging tools such as laser-capture micro-dissection can also be utilized in conjunction with molecular and genomic techniques to isolate cell types and gain a more comprehensive understanding of their specific metabolism and contributions in AT and SM depots. It may also be prudent to determine IMF adipocyte number in order to determine the relative contribution of these cells to gene expression especially when attempting to compare expression with larger AT depots such as SC. The advent of gene silencing techniques is also certain to be a major contributor to our understanding of specific genes and pathways to economically valuable phenotypes.

While the current studies involved very limited snap shots of what are inherently dynamic processes, a more comprehensive approach must be taken to integrate animal performance with functional genomics (transcriptomic, proteomic, metabolomics) data in order to gain a more panoptic understanding of the molecular regulation of economically relevant traits. Genome wide association studies are becoming *in vogue* in terms of understanding the interconnection of genomic regions with metabolic pathways at the organismal level. Systems biology approaches have proven valuable in studying how physiological state, nutrition, etc. can affect tissue function and development in a depot-specific fashion. Next generation sequencing technologies are generating genomic data at unprecedented rates at a much cheaper cost which will aid in wide scale adoption in the industry. In addition to these high-throughput approaches, a reductionist perspective will be critical to understanding how molecular machinery governs

cell/tissue-specific alterations, leads to functional changes, and contributes to the desirable phenotypes in beef cattle. Therefore microarray, GWAS, and other high-throughput techniques must be used in conjunction with targeted transcriptomic approaches to identify and validate putative and novel pathways and their contributing regulatory factors.

The current work did not elucidate any specific regulatory targets but did indicate that there was some association of specific genes with various efficiency and performance parameters. Moreover, these studies demonstrated the ability to target and monitor the expression of specific regulatory genes in beef cattle indicative of the advancing state and availability of genomic information and technological platforms for use in beef cattle and other livestock species. This will undoubtedly foster future hypothesis driven research in this field that is likely to validate the transcriptomic approach as a valuable asset in improving production efficiency through targeted regulation of molecular processes in a tissue- and depot- specific manner. Also it should be emphasized that while the expression of individual candidate genes was characterized in the current project, these genes are representative of putative and novel metabolic regulatory junctions, so it is important not to over speculate based on individual genes. The influence of other cellular modifications and participation of micro-RNAs and other novel regulatory factors is also like to compound the issue. Instead this information should be used to glean into the overall mechanisms and pathways that can be targeted with management strategies and pharmacological agents such as BAA to improve animal performance. Ultimate therapeutic approaches may or may not include these individual targets specifically but are likely to involve these and other relevant metabolic pathways. These advances will result from a combination of other molecular and biochemical data as well as performance data to understand more clearly

how biological processes contribute to producing a valuable beef cattle phenotype in an efficient manner.

The genomic revolution has brought with it a wealth of useful knowledge in livestock production but has also produced more questions than answers in many cases. Perhaps victims of the technologies success, researchers are generating information at rates that exceed our current capacity to understand and/or make use of. On one hand the resulting information bottleneck is fertile ground for future hypothesis driven research. Further such logjams can appear to be meritless with no tangible practical advantage in the eyes of producers and industry leaders. This dampens the incentive for industry and shareholder investment in, and hampers the application these technologies. Hopefully, the advent of dedicated genomic platforms will break through this impasse and expedite progress given the wealth of information that has yet to be discovered in this burgeoning field. The success of this is however dependent on the willingness for industry stakeholders, academic institutions, and funding agencies to cooperate to collect and share data, refine technologies, and implement techniques in a practical manner suitable for large scale beef cattle production.

As a whole, this body of work provides the basis for future studies and has established several findings. First off, transcriptomic/gene expression profiling in beef cattle determined by quantitative RT-PCR of skeletal muscle and adipose tissue samples can be an effective approach in conducting a performance/phenotypic evaluation of individual cattle particularly in grazing systems in the SE. This will increase our understanding of mechanisms at the genomic/molecular regulation level that regulate production efficiency, and such results can be used to design improved beef cattle feeding strategies for production systems in the SE. Finally these experimental methods can be used in conjunction with more elaborate approaches to identify and

manipulate molecular distinctions in a manner that improves production efficiency and carcass value by augmenting specific tissues independently of less valuable ones. These findings are aimed at improving production efficiency through molecular mechanisms which dictate animal performance and phenotype. These molecular subtleties have the potential to orchestrate large-scale phenotypic changes and reshape our approach to genetic selection and performance evaluation. Ultimately this will improve the viability and sustainability of the beef cattle industry across production systems.

References

Albrecht, E., T. Gotoh, et al. 2011. Cellular conditions for intramuscular fat deposition in Japanese Black and Holstein steers. Meat Sci. 89:13-20.

Albrecht, E., T. Gotoh, F. Ebara, J. Wegner, and S. Maak. 2011. Technical note: Determination of cell-specific gene expression in bovine skeletal muscle tissue using laser microdissection and reverse-transcription quantitative polymerase chain reaction. J Anim Sci. 89(12):4339-43.

Allen, V. G., J. P. Fontenot, R. F. Kelly, and D. R. Notter. 1996. Forage systems for beef production from conception to slaughter: III. Finishing systems. J Anim Sci. 74:625-638.

Attaix D, Aurousseau E, Combaret L, Kee A, Larbaud D, Ralliere C, Souweine B, Taillandier D, and Tilignac T. 1998.Ubiquitin-proteasome dependent proteolysis in skeletal muscle. Reprod Nurt Dev 38:153-165.

Barendse, W., A. Reverter, et al. 2007. A validated whole-genome association study of efficient food conversion in cattle. Genetics. 176:1893-1905.

Basarab, J. A., M. A. Price, et al. 1996. Nutrient requirements of beef cattle residual feed intake and body composition in young growing cattle. Can. J. Anim. Sci. 83:189-204.

Bauman, D. E, K. J. Harvatine, and A.L. Lock. 2011. Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. Annu. Rev. Nutr. 31:299-319.

Bene, H., D. Lasky, and J.M. Ntambi. 2001. Cloning and characterization of the human stearoyl-CoA desaturase gene promoter: transcriptional activation by sterol regulatory element binding protein and repression by polyunsaturated fatty acids and cholesterol. Biochem. Biophys. Res. Commun. 284:1194–1198.

Bennett, M. K., Y. K. Seo, et al. 2008. Selective binding of sterol regulatory element-binding protein isoforms and co-regulatory proteins to promoters for lipid metabolic genes in liver. J. Biol. Chem. 283:15628-15637.

Benoit, S. C., D.J. Clegg, R. J. Seeley and S. C. Woods. 2004. Insulin and Leptin as Adiposity Signals. Recent Progress in Hormone Research 59:267-285.

Benton, C. R., J. G. Nickerson, et al. 2008. Modest PGC-1alpha overexpression in muscle in vivo is sufficient to increase insulin sensitivity and palmitate oxidation in subsarcolemmal, not intermyofibrillar, mitochondria. J. Biol. Chem. 283:4228-4240.

Bergen, W. G., S. E. Johnson, et al. 1989. Muscle protein metabolism in finishing pigs fed ractopamine. J. Anim. Sci. 67:2255-2262.

Bergen, W. G. and R. A. Merkel. 1991. Body composition of animals treated with partitioning agents: implications for human health. The FASEB J. 5:2951-2957.

Bergen, W. G., and R. A. Merkel. 1991. Protein accretion. Pages 169–202 in Growth Regulation in Farm Animals. A. M. Pearson and T. R. Dutson, ed. Elsevier Applied Science, London, UK.

Berthiaume, R., I. Mandell, L. Faucitano, and C. Lafrenière. 2006. Comparison of alternative beef production systems based on forage finishing or grain-forage diets with or without growth promotants: 1. Feedlot performance, carcass quality, and production costs. J. Anim. Sci. 84:2168-2177.

Biddinger, S. B. , K. Almind, M. Miyazaki, E. Kokkotou, J. M. Ntambi and C. R. Kahn. 2005. Effects of diet and genetic background on sterol regulatory element-binding protein-1c, stearoyl-CoA desaturase 1, and the development of the metabolic syndrome. Diabetes. 54: 1314–1323.

