Effect of Early Grain Feeding of Steers on Postabsorptive Capacity to Utilize Acetate and Glucose

Deepthi Nayananjalie Wettathperuma Arachchige

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirement for the degree of

Doctor of Philosophy

In

Animal Sciences, Dairy

Mark D. Hanigan, Committee Chair

Robert M. Akers

Michael L. McGilliard

Katharine F. Knowlton

Mark A. McCann

6th November, 2012

Blacksburg, Virginia

Key words: acetate, adipose tissue, clearance, fractional synthesis rate, glucose

Effect of Early Grain Feeding of Steers on Postabsorptive Capacity to Utilize Acetate and Glucose

Deepthi Nayananjalie Wettathperuma Arachchige

ABSTRACT

The aims of the first study were to determine the effects of early weaning followed by a period of high-grain feeding on signaling protein phosphorylation in muscle and on acetate clearance in growing steers. Twelve Angus X Simmental steers were used in the study. Six steers were weaned early (EW) and fed a high-gain diet for 148 d and the remainder were weaned at the normal age (NW). Longissimus muscle tissue biopsies were collected to determine total and phosphorylated forms of AMP kinase and downstream proteins of the mTOR signaling pathway. Of the 12, 8 steers were used to assess acetate clearance at different age points. During early grain feeding, phosphorylated:total ratios of rpS6 and S6K1 were greater in EW steers and they had lower acetate clearance rates and greater synthesis rates.

The objectives of second study were to assess the effects of early grain feeding on acetate and glucose turnover rates, palmitate synthesis, and on acetate and glucose preference by subcutaneous, intramuscular and visceral adipose tissues in finishing steers. Sixteen Angus x Simmental steers were infused continuously with [$^{2}H_3$] acetate (n = 8) or [U-$^{13}C_6$] glucose (n = 8), over a 12 h period immediately prior to harvest. Plasma acetate and glucose enrichment, and palmitate enrichment in different adipose tissue locations were determined. There were no treatment effects on acetate or glucose turnover rates or palmitate fractional synthesis rates (FSR). Acetate turnover and palmitate FSR from acetate were greater than the corresponding

rates from glucose. There were no differences in preference for acetate or glucose among the fat depots.

In conclusion, phosphorylation ratios of signaling proteins were not affected treatment. Acetate clearance increased when steers were heavier and older. Thus older calves are able to clear more acetate per unit of time and body mass than younger calves reflective of an enhanced ability to utilize the substrate. There were no differences in preference for acetate and glucose among the major fat depots. Thus diets leading to high glucose supply will not preferentially direct energy storage to intramuscular stores.

DEDICATION

I dedicated my dissertation to my parents and teachers for their effort and contribution to my education.

ACKNOWLEDGEMENT

While pursing my doctorate, there are many individuals who contributed their advice, guidance, time, expertise, friendship and love. To begin, I would like to thank my advisor, Dr. Mark Hanigan for his advice, guidance, support, encouragement, and confidence throughout the course of my time spent here at Virginia Tech. He was always there to answer my questions and I have acquired a wealth of knowledge. He has helped to mold me into the individual I am today. I would like to express my gratitude to my committee members, Drs. Akers, Knowlton, McCann and McGilliard for their assistance, encouragement and for their valuable inputs and suggestions. It has been great pleasure to get to know and work so closely with each of you. Dr. McGilliard, thank you for your expertise, time and guidance in statistical analysis.

I am grateful to ADM for the partial funding of my research. My sincere thanks go to the VT Beef center crew and Mark Stevenson from the Meat Science lab for their outstanding help during the experiments. I could not have done it without your assistance and support. To the VT dairy farm crew; Shane, Woody and Curtis, I greatly appreciate your help while experiments run at Dairy. My sincere thanks go to Drs. Ondrej Becvar and Hollie Schramm for assisting in catheterization. I also thank Drs. Scheffler and Jiang for the assistance during experiments. The support of Cathy Parsons and Dr. Akers in the analysis of insulin is gratefully acknowledged. Special thank goes to Dr. Corl for the guidance on sample preparation for GC-MS. I am grateful to Tara for her support and making sure things get done right and for helping with laboratory analysis. Thank you for running all my GC-MS analysis throughout my research.

Many thanks goes to Ranga, who pointed me in the right direction, when I first spoke to him about graduate school and who taught me all the basic analysis being conducted when I joined the laboratory. My lab mates; Sebastian, Sandip, Jason, Michelle and Tyler, thank you for

your help during my experiments, company and friendship. I would also like thank the undergraduate students; Katie, Carenda, Morgan, Jessica, Makayla, Erica, Roxanne and Chris who worked with me during the animal experiments. I appreciate the company and friendship of my fellow graduate students in the Department of Dairy Science and the Department of Animal and Poultry Sciences. I would like to thank my office mates; Partha and Liying for sharing things with me during my stay. Also my gratitude goes to all members of Department of Dairy Science and Animal and Poultry Sciences for being friendly and so warm.

And last but certainly not least, a very special thanks to my husband Jayantha for all his love, devotion, care and endless support and also who encouraged me to pursue this path. I wish to thank my adorable son Sanuka for his love, patience, support and understanding. Finally, I thank my parents, even though we were thousands of miles away while in the USA, I had your blessings and love, always encouraged me to pursue my dreams.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Introduction

Rising global energy demand has dramatically increased petroleum prices and thereby resulted in significant growth of the bio-fuel industries such as corn ethanol and soy-diesel. The bio-fuel industries have provided significant economic benefits to grain producers and underwent a rapid expansion causing unprecedented changes in animal agriculture in the recent past (Doering and Hurt, 2006). Low availability and high cost of feed ingredients has significantly challenged the profitability of today's animal production systems. Improving the feed efficiency and grain usage by selective timing of high nutrient feeding periods and use of by-products from the bio-fuel industry may improve food system efficiency as indicated by less grain per kg of product derived from both fat and protein deposition. This may be more feasible for ruminants where high forage diets can be used.

Beef calves are usually weaned from a milk- and grass-based diet at 205 d of age in the USA. Early weaning has been tried as a method to improve cow condition for rebreeding (Arthington and Kalmbacher, 2003). Further, peak milk production in beef cows occurs between 60 - 80 d of lactation (NRC, 2000). Therefore, decreasing milk availability to calves after 80 d would limit the energy intake and average gain during the growth period, if supplements are not provided. Myers et al. (1999c) compared three weaning ages (90, 152 and 215d of age) followed by grain feeding and observed linear increases in average daily gain and feed efficiency as weaning age decreased. Early weaning followed by grain feeding can therefore be a good alternative management practice. However, early weaning followed by high grain feeding until harvest appeared to yield mixed results in terms of production efficiency. Although early

weaning increased weight gain, enhanced feed conversion efficiency, and improved carcass quality grade (Fluharty et al., 2000; Myers et al., 1999a; Myers et al., 1999b; Myers et al., 1999c; Wertz et al., 2002), it is associated with early-maturing cattle yielding smaller carcasses, greater number of days on the concentrate diets and increased feed costs (Schoonmaker et al., 2003; Schoonmaker et al., 2004). In a conventional system, calves are weaned around 200 d and raised on pasture, and fed a grain-based diet for 100 to 120 d prior to harvest. In this system, approximately 900 kg of grain/steer is required in the feedlot (Scheffler et al., Personnel communication). However, when steers were fed grain from weaning at 215 d through harvest, consumption increased to 1758 kg of grain/steer. Grain consumption was further increased up to 1984 kg of grain/steer with early weaning (90 d) (Myers et al., 1999c).

Hanley et al. (2010) defined 'metabolic imprinting' as the concept that changes in nutrition and growth at early life development affect later physiological events. Barker (1995) showed that prepubertal metabolic imprinting affected hormone profiles, food intake, energy metabolism and adipocyte development in humans and rodents later in life. There is little research reported for metabolic imprinting in beef production. Gotoh et al. (2010) showed that Japanese black cattle had higher intramuscular fat (**IMF**), when they were fed high energy diets during the early growth phase while finishing them on grass. Scheffler et al. (Personnel communication) further tested this phenomenon. They demonstrated that calves weaned early and fed a high energy diet in the early growing period from 100 to 250 d of age followed by a low input feeding period (grazing) before feedlot entry had similar yield grades and higher marbling scores than conventionally weaned calves. Therefore, feeding calves a high energy diet in the early growing period and transfer later to a low input feeding period (grazing) before feedlot entry influenced IMF formation and avoided the early-maturing cattle associated with

continuous grain feeding. Further, Scheffler et al. (Personnel communication) suggested that early grain feeding could create metabolic imprinting partly by enhancing proliferation of preadipocytes and thereby establishing a large population of undifferentiated cells maintained during the forage phase and readily matured during the feedlot phase.

Metabolic imprinting may also be associated with modulation of nutrient partitioning and utilization efficiencies. In ruminants, acetate and glucose are the major precursors used in biosynthesis of fatty acids such as palmitate which is subsequently used in triglyceride synthesis (Smith and Crouse, 1984) . Therefore, investigations on blood acetate and glucose turnover rates, and palmitate fractional synthesis rates in different adipose tissues could widen our understanding about the proposed metabolic imprinting phenomenon.

Smith and Crouse (1984) demonstrated that intramuscular adipocytes prefer glucose as a substrate for fat synthesis while subcutaneous adipocytes prefer acetate when assessed using an *in vitro* system. The ratio of fatty acids synthesized from acetate to those from glucose was 0.3 in IMF but 33 in SCF. In contrast, Lee et al. (2000) observed incorporation ratios for acetate and glucose of 1.61 and 1.23 for subcutaneous and intramuscular adipose tissues, respectively using a similar culture system, and Song et al. (2001) observed 1.25 and 1.27, respectively. Schoonmaker et al. (2004) studied the effects of feeding diets differing in starch and fiber concentrations with equal energy for growing steers, but they did not detect significant differences in fat deposition between subcutaneous fat (**SCF**) and IMF depots. It is unclear whether the observed inconsistency across *in vitro* studies is due to breed effects, but the lack of concordance between *in vitro* and *in vivo* results casts doubt on the validity of the *in vitro* approach. Thus it is important to examine this question *in vivo* to determine whether the *in vitro* results apply for breeds used in North America and under prevailing management practices.

The beef industry also desires to produce lean tissue as efficiently as possible. High grain feeding leads to the absorption of more glucose precursors (Huntington et al., 1980, 1981) which can regulate muscle protein synthesis by signaling through protein kinase B (**Akt**) and AMP-activated protein kinase (**AMPK**) (Bolster et al., 2002). The AMP-activated protein kinase pathway is known to be an efficient sensor of cellular energy status. When cells are in an energy deficient state, the activity of AMPK is increased. Activated AMPK in turn triggers catabolic pathways which produce ATP and turns off anabolic pathways which consume ATP (Hardie et al., 2006). Protein synthesis is energetically costly, and is regulated by amino acids and insulin via the phosphorylation of mammalian target of rapamycin (**mTOR**). If cellular energy status is low, AMPK inhibits mTOR signaling by phosphorylating tuberous sclerosis 2 (**TSC2**) (Drummond et al., 2009; Hardie et al., 2006).

Since acetate serves as the major energy source for post-weaning growth of calves, the rate of acetate clearance may be indicative of their growth capacity. Further, supplementation of energy substrates such as glucose or acetate can enhance protein synthesis by regulating AMPK/mTOR signaling. Therefore, understanding AMPK/mTOR signaling with different feeding strategies may explain the effects of acetate and glucose metabolism on muscle tissue.

We hypothesized that signaling protein phosphorylation and acetate clearance rates would be affected by a period of high-grain feeding after early weaning as compared to normal weaning. Further, we hypothesized that carry over effects of early grain feeding may affect acetate and glucose turnover rates and hence fat synthesis but differential preference for acetate and glucose may not exist in different depots SCF, IMF and visceral fat (**VF**). If periods of high energy feeding early in life alter the response to nutrients later in life, there may be potential for improving feed efficiency and grain usage by selective timing of high nutrient feeding periods.