Bionaz, M. and J. J. Loor. 2007. Identification of reference genes for quantitative real-time PCR in the bovine mammary gland during the lactation cycle. Physiol. Genomics. 29:312-319.

Bionaz, M. and J. J. Loor. 2008. ACSL1, AGPAT6, FABP3, LPIN1, and SLC27A6 are the most abundant isoforms in bovine mammary tissue and their expression is affected by stage of lactation. J. Nutr. 138:1019-1024.

Bionaz, M., and J.J. Loor. 2011. Gene networks driving bovine mammary protein synthesis during the lactation cycle. Bioinformatics and Biology Insights 5:83-98

Boddicker, N., N.K. Gabler, M.E. Spurlock, D. Nettleton, and J.C. Dekkers. 2011. Effects of ad libitum and restricted feeding on early production performance and body composition of Yorkshire pigs selected for reduced residual feed intake. Animal. 5:1344-53.

Bodine S.C., E. Latres, S. Baumhueter, V.K. Lai, L. Nunez, B.A. Clarke, W.T. Poueymirou, F.J. Panaro, E. Na, K. Dharmarajan, Z.Q. Pan, D.M. Valenzuela, T.M. DeChiara, T.N. Stitt, G.D. Yancopoulos, and D.J. Glass. 2001. Identification of ubiquitin ligases required for skeletal muscle atrophy. Science 294: 1704–1708.

Bolormaa, S., B. J. Hayes, et al. 2011. Genome-wide association studies for feedlot and growth traits in cattle. J. Anim. Sci. 89:1684-1697.

Bonanome, A., S. M. Grundy. 1988. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. N. Engl. J. Med. 318:1244-48

Bonen, A., X. X. Han, et al. 2007. A null mutation in skeletal muscle FAT/CD36 reveals its essential role in insulin- and AICAR-stimulated fatty acid metabolism. Am. J. Physiol. Endocrino.l Metab. 292:E1740-1749.

Bonnet, M., Y. Faulconnier, et al. 2007. Glucose-6-phosphate dehydrogenase and leptin are related to marbling differences among Limousin and Angus or Japanese Black x Angus steers. J.

Anim. Sci. 85:2882-2894.

Boord, J. B., S. Fazio, and M. F. Linton. 2002. Cytoplasmic fatty acid-binding proteins: emerging roles in
metabolism and atherosclerosis. Curr. Opin. Lipidol. 13:141-147.

Brackebusch, S.A., F. K. McKeith, T. R. Carr, and D. G. McLaren. 1991 .Relationship between longissimus composition and the composition of other major muscles of the beef carcass. J ANIM SCI 69:631-40

Byerly, T. C. 1941. Feed and other costs of producing market eggs. Tech. Bull. No. A1. The University of Maryland Agric. Exp., College Park, Maryland.

Byrne, K. A., Y. H. Wang, et al. 2005. Gene expression profiling of muscle tissue in Brahman steers during nutritional restriction. J. Anim. Sci. 83:1-12.

Canovas, A., J. Estany, M. Tor, R. N. Pena, and O. Doran. 2009. Acetyl-CoA carboxylase and stearoyl-CoA desaturase protein expression in subcutaneous adipose tissue is reduced in pigs selected for decreased backfat thickness at constant intramuscular fat content. J. Anim. Sci. 87:3905–3914.

Chaosap, C., T. Parr, and J. Wiseman. 2011. Effect of compensatory growth on performance, carcass composition and plasma IGF-1 in grower finisher pigs. Animal. 5:749-56.

Chen, J., M. V. Dodson, and Z. Jiang. 2010. Cellular and molecular comparison of redifferentiation of intramuscular- and visceral-adipocyte derived progeny cells. Int. J. Biol. Sci. 6:80-88.

Cho, I., J. Kim, H. Y. Seo, D. Lim, J. S. Hong, Y. H. Park, D. C. Park, K. C. Hong, K.Y. Whang, and Y. S. Lee. 2010. Cloning and characterization of microRNAs from porcine skeletal muscle and adipose tissue. Mol. Biol. Rep. 37:3567–74.

Chung, K. Y. and B. J. Johnson. 2008. Application of cellular mechanisms to growth and development of food producing animals. J. Anim. Sci. 86:E226-E235.

Cianzio, D. S., D. G. Topel, et al. 1985. Adipose tissue growth and cellularity: changes in bovine adipocyte size and number. J. Anim. Sci. 60:970-976.

Connor, E. E., S. Kahl, T. H. Elsasser, J. Parker, R. W. Li, C. P. Van Tassell, R. L. Baldwin, and S. M. Barao. 2009. Enhanced mitochondrial complex gene function and reduced liver size may mediate improved feed efficiency of beef cattle during compensatory growth. Funct. Integr. Genomic. 10:39-51.

Coutinho, L. L., W. G. Bergen, et al. 1993. Alpha-2 adrenergic receptor activity in porcine adipocytes is androgen and age/cell size dependent. Comp. Biochem. Physiol. Part A: Physiology. 105:333-339.

Coux, O., K. Tanaka, and A.L. Goldberg. 1996. Structure and functions of the 20S and 26S proteasomes. Annu. Rev. Biochem. 65:801-47.

Daley, C. A., A. Abbott, P. S. Doyle, G. A. Nader, and S. Larson. 2010. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. Nutr. J. 9:10.

Dayton W.R., and M.E. White. 2008. Cellular and molecular regulation of muscle growth and development in meat animals. J. Anim Sci. 86(14 Suppl):E217-25.

de Jong, H., A. C. Neal, et al. 2007. Ontogeny of mRNA expression and activity of long-chain acyl-CoA synthetase (ACSL) isoforms in Mus musculus heart. Biochim. Biophys. Acta. 1771:75-82.

De la Fuente, J., M. T. Diaz, I. Alvarez, M. A. Oliver, M. Font i Furnols, C. Sanudo, M. M. Campo, F. Montossi, G. R. Nute, and V. Caneque. 2009. Fatty acid and vitamin E composition of intramuscular fat in cattle reared in different production systems. Meat Sci. 82:331-337.

Delavaud, C., A. Ferlay, Y. Faulconnier, F. Bocquier, G. Kann, and Y. Chilliard. 2002. Plasma leptin concentration in adult cattle: effects of breed, adiposity, feeding level, and meal intake. J. Anim. Sci. 80:1317-28.

Delavaud C, F. Bocquier, R. Baumont, E. Chaillou, T. Ban-Tokuda, and Y. Chilliard. 2007. Body fat content and feeding level interact strongly in the short- and medium-term regulation of plasma leptin during underfeeding and re-feeding in adult sheep. Br J Nutr. 98 (1):106-15.

Dekkers, J. C. 2004. Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. J. Anim. Sci. 82 E-Suppl:E313-328.

Descalzo, A. M. and A. M. Sancho. 2008. A review of natural antioxidants and their effects on oxidative status, odor and quality of fresh beef in Argentina. Meat Sci. 79:423-436.

Descalzo, A. M, L. Rossetti, G. Grigioni, M. Irurueta, A. M. Sancho, J. Carrete, N. A. Pensel. 2007. Antioxidant status and odor profile in fresh beef from pasture or grain-fed cattle. Meat Sci. 75:299-307.

Descalzo, A. M., E. M. Insani, A. Biolatto, A. M. Sancho, P. T. Garcia, N. A. Pensel, J. A. Josifovich. 2005. Influence of pasture or grain-based diets supplemented with vitamin E on antioxidant/oxidative balance of Argentine beef. Meat Sci. 70:35-44.

De Spiegelaere W, Filliers M, Van Soom A. 2012. Laser capture microdissection for gene expression analysis of specific cell populations in single blastocysts. Methods Mol Biol. 853:29-37.

Desvergne, B., L. Michalik, et al. 2006. Transcriptional regulation of metabolism. Physiol. Rev. 86:465-514.

Dikeman, M.E. 2007. Effects of metabolic modifiers on carcass traits and quality. Meat Sci. 77:121-135.

Dimopoulos, N., M. Watson, et al. 2006. Differential effects of palmitate and palmitoleate on insulin action and glucose utilization in rat L6 skeletal muscle cells. Biochem. J. 399:473-481.

Dodson M. V., G. J. Hausman, L. Guan, T. P. Du M, Rasmussen, S. P. Poulos, P. Mir, W. G.Bergen, M. E. Fernyhough, D. C. McFarland, R. P. Rhoads, B. Soret, J. M. Reecy, S. G. Velleman, and Z. Jiang. 2010. Lipid metabolism, adipocyte depot physiology and utilization of meat animals as experimental models for metabolic research. Int. J. Biol. Sci. 6:691-699.

Dodson, M. V., G. J. Hausman, L. Guan, M. Du, T. P. Rasmussen, S. P. Poulos, P. Mir, W. G. Bergen, M. E. Fernyhough,D. C. McFarland, R. P. Rhoads, B. Soret, J. M. Reecy, S. G. Velleman, and Z. Jiang. 2010. Skeletal muscle stem cells from animals I. basic cell biology. Int. J. Biol. Sci. 6:465-474.

Dodson, M. V., Z. Jiang, et al. 2010. Allied industry approaches to alter intramuscular fat content and composition in beef animals. J. Food Sci. 75:R1-R8.

Doege, H. and A. Stahl. 2006. Protein-mediated fatty acid uptake: novel insights from in vivo models. Physiology (Bethesda). 21:259-268.

Drouillard J.S., and G. L. Kuhl. 1999. Effects of Previous Grazing Nutrition and Management on Feedlot Performance of Cattle. J. Anim Sci 77:136.

Du M., J. Tong, J.F. Zhao, J. Zhao, K.R. Underwood, M. Zhu, S.P. Ford and P.W. Nathanielsz. 2010. Fetal programming of skeletal muscle development in ruminant animals. J. Anim. Sci. 88:E51–E60.

Du, M., J. Yin, et al. 2010. Cellular signaling pathways regulating the initial stage of adipogenesis and marbling of skeletal muscle. Meat Sci. 86:103-109.

Dunne P. G., F. J. Monahan, F. P. O'Mara, and A. P. Moloney. 2009. Colour of bovine subcutaneous adipose tissue: A review of contributory factors, associations with carcass and meat quality and its potential utility in authentication of dietary history. Meat Sci. 81:28-45.

Dunshea, F. R., D. N. D'Souza, et al. 2005. Effects of dietary factors and other metabolic modifiers on quality and nutritional value of meat. Meat Sci. 71:8-38.

Evans, M., C. Geigerman, J. Cook, L. Curtis, B. Kuebler, and M. McIntosh. 2000. Conjugated linoleic acid suppresses triglyceride accumulation and induces apoptosis in 3T3-L1 preadipocytes. Lipids. 35:899-910.

Fernyhough, M. E., E. Okine, et al. 2007. PPARgamma and GLUT-4 expression as developmental regulators/markers for preadipocyte differentiation into an adipocyte. Domest

Anim. Endocrinol. 33:367-378.

FAO. 2009. How to Feed the World in 2050. Food and Agriculture Organization of the United Nations, Rome, Italy

Feve, B. 2005. Adipogenesis: Cellular and molecular aspects. Best Pract. Res. CLin. Endocrinol. Metab. 19:483-499.

Finck, B. N., M. C. Gropler, et al. 2006. Lipin 1 is an inducible amplifier of the hepatic PGC-1alpha/PPARalpha regulatory pathway. Cell Metab. 4:199-210.

Foote, M. R., S. L. Giesy, G. Bernal-Santos, D. E. Bauman, and Y. R. Boisclair. 2010. t10,c12-CLA decreases adiposity in peripubertal mice without dose-related detrimental effects on mammary development, inflammation status, and metabolism. Am. J. Physiol. Regul. Integr. Comp. Physiol. 299:R1521-8.

Fox, D. G., R. R. Johnson, R. L. Preston, T. R. Dockerty, and E. W. Klosterman. 1972. Protein and energy utilization during compensatory growth in beef cattle. J. Anim. Sci. 34:310-318.

Freake, H. C. and Y. K. Moon. 2003. Hormonal and nutritional regulation of lipogenic enzyme mRNA levels in rat primary white and brown adipocytes. J. Nutr. Sci. Vitaminol. (Tokyo). 49:40-46.

Freyssenet, D. 2007. Energy sensing and regulation of gene expression in skeletal muscle. J. Appl. Physiol. 102:529-540.

Gao S.Z., and S.M. Zhao. 2009. Physiology, affecting factors and strategies for control of pig meat intramuscular fat. Recent Patents on Food, Nutrition & Agriculture, 1:59–74.

Gatellier, P., Y. Mercier, and M. Renerre. 2004. Effect of diet finishing mode (pasture or mixed diet) on antioxidant status of Charolais bovine meat. Meat Sci. 67:385–394.

Gaylean, M. L., C. Ponce, and J. Schutz. 2011. The future of beef production in North America. Animal Frontiers. 1:29-36.

Geiduschek, E. P. 2010. An introduction to transcription and gene regulation. J. Biol. Chem. 285:25885-25892.

Gesta, S., Y. H. Tseng, and C. R. Kahn. 2007. Developmental origin of fat: tracking obesity to its source. *Cell.* 131:242–256.

Gibbs, R., G. Weinstock, S. Kappes, L. Schook, L. Skow, and J. Womack. 2004. Bovine Genomic Sequencing Initiative: Cattle-izing the Human Genome. http://www.genome.gov/Pages/Research/ Sequencing/SeqProposals/BovineSEQ.pdf Accessed August 18, 2008.

Gilbert, H., J. P. Bidanel, J. Gruand, J. C. Caritez, Y. Billon, P. Guillouet, H. Lagant, J. Noblet, and P. Sellier. 2007. Genetic parameters for residual feed intake in growing pigs, with emphasis on genetic relationships with carcass and meat quality traits. J. Anim. Sci. 85: 3182-3188.

Giorgino F., L. Laviola, and J. W. Eriksson. 2005. Regional differences of insulin action in adipose tissue: insights from *in vivo* and *in vitro* studies. Acta. Physiol. Scand. 183:13-30.

Goll, D. E., G. Neti, S. M. Mares, and V. F. Thompson. 2008. Myofibrillar protein turnover: The proteasome and the calpains. J. Anim. Sci. 86(E Suppl.):E19–E35.

Gomes M.D., S.H. Lecker, R.T. Jagoe, A. Navon, and A.L. Goldberg. 2001. Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. Proc Natl Acad Sci USA 98: 14440–14445.

Gonçalves D.A., W.A. Silveira, E.C. Lira, F.A. Graça, S. Paula-Gomes, N.M. Zanon, I.C. Kettelhut, and L.C. Navegantes. Clenbuterol suppresses proteasomal and lysosomal proteolysis and atrophy-related genes in denervated rat soleus muscles independently of Akt. Am J Physiol Endocrinol Metab.; 302(1):E123-33.

Gondret, F., N. Guitton, C. Guillerm-Regost, and I. Louveau. 2008. Regional differences in porcine adipocytes isolated from skeletal muscle and adipose tissues as identified by a proteomic approach. Journal of Ani Sci., 86:2115–2125.

Gnanalingham, M. G., A. Mostyn, M. E. Symonds, and T. Stephenson. 2005. Ontogeny and nutritional programming of adiposity in sheep: Potential role of glucocorticoid action and uncoupling protein-2. Am. J. Physiol. Regul. Integr. Comp. Physiol. 289:R1407–R1415.

Gonzalez, J. M., S. E. Johnson, et al. 2010. Effect of ractopamine-HCl supplementation for 28 days on carcass characteristics, muscle fiber morphometrics, and whole muscle yields of six distinct muscles of the loin and round. Meat Sci. 85:379-384.

Grant, A. C., G. Ortiz-ColÃ³n, et al. 2008. Differentiation of bovine intramuscular and subcutaneous stromal-vascular cells exposed to dexamethasone and troglitazone. J. Anim. Sci. 86:2531-2538.

Grant, A. L., D. M. Skjaerlund, et al. 1993. Skeletal muscle growth and expression of skeletal muscle alpha-actin mRNA and insulin-like growth factor I mRNA in pigs during feeding and withdrawal of ractopamine. J. Anim. Sci. 71:3319-3326.

Graugnard, D. E., L. L. Berger, D. B. Faulkner, and J. J. Loor. 2010. High-starch diets induce precocious adipogenic gene network up-regulation in longissimus lumborum of early-weaned Angus cattle. Br. J. Nutr. 103:953-963.