Moreover, an understanding of the metabolic fuel preferences of adipocytes in subcutaneous and intramuscular depots is critical to designing feeding programs to meet desired adipocyte stores while minimizing starch and grain use. Therefore, the objectives of this dissertation were to determine the effects of early weaning followed by a period of high-grain feeding on:

- signaling protein phosphorylation in muscle,
- acetate clearance in growing steers,
- acetate and glucose turnover rates, and palmitate synthesis rates in finishing steers,
- acetate and glucose preference by SCF, IMF and VF in finishing steers.

CHAPTER 2

Literature Review

Metabolic imprinting

In general metabolic imprinting refers to "early life events which impact later physiological outcomes via genomic level control" (Hanley et al., 2010). Barker (1992) showed that fetal growth later affecting adult diseases. These observations are supported by epidemiological studies that observed a relationship between early diets and subsequent health events. Further, prepubertal metabolic imprinting affect changes in hormone profiles, food intake, energy metabolism and adipocyte development in humans and rodents later in life (Barker, 1995).

In the beef industry, different feed management systems have been studied with the objective of producing high quality meat and improving the yield while minimizing cost of production. These strategies include adjustments in nutrition and growth at specific development points. Alterations in nutrition may change the supply of nutrients, absorption and metabolism. It may also affect growth and development of adipose and lean tissues. After weaning, during the early growing phase, calves can be raised under different feeding management systems to test effects on carcass yield and quality, thereby also testing the concept of metabolic imprinting. Gotoh et al. (2010) showed that feeding calves high energy diets during the early growth phase while finishing them on grass influenced carcass characteristics and IMF content in Japanese black cattle (metabolic imprinting). Hence, changes in dietary nutrients early in the life can affect subsequent deposition of nutrients in muscle and adipose tissues.

Acetate and glucose production in ruminants

Ruminant diets mainly consist of carbohydrates which are generally composed of structural (cellulose and hemicelluloses), and non-structural carbohydrates (starch and sugars). The main end products of carbohydrate digestion in ruminants are VFAs which provide 70% of the energy supply to the animal. Acetate, propionate and butyrate are the major VFAs produced in the rumen but the ratio varies from 75:15:10 to 40:40:20 (Bergman, 1990). For an average forage-based diet, the proportions of VFAs produced in the rumen are 65 - 70% acetate, 15 - 25% propionate and 5 - 10% butyrate. Feeding high starch diets increases the proportion of propionate and reduces the proportions of the other VFAs (Fluharty, 2003). Sutton et al. (2003) reported that net energy supplied by of acetate, propionate and butyrate production in rumen were 49.5, 25.8 and 14.2 MJ/d with normal diets (concentrate:hay = 60:40), respectively but 42.9, 55.6 and 10.6 MJ/d for low roughage diets (concentrate:hay = 90:10).

Absorption of VFAs across the rumen wall is rapid. About 90% of butyrate is metabolized by the ruminal epithelium and converted to ketone bodies or CO_2. The remaining butyrate and most of the acetate and propionate is transported to the liver (Bergman, 1990). Almost all of the propionate and butyrate are removed by the liver and insignificant amounts appear in peripheral circulation. Therefore, more than 95% of the VFAs present in the peripheral circulation are acetate. Most of the propionate taken up from the portal blood by the liver is used to synthesize glucose. Propionate is the most important carbon source for gluconeogenesis in ruminants (Bergman, 1990). However, feeding ruminants high starch diets also increases direct glucose absorption from the gut (Huntington, 1997). In ruminants, acetate and glucose are the major substrates utilized as precursors for lipogenesis in adipose tissues, and availability of these

precursors regulates protein metabolism directly or indirectly through hormones in skeletal muscle (Hocquette et al., 2001).

Adipose tissue growth and development

Adipose tissue can be defined loosely as connective tissue containing mainly adipocytes (Ahima, 2006). The remaining cells are blood cells, endothelial cells, pericytes, macrophages, fibroblasts, and adipocyte precursor cells. Adipocytes are also called fat cells or lipocytes. These are specialized for the storage of energy in the form of fat (Ahima, 2006; Ailhaud et al., 1992; Kershaw and Flier, 2004). Adipocytes are derived from multi-potential stem cells. These stem cells form adipoblasts. Adipoblast formation leads to formation of pre-adipocytes which subsequently differentiate into adipocytes. Hence, pre-adipocyte division increases the number of adipose cells. Adipose tissue mass can expand by formation of new adipocytes from precursor cells and cell enlargement through lipid filling.

There are two types of adipose tissues: white adipose tissue and brown adipose tissue (Laplante and Sabatini, 2009). Brown adipose tissue is present pre-natally but generally disappears after birth. White adipose tissue development occurs after birth and continuous throughout life making it the predominate form (Ailhaud et al., 1992). Postnatal adipose tissue growth consists of 3 phases: initially hyperplastic, followed by a mix of hyperplastic and hypertrophic growth, and finally hypertrophic growth (Beitz, 1985). Adipocytes are found primarily in four major depots: IMF, SCF, VF, and intermuscular fat.

Intramuscular fat results from accumulation of triglycerides primarily within adipocytes located between bundles of muscle fibers in muscle tissue (Pethick et al., 2004). This fat is also

known as marbling. It is used as an indicator of beef quality, and amount and distribution of marbling in the ribeye muscle (between 12th and 13th ribs) cross section is evaluated as a determinant of quality grade (USDA, 1997) . Since marbling enhances tenderness, juiciness, flavor and overall palatability of beef, consumers are willing to pay a premium for additional marbling (Feuz et al., 2004). Therefore, higher IMF deposition increases market value of beef.

Several developmental studies concluded that IMF is a late developing adipose tissue which is following the generally accepted development order of fat: abdominal, intermuscular, SCF and IMF (Vernon, 1980). May et al. (1994) also showed that IMF is late developing compared to SCF. Hood and Allen (1973) showed that IMF continues expansion as the animal ages reaching maximum development during adult life in ruminants. However, decreased rate of lean deposition in older animals contributes to the relative amount of fat when it is expressed as a percent of carcass weight. Thus the apparent increase in the proportion of IMF late in maturity is not totally a function of IMF accretion leading Pethick et al. (2004) to suggest that IMF accumulation is not late maturing. Bruns et al. (2004) studied the relationship among body weight, body composition and IMF content in steers using Angus steers harvested at five different targeted hot carcass weight (**HCW**; 204, 250, 295, 340 and 386 kg). The data showed that longissimus muscle area, marbling, scores and IMF at the 12th rib were linearly increased between 200 – 400 kg HCW in steers fed a high grain diet. However, the marbling response was not parallel to the response for total fatness. Their data showed that early in the growth curve, quality grade increased more rapidly than yield grade whereas, later in the growth curve, yield grade increased more rapidly than quality grade. Therefore, they suggested that it is possible to manipulate final quality grade with early growth management.

Duckett et al. (1993) studied the effect of time on feed on beef nutrient composition using Angus x Hereford steers. They fed a high concentrate diet for 196 d, and serially harvested in 28 d intervals from 0 to 196-d, where 0-d group served as grass fed control. They showed that the most significant increase in IMF deposition occurred between 84 - 112 d of feeding period. Therefore, they suggested that initial feeding level is a key factor which determines the final level of IMF. Further, Pethick et al. (2004) noted that the starting weight relative to mature weight, genetic propensity to marble, mature body size, pre-feedlot growth rate, and pattern of growth were factors affecting the initial level of IMF.

Subcutaneous fat is laid down under the skin. Yield grade is determined by SCF (back fat) measured at the 12^{th} rib (Owens and Gardner, 2000) which reflects total carcass SCF. Normally, excess SCF is trimmed thus it reduces profit margin. It also reduces production efficiencies because feed consumed to synthesize SCF does not contribute to final carcass yield. Further, human health concerns lead to consumer demand for lean beef (Vernon, 1980).

Hood and Allen (1973) reported that subcutaneous adipocyte proliferation is completed around 8 m of age in cattle. Therefore, further increases in adipose tissue mass occur by cell enlargement. Compared to the IMF, SCF as a proportion of mature composition was approximately 60% at 11 to 19 m age steers. The number of adipocytes per gram of subcutaneous adipose tissue was decreased with the age during this period and diameter of adipocytes increased after 13 m of age (Cianzio et al., 1985).

Lipogenesis

Lipogenesis is the process of fatty acid synthesis and subsequent synthesis of triglycerides. Mainly, it occurs in adipose tissue and liver. Studies conducted by Ingle et al. (1972) with a single injection of labeled acetate and Broad and Ulyatt (1980) with continuous infusions of labeled acetate demonstrated that adipose tissue was the most important site for lipogenesis in ruminants. One glycerol molecule and 3 fatty acid molecules (as fatty acyl CoA derivatives) are the precursors of triglycerides (Medeiros and Wildman, 2012). Fatty acids can be supplied by either gut absorption or *de novo* synthesis from the other metabolites such as acetate, glucose and lactate (Bauman, 1976). Acetate is converted to acetyl CoA in the cytoplasm. Glucose enters fatty acid synthesis via glycolysis which produces pyruvate and is further transformed into acetyl CoA. Lactate follows the same pathway as glucose. Acetyl CoA is converted into malonyl CoA under the catalytic action of acetyl CoA carboxylase. Fatty acid synthase catalyzes biosynthesis of fatty acids (such as palmitate, myristic acid, stearic acid) in the cytosol (Laliotis et al., 2010). Glycerol either derived from partial breakdown of glucose or gluconeogenesis: formation of glycerol 3-phosphate from precursors such as lactate, amino acid carbon skeletons. Further, lipogenesis requires reducing power from NADPH derived from oxidation of isocitrate and malate in the cytosol, and glucose in the pentose phosphate cycle (Smith, 1983). Therefore, cytosolic NADP malate dehydrogenase, cytosolic NADP isocitrate dehydrogenase, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are the major enzymes responsible for the NADPH production in ruminants (Laliotis et al., 2010).

Substrate utilization by adipose tissues

Acetate and glucose are the major substrates utilized as precursors for lipogenesis in ruminant adipose (Smith and Crouse, 1984). Prior and Jacobson (1979) reported that lactate is also utilized in lipogenesis. Many studies have been conducted on substrate utilization by different adipose tissues (Ballard et al., 1972 ; Lee et al., 2000; Prior and Scott, 1980; Smith and Crouse, 1984; Song et al., 2001; Yang and Baldwin, 1973).

Ballard et al. (1972) studied the effects of carbohydrate availability on lipogenesis in sheep by infusing glucose into the abomasum, direct intravenous glucose infusion, or feeding 70% soluble carbohydrates to sheep. They observed greater incorporation of glucose into glycerol of triglyceride in adipose tissue slices of carbohydrate fed and abomasal glucose infused animals compared to the control treatment. Abomasal and intravenous infusions of glucose increased glucose incorporation into fatty acids in adipose tissues. Lipogenesis from acetate was increased for all high carbohydrate treatments with addition of glucose and insulin to the incubation medium. Activities of ATP citrate lyase and NADP-malate dehydragenase in adipose tissue also increased in carbohydrate treated and intravenous glucose infused groups (Ballard et al., 1972).

Later, Yang and Baldwin (1973) reported that glucose is an important stimulant in incorporation of acetate into fatty acids in ruminant adipose tissues. When they incubated fat cells from adipose tissues in media containing acetate with different concentrations of glucose with or without insulin, they observed lower acetate oxidation and lipogenesis with the absence of glucose in the media. Further, when subcutaneous adipose tissues were incubated with acetate in the absence and presence of glucose, lactate and insulin, the incorporation was significantly increased in the presence of glucose and lactate in the media (Smith, 1984). They showed that

incubation of these adipose tissues with glucose increased the glycolytic intermediates: glucose-6-phosphate and fructose-6-phosphate.