Graugnard, D. E., P. Piantoni, et al. 2009. Adipogenic and energy metabolism gene networks in longissimus lumborum during rapid post-weaning growth in Angus and Angus x Simmental cattle fed high-starch or low-starch diets. BMC Genomics. 10:142.

Graugnard, D. E., S. L. Rodriguez-Zas, et al. 2007. Temporal longissimus muscle gene expression profiles due to plane of dietary energy in early-weaned Angus steers. J. Anim. Sci. 85(Suppl 1):415.

Grubbs J.K., A.N. Fritchen, E. Huff-Lonergan, J.C. Dekkers, N.K. Gabler, and S.M. Lonergan. 2013.Divergent genetic selection for residual feed intake impacts mitochondria reactive oxygen species production in pigs. J Anim Sci. 91(5):2133-40.

Gunawan, A. M., B. T. Richert, et al. 2007. Ractopamine induces differential gene expression in porcine skeletal muscles. J. Anim. Sci. 85:2115-2124.

Halsey, C. H. C., P. S. Weber, et al. 2011. The effect of ractopamine hydrochloride on gene expression in adipose tissues of finishing pigs. J. Anim. Sci. 89:1011-1019.

Hammond, A. C, T. H. Elsasser, W. E. Kunkle, T. S. Rumsey, M. J. Williams, and W. T. Butts. 1990. Effects of winter nutrition and summer pasture or a feedlot diet on plasma insulin-like growth factor I (IGF-I) and the relationship between circulating concentrations of IGF-I and thyroid hormones in steers. Domest. Anim. Endocrinol. 7:465-75.

Hardie, D. G. 2008. AMPK and Raptor: matching cell growth to energy supply. Mol. Cell. 30:263-265.

Harper, G. S. and D. W. Pethick. 2004. How might marbling begin?. Aust. J. Exp. Agr. 44:653-662.

Hashimoto, K., M. Yamada, et al. 2006. Mouse sterol response element binding protein-1c gene expression is negatively regulated by thyroid hormone. Endocrinology. 147:4292-4302.

Hasselgren PO, Fischer JE. The ubiquitin-proteasome pathway: review of a novel intracellular mechanism of muscle protein breakdown during sepsis and other catabolic conditions. Ann Surg. 225(3):307-

Hausman, G. J., M. V. Dodson, et al. 2009. BOARD-INVITED REVIEW: The biology and regulation of preadipocytes and adipocytes in meat animals. J. Anim. Sci. 87:1218-1246.

Hausman, G. J., S. P. Poulos, et al. 2008. The influence of thiazolidinediones on adipogenesis in vitro and in vivo: Potential modifiers of intramuscular adipose tissue deposition in meat animals. J. Anim. Sci. 86(14 suppl):E236-E243.

Hausman, G. J. and R. L. Richardson. 2004. Adipose tissue angiogenesis. J. Anim. Sci. 82:925-934.

Hayden J. M., J. E. Williams, and R. J. Collier. 1993. Plasma growth hormone, insulin-like growth factor, insulin, and thyroid hormone association with body protein and fat accretion in

steers undergoing compensatory gain after dietary energy restriction. J. Anim. Sci. 71:3327-3338.

Hayes, B. and M. Goddard. 2010. Genome-wide association and genomic selection in animal breeding. Genome. 53:876-883.

Hegarty, B. D., S. M. Furler, et al. 2003. The role of intramuscular lipid in insulin resistance. Acta. Physiol. Scand. 178:373-383.

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. 82:2059-68.

Herrmann J, L.O. Lerman, and A. Lerman. 2007. Ubiquitin and ubiquitin-like proteins in protein regulation. Circ Res. 100(9):1276-91.
Hertzel A. V., Smith L. A., Berq A. H., Cline G. W., Shulman G. I., Scherer P. E., Bernlohr D. A.2006. Lipid metabolism and adipokine levels in fatty acid-binding protein null and transgenic mice. Am. J. Physiol. Endocrinol. Metab. 290:E814–E823

Higami, Y., T. D. Pugh, G.P. Page, D. B. Allison, T. A. Prolla, and R. Weindruch. 2004. Adipose tissue energy metabolism: altered gene expression profile of mice subjected to long-term caloric restriction. FASEB J. 18:415-417.

Hirwa, C. A., P. Wallce, X. Shen, Q. Nie, G. Yang, and X. Zhang. 2011. Genes related to economically important traits in beef cattle. Asian J. Anim. Sci. 5:34-45.

Hocquette, J. F., I. Ortigues-Marty, et al. 1998. Nutritional and hormonal regulation of energy metabolism in skeletal muscles of meat-producing animals. Livest. Prod. Sci. 56:115-143.

Hocquette, J.F., Gondret, F., Baeza, E., Medale, F., Jurie, C., Pethick, D.W., 2010. Intramuscular fat content in meat-producing animals: development, genetic and nutritional control, identification of putative markers. Animal 4, 303–319.

Hornick J. L., C. Van Eenaeme, A. Clinquart, M. Diez, and L. Istasse. 1998. Different periods of feed restriction before compensatory growth in Belgian Blue bulls: I. animal performance, nitrogen balance, meat characteristics, and fat composition. J. Anim. Sci. 76:249-259.

Horton, J. D., J. L. Goldstein, et al. 2002. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J. Clin. Invest. 109:1125-1131.

Horton, J. D., N. A. Shah, et al. 2003. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. Proc. Natl. Acad. Sci. USA 100:12027 -12032.

Hotamisligil, G. S., R. S. Johnson, R. J. Distel, R. Ellis, V. E. Papaioannou, and B. M. 1996. Spiegelman. Uncoupling of obesity from insulin resistance through a targeted mutation in aP2, the adipocyte fatty acid binding protein. Science. 274:1377-1379.

Hulsmans, M., D. De Keyzer, et al. 2011. MicroRNAs regulating oxidative stress and inflammation in relation to obesity and atherosclerosis. FASEB J. 25:2515-2527.

Hunter, J. E., J. Zhang, P. M. Kris-Etherton. 2010. Cardiovascular disease risk of dietary stearic acid compared with trans, other saturated, and unsaturated fatty acids: a systematic review. Am. J. Clin. Nutr. 91(1):46-63.

http://beefmagazine.com/genetics/0201-increased-beef-cows

http://www.wvu.edu/~agexten/forglvst/pasturemang.pdf

Ibrahimi, A., A. Bonen, et al. 1999. Muscle-specific overexpression of FAT/CD36 enhances fatty acid oxidation by contracting muscle, reduces plasma triglycerides and fatty acids, and increases plasma glucose and insulin. J. Biol. Chem. 274:26761-26766.

Iizuka, K., R. K. Bruick, G. Liang, J. D. Horton, and K. Uyeda. 2004. Deficiency of carbohydrate response element binding protein (ChREBP) reduces lipogenesis as well as glycolysis. Proc. Nat. Acad. Sci. U. S. A. 101(19):7281–7286.

Iizuka, K., R. K. Bruick, G. Liang, J. D. Horton, and K. Uyeda. 2004. Carbohydrate response element binding protein directly promotes lipogenic enzyme gene transcription. Proc. Natl. Acad. Sci. USA. 101(44):15597-15602.

Ireland, J. J., R. M. Roberts, G. H. Palmer, D. E. Bauman, and F. W. Bazer. 2008. A commentary on domestic animals as dual-purpose models that benefit agricultural and biomedical research. J. Anim. Sci. 86:2797–2805.

Jiang, Z., J. J. Michal, J. Chen, T. F. Daniels, T. Kunej, M. D. Garcia, C. T. Gaskins, J. R. Busboom, L. I. Alexander, R. W. Wright Jr., and M. D. MacNeil. 2009. Discovery of novel genetic networks associated with 19 economically important traits in beef cattle. Int. J. Biol. Sci. 5:528-542.
Jin, W., M. Dodson, S. Moore, J. Basarab, L. L. Guan. 2010. Characterization of microRNA expression in bovine adipose tissues: a potential regulatory mechanism of subcutaneous adipose tissue development. BMC Mol Biol 11:29.

Jéquier E. 2002. Leptin Signaling, Adiposity, and Energy Balance. Ann N Y Acad Sci. 967:379-88.

Jeong, J., E. G. Kwon, S. K. Im, K. S. Seo, and M. Baik. 2012. Expression of fat deposition and fat removal genes is associated with intramuscular fat content in longissimus dorsi muscle of Korean cattle steers. J. Anim. Sci. 90:2044-2053.Jin, W., J. Grant, P. Stothard, S. Moore, and L.

L. Guan. 2009. Characterization of bovine miRNAs by sequencing and bioinformatics analysis. BMC Mol. Biol. 10:90.

Joseph, S. J., S. L. Pratt, et al. 2010. "Omega-6 fat supplementation alters lipogenic gene expression in bovine subcutaneous adipose tissue. Gene Regulation and Systems Biology 91-101.

Jurie, C., I. Cassar-Malek, et al. 2007. Adipocyte fatty acid-binding protein and mitochondrial enzyme activities in muscles as relevant indicators of marbling in cattle. J. Anim. Sci. 85:2660-2669.

Kabbali, A., W. L. Johnson, D. W. Johnson, R. D. Goodrich, and C. E. Allen. 1992. Effects of compensatory growth on some body component weights and on carcass and noncarcass composition of growing lambs. J. Anim. Sci. 70:2852-2858.

Kadegowda, A. K. G., M. Bionaz, et al. 2008. Lipogenic gene expression in MAC-T cells is affected differently by fatty acids and enhanced by PPAR-gamma activation. J. Anim. Sci. 86(E-Suppl 2):566.

Karbiener M, C. Fischer, S. Nowitsch, P. Opriessnig , C. Papak, G. Ailhaud, C. Dani, E.Z. Amri, and M. Scheideler. 2009. microRNA miR-27b impairs human adipocyte differentiation and targets PPARgamma. Biochem Biophys Res Commun. 390:247-251.

Kast-Woelbern, H. R., S. L. Dana, et al. 2004. Rosiglitazone induction of Insig-1 in white adipose tissue reveals a novel interplay of peroxisome proliferator-activated receptor gamma and sterol regulatory element-binding protein in the regulation of adipogenesis. J. Biol. Chem. 279:23908-23915.

Kelemen, L. E., L. H. Kushi, D. R. Jacobs Jr, and J. R. Cerhan. 2005. Associations of dietary protein with disease and mortality in a prospective study of postmenopausal women. Am. J. Epidemiol. 161:239-249.

Key C. N., S. D. Perkins, C. L. Bratcher, L. A. Kriese-Anderson, and T. D. Brandebourg. 2013. Grain feeding coordinately alters expression patterns of transcription factor and metabolic genes in subcutaneous adipose tissue of crossbred heifers J Anim Sci. 91:2616-2627

Keyzer, M. A., M. D. Merbis, I. F. P. W. Pavel, C. F. A. van Wesenbeeck. 2005. Diet shifts towards meat and the effects on cereal use: Can we feed the animals in 2030?. Ecol. Econ. 55:187–202.

Killinger, K. M., C. R. Calkins, W. J. Umberger, D. M. Feuz, and K. M. Eskridge. 2004a. Consumer sensory acceptance and value for beef steaks of similar tenderness, but differing in marbling level. J. Anim. Sci. 82:3294–3301.

Killinger, K. M., C. R. Calkins, W. J. Umberger, D. M. Feuz, and K. M. Eskridge. 2004b. A comparison of consumer sensory acceptance and value of domestic beef steaks and steaks from a branded, Argentine beef program. J. Anim. Sci. 82:3302–3307.

Kim, J. B., H. M. Wright, et al. 1998. ADD1/SREBP1 activates PPARgamma through the production of endogenous ligand. Proc. Natl. Acad. Sci. USA 95:4333-4337.

Kinlaw, W. B., J. L. Church, et al. 1995. Direct evidence for a role of the "spot 14" protein in the regulation of lipid synthesis. J. Biol. Chem. 270:16615-16618.

Kokta, T. A., M. V. Dodson, et al. 2004. Intercellular signaling between adipose tissue and muscle tissue. Domest. Anim. Endocrin. 27:303-331.

Kontogianni, M. D., D. B. Panagiotakos, C. Pitsavos, C. Chrysohoou and C. Stefanadis. 2008. Relationship between meat intake and the development of acute coronary syndromes: The CARDIO2000 case–control study. Eur. J. Clin. Nutr. 62:171–177

Kouba, M. and J. Mourot. 2011. A review of nutritional effects on fat composition of animal products with special emphasis on n-3 polyunsaturated fatty acids. Biochimie. 93:13-7.

Kritchevsky D. 2002. Caloric restriction and experimental carcinogenesis. Hybridoma and hybridomics. 21:147–151.

Kruger, M., I. Kratchmarova, et al. 2008. Dissection of the insulin signaling pathway via quantitative phosphoproteomics. Proc. Natl. Acad. Sci. USA 105:2451-2456.

LaFave, L. T., L. B. Augustin, et al. 2006. S14: insights from knockout mice. Endocrinology 147:4044-4047.

Lefaucheur L., B. Lebret, P. Ecolan, I. Louveau, M. Damon, A. Prunier, Y. Billon, P. Sellier, and H. Gilbert 2011. Muscle characteristics and meat quality traits are affected by divergent selection on residual feed intake in pigs. J. Anim. Sci. 89:996-1010

Lehnert, S. A., K. A. Byrne, et al. 2006. Gene expression profiling of bovine skeletal muscle in response to and during recovery from chronic and severe undernutrition. J. Anim. Sci. 84:3239-3250.

Lehnert, S. A., Y. H. Wang, et al. 2006. Gene expression-based approaches to beef quality research. Aust. J. Exp. Agr. 46:165-172.

Lemay D. G., Hwang D. H.2006. Genome-wide identification of peroxisome proliferator response elements using integrated computational genomics. J. Lipid Res. 47:1583–1587

Li B., Zerby H. N., Lee K.2007. Heart fatty acid binding protein is upregulated during porcine adipocyte development. J. Anim. Sci. 85:1651–1659

Li, J., K. Takaishi, et al. 2003. Insig-1 "brakes" lipogenesis in adipocytes and inhibits differentiation of preadipocytes. Proc. Natl. Acad. Sci. USA 100:9476-9481.

Li, L. O., D. G. Mashek, et al. 2006. Overexpression of rat long chain acyl-CoA synthetase 1 alters fatty acid metabolism in rat primary hepatocytes. J. Biol. Chem. 281:37246-37255.

Lin, Q., Z. Gao, R. M. Alarcon, J. Ye, and Z. Yun. 2009. A role of miR-27 in the regulation of adipogenesis. FEBS J. 276:2348–2358.

Littell, R. C., P. R. Henry, et al. 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76:1216-1231.

Lkhagvadorj, S., L. Qu, et al. 2010. Gene expression profiling of the short-term adaptive response to acute caloric restriction in liver and adipose tissues of pigs differing in feed efficiency. Am. J. Physiol. Regul. Integr. Comp. Physiol. 298:R494-R507.

Lopez-Carlos, M. A., R. G. Ramirez, et al. 2011. Effect of two beta adrenergic agonists and feeding duration on feedlot performance and carcass characteristics of finishing lambs. 138:251-258.

Lucy, M. C. 2008. Functional Differences in the Growth Hormone and Insulin-like Growth Factor Axis in Cattle and Pigs: Implications for Post-partum Nutrition and Reproduction. Reprod. Domest. Anim. 43 Suppl 2:31-39.

Lulu Strat, A., T. A. Kokta, et al. 2005. Early signaling interactions between the insulin and leptin pathways in bovine myogenic cells. Biochim. Biophys. Acta. 1744:164-175.

MacDougald, O. A., and M. D. Lane. 1995. Transcriptional regulation of gene expression during adipocyte differentiation. Annu. Rev. Biochem. 64:345–373.

Mann, N. J., E. N. Ponnampalam, Y. Yep, and A. J. Sinclair. 2003. Feeding regimes affect fatty acid composition in Australian beef cattle. Asia Pac. J. Clin. Nutr. 12 Suppl:S38.

Mannen, H. 2011. Identification and utilization of genes associated with beef qualities. J. Anim. Sci. 82: 1-7.