Smith (1983) showed that glucose is required as a precursor for glycerol and a source of NADPH required for fatty acid synthesis in ruminant adipose tissue. Data from his *in vitro* study indicated that without other exogenous substrates in the media, 12% of total glucose was metabolized in the pentose cycle and it was increased up to 22 - 37% when acetate and lactate was included in the media. With glucose in the media, 30% of NADPH was derived from the pentose cycle, and this increased to 72% when acetate and lactate were added to the media. The presence of glucose in the media stimulated acetate incorporation into fatty acids by 90% in the adipose tissue slices (Smith, 1983).

Prior and Scott (1980) showed that *in vitro* rates of lactate and glucose incorporation into fatty acids in subcutaneous adipose tissue were increased by *in vivo* glucose, lactate and propionate infusions. Therefore, they suggested that feeding steers with high concentrate diets increase the availability of glucose or glucogenic precursors which may induce lipogenesis. A study conducted by Smith et al. (1984) showed that feeding high concentrate diets increased lipogenic enzyme activities in subcutaneous adipose tissue compared to steers fed forage based diets. When the tissues samples were incubated with different substrates and they did not see any difference in ^{14}C-labeled acetate or lactate incorporation in to the fatty acids.

Smith and Crouse (1984) observed that acetate and glucose utilization by the intramuscular and subcutaneous adipose tissues were different. They showed that intramuscular adipocytes prefer glucose as a substrate for fat synthesis while subcutaneous adipocytes prefer acetate. Acetate contributed 70 to 80% of acetyl units for *in vitro* lipogenesis in subcutaneous

adipose tissue whereas, it was only 10 to 25% in intramuscular adipose tissue. On the other hand, glucose provides 50 to 75% of acetyl units for *in vitro* lipogenesis in the intramuscular adipose tissue but, it was only 1 to 10% in subcutaneous adipose tissue. The observed ratio for fatty acid synthesis from acetate:glucose was 0.28 in IMF but 32.95 in SCF (Smith and Crouse, 1984). Therefore, they suggested that *de novo* fatty acid synthesis in intramuscular and subcutaneous adipose tissue is controlled by different regulatory mechanisms. Song et al. (2001) also examined lipogenic substrate utilization in different adipose tissues (*in vitro*) from Hanwoo steers. They reported that the ratio of acetate:glucose use for lipogenesis in SCF vs. IMF averaged 1.25 and 1.27 respectively. Ratios for incorporation of acetate and glucose into adipose tissue of Hanwoo steers were also studied by Lee et al. (2000). They reported acetate:glucose incorporation ratios for SCF and IMF of 1.61 and 1.23, respectively.

Forage based diets are lower in the proportion of propionate to acetate compared to grain based diets (Bergman, 1990). This may decrease the glucose supply compared to acetate supply and therefore decrease the rate of lipogenesis and rates of acetate clearance (Cronjé et al., 1991). Therefore, Cronjé et al. (1991) studied the interaction between acetate and glucose metabolism in sheep fed a basal diet, or the basal diet supplemented with acetate or acetate and propionate. They showed that acetate clearance rate was increased with supplementation of propionate. When they continued the experiment with different propionate to acetate ratios, they found that acetate clearance was increased when the propionate:acetate ratio increased. Therefore, they concluded that metabolism of excess acetate is responsive to the dietary supply of glucose precursor. However, Chakeredza et al. (2002) showed that plasma acetate concentrations did not significantly differ with diet in weaned lambs fed maize stover supplemented with cowpea hay,

groundnut hay, cotton seed meal or maize meal. Further, they observed similar acetate clearance rates after acetate infusion across the diets.

Greathead et al. (2001) conducted an *in vivo* study to measure the rate of lipogenesis in ruminants. They infused labeled acetate continuously for 6 h and showed that isotopic equilibrium for labeled acetate in plasma was reached within 45 min for both sheep and steers. The rate of labeled acetate incorporated into adipose tissue lipids was constant. They pointed out that *in vitro* incubation of adipose tissues tends to be in a net negative state and may underestimate the rates of lipogenesis. However, the method described Greathead et al. (2001) allows dynamic measurements of the rates of lipogenesis. Dunshea et al. (1992) also used this method to measure rates of lipogenesis in pigs.

Skeletal muscle growth and development

Skeletal muscle makes up 35 – 65% of the carcass weight of an animal and, is of course the key for meat production. One of the primary goals in the beef industry is to increase muscle growth. Muscle tissue growth is accomplished by cell proliferation and protein accretion. Cell proliferation occurs in both prenatal and postnatal phases. Proliferation of myogenic precursor cells and their differentiation form mature muscle cells. Proliferation of satellite cells (postnatal myogenic cells) also form mature muscle cells. Protein accretion is the net result of protein synthesis and protein degradation. Protein accretion can be increased by increasing protein synthesis and decreasing protein degradation (Allen et al., 1979). Protein synthesis mainly depends on amino acid supply, and it is an energy costly process. Protein synthesis is also

regulated by energy yielding substrates which act directly or indirectly through hormones (Hocquette et al., 2001).

Nutrients, hormones, growth factors, cytokines, and mechanical loading are major factors which regulate the growth, development and functions of skeletal muscle. All of these factors regulate at the level of cell signaling (Miyazaki and Esser, 2009). AMP-kinase, mTOR and protein kinase B (**Akt**) are important signaling proteins in skeletal muscle.

AMPK signaling pathway

AMP-activated protein kinase is a heterotimeric protein kinase composed of a catalytic subunit (α) and two regulatory subunits (β and γ). In the presence of AMP, the activity of AMPK is increased by regulating the level of phosphorylation: when AMP concentration is increased, it binds to the γ subunit which makes the conformational changes in γ subunit and as a result, phosphorylation of α subunit occurs at the threonine 172 residue. AMP-activated protein kinase is thus a sensor of cellular energy status and controls metabolism to balance the energy supply and demand. It activates under conditions of energy stress when intracellular ATP:AMP ratio decreases (Hardie et al., 2003). AMP-activated protein kinase monitors intracellular energy status and regulates fatty acid, glucose, cholesterol, glucogen and protein metabolism to maintain cellular energy status. When cells are in energy deficient states the activity of AMPK increases by phosphorylation. This triggers catabolic pathways which produce ATP and turned off anabolic pathways which consume ATP (Hardie et al., 2006). Hence, activation of AMPK inhibits lipogenesis and enhances lipid oxidation (Carey et al., 2006; Kim et al., 2011; Yoon et al., 2006).

Once AMPK is activated, it phosphorylates acetyl CoA carboxylase (**ACC**) (Carey et al., 2006; Kim et al., 2011; Yoon et al., 2006). Ingle et al. (1973) reported that ACC is a key enzyme in fatty acid synthesis. The ACC α-form catalyzes the carboxylation of acetyl-CoA to produce malonyl-CoA whereas the ACC β-form acts on malonyl-CoA to regulate carnitine palmitoyl transferase 1 (**CPT1**) and control β-oxidation. Phosphorylation of ACC results in inhibition of activity of ACC (Carlson and Kim, 1973). As a result, it decreases the formation of malonyl-CoA and synthesis of fatty acids. Finally, it decreases lipogenesis. Malonyl-CoA is an inhibitor of CPT1. Therefore, decreased malonyl-CoA concentration in cytoplasm activates CPT1. This leads to increased uptake of fatty acids into mitochondria and increases fatty acid oxidation which generates more ATP (Hardie and Sakamoto, 2006).

AMPK is active under nutrient poor conditions which follows an inverse pattern to mTOR (Wullschleger et al., 2006). Therefore, the energy state of the cell signals to mTOR through AMPK. Activated AMPK under low ATP levels or energy depleted conditions, decreases the mTOR activity by phosphorylating mTORC1 component regulatory associated protein (**Raptor**) (Gwinn et al., 2008). Moreover, Inoki et al. (2003) showed that under energy depletion, mTOR activity can be decreased by activated AMPK by phosphorylating TSC2 at Thr1227 and Ser1345, which increases the GTPase activated protein (**GAP**) activity (Finlay and Cantrell, 2011).

Kuhla et al. (2009) showed that restricted feeding increased the phosphorylation of AMPK compared to the *ad libitum* feeding. At the same time, glucose, glucogen, and protein contents in liver (as a percent in dry matter) also decreased in the restricted fed group (Kuhla et al., 2009). Moreover, McFadden and Corl (2009) reported that activated AMPK significantly decreased the *de novo* fatty acid synthesis in MAC-T cells by inactivating ACC. Further, they

noted that activated AMPK decreases the $NaHCO_3$ incorporation in to malonyl-CoA and the gene expression of fatty acid synthase.

Underwood et al. (2008) studied the association of AMPK with IMF deposition using steers with high and low IMF contents. The data showed that AMPK and ACC phosphorylation in high IMF steers were lower compared to low IMF steers. These revealed that phosphorylation of ACC is positively correlated with AMPK activity.

mTOR signaling pathway

Mammalian target of rapamycin is a serine/threonine protein kinase and mTOR pathway regulates a wide range of functions such as protein synthesis and transcription, cell growth, cell proliferation and cell survival. Mammalian target of rapamycin is responsive to growth factors, energy status, oxygen and amino acids and regulates the above processes (Laplante and Sabatini, 2009). Wullschleger et al. (2006) stressed that mTOR is highly sensitive to nutrients and it is active under nutrient rich conditions. Mammalian target of rapamycin regulates mRNA translation by phosphorylation of S6 kinase 1 (**S6K1**) and eukaryotic initiation factor 4E binding protein 1 (**4E-BP1**) (Suryawan et al., 2006). Activated S6K1 phosphorylates ribosomal protein S6 (rpS6) which, increases protein synthesis by increasing the translation of mRNA (Kimball and Jefferson, 2004). Further, S6K1 reduces phosphorylation of eucaryotic elongation factor 2 (eEF2) which facilitates the elongation of peptide chains (Browne and Proud, 2002).

Akt signaling pathway

Plasma glucose concentration is regulated by insulin (Saltiel and Kahn, 2001). Increased plasma glucose concentrations enhance insulin release. Moreover, propionate is a potent stimulant of insulin release in ruminants (Sano et al., 1993). Binding of insulin to its receptor induces receptor dimerization and autophosphorylation at a specific tyrosine receptor residue. Phosphorylated insulin receptors activate phosphatidylinositol-3-kinase (**PI3K**) through phosphorylation of adaptor proteins such as insulin receptor substrate 1 (IRS1) (Fujita et al., 2007). Phosphorylated PI3K activates Akt by catalyzing phosphorylation at residues Thr308 or Thr473. Akt phosphorylates other proteins such as TSC2 at Ser1462 which result in dissociation of TSC1 and TSC2 complex. This prevents stimulation of GAP activity which leads to activation of mTOR (Finlay and Cantrell, 2011). Hence, mTOR and Akt are downstream signaling pathways of insulin signaling (Latres et al., 2005).

Jakobsen et al. (2001) also noted that AMPK can regulate insulin and insulin like growth factor-1 (IGF1) signaling which plays a major role in lean growth. Therefore, AMPK may regulate lean growth. When Underwood et al. (2008) studied the association of AMPK with muscle growth using steers with high and low IMF contents, phosphorylation of Akt tended to increase in high IMF steers. However, they did not see any differences in phosphorylation status in mTOR, rpS6 or 4E-BP1.

Feeding strategies

In the USA, beef calves are traditionally weaned around 205 days of age. Attempts at early weaning have led to mixed results in production efficiency. Many studies have been done

to evaluate different production systems by placing the early weaned calves on different energy diets to evaluate their production efficiencies. The rumen has an inherent ability to develop; feeding calves only with milk decreases the rate of rumen development. Tamate et al. (1962) observed that in addition to milk, feeding hay and grain enhances rumen development in calves. Benschop and Cant (2009) also showed that rumen morphology and functionality change with age and diet in calves, and that glucose remained the major energy source until 8 weeks of age in Holstein calves. Therefore, calves are initially dependent upon glucose as the major energy source. However, at weaning, they must adapt to VFAs produced from ruminal fermentation as the major energy source.