McDonald, J. M., J. J. Ramsey, J. L. Miner, and M. K. Nielsen. 2009. Differences in mitochondrial efficiency between lines of mice divergently selected for heat loss. J Anim Sci 87:3105-3113.

McCurdy M. P., G. W. Horn, J. J. Wagner, P. A. Lancaster, C. R. Krehbiel. 2010. Effects of winter growing programs on subsequent feedlot performance, carcass characteristics, body composition, and energy requirements of beef steers. J. Anim. Sci. 88:1564-76.

McMurry, B. 2009. Cow size is growing. Beef Magazine. Accessed May 23, 2011.

Melton, S. L., M. Amiri, G. W. Davis, and W. R. Backus. 1982. Flavor and chemical characteristics of ground beef from grass-, forage-grain-, and grain-finished steers. J. Anim. Sci. 55:77–87.

Mersmann, J., M.D. MacNeil, S. C. Seideman, and W. G. Pond. 1987.Compensatory growth in finishing pigs after feed restriction. J. Anim. Sci. 64:752-764.

Merry, B. J. 2002. Molecular mechanisms linking calorie restriction and longevity. Int. J. Biochem. Cell Biol. 34:1340-54.

Meyer, D. L., M. S. Kerley, et al. 2005. Growth rate, body composition, and meat tenderness in early vs. traditionally weaned beef calves. J. Anim. Sci. 83:2752-2761.

Michal, J.J., Z.W. Zhang, C.T. Gaskins, and Z. Jiang. 2006. The bovine fatty acid binding protein 4 gene is significantly associated with marbling and subcutaneous fat depth in Wagyu x Limousin F2 crosses. Anim Genet. 37(4):400-2.

Mills, S. E. 2002. Biological basis of the ractopamine response. J. Anim. Sci. 80(E-Suppl 2):E28-E32.

Mills, S. E., J. Kissel, et al. 2003. Stereoselectivity of porcine Î²-adrenergic receptors for ractopamine stereoisomers. J. Anim. Sci. 81:122-129.

Mills, S. E., M. E. Spurlock, et al. 2003. Î²-Adrenergic receptor subtypes that mediate ractopaminestimulation of lipolysis. J. Anim. Sci. 81:662-668.

Mimbs, K. J., T. D. Pringle, et al. 2005. Effects of ractopamine on performance and composition of pigs phenotypically sorted into fat and lean groups. J. Anim. Sci. 83:1361-1369.

Mir, P. S., D. R. C. Bailey, Z. Mir, T. Entz, S. D. M. Jones, W. M. Rober Tson, R. J. Weselake, F. J. Lozeman. 1999. Growth, carcass and meat quality characteristics of beef cattle with 0, 50 and 75 percent Wagyu genetic influence. Can. J. Anim. Sci. 79:129-137.

Moore S.S., F.D. Mujibi, and E.L. Sherman. 2009. Molecular basis for residual feed intake in beef cattle. J Anim Sci. 87(14 Suppl):E41-7.

Muoio, D. M., P. S. MacLean, et al. 2002. Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) alpha knock-out mice. Evidence for compensatory regulation by PPAR delta. J. Biol. Chem. 277:26089-26097.

Myers, S. E., D. B. Faulkner, et al. 1999. Production systems comparing early weaning to normal weaning with or without creep feeding for beef steers. J. Anim. Sci. 77:300-310.

Myers, S. E., D. B. Faulkner, et al. 1999. Performance and carcass traits of early-weaned steers receiving either a pasture growing period or a finishing diet at weaning. J. Anim. Sci. 77:311-

322.

Nakajima, I., M. Oe, et al. 2011. Cellularity of developing subcutaneous adipose tissue in Landrace and Meishan pigs: Adipocyte size differences between two breeds. J. Anim. Sci. 82:144-149.

Nishimura, T., A. Hattori, et al. 1999. Structural changes in intramuscular connective tissue during the fattening of Japanese black cattle: effect of marbling on beef tenderization. J. Anim. Sci. 77:93-104.

Norrelund, H., K. S. Nair, J. O. Jorgensen, J. S. Christiansen, and N. Moller. 2001. The protein-retaining effects of growth hormone during fasting involve inhibition of muscle-protein breakdown. Diabetes. 50:96-104

Novakofski, J. 2004. Adipogenesis: Usefulness of in vitro and in vivo experimental models. J. Anim. Sci. 82:905-915.

Noviandi, C. T., R. E. Ward, D. R. ZoBell, R. D. Stott, B. L. Waldron, M. D. Peel, and J. -S. Eun. 2012. Fatty acid composition in adipose tissue of pasture- and feedlot-finished beef steers. PAS. 28:184-193.

Obregon, M. J. 2008. Thyroid hormone and adipocyte differentiation.Thyroid 18:185-195.

Ohsaki, H., T. Sawa, S. Sasazaki, K. Kano, M. Taniguchi, F. Mukai, and H. Mannen. 2007. Stearoyl-CoA desaturase mRNA expression during bovine adipocyte differentiation in primary culture derived from Japanese Black and Holstein cattle. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 148:629-34.

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

Oksbjerg, N., M. T. Sorensen, and M. Vestergaard. 2002. Compensatory growth and its effect on technological meat quality in growing pigs. Acta. Agric. Scand. Sect. A. Anim. Sci. 52:85–90.

Okine, E. K., J. A. Basarab, L. A. Goonewardene, and P. Mir. 2004. Residual feed intake and feed efficiency: Differences and implications. In: Florida Ruminant Nutrition Symposium

Osborne, T. B. and L. B. Mendel. 1916. Acceleration of growth after retardation. Amer. J. Physiol. 40:16.

Owens, F. N., P. Dubeski, and C. F. Hanson. 1993. Factors that alter the growth and development of ruminants. J. Anim. Sci. 71:3138-50.

Park, G. B., S. S. Moon, Y. D. Ko, J. K. Ha, J. G. Lee, H. H. Chang, and S. T. Joo. 2002. Influence of slaughter weight and sex on yield and quality grades of Hanwoo (Korean native cattle) carcasses. J.Anim. Sci. 80:129–136.

Pascual M., A.S. Del Barrio, M.P. Portillo, J.A. Martinez, and J. Larralde. 1993. Tissue protein turnover in animals treated with the mixed beta-agonist metaproterenol: influence of dose, route and pattern of administration. Biochimie. 75(10):879-83.

Pethick, D. W., G. S. Harper, et al. 2004. Growth, development and nutritional manipulation of marbling in cattle: a review. Aust. J. Exp. Agr. 44:705-715.

Piantoni, P., M. Bionaz, et al. 2008. Gene expression ratio stability evaluation in prepubertal bovine mammary tissue from calves fed different milk replacers reveals novel internal controls for quantitative polymerase chain reaction. J. Nutr. 138:1158-1164.

Pickworth, C. L., S. C. Loerch, et al. 2011. Adipogenic differentiation state-specific gene expression as related to bovine carcass adiposity. J. Anim. Sci. 89:355-366.

Pitchford, W. S. 2004. Genetic improvement of feed efficiency of beef cattle: What lessons can be learnt from other species? Aust. J. Exp. Agric. 44:371–382.

Pond, C. M. 2003. Paracrine interactions of mammalian adipose tissue. J. Exp. Zool. Comp. Exp. Biol. 295A1:99-110.

Preston, R. L. 1999. Hormone containing growth promoting implants in farmed livestock. Adv. Drug Delivery Rev. 38:123-138.

Preston, R. L. 2004. Typical composition of feeds for cattle and sheep, 2004. Beef Mag.

Prior, R. L. and R. A. Scott. 1980. Effects of intravenous infusions of glucose, lactate, propionate or acetate on the induction of lipogenesis in bovine adipose tissue. J. Nutr. 110:2011-2019.

Pyatt, N. A. and L. L. Berger. 2005. Review: potential effects of vitamins a and D on marbling deposition in beef cattle. PAS. 21:174-181.

Reecy, J. M., D. M. Spurlock, et al. 2006. Gene expression profiling: Insights into skeletal muscle growth and development. J. Anim. Sci. 84(13 suppl):E150-E154.