Peak milk production in beef cows occurs between 60 - 80 d of lactation (NRC, 2000). Therefore, decreasing milk yield after 80 d would potentially limit energy intake and calf growth if energy supplements are not provided. Weaning calves early is one alternative management practice to overcome the situation. Placing early weaned calves immediately on the high grain diets positively influences growth performance. When Myers et al. (1999c) compared steers weaned at 90, 152 and 215 d of age placed in the feedlot thereafter, they observed a linear increase in ADG and feed efficiency with decreased weaning age. However, the number of total days in the feedlot increased linearly with decreasing weaning age when steers were harvested at constant fat end point. Further, total grain consumption increased with decreasing weaning age (90 d = 1984 kg of grain/steer, 152 d = 1826 kg of grain/steer and 215 d = 1758 kg of grain/steer).

Fluharty et al. (2000) also studied the effect of weaning age on steers: weaned early (100 d of age) fed high grain vs. normal weaned (at 205 d of age). Both groups were placed in the feedlot at 205 d of age. They observed that early weaned steers had greater ADG compared to

normal weaned steers and early weaned steers reached market weight earlier than normal weaned steers. Consumption of concentrates was approximately 1915 kg and 1620 kg of grain/steer for early weaned and normal weaned steers, respectively.

Myers et al. (1999a) compared the effects of three weaning management systems on steer performance with a 2 yr study: weaned at 177 d of age (year 1) or 158 d of age (year 2) and fed a finishing diet, weaned on 177 d of age (year 1) or 158 d of age (year 2) and placed on pasture with grain supplementation for 55 d and then placed on a finishing diet, or on pasture for 55 d while nursing their dams and then placed on a finishing diet. They showed that steers fed finishing diets after weaning gained 100% faster than the average of other two treatments. Further, they showed that steers supplemented with grain after weaning gained 32% faster than pasture fed steers before starting the finishing diet. Moreover, they observed that steers fed a finishing diet after weaning had better feed conversion efficiencies and marbling scores compared to the other two treatments, however, steers fed the finishing diet after weaning spent more time in the feedlot and consumed higher amounts of concentrates (2033 kg of grain/steer). Consumption of concentrates was similar between the concentrated supplement group (1853 kg of grain/steer) and pasture fed group (1728 kg of grain/steer).

Schoonmaker et al. (2003) studied the effect of source of energy on performances and carcass characteristics in early weaned steers. After early weaning (119 d) steers received grain *ad libitum*, restricted grain to achieve 0.8 kg/d, restricted grain to achieve 1.2 kg/d, or a high fiber diet *ad libitum*. All steers were placed in the feedlot from 218 d of age until harvest. Age at harvest and days in feedlot were greatest for restricted grain fed steers compared to *ad libitum* grain and fiber fed steers. However, the greatest ADG was observed for the *ad libitum* grain fed group whereas the lowest feed efficiency was observed for the *ad libitum* fiber fed group. They

showed that restricted grain feeding did not extend the growth curve or IMF deposition at harvest compared to the *ad libitum* feeding of grain or forage (Schoonmaker et al., 2003).

Schoonmaker et al. (2004) further studied growing steers fed different sources and amounts of energy. Groups of steers were weaned at 119 d of age and fed a 50% grain diet *ad libitum,* a 70 % grain diet restricted or haylage diet (60% haylage from 119 to 192 d of age followed by a 25% haylage from 193 to 259 d age) *ad libitum*. The other group of steers was weaned at 204 d of age and fed a silage diet until 259 d of age. All steers were fed a feedlot ration from d 260 to harvest. They showed that *ad libitum* feeding of a 50% grain diet accelerated early physiological maturity compared to other treatments at d 260. However, there was no difference in marbling scores among treatments. The approximate grain consumptions were 2286, 2645, 2397 and 1751 kg of grain/steer for the 50% grain diet, 70% grain diet, haylage diet and normal weaned steers, respectively (Schoonmaker et al., 2004).

Moriel et al. (2012) compared normal weaning (control) and three early weaned (72 d of age) treatments: early weaning followed by grazing pasture until feedlot entry at d 260 of age, early weaning followed by feeding concentrates until feedlot entry and early weaning followed by concentrate feeding for 94 d and then grazed until feedlot entry (metabolically imprinted). They showed that at the day of feedlot entry, body weights were greater in concentrate fed steers compared to other treatments. Control and grass fed groups had similar body weights but were lighter than the metabolically imprinted group. Back fat thickness was similar among control, grass fed and metabolically imprinted groups but all 3 were lower than for the concentrate fed group. Area of LM was greatest in the concentrate fed group, least in the grass fed group and similar between control and metabolically imprinted groups.

Scheffler et al. (Personnel communication) studied the supply of high energy diet prior to a typical grazing period to manipulate IMF deposition in finishing cattle using normal weaned (weaned at 253 d of age) and metabolically imprinted (weaned at 105 d of age and fed high energy diet for 148 d) steers. Both groups were on pasture for 150 d from 253 d of age to feedlot entry and then fed a high grain diet. They showed that metabolically imprinted steers had greater ADG during the early grain feeding period and NW steers had a greater ADG during the grazing period. Though feedlot performance and yield grades were similar between the treatments, metabolically imprinted steers had higher marbling scores. Approximately 1004 kg of grain/steer was fed to the metabolically imprinted group during the early grain feeding period. Feedlot grain consumptions were 876 and 957 kg of grain/steer for NW and metabolically imprinted groups, respectively. Therefore, total grain consumption for the metabolically imprinted group was 1961 kg/steer.

Summary

In order to minimize grain use while improving carcass quality and yield, it is important to exploit the biological processes that can be configured by dietary nutrients. Alterations in nutrition may change supply of nutrients, absorption and metabolism. As a result, it may affect the growth and development of adipose and lean tissues. Changes in dietary nutrients early in life likely affect subsequent deposition of nutrients in muscle and adipose tissues.

Hence, chapter 2 summarizes the utilization of acetate and glucose by ruminants. First, it has been discussed that how acetate and glucose produce in ruminants since they are major precursors in lipogenesis and regulators in protein synthesis. Second, what has already being known about formation of adipose tissues and how these tissues use acetate and glucose for their

development have also been reviewed. Other than energy supply, acetate and glucose are used in regulating protein synthesis in skeletal muscle. Therefore, skeletal muscle development and cell signaling pathways regulated by acetate and glucose have also been covered. Considering the above information on metabolism, different feeding strategies have been tried in the recent past and their effects on carcass yield and quality have been discussed.

References

Ahima, R. S. 2006. Adipose tissue as an endocrine organ. Obesity 14: 242S-249S.

Ailhaud, G., P. Grimaldi, and R. Négrel. 1992. Cellular and molecular aspects of adipose tissue development. Annu. Rev. Nutr. 12: 207-233.

Allen, R. E., R. A. Merkel, and R. B. Young. 1979. Cellular aspect of muscle growth: Myogenic cell proliferation. J. Anim. Sci. 49: 115-127.

Arthington, J. D., and R. S. Kalmbacher. 2003. Effect of early weaning on the performance of three-year-old, first-calf beef heifers and calves reared in the subtropics. J. Anim. Sci. 81: 1136-1141.

Ballard, F. J., O. H. Filsell, and I. G. Jarrett. 1972 Effects of carbohydrate availability on lipogenesis in sheep. Biochem. J. 126: 193–200.

Barker, D. J. P. 1992. The effect of nutrition of the fetus and neonate on cardiovascular disease in adult life. Proc. Nutr. Soc. 51: 135-144.

Barker, D. J. P. 1995. The fetal and infant origins of disease. Eur. J. Clin. Invest. 25: 457-463.

Bauman, D. E. 1976. Intermediary metabolism of adipose tissue. Fed. Proc. 35: 2308-2313.

Beitz, D. C. 1985. Physiological and metabolic systems important to animal growth: An overview. J. Anim. Sci. 61: 1-20.

Benschop, D. L., and J. P. Cant. 2009. Developmental changes in clearance of intravenous doses of glucose, acetate and [beta]-hydroxybutyrate from plasma of calves. Livest. Sci. 122: 177-185.

Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol. Rev. 70: 567-590.

Bolster, D. R., S. J. Crozier, S. R. Kimball, and L. S. Jefferson. 2002. Amp-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mtor) signaling. J. Biol. Chem. 277: 23977-23980.

Broad, T. E., and M. J. Ulyatt. 1980. The effect of level of food intake on the incorporation of acetate into lipids and its distribution among various tissues in sheep. Br. J. Nutr. 44: 71-79.

Browne, G. J., and C. G. Proud. 2002. Regulation of peptide-chain elongation in mammalian cells. Eur. J. Biochem. 269: 5360-5368.

Bruns, K. W., R. H. Pritchard, and D. L. Boggs. 2004. The relationships among body weight, body composition, and intramuscular fat content in steers. J. Anim. Sci. 82: 1315-1322.

Carey, A. L., G. R. Steinberg, S. L. Macaulay, W. G. Thomas, A. G. Holmes, G. Ramm, O. Prelovsek, C. Hohnen-Behrens, M. J. Watt, D. E. James, B. E. Kemp, B. K. Pedersen, and M. A. Febbraio. 2006. Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via amp-activated protein kinase. Diabetes 55: 2688-2697.

Carlson, C. A., and K.-H. Kim. 1973. Regulation of hepatic acetyl coenzyme a carboxylase by phosphorylation and dephosphorylation. J. Biol. Chem. 248: 378-380.

Chakeredza, S., U. ter Meulen, and L. R. Ndlovu. 2002. Effect of cowpea hay, groundnut hay, cotton seed meal and maize meal supplementation to maize stover on intake, digestibility, microbial protein supply and acetate kinetics in weaner lambs. Trop. Anim. Health Prod. 34: 49-64.

Cianzio, D., D. Topel, G. Whitehurst, D. Beitz, and H. Self. 1985. Adipose tissue growth and cellularity: Changes in bovine adipocyte size and number. J. Anim. Sci. 60: 970 - 976.

Cronjé, P. B., J. V. Nolan, and R. A. Leng. 1991. Acetate clearance rate as a potential index of the availability of glucogenic precursors in ruminants fed on roughage-based diets. Br. J. Nutr. 66: 301-312.

Doering, O., and C. Hurt. 2006. Competition for feed resources from other applications:Ethanol and bio-diesel. National Conference of State Legislatures, http://www.ncsl.org/programs/agri/EthanolBio.

Drummond, M. J., H. C. Dreyer, C. S. Fry, E. L. Glynn, and B. B. Rasmussen. 2009. Nutritional and contractile regulation of human skeletal muscle protein synthesis and mtorc1 signaling. J. Appl. Physiol. 106: 1374-1384.

Duckett, S. K., D. G. Wagner, L. D. Yates, H. G. Dolezal, and S. G. May. 1993. Effects of time on feed on beef nutrient composition. J. Anim. Sci. 71: 2079-2088.

Dunshea, F. R., D. M. Harris, D. E. Bauman, R. D. Boyd, and A. W. Bell. 1992. Effect of porcine somatotropin on in vivo glucose kinetics and lipogenesis in growing pigs. J. Anim. Sci. 70: 141-151.

Feuz, D. M., W. J. Umberger, C. R. Calkins, and B. M. Sitz. 2004. U.S. Consumers' willingness to pay for flavor and tenderness in steaks as determined with an experimental auction. J. Agr. Resour. Econ. 29: 501-516.

Finlay, D., and D. A. Cantrell. 2011. Metabolism, migration and memory in cytotoxic t cells. Nat. Rev. Immunol. 11: 109-117.

Fluharty, F. L. 2003. Interaction of management and diet on final meat characteristics on beef animal. Http://beef.Osu.Edu/library/mgtdiet.Html.

Fluharty, F. L., S. C. Loerch, T. B. Turner, S. J. Moeller, and G. D. Lowe. 2000. Effects of weaning age and diet on growth and carcass characteristics in steers. J. Anim. Sci. 78: 1759-1767.

Fujita, S., H. C. Dreyer, M. J. Drummond, E. L. Glynn, J. G. Cadenas, F. Yoshizawa, E. Volpi, and B. B. Rasmussen. 2007. Nutrient signalling in the regulation of human muscle protein synthesis. J. Physiol. 582: 813-823.