Raimbault, S., S. Dridi, F. Denjean, J. Lachuer, E. Couplan, F. Bouillaud, A. Bordas, C. Duchamp, M. Taouis, and D. Ricquier. 2001. An uncoupling protein homologue putatively involved in facultative muscle thermogenesis in birds. Biochem. J. 353:441–444.

Rajaei Sharifabadi H., M. J. Zamiri, E. Rowghani, and W. G. Bottje. 2011. Relationship between the activity of mitochondrial respiratory chain complexes and feed efficiency in fat-tailed Ghezel lambs. J Anim Sci 90:1807-1815

Realini, C. E., S. K. Duckett, G. W. Brito, M. D. Rizza, and D. De Mattos. 2004. Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. Meat Sci. 66:567-77.

Reinhardt, C. 2007. Growth-Promotant Implants: Managing the Tools. Vet. Clin. N. Am. Food Anim. Pract. 232:309-319.

Reinstein, E., and A. Ciechanover. 2006. Narrative Review: Protein degradation and human diseases: The ubiquitin connection.Ann. Intern. Med. 145:676–684.

Riedmaier, I., M. W. Pfaffl , and H. H. Meyer. 2011. The analysis of the transcriptome as a new approach for biomarker development to trace the abuse of anabolic steroid hormones. Drug Test Anal. 3:676-681.

Reinstein, E., and A. Ciechanover. 2006. Narrative Review: Protein degradation and human diseases: The ubiquitin connection.Ann. Intern. Med. 145:676–684.

Reverter, A., K. A. Byrne, et al. 2003. A mixture model-based cluster analysis of DNA microarray gene expression data on Brahman and Brahman composite steers fed high-, medium-, and low-quality diets. J. Anim. Sci. 81:1900-1910.

Rhoades, R. D., J. E. Sawyer, et al. 2007. Effect of dietary energy source on in vitro substrate utilization and insulin sensitivity of muscle and adipose tissues of Angus and Wagyu steers. J. Anim. Sci. 85:1719-1726.

Romao, J. M., W. Jin, M. V. Dodson, G. I. Hausman, S. S. Moore, L. Guan le. 2011. MicroRNA regulation in mammalian adipogenesis. Exp. Biol. Med. (Maywood). 236:997-1004.

Rosegrant, M. W., N. Leach, and R. V. Gerpacio. 1999. Meat or wheat for the next millennium? Alternative futures for world cereal and meat consumption. Proc. Nutr. Soc. 58:219–234.

Robelin, J. 1981. Cellularity of bovine adipose tissues: developmental changes from 15 to 65 percent mature weight. J. Lipid Res. 22:452-457.

Rosen, E. D. and O. A. MacDougald 2006. Adipocyte differentiation from the inside out. Nat. Rev. Mol. Cell Biol. 7:885-896.

Roy R., L. Ordovas, S. Taourit, P. Zaragoza, A. Eggen, and C. Rodellar. 2006. Genomic structure and an alternative transcript of bovine mitochondrial glycerol-3-phosphate acyltransferase gene (GPAM). Cytogenet Genome Res 112:82–89.

Solomon V and Goldberg AL. Important of the ATP-ubiquitin-proteasome pathway in the degradation of soluble and myofibrillar proteins in rabbit muscle extracts. J Biochem 271: 26690-26697, 1996.

Schmitt, E., M. A. Ballou, M. N. Correa, E. J. DePeters, J. K. Drackley, and J. J. Loor. 2011. Dietary lipid during the transition period to manipulate subcutaneous adipose tissue peroxisome proliferator-activated receptor-γ co-regulator and target gene expression. J. Dairy Sci. 94:5913-25.

Schoonmaker, J. P., V. M. Cecava, et al. 2003. Effect of source of energy and rate of growth on performance, carcass characteristics, ruminal fermentation, and serum glucose and insulin of early-weaned steers. J. Anim. Sci. 81 843-855.

Schoonmaker, J. P., F. L. Fluharty, et al. 2004. "Effect of source and amount of energy and rate of growth in the growing phase on adipocyte cellularity and lipogenic enzyme activity in the intramuscular and subcutaneous fat depots of Holstein steers." J. Anim. Sci. 82:137-148.

Scollan, N. D., N. J. Choi, E. Kurt, A. V. Fisher, M. Enser, J. D. Wood. 2001. Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. Br. J. Nutr. 85:115-24.

Sekiya, M., N. Yahagi, et al. 2007. SREBP-1-independent regulation of lipogenic gene expression in adipocytes. J. Lipid Res. 48:1581-1591.

Sellner, E. M., J. W. Kim, M. C. McClure, K. H. Taylor, R. D. Schnabel, and J. F. Taylor. 2007. Board-invited review: Applications of genomic information in livestock. J. Anim Sci. 85:3148-3158.

Sensky, P. L., K. K. Jewell, et al. 2006. Effect of anabolic agents on calpastatin promoters in porcine skeletal muscle and their responsiveness to cyclic adenosine monophosphate-and calcium-related stimuli. J. Anim. Sci. 84:2973-2982.

Shimano, H. 2001. Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. Prog. Lipid. Res. 40:439–452.

Sillence, M. N. 2004. Technologies for the control of fat and lean deposition in livestock. The Veterinary Journal 167:242-257.

Simonne, A. H., N. R. Green, D. I. Bransby. 1996. Consumer acceptability and beta-carotene content of beef as related to cattle finishing diets. J. Food Sci. 61:1254–1256.

Sluka P., L. O'Donnell, R.I. McLachlan, and P.G. Stanton. 2008. Application of laser-capture microdissection to analysis of gene expression in the testis. Prog Histochem Cytochem. 42(4):173-201.

Smith, S. B. and J. D. Crouse 1984. Relative contributions of acetate, lactate and glucose to lipogenesis in bovine intramuscular and subcutaneous adipose tissue. J. Nutr. 114:792-800.

Smith, S. B., H. Kawachi, et al. 2009. Cellular regulation of bovine intramuscular adipose tissue development and composition. J. Anim. Sci. 87(14 suppl):E72-E82.

Smith, S. B., R. L. Prior, et al. 1984. Interrelationships among diet, age, fat deposition and lipid metabolism in growing steers. J. Nutr. 114(1):153-162.

Soukas, A., P. Cohen, N.D., Socci, and J.M. Friedman. 2000. Leptin-specific patterns of gene expression in white adipose tissue. Genes and Development. 14: 963–980.

Sturn, A., J. Quackenbush, et al. 2002. Genesis: cluster analysis of microarray data. Bioinformatics 18: 207-208.

Surai, P. F. and N. H. C. Sparks. 2001. Designer eggs: from improvement of egg composition to functional food. Trends Food Sci. Tech., Volume 12:1 7-16

Sweeten, M. K., H. R. Cross, G. C. Smith, J. W. Savell, and S. B. Smith. 1990. Lean beef: impetus for lipid modifications. J. Am. Diet Assoc. 90:87-92.

Szewczyk, A., and L. Wojtczak. 2002. Mitochondria as a Pharmacological Target Pharmacol Rev. 54:101-127.

Tan, S. H., A. Reverter, et al. 2006. Gene expression profiling of bovine in vitro adipogenesis using a cDNA microarray. Funct. Integr. Genomics. 6:235-249.

Tang, Q. Q., T. C. Otto, and M. D. Lane. 2003. Mitotic clonal expansion: a synchronous process required for adipogenesis. Proc. Natl. Acad. Sci. 100:44-49.

Tang, Q. Q., T. C. Otto, and M. D. Lane. 2003. CCAAT/enhancer-binding protein β is required for mitotic clonal expansion during adipogenesis. Proc. Natl. Acad. Sci. 100:850–855.

Taniguchi, M., L. Guan le, et al. 2008. Adipogenesis of bovine perimuscular preadipocytes. Biochem. Biophys. Res. Commun. 366:54-59.

Taniguchi, M., L. L. Guan, et al. 2008. Comparative analysis on gene expression profiles in cattle subcutaneous fat tissues. Comp. Biochem. Physiol. D Genomics Proteomics. 3:251-256.

Taniguchi, M., L. L. Guan, et al. 2008. Gene expression patterns of bovine perimuscular preadipocytes during adipogenesis. Biochem. Biophys. Res. Commun. 366:346-351.