Gotoh, T., K. Etoh, K. Saitoh, K. Metoki, S. Kaneda, T. Abe, T. Etoh, Y. Shiotsuka, R. Fujino, K. Matsuda, H. Suzuki, H. Hasebe, F. Ebara, J. Wegner, and S. Tabata. 2010. Metabolic imprinting effect in beef production: Influence of nutrition manipulation during an early growth stage on carcass characteristics and intramuscular fat content of longissimus muscle in wagyu (japanese black) Eaap publication no. 127. p 669-670. Wageningen Academic Publishers, Wageningen.

Greathead, H. M. R., J. M. Dawson, N. D. Scollan, and P. J. Buttery. 2001. In vivo measurement of lipogenesis in ruminants using [1-c]acetate. Br. J. Nutr. 86: 37-44.

Gwinn, D. M., D. B. Shackelford, D. F. Egan, M. M. Mihaylova, A. Mery, D. S. Vasquez, B. E. Turk, and R. J. Shaw. 2008. Ampk phosphorylation of raptor mediates a metabolic checkpoint. Mol. Cell 30: 214-226.

Hanley, B., J. Dijane, M. Fewtrell, A. Grynberg, S. Hummel, C. Junien, B. Koletzko, S. Lewis, H. Renz, M. Symonds, M. Gros, L. Harthoorn, K. Mace, F. Samuels, and E. M. van Der Beek. 2010. Metabolic imprinting, programming and epigenetics – a review of present priorities and future opportunities. Br. J. Nutr. 104: S1-S25.

Hardie, D. G., S. A. Hawley, and J. W. Scott. 2006. Amp-activated protein kinase – development of the energy sensor concept. J. Physiol. 574: 7-15.

Hardie, D. G., and K. Sakamoto. 2006. Ampk: A key sensor of fuel and energy status in skeletal muscle. J. Physiol. 21: 48-60.

Hardie, D. G., J. W. Scott, D. A. Pan, and E. R. Hudson. 2003. Management of cellular energy by the amp-activated protein kinase system. FEBS Lett. 546: 113-120.

Hocquette, J. F., I. Ortigues-Marty, and M. Vermorel. 2001. Manipulation of tissue energy metabolism in meat producing animals. Asian-Aust. J. Anim. Sci. 14: 720-732.

Hood, R. L., and C. E. Allen. 1973. Cellularity of bovine adipose tissue. J. Lipid Res. 14: 605-610.

Huntington, G. B. 1997. Starch utilization by ruminants: From basics to the bunk. J. Anim. Sci. 75: 852-867.

Huntington, G. B., R. L. Prior, and R. A. Britton. 1980. Glucose and lactate absorption and metabolic interrelationships in lambs switched from low to high concentrate diets. J. Nutr. 110: 1904-1913.

Huntington, G. B., R. L. Prior, and R. A. Britton. 1981. Glucose and lactate absorption and metabolic interrelationships in steers changed from low to high concentrate diets. J. Nutr. 111: 1164-1172.

Ingle, D. L., D. E. Bauman, and U. S. Garrigus. 1972. Lipogenesis in the ruminant: In vivo site of fatty acid synthesis in sheep. J. Nutr. 102: 617-623.

Ingle, D. L., D. E. Bauman, R. W. Mellenberger, and D. E. Johnson. 1973. Lipogenesis in the ruminant: Effect of fasting and refeeding on fatty acid synthesis and enzymatic activity of sheep adipose tissue. J. Nutr. 103: 1479-1488.

Inoki, K., T. Zhu, and K.-L. Guan. 2003. Tsc2 mediates cellular energy response to control cell growth and survival. Cell 115: 577-590.

Jakobsen, S. N., D. G. Hardie, N. Morrice, and H. E. Tornqvist. 2001. 5′-amp-activated protein kinase phosphorylates irs-1 on ser-789 in mouse c2c12 myotubes in response to 5-aminoimidazole-4-carboxamide riboside. J. Biol. Chem. 276: 46912-46916.

Kershaw, E. E., and J. S. Flier. 2004. Adipose tissue as an endocrine organ. J. Clin. Endocrinol. Metab. 89: 2548-2556.

Kim, E., J.-H. Lee, J. M. Ntambi, and C.-K. Hyun. 2011. Inhibition of stearoyl-coa desaturase1 activates ampk and exhibits beneficial lipid metabolic effects in vitro. Eur. J. Pharmacol. 672: 38-44.

Kimball, S. R., and L. S. Jefferson. 2004. Molecular mechanisms through which amino acids mediate signaling through the mammalian target of rapamycin. Curr. Opin. Clin. Nutr. Metab. Care 7: 39-44.

Kuhla, B., D. Albrecht, S. Kuhla, and C. C. Metges. 2009. Proteome analysis of fatty liver in feed-deprived dairy cows reveals interaction of fuel sensing, calcium, fatty acid, and glycogen metabolism. Physiol. Genomics 37: 88-98.

Laliotis, G. P., I. Bizelis, and E. Rogdakis. 2010. Comparative approach of the de novo fatty acid synthesis (lipogenesis) between ruminant and non ruminant mammalian species: From biochemical level to the main regulatory lipogenic genes. Curr. Genomics 11: 168-183.

Laplante, M., and D. M. Sabatini. 2009. Mtor signaling at a glance. J. Cell Sci. 122: 3589-3594.

Latres, E., A. R. Amini, A. A. Amini, J. Griffiths, F. J. Martin, Y. Wei, H. C. Lin, G. D. Yancopoulos, and D. J. Glass. 2005. Insulin-like growth factor-1 (igf-1) inversely regulates atrophy-induced genes via the phosphatidylinositol 3-kinase/akt/mammalian target of rapamycin (pi3k/akt/mtor) pathway. J. Biol. Chem. 280: 2737-2744.

Lee, S. C., H. J. Lee, D. W. Kim, J. G. Park, and I. K. Han. 2000. Effects of carbon precursors and hormones on the lipogenesis and lipolysis of hanwoo cattle adipose tissues. Asian-Australasian J. Anim. Sci. 13: 300-306.

May, S. G., J. W. Savell, D. K. Lunt, J. J. Wilson, J. C. Laurenz, and S. B. Smith. 1994. Evidence for preadipocyte proliferation during culture of subcutaneous and intramuscular adipose tissues from angus and wagyu crossbred steers. J. Anim. Sci. 72: 3110-3117.

McFadden, J. W., and B. A. Corl. 2009. Activation of amp-activated protein kinase (ampk) inhibits fatty acid synthesis in bovine mammary epithelial cells. Biochem. Biophys. Res. Commun. 390: 388-393.

Medeiros, D. M., and R. E. C. Wildman. 2012. Advanced human nutrition. 2nd ed. Higher Education, Cathleen Sether.

Miyazaki, M., and K. A. Esser. 2009. Cellular mechanisms regulating protein synthesis and skeletal muscle hypertrophy in animals. J. Appl. Physiol. 106: 1367-1373.

Moriel, P., V. Mercadante, A. D. Aguiar, S. E. Johnson, M. J. Hersom, J. M. B. Vendramini, and J. D. Arthington. 2012. Effects of metabolic imprinting on growth performance of early-weaned beef steers. J. Anim. Sci. 90 (Suppl. 3): 193(Abstr.).

Myers, S., D. Faulkner, F. Ireland, L. Berger, and D. Parrett. 1999a. Production systems comparing early weaning to normal weaning with or without creep feeding for beef steers. J. Anim. Sci. 77: 300 - 310.

Myers, S., D. Faulkner, T. Nash, L. Berger, D. Parrett, and F. McKeith. 1999b. 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.

Myers, S. E., D. B. Faulkner, F. A. Ireland, and D. F. Parrett. 1999c. Comparison of three weaning ages on cow-calf performance and steer carcass traits. J. Anim. Sci. 77: 323-329.

NRC. 2000. Nutrient requirements of beef cattle, 7th ed. Nati. Acad. Press, Washington, D.C. .

Owens, F. N., and B. A. Gardner. 2000. A review of the impact of feedlot management and nutrition on carcass measurements of feedlot cattle. J. Anim. Sci. 77: 1-18.

Pethick, D. W., G. S. Harper, and V. H. Oddy. 2004. Growth, development and nutritional manipulation of marbling in cattle: A review. Aust. J. Exp. Agric. 44: 705-715.

Prior, R. L., and J. J. Jacobson. 1979. Lactate, pyruvate and acetate interactions during in vitro lipogenesis in bovine adipose tissue. J. Anim. Sci. 49: 1410-1416.

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.

Saltiel, A. R., and C. R. Kahn. 2001. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414: 799-806.

Sano, H., N. Hattori, Y. Todome, J. Tsuruoka, H. Takahashi, and Y. Terashima. 1993. Plasma insulin and glucagon responses to intravenous infusion of propionate and their autonomic control in sheep. J. Anim. Sci. 71: 3414-3422.

Scheffler, J. M., M. A. McCann, S. P. Greiner, H. Jiang, M. D. Hanigan, G. A. Bridges, S. L. Lake, and D. E. Gerrard. Personnel communication. Early metabolic imprinting events increase marbling scores in fed cattle.

Schoonmaker, J. P., M. J. Cecava, D. B. Faulkner, F. L. Fluharty, H. N. Zerby, and S. C. Loerch. 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., M. J. Cecava, F. L. Fluharty, H. N. Zerby, and S. C. Loerch. 2004. Effect of source and amount of energy and rate of growth in the growing phase on performance and carcass characteristics of early- and normal-weaned steers. J. Anim. Sci. 82: 273-282.

Smith, S., R. Prior, C. Ferrell, and H. Mersmann. 1984. Interrelationships among diet, age, fat deposition and lipid metabolism in growing steers. J. Nutr. 114: 153 - 162.

Smith, S. B. 1983. Contribution of the pentose cycle to lipogenesis in bovine adipose tissue. Arch. Biochem. Biophys. 221: 46-56.

Smith, S. B. 1984. Evidence that phosphofructokinase limits glucose utilization in bovine adipose tissue. J. Anim. Sci. 58: 1198-1204.

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.

Song, M. K., H. J. Sohn, S. K. Hong, and H. C. Kim. 2001. Utilization of substrate for in vitro lipid synthesis in adipose tissue of hanwoo steers. Asian-Aust. J. Anim. Sci. 14: 1560-1563.

Suryawan, A., J. Escobar, J. W. Frank, H. V. Nguyen, and T. A. Davis. 2006. Developmental regulation of the activation of signaling components leading to translation initiation in skeletal muscle of neonatal pigs. Am. J. Physiol. Endocrinol. Metab. 291: E849-E859.

Sutton, J. D., M. S. Dhanoa, S. V. Morant, J. France, D. J. Napper, and E. Schuller. 2003. Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. J. Dairy Sci. 86: 3620-3633.

Tamate, H., A. D. McGilliard, N. L. Jacobson, and R. Getty. 1962. Effect of various dietaries on the anatomical development of the stomach in the calf. J. Dairy Sci. 45: 408-420.

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: 394-402.

USDA. 1997. United states standards for grades of carcass beef, AMS, USDA, Washington, DC.

Vernon, R. G. 1980. Lipid metabolism in the adipose tissue of ruminant animals. Prog. Lipid Res. 19: 23-106.

Wertz, A., L. Berger, P. Walker, D. Faulkner, F. McKeith, and S. Rodriguez-Zas. 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.

Wullschleger, S., R. Loewith, and M. N. Hall. 2006. Tor signaling in growth and metabolism. Cell 124: 471-484.

Yang, Y. T., and R. L. Baldwin. 1973. Preparation and metabolism of isolated cells from bovine adipose tissue. J. Dairy Sci. 56: 350-365.

Yoon, M. J., G. Y. Lee, J.-J. Chung, Y. H. Ahn, S. H. Hong, and J. B. Kim. 2006. Adiponectin increases fatty acid oxidation in skeletal muscle cells by sequential activation of amp-activated protein kinase, p38 mitogen-activated protein kinase, and peroxisome proliferator–activated receptor α. Diabetes 55: 2562-2570.

CHAPTER 3

Effect of Early Grain Feeding of Steers on Postabsorptive Capacity to Utilize Acetate

Abstract

The objectives of this study were to determine the effects of early weaning followed by a period of high-grain feeding on signaling protein phosphorylation in muscle and on acetate clearance in growing steers. Twelve fall-born Angus X Simmental steers were either weaned at 106 ± 4 d of age (EW, n=6) and fed a high-grain diet for 148 d or remained with their dams (NW, n = 6) on pasture until weaning at 251± 5 d of age. Following weaning of the NW group, both treatments were combined and grazed mixed summer pasture from 253 ± 5 d to 394 ± 5 d of age and a feedlot ration until harvest at 513 ± 5 d of age. Longissimus muscle tissue biopsies were collected at 253 ± 5 and 394 ± 5 d of age and longissimus muscle tissue samples were obtained at harvest. Total and phosphorylated forms of AMP kinase (AMPK) and downstream proteins of the mTOR signaling pathway were determined by Western immunoblotting. Of the 12, 8 steers were used to assess acetate clearance at different age points. A bolus of acetate (4 mmol/kg of BW) was infused into a jugular vein and blood samples were collected before and after the bolus infusion. Plasma acetate, glucose, insulin and β-hyroxybutyrate (BHBA) concentrations were determined. Acetate clearance and appearance rate constants were determined using a one-pool model. Early weaned steers had greater ADG ($P <$ 0.05) during the early grain feeding period than did NW steers still on their dams during that time frame. Normal weaned steers had greater ADG during the subsequent grazing period ($P <$ 0.05). However, ADG during the feedlot period and overall ADG were not different between

treatment groups. There were no treatment differences in signaling protein phosphorylation ratios for any sampling times except ribosomal protein S6 and ribosomal protein S6 kinase 1. Phosphorylated:total ratios of S6K1, rpS6 and 4EBP1 were positively correlated with ADG. Acetate clearance rate constants were less ($P = 0.06$) and synthesis rate constants were greater ($P < 0.05$) in EW steers during early grain feeding. Acetate synthesis was greater ($P < 0.05$) in NW steers at harvest. Neither treatment nor acetate infusion significantly affects plasma glucose or insulin concentrations. Plasma BHBA concentrations increased with acetate infusion ($P < 0.05$). In conclusion, phosphorylation ratios of observed signaling proteins were not affected by treatment. Early grain feeding increased rates of acetate appearance and there was a trend to reduce acetate clearance during the early grain feeding period. Acetate clearance increased when steers were heavier and older. Neither treatment nor acetate infusion significantly affected plasma glucose or insulin concentrations. Plasma BHBA concentrations increased with jugular acetate infusion.

Key words: acetate, average daily gain, clearance, signaling proteins, steers, early weaning

Introduction

Rapid expansion of the bio-fuel industry has caused unprecedented changes in animal agriculture (Doering and Hurt, 2006). The resulting reduction in availability and increases in cost of feed have significantly challenged profitability of livestock production. Improving feed efficiency and reducing grain usage by selective timing of high nutrient feeding periods and use of by-products from the bio-fuel industry should reduce the cost of production. Beef calves are normally weaned around 205 days of age in the USA. Attempts at early weaning where steers were fed grain from weaning to harvest have had mixed results in production efficiency and fat

deposition (Fluharty et al., 2000; Schoonmaker et al., 2004). However, efficiency and carcass quality can be managed by growing calves on pasture following the early grain feeding before entering the feedlot (Scheffler et al., Personnel communication).

Calves are initially dependent upon glucose as the major energy source, and must adapt to volatile fatty acids produced from ruminal fermentation (Young et al., 1965). Acetate is the most important energy source for peripheral tissues of the ruminant (Ballard, 1972). However feeding high-starch diets increases the proportion of propionate (Fluharty, 2003) which leads to absorption of more glucose precursors (Huntington et al., 1980, 1981). Greater glucose supply improves cellular energy levels by decreasing the AMP:ATP ratio which decreases the activation of AMP-activated protein kinase (**AMPK**). This increases the lipogenesis by decreases phosphorylation of acetyl CoA carboxylase (**ACC**) (Carlson and Kim, 1973) which is a key enzyme in fatty acid synthesis (Ingle et al., 1973). The latter induces mammalian target of rapamycin (**mTOR**) signaling which regulates translation initiation and elongation of mRNA (Bolster et al., 2002). The objectives of this study were to determine the effects of early weaning followed by a period of high-grain feeding on signaling protein phosphorylation in muscle and on acetate clearance in growing steers.

Materials and Methods

Animals, diets and treatments

All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Virginia Tech. The experiment was conducted using 12 fall-born, Angus X Simmental cross bred-steers either weaned at 106 ± 4 d of age (139 ± 7 kg BW) in

December (**EW**; n = 6), and fed a high-grain diet (Table 3. 1) as a group for 148 d or remained with their dams (**NW**; n = 6) on pasture until weaning at 251 ± 5 d of age (271 ± 6 kg BW) in May. Groups were combined and grazed, predominantly on tall Fescue *(Festuca arundinacea)* from 253 ± 5 d to 394 ± 5 d of age. Thereafter, they were placed in the feedlot and fed corn silage plus a grain supplement which transitioned from 75% silage at the start to 25% silage at harvest (Table 3. 1). Body weights were recorded during different transitions and sampling times. Hot carcass weights (**HCW**), rib eye area (**REA**), 12th rib fat thickness (**BF**), kidney, pelvic and heart (**KPH**) fat percentage, and marbling scores were also recorded after harvest.

Tissue collection, sample preparation for Western blotting and analysis

Longissimus muscle (**LM**) tissue biopsies were collected on two occasions from all 12 steers, and LM tissues were collected at harvest (Figure 3. 1). For biopsies, the site between the 10th and 11th rib was shaved, cleaned with betadine followed by 70% ethanol, and lidocaine (10 mL) was administrated subcutaneously. Under local anesthesia, a small incision was made, muscle biopsies were collected using a biopsy needle (inner diameter = 6 mm), and the incision was sutured. Tissue samples (50 -100 mg) were processed for Western blot analysis immediately after taking the biopsies as described by Escobar et al. (2006). Briefly, tissue samples were mixed 1:7 (wt/vol) with lysis buffer containing protease and phosphatase inhibitors (1861281, Thermo Scientific, Rockford, IL) and homogenized for 60 s. Tissue lysates were centrifuged at 14,000 × g for 10 min at 4°C. The resulting supernatants were mixed with Laemmli-sodium dodecyl sulfate (SDS) sample buffer in a 1:1 ratio, boiled at 80°C for 10 min and stored at -80°C until immunoblot analysis.

Tissue lysates (approximately 20 μg of protein) were electrophoretically separated in polyacrylamide gels, transferred to Immobilon-FL membranes (IPFL00010, Millipore Corporation, Billerica, MA), blocked with blocking buffer (37542; Starting block, Thermo Scientific, Rockford, IL), and incubated with primary antibodies (from Cell Signaling Technology, Beverly, MA, unless otherwise indicated). Primary antibodies used recognized phosphorylated forms of protein kinase B (Akt, Ser473, sc-101629, Santa Cruz), eukaryotic initiation factor 4E binding protein 1 (4EBP1, Thr37/46, 9459), S6 kinase 1 (S6K1, Thr389, 9206), eucaryotic elongation factor 2 (eEF2, Thr56, 2331), ribosomal protein S6 (rpS6, Ser 235/236, 2211), AMPK (Thr172, 2531), and ACC β (Ser79, 3661) and total forms of Akt (2967), 4E-BP1 (sc-81149, Santa Cruz), S6K1 (9202), eEF2 (sc-166415, Santa Cruz), rpS6 (2317), AMPK (2532), and ACCβ (3662). All blots were also probed for α-Tubulin (2144) to correct for loading variation. Blots were visualized by incubating with secondary antiboidies bound to florescent dyes. Secondary antibodies used were IRDye 680 goat anti-mouse or IRDye 800 goat anti-rabbit (LI-COR Bio Science, Lincoln, NE). Spot density was quantified using an Odessey infrared imaging system (model 9120, LI-COR Biosciences, Lincoln, NE). In general, analyses for phospho and total proteins were done simultaneously. Where different species of primary antibodies were not available for the phospho and total forms, the visualized blots were initially probed for the phospho-protein, stripped (N552-IL; Gentle ReView buffer, aMReSCO, Solon, OH), and re-probed for the total form. Phospho and total densities were divided from average of NW treatment density of each gel to correct for the gel to gel variation and standardized the data to the NW treatment. Then Phospho and total densities were corrected for loading differences by dividing by the tubulin density. The corrected data were used to calculate the ratio of phosphorylated:total forms of each protein.

Infusions of acetate, blood sampling and sample analysis

Eight steers were randomly selected (4 steers from each treatment group) and given a bolus injection of acetate via an indwelling jugular catheter to assess acetate clearance rates at different stages of their growth cycle (Figure 3. 1). Acetate solutions were prepared on the day prior to each infusion. Saline bags (500 mL, 0.9%-NaCl , USP, Hospira Inc., Lake Forest, IL) were emptied and the required amount of sodium acetate (4 mmol acetate/kg of BW; Sigma Aldrich, St. Louis, MO) for each animal (Chakeredza et al., 2002; Cronjé et al., 1991) was dissolved in the decanted saline solution and adjusted to a pH of 7.0. The resulting acetate solution was sterile filtered (0.22 μm; Millipore Corporation, Billerica, MA) back into the original saline bag using a peristaltic pump (Harvard Apparatus Co. Inc., Millis, MA). Approximately 30 min prior to the start of the infusion, sterile polyurethane catheters (o.d. = 2.03 mm, i.d. = 1.02 mm, Braintree Scientific Inc., Braintree, MA) were inserted into a jugular vein under local anesthesia. The acetate solution was subsequently infused using clinical infusion pumps (Abbott laboratories, North Chicago, IL) and starting and ending infusion times and infusate weights were recorded for each animal.

Jugular blood samples (5 mL) were drawn using a heparinized syringe prior to infusion (-5 min), at 5 min intervals for the first 30 min post-infusion, and at 15 min intervals for the next 60 min post-infusion. After each blood sampling, catheters were flushed with 20 units/mL of heparin in sterile saline. Immediately after collection, blood samples were transferred to tubes containing 125 units of heparin, gently mixed by inversion, and placed on ice. Plasma was prepared by centrifugation at 1,200 x g for 10 min at 4 ^{0}C and stored at -80 ^{0}C until analysis of plasma acetate, glucose, insulin and β-hydroxylbutyric acid (**BHBA**) concentrations.

To determine the plasma acetate concentrations**,** 2-chloroethyl esters of acetate were prepared as described by Kristensen (2000) with minor modifications. Plasma (450 μL) and 100 μL of a 2.5 mM [1-^{13}C, $^{2}H_3$]sodium acetate standard solution (Cambridge isotope laboratories, Inc., Andover, MA) were gravimetrically transferred in to 100 x 16 mm, screw-capped glass tubes. A volume of 750 μL 2-chloroethanol (Acros Organics, Fair Lawn, NJ) and 750 μL acetonitrile (Fisher Scientific, Fair Lawn, NJ) were added, and the sample was vortexed and centrifuged at 2,550 x g for 30 min at 4°C. The 2-chloroethyl esters were extracted in 100 μL chloroform (Fisher Scientific, Fair Lawn, NJ). Eighty micro liters of the organic phase was transferred to an auto-sampler vial and 1μL was injected onto a gas chromatograph mass spectrometer (**GC-MS**; Thermo PolarisQ mass spec in tandem with a Thermo electron Focus GC, Thermo Scientific, Waltham, MA). The GC was operated in split mode (split ratio 80) with an injection temperature of 225°C. The compounds were separated on a 30 m x 0.25 mm i.d. (with a 0.25 μm film) Factor FOUR VF-170ms column (Varian Inc., Palo Alto, CA) at a constant helium flow of 1.2 mL/min. The column temperature was set at 75°C and ramped at 5°C/min to 135°C, then at 40°C/min to 225°C. The mass spectrometer was operated in selective ion monitoring mode with positive electron ionization. Molecular ions of mass to charge ratios of 43 and 47 were monitored to determine normal and enriched ion abundance, respectively. Peak detection and area under curve integration were conducted using the ICIS algorithm encoded in the XCalibur software (Ver. 1.4, Thermo Scientific, Waltham, MA). Peak area ratios of unlabeled (m+0) to labeled (m+4) ion intensities were calculated and plasma acetate concentrations were determined using MS data derived from use of a gravimetric standard curve prepared from unlabeled and labeled acetate.