Taniguchi, M., T. Utsugi, H. Mannen and S. Tsuji. 2004. Genotype of stearoyl-CoA desaturase is associated with fatty acid composition in Japanese Black cattle. Mamm. Genome. 14:142–148.

Tchoukalova, Y. D., S. B. Votruba, et al. 2010. Regional differences in cellular mechanisms of adipose tissue gain with overfeeding. Proc. Nat. Acad. Sci. 107:18226-18231.

Therkildsen, M., M. Vestergaard, H. Buska, M. T. Jensena, B. Riisa, A. H. Karlssona, L. Kristensenb, P. Ertbjergb, N. Oksbjerg. Compensatory growth in slaughter pigs—in vitro muscle protein turnover at slaughter, circulating IGF-I, performance and carcass quality. Livest. Prod. Sci. 88:63-75.

Tontonoz, P., Hu, E. & Spiegelman, B. M.1994. Stimulationof adipogenesis in fibroblasts by PPARγ2, a lipid activatedtranscription factor. Cell 79, 1147–1156.

Torii, S., T. Kawada, K. Matsuda, T. Matsui, T. Ishihara, and H. Yano. 1998. Thiazolidinedione induces the adipose differentiation of fibroblast-like cells resident within bovine skeletal muscle. Cell Biol. Int. 22: 421-27.

Trenkle, A. 1978. Relation of hormonal variations to nutritional studies and metabolism of ruminants. J. Dairy Sci. 61:281-293.

Tramontana, S., M. Bionaz, et al. 2008. Internal controls for quantitative polymerase chain reaction of swine mammary glands during pregnancy and lactation. J. Dairy Sci. 91:3057-3066.

Tsai S, Cassady JP, Freking BA. et al. 2006. Annotation of the Affymetrix porcine genome microarray. Anim. Genet. 37:423-424.

Tseng, Y. H., A. J. Butte, et al. 2005. Prediction of preadipocyte differentiation by gene expression reveals role of insulin receptor substrates and necdin. Nat. Cell Biol. 7:601-611.

Underwood, K.R., W.J. Means, M.J. Zhu, S.P. Ford, B.W. Hess, and M. Du. 2008. AMP-activated protein kinase is negatively associated with intramuscular fat content in longissimus dorsi muscle of beef cattle. Meat Sci. 79(2):394-402.

Upritchard, J. E., M. J. Zeelenberg, H. Huizinga, P. M. Verschuren, and E. A. Trautwein. 2005. Modern fat technology: what is the potential for heart health? Proc. Nutr. Soc. 64:379-86.

Uysal, K. T., L. Scheja, S. M. Wiesbrock, S. Bonner-Weir, and G. S. Hotamisligil. 2000. Improved glucose and lipid metabolism in genetically obese mice lacking a P2. Endocrinology. 141:3388-3396.

US Census Bureau. 2008. Total Midyear Population for the World: 1950–2050

United States Department of Agricultrue. Grass (Forage) Fed Marketing Claim Standard (October 16, 2007, Federal Register Notice (72 FR 58631)) - Federal Register / Vol. 72, No. 199 /Tuesday, October 16, 2007.

Valaja, J., T. Alaviuhkola, K. Suomi, and I. Immonen. 1992. Compensatory growth after feed restriction during the rearing period in pigs. Agric. Sci. Finland 1:15-20.

van Oort, M. M., J. M. van Doorn, et al. 2008. Insulin-induced translocation of CD36 to the plasma membrane is reversible and shows similarity to that of GLUT4. Biochim. Biophys. Acta. 1781:61-71.

Vandesompele, J., K. De Preter, et al. 2002. Accurate normalization of real-time quantitative RT-PCR data

by geometric averaging of multiple internal control genes. Genome Biol. 3:RESEARCH0034.

Verde, L. S. and A. Trenkle 1987. Concentrations of hormones in plasma from cattle with different growth potentials. J. Anim. Sci. 64:426-432.

Verhoeckx, K. C. M., R. P. Doornbos, et al. 2005. Inhibitory effects of the β2-adrenergic receptor agonist zilpaterol on the LPS-induced production of TNF-α in vitro and in vivo. J. Vet. Pharmacol. Ther. 28:531-537.

Viguerie, N., L. Millet, et al. 2002. Regulation of human adipocyte gene expression by thyroid hormone. J. Clin. Endocrinol. Metab. 87:630-634.

Wabitsch M., W. F. Blum, R. Muche, M. Braun, F. Hube, W. Rascher, E. Heinze, W. Teller, H. Hauner. 1997. Contribution of androgens to the gender difference in leptin production in obese children and adolescents. J. Clin. Invest. 100(4): 808-813.

Wang, D., N. Wang, et al. 2009. Identification of differentially expressed proteins in adipose tissue of divergently selected broilers. Poult. Sci. 88:2285-2292.

Wang, Y., N. I. Bower, A. Reverter, et al. 2009. Gene expression patterns during intramuscular fat development in cattle. J. Anim. Sci. 87:119-30.

Wang, Y. H., K. A. Byrne, et al. 2005. Transcriptional profiling of skeletal muscle tissue from two breeds of cattle. Mamm. Genome 16:201-210.

Welchman R.L., C. Gordon, and R.J. Mayer. Ubiquitin and ubiquitin-like proteins as multifunctional signals. Nat Rev Mol Cell Biol 6: 599–609.

Wertz, A. E., L. L. Berger, et al. 2002. Early-weaning and postweaning nutritional management affect feedlot performance, carcass merit, and the relationship of 12th-rib fat, marbling score, and feed efficiency among Angus and Wagyu heifers. J. Anim. Sci. 80:28-37.

Whitehurst, G. B., D. C. Beitz, et al. 1981. Fatty acid synthesis from lactate in growing cattle. J. Nutr. 111(8): 1454-1461.

Wilkins, P.W. 1991. Breeding perennial ryegrass for agriculture. Euphytica. 52:201–214.

Wood, J. D., M. Enser, A. V. Fisher, G. R. Nute, R. I. Richardson, and P. R. Sheard. 1999. Manipulating meat quality and composition. Proc. Nutr. Soc. 58:363-70.

Wood, J. D., R. I. Richardson, G. R. Nute, A. V. Fisher, M. M. Campo, E. Kasapidou, P. R. Sheard, and M. Enser. 2003. Effects of fatty acids on meat quality: review. Meat Sci. 66:21-32.

Wood, W. B. 1988. Determination of pattern and fate in early embryos of *Caenorhabditis elegans*. *Dev. Biol. (N Y 1985).* 5:57–78.

Wright, I. A. and A. J. F. Russel. 1991. Changes in the body composition of beef cattle during compensatory growth. Anim. Prod. 52:105-113

Wu, R. and M. Lin. 2006. Functional mapping - how to map and study the genetic architecture of dynamic complex traits. Nat. Rev. Genet. 7:229-237.

Wu, P., K. Sato, F. Suzuta, Y. Hikasa, and K. Kagota. 2000. Effects of lipid-related factors on adipocyte differentiation of bovine stromal-vascular cells in primary culture. J. Vet. Med. Sci. 62:933-939.

Xu, J. X., E. Albrecht, et al. 2009. Perilipin, C/EBP[alpha], and C/EBP[beta] mRNA abundance in longissimus muscle and different adipose tissues of Holstein and Charolais cattle. Meat Sci. 83:120-126.

Yabe, D., M. S. Brown, et al. 2002. Insig-2, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol regulatory element-binding proteins. Proc. Natl. Acad. Sci. USA. 99:12753-12758.

Yamada, T., S. I. Kawakami, et al. 2009. Effects of dietary roughage levels on the expression of adipogenic transcription factors in Wagyu steers. Meat Sci. 83:775-781.

Yambayamba E. S., 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.

Zembayashi, M., K. Nishimura, et al. 1995. Effect of breed type and sex on the fatty acid composition of subcutaneous and intramuscular lipids of finishing steers and heifers. J. Anim. Sci. 73:3325-3332.

Zimmerman, A. W. and J. H. Veerkamp. 2002 New insights into the structure and function of fatty acid-binding proteins. Cell. Mol. Life Sci. 59:1096-1116

Zhang, Y., R. Proenca, et al. 1994. Positional cloning of the mouse obese gene and its human homologue. Nature. 372:425-432.