Plasma glucose concentrations were determined using a YSI glucose analyzer according to the manufacturor's guidelines (model 2300 STAT Plus, YSI Inc., Yellow Springs, OH). Plasma insulin concentrations were determined for -5, 5, and 30 min post-infusion samples using the COAT-A-COUNT insulin radioimmunoassay kit (TKIN2, Siemens Healthcare diagnostics, Inc., Los Angeles, CA) according to the manufacturor's guidelines. All analyses were completed in a single assay and the intra-assay CV was 4%. Plasma BHBA concentrations were determined using a BHBA assay kit (Cayman, Ann Arbor, MI) according to the manufacturor's guidelines. The inter-assay CV was 7%.

Model development

The one pool model used to interpret acetate infusion data is presented in Figure 3. 2. The change in blood acetate with respect to time ($dQ_{Acetate\ (Blood)}/dt$) was represented as the difference between acetate influx from the gut ($F_{Synthesis}$) plus infused acetate (F_{Bolus}) and acetate clearance by peripheral tissues ($F_{Clearance}$) (all mmol/min):

$$Q_{\mathrm{Acetate(Blood)}}/\,\mathrm{dt} = F_{Synthesis} + F_{Bolus} - F_{Clearance}.$$

The rate of acetate synthesis was represented as a function of metabolic body weight **(MBW)**:

$$\mathrm{F}_{Synthesis} = \mathrm{MBW} \times K_{\mathrm{Synthesis}},$$

where $K_{Synthesis}$ represented the rate constant (mmol. min^{-1}.MBW^{-1}) for acetate synthesis. The flux of acetate clearance was modeled as a mass action function of blood acetate concentration adjusted for MBW:

$$\mathrm{F}_{Clearance} = \mathrm{C}_{Acetate\ (Blood)} \times (K_{Clearance} \times MBW)$$

where $K_{Clearance}$ represented the rate constant for acetate clearance ($min^{-1}.MBW^{-1}$).

Blood acetate pool size was calculated by numerical integration of $Q_{Acetate\ (Blood)}/dt$ from a given initial pool size for $Q_{Acetate\ (Blood))}$.

$$\mathrm{Q}_{Acetate\ (Blood)} = \int \frac{d\mathrm{Q}_{Acetate\ (Blood)}}{\mathrm{dt}} + \mathrm{i}Q_{\mathrm{Acetate\ (Blood)}}$$

Since the observed data were blood concentrations of acetate, blood acetate concentration was calculated from the predicted pool size assuming blood volume ($V_{Acetate\ (Blood)}$) was 8% of body weight (Ganong, 1997):

$$\mathrm{C}_{Acetate\ (Blood)} = \mathrm{Q}_{Acetate\ (Blood)} \,/\, \mathrm{V}_{Acetate\ (Blood)}$$

Infusions required about 30 min, varying slightly by animal. Therefore, the model was configured to reproduce the observed rate and duration of infusion from inputs of the actual start and end time of infusion and the quantity of acetate infused.

$K_{Clearance}$ and $K_{Synthesis}$ were derived by animal and period by fitting the model to observed blood acetate concentrations using the Nelder Mead algorithm encoded in acslXtreme (Ver. 3.0, AEgis Technologies, Huntsville, AL).

Statistical analysis

Data were analyzed according to a randomized complete block design. During the early grain feeding period, average daily gain (**ADG**) of EW and NW steers were 1.4 ± 0.1 kg/d and

0.9 ± 0.02 kg/d, respectively. The variation in ADG among animals within each treatment group were very small (EW = 8% and NW = 2%). Thus we assumed access to feed was similar for the steers within each treatment group. Further, EW steers did not escape from the pen and nor did NW steers have access to grain during the period. Therefore, animal was considered as the experimental unit. Statistical analyses were conducted using the GLIMMIX procedure of SAS (Ver. 9.2, SAS Institute Inc., Cary, NC) unless specified otherwise. Statistical significance was declared at $P < 0.05$. Initially sire and its interactions with other main effects were tested. If it was not significant, it was removed from models (unless specified).

The effect of treatment on HCW, BF, REA, KPH fat percentage, yield grade and marbling scores were analyzed using the following model. Rib eye area was adjusted for HCW.

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ijk} = HCW, BF, REA, KPH, yield grade or marbling scores, μ = mean HCW, BF, REA, KPH, yield grade or marbling scores, T_i = fixed effect of treatment i (i =1, 2) and e_{ij} = random error.

The effect of treatment on ADG, phosphorylation of signaling proteins and estimates derived from the acetate clearance model were analyzed using the following model. The time effect was considered a repeated measure.

$$Y_{ijk} = \mu + T_i + A_{(i)j} + P_k + (T \times P)_{ik} + e_{ijk}$$

where Y_{ijk} = ADG, phosphorylation status, acetate clearance or synthesis rate constants, μ = mean ADG, phosphorylation status, acetate clearance or synthesis rate constant, T_i = fixed effect of treatment i (i =1, 2), $A_{(i)j}$ = random effect of animal j (j = 1,6) for ADG and

phosphorylation status, and j (j = 1,4) for acetate clearance and synthesis rate constant, P_k = fixed effect of period k (k = 1, 3) for ADG and phosphorylation status, and k (k = 1, 5) for acetate clearance and synthesis rate constant, $(T \times P)_{ik}$ = fixed interaction between treatment and period, and e_{ijk} = random error. Compound symmetry (**CS**) was used to model the covariance structure. If the interaction effect was significant or if there was a trend for significance ($P < 0.10$), the test effect was sliced by period. In addition, rate parameters were analyzed for the fixed effect of treatment only for the early grain feeding period.

Linear relationships between phosphorylation status of each signaling protein and ADG was determined using the regression procedure with the effect of age included in the model,

$$ADG = \beta_0 + \beta_1 \times \text{Phosphorylation status} + \beta_2 \times \text{Age} + \text{Error}$$

Effects of treatments and acetate infusion on plasma glucose, insulin and BHBA concentrations were statistically analyzed using the following statistical model. The time effect was considered as a repeated measure.

$$Y_{ijkl} = \mu + T_i + A_{(i)j} + P_k + (T \times P)_{ik} + (A \times P)_{(i)jk} + M_l + (T \times M)_{il} + (P \times M)_{kl} + (T \times P \times M)_{ikl} + e_{ijkl}$$

where Y_{ijkl} = plasma glucose, insulin or BHBA concentrations, μ = mean plasma glucose, insulin or BHBA concentrations, T_i = fixed effect of treatment i (i = 1, 2), $A_{(i)j}$ = random effect of animal, P_k = fixed effect of period k (k = 1, 5), $(T \times P)_{ik}$ = fixed interactive effect between treatment and period, $(A \times P)_{(i)jk}$ = error term for animal period interaction, M_l = fixed effect of time l (l = 1, 11), $(T \times M)_{il}$ = fixed interactive effect of treatment and time, $(P \times M)_{kl}$ = fixed interactive effect of period and time, $(T \times P \times M)_{ikl}$ = fixed interactive effect of treatment, period and time, and e_{ijkl} = random error. Compound symmetry (CS) was used as the covariance

structure for periods. The test effect was sliced by the period if the treatment by period interaction effect was significant.

Results

Performances and phosphorylation of signalling proteins

Early weaned steers had significantly greater carcass weight and marbling scores compared to NW steers ($P < 0.001$; Table 3. 2). Normal weaned steers had significantly greater KPH fat percentage compared to EW steers ($P < 0.01$). However, BF, REA and yield grades were similar between the two treatment groups. Early weaned calves had greater ADG (1.37 ± 0.05 kg/d) during the early grain feeding period than NW steers (0.91 ± 0.05 kg/d; $P < 0.001$, Figure 3. 3). However, NW steers had greater ADG during the subsequent grazing period (0.72 ± 0.05 vs 0.35 ± 0.05 kg/d; $P < 0.001$). There were no significant differences in ADG during the feedlot feeding period, and overall ADG (early-grain feeding to harvest) was similar between the two treatment groups.

There were no differences in signaling protein phosphorylation ratios between EW and NW treatment groups during the early grain feeding period except for the phosphorylation ratio of rpS6 and S6K1 which were greater in EW steers (Table 3. 3). We did not see any significant treatment effects on signaling protein phosphorylation during grazing and feedlot feeding periods. Representative Western immunoblots are shown in Figure 3. 4. Phosphorylated: total S6K1, rpS6 and 4EBP1 were positively correlated with ADG whereas phosphorylated APMK:Tubulin was negatively correlated with ADG (Table 3. 4).

Acetate clearance and synthesis

Coefficient of variation for the parameter estimates were less than 20% for each group in respective periods except $K_{Synthesis}$ at 89 d of age where, we observed a very low synthesis rate (Table 3. 5). Therefore, data were adequate to describe the model parameters. Average data for one period (A3) are presented in Figure 3. 5. As can be seen the model tended to slightly over-predict blood acetate concentrations just after the infusion was stopped and under-predict for the next 15 min. Attempts to fit a 2-pool model to the data to address the bias proved unsuccessful due to lack of model identifiability. Average root mean square prediction errors (**RMSPE**) were 22% of the observed mean (Table 3. 6). Average mean and slope bias accounted for 1% and 28% of the mean square prediction error, respectively.

The interaction of treatment and period for acetate clearance was not significant (P = 0.09). Acetate clearance rates were significantly greater in NW steers compared to EW steers at 114 and 516 d of age ($P < 0.05$, Table 3. 7). There was a significant difference in acetate clearance among periods ($P < 0.001$) with clearance rates increasing with age. There was an interaction between treatment and period ($P = 0.002$) with greater acetate synthesis in NW steers at harvest (516 d of age, $P = 0.002$).

There was a trend for lower acetate clearance rates (0.04 ± 0.006 L x min^{-1}x MBW^{-1}) for EW steers compared to NW steers (0.06 ± 0.006 L x min^{-1}x MBW^{-1}, $P = 0.06$) during the early grain feeding period. However, acetate synthesis were greater ($P < 0.02$) in EW steers (0.08 ± 0.007 mmol x min^{-1}x MBW^{-1}) compared to NW steers (0.05 ± 0.007 mmol x min^{-1}x MBW^{-1}) during the early grain feeding.

The two-way interaction between treatment and period was significant ($P < 0.05$) with greater plasma glucose concentrations in NW steers during the early weaning period (A2; $P < 0.05$, Table 3. 8). There were no significant time effects associated with the bolus acetate infusion. There were no significant interactions between treatment, period, and time after the bolus acetate infusion on plasma insulin concentrations. However, plasma insulin concentrations were significantly greater ($P < 0.05$) at harvest (516 d of age) than the other sampling periods (Table 3. 8).

There was a significant interaction between treatment and period ($P < 0.05$) with greater BHBA concentrations in EW steers at 114 and 141 d of age ($P < 0.05$). Plasma BHBA concentrations were significantly greater ($P < 0.05$) in both treatment groups and across periods from 30 min post acetate infusion until 50 min post infusion compared to basal concentrations. Basal concentrations of plasma BHBA were also greater in EW steers compared to NW steers during the early grain feeding period ($P < 0.05$; EW = 0.60 ± 0.06 vs. NW = 0.30 ± 0.05 mM).

Discussion

Acetate utilization

Early weaned steers had greater ADG compared to the NW steers during the early grain feeding as we expected. Similar results were observed in studies conducted by Fluharty et al. (2000), Schoonmaker et al. (2004) and Myers et al. (1999b) under similar management conditions. Since we observed differences in ADG between the two treatment groups during early grain feeding and grazing periods, we speculated that there may be a correlation between signaling protein phosphorylation and ADG. Phosphorylated: total S6K1, rpS6 and 4EBP1 were

positively correlated with ADG suggesting that increase phosphorylation of these signaling proteins increase protein synthesis.

We anticipated lower phosphorylation of AMPK in EW steers during the early grain feeding period compared to the NW steers given the difference in energy intakes of the 2 groups during that time. The lack of a difference may simply reflect the variation in the assay particularly given the observation that the phosphorylated AMPK to tubulin ratio was negatively correlated with ADG. Since, increased phosphorylation of S6K1 and rpS6 results in increased protein synthesis rates, the increase in phosphorylation for the EW steers is consistent with their increased ADG. Moreover, the observed positive correlation between phosphorylated:total rpS6, S6K1 and 4EBP1, and ADG further confirmed this relationship. However, there were no treatment differences in signaling protein phosphorylation ratios between EW and NW treatment groups during the early grain feeding period for ACC, eEF2, Akt and 4EBP1. Rius et al. (2012) also examined the phosphorylation ratios of different signaling proteins from muscle tissue of dairy calves fed different amounts of dietary protein and fat, but did not see any differences.

Acetate clearance and plasma metabolites

The model generally fitted the data well across periods (Table 3. 5). However, at 89 d of age the standard deviation for the acetate synthesis rate constant was relatively large compared to the parameter estimate. However, it did not deviate from the other standard deviations. At 89 d of age, steers were still with their dams and they did not have much acetate synthesis in the gut. Therefore, acetate appearance was very low. Thus observed CV at 89 d was higher relative to other periods. However, treatments had not been applied at the time of sampling at 89 d of age

and these parameter estimates were not included in the statistical analyses. Thus it did not affect the interpretation.

In an attempt to reduce slope error for the acetate clearance model, another pool was introduced to represent the interstitial pool of acetate which exchanges with blood and the intracellular space. Since the model tended to over-predict acetate concentrations just after infusion, acetate might be clear faster than the predicted values. Then for about 15 minutes, model tended to under-predict acetate concentration indicating acetate cleared slower than the predicted values. Thus acetate might be exchanged with another pool during this time. However, parameters in the modified model were not identifiable from the data, and thus further attempts to improve the model were abandoned. Given the fact that plasma acetate concentrations returned to baseline by 60 min, more sampling points within the first 30 min may have provided the needed power to uniquely derive the additional parameters.

However, RMSPE was 22% of the observed values which was not large enough to significantly impact the parameter estimates. Further, model was able to predict acetate concentrations well about 20 min after infusion suggesting one pool model appeared to be good despite the minor slope error. If two-pool model could have been successful, we would have not seen widely different clearance and appearance parameter estimates.

We speculated that EW calves may clear blood acetate faster due to utilization in peripheral tissues, since previous studies (Fluharty et al., 2000; Myers et al., 1999a; Schoonmaker et al., 2003; Schoonmaker et al., 2004) reported that feeding high concentrate diets to EW beef calves accelerated growth rate and fat deposition. However, we observed a trend for the opposite response during the early grain feeding period. Chakeredza et al. (2002) observed

no differences in plasma acetate concentrations and clearance rates in weaned lambs supplemented with different nitrogen or energy sources. Cronjé et al. (1991) reported that acetate clearance rate was 0.006 – 0.010 L x min^{-1}x MBW^{-1} in sheep fed a wheat straw diet supplemented with urea and 80 g/d of protein meal, and was increased by dietary supplementation with acetate or acetate plus propionate. It is unclear whether the trend we observed for increased clearance by NW steers during early grain feeding is biologically meaningful. Benschop and Cant (2009) did not see any differences in acetate clearance rate constants by time or treatment in dairy calves. They reported that the average rate constant ranged from 0.05 to 0.06 L x min^{-1}x MBW^{-1} compared to 0.02 to 0.09 L x min^{-1}x MBW^{-1} in the present study.

Surprisingly, the rate constants for acetate synthesis were only different between treatments at harvest with EW steers having a slower rate. Because the groups were fed the same diet for the least 120 d and feed intake was not significantly different, the difference in synthesis rates suggests some type of an imprinting or metabolic programming event. But the programming must have occurred with the gut microflora because acetate primarily arises from ruminal fermentation. Fernando et al. (2010) observed that microbial populations shifted when feedlot steers adapted from high-forage diets to high-grain diets. Total population of rumen epithelial bacteria was positively correlated with molar proportions of acetate and isobutyrate (Chen et al., 2011). Early weaned steers reduced their ADG after transition from grain to forage possibly EW steers have not adapted well to the diet change. Thus reduced acetate synthesis in EW steers at harvest may be due to carryover effect of microflora changes resulting shift in ratios of VFA production with transitions of diets at early stages.

We speculated that acetate infusion may increase plasma glucose concentrations. Higher plasma acetate concentrations increase acetate uptake and use for energy production. Resulting high ATP levels in the cell inhibit phosphofructokinase which is the key enzyme in the control of glycolysis (Berg et al., 2002). However, acetate infusion did not affect plasma glucose concentrations. Plasma glucose concentration is regulated by insulin (Saltiel and Kahn, 2001) and increased plasma glucose concentration enhances insulin release and glucose uptake by cells. Thus it is possible to see unchanged glucose concentration. Then we expected to observe higher plasma insulin concentrations with acetate infusion. Trenkle (1978) observed enhanced insulin responses due to the glucogenic effect of the high grain feeding. However, plasma insulin concentrations were also unaffected by treatment or acetate infusion suggesting that any shift in acetate to propionate fermentation ratios did not result in a significant shift in glucose metabolism. However, plasma insulin concentrations were greater at harvest than at any other time points. Matsuzaki et al. (1997) also reported increased plasma insulin concentrations as BW and age increased across breeds.

We examined BHBA concentrations because acetate can easily be converted to BHBA. Before acetate infusion, concentrations of BHBA were 0.36 mM, and this increased to as much as 0.55 mM 5 min after acetate infusion suggesting that some of the acetate was converted to BHBA. DiCostanzo et al. (1999) also showed that plasma concentrations of BHBA increased with ruminal acetate infusion in beef heifers. Further, BHBA concentrations were greater in EW steers compared to NW, across periods suggesting more acetate is converted in to BHBA in EW steers.

Approximately 1004 kg of grain/steer was fed for early weaned steers during the early grain feeding period. Feedlot grain consumptions were 876 and 957 kg of grain/steer for NW

and EW groups, respectively. Therefore, total grain consumption for the EW group was 1961 kg/steer. Early weaned steers had greater carcass weight and marbling scores as we anticipated. Three of six early weaned steers had marbling scores sufficient for USDA choice+ (≥ 600). However, the added return from carcass quality was not adequate to cover the cost of the additional grain feeding during early grain feeding period. However, our original intent was to feed grain for 100 d during the early period, and this time was extended due to delays in gaining IACUC approval for additional work. If the steers had been fed for the intended 100 d during the early grain feeding period, grain consumption would have been 643 kg/steer giving a total grain consumption of 1600 kg/steer. This would have reduced the cost of the treatment. When weMcCann et al. (Personnel communication) repeated the experiment in the spring, grain consumption was 545 kg/steer during the 100 d early grain feeding period. The total grain consumption was 1362 kg/steers for the early weaned group and it was 1115 kg/steer for normal weaned group. However, we did not see any significant differences in marbling and carcass weight for the spring groups.

In conclusion, phosphorylation ratios of observed signaling proteins were not affected by treatment. Early grain feeding increased rates of acetate appearance and there was a trend to reduce acetate clearance during early grain feeding period. Acetate clearance increased when steers were heavier and older. Neither treatment nor acetate infusion significantly affected plasma glucose or insulin concentrations. Plasma BHBA concentrations increased with jugular acetate infusion.

References

Ballard, F. J. 1972. Supply and utilization of acetate in mammals. Am. J. Clin. Nutr. 25: 773-779.

Benschop, D. L., and J. P. Cant. 2009. Developmental changes in clearance of intravenous doses of glucose, acetate and [beta]-hydroxybutyrate from plasma of calves. Livest. Sci. 122: 177-185.

Berg, J. M., T. J.L., and S. L. 2002. Biochemistry. 5th edition ed, New York: W H Freeman.

Bolster, D. R., S. J. Crozier, S. R. Kimball, and L. S. Jefferson. 2002. Amp-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mtor) signaling. J. Biol. Chem. 277: 23977-23980.

Carlson, C. A., and K.-H. Kim. 1973. Regulation of hepatic acetyl coenzyme a carboxylase by phosphorylation and dephosphorylation. J. Biol. Chem. 248: 378-380.

Chakeredza, S., U. ter Meulen, and L. R. Ndlovu. 2002. Effect of cowpea hay, groundnut hay, cotton seed meal and maize meal supplementation to maize stover on intake, digestibility, microbial protein supply and acetate kinetics in weaner lambs. Trop. Anim. Health Prod. 34: 49-64.

Chen, Y., G. B. Penner, M. Li, M. Oba, and L. L. Guan. 2011. Changes in bacterial diversity associated with epithelial tissue in the beef cow rumen during the transition to a high-grain diet. Appl. Environ. Microbiol. 77: 5770-5781.

Cronjé, P. B., J. V. Nolan, and R. A. Leng. 1991. Acetate clearance rate as a potential index of the availability of glucogenic precursors in ruminants fed on roughage-based diets. Br. J. Nutr. 66: 301-312.

DiCostanzo, A., J. E. Williams, and D. H. Keisler. 1999. Effects of short- or long-term infusions of acetate or propionate on luteinizing hormone, insulin, and metabolite concentrations in beef heifers. J. Anim. Sci. 77: 3050-3056.

Doering, O., and C. Hurt. 2006. Competition for feed resources from other applications:Ethanol and bio-diesel. National Conference of State Legislatures, http://www.ncsl.org/programs/agri/EthanolBio.

Escobar, J., J. W. Frank, A. Suryawan, H. V. Nguyen, S. R. Kimball, L. S. Jefferson, and T. A. Davis. 2006. Regulation of cardiac and skeletal muscle protein synthesis by individual branched-chain amino acids in neonatal pigs. Am. J. Physiol. Endocrinol. Metab. 290: E612-E621.

Fernando, S. C., H. T. Purvis, F. Z. Najar, L. O. Sukharnikov, C. R. Krehbiel, T. G. Nagaraja, B. A. Roe, and U. DeSilva. 2010. Rumen microbial population dynamics during adaptation to a high-grain diet. Appl. Environ. Microbiol. 76: 7482-7490.

Fluharty, F. L. 2003. Interaction of management and diet on final meat characteristics on beef animal. Http://beef.Osu.Edu/library/mgtdiet.Html.

Fluharty, F. L., S. C. Loerch, T. B. Turner, S. J. Moeller, and G. D. Lowe. 2000. Effects of weaning age and diet on growth and carcass characteristics in steers. J. Anim. Sci. 78: 1759-1767.

Ganong, W. F. 1997. The general and cellular basis of medical physiology Review of medical physiology. p 1, Appleton and Lange, Stamford, Connecticut.

Huntington, G. B., R. L. Prior, and R. A. Britton. 1980. Glucose and lactate absorption and metabolic interrelationships in lambs switched from low to high concentrate diets. J. Nutr. 110: 1904-1913.

Huntington, G. B., R. L. Prior, and R. A. Britton. 1981. Glucose and lactate absorption and metabolic interrelationships in steers changed from low to high concentrate diets. J. Nutr. 111: 1164-1172.

Ingle, D. L., D. E. Bauman, R. W. Mellenberger, and D. E. Johnson. 1973. Lipogenesis in the ruminant: Effect of fasting and refeeding on fatty acid synthesis and enzymatic activity of sheep adipose tissue. J. Nutr. 103: 1479-1488.

Kristensen, N. B. 2000. Quantification of whole blood short chain fatty acids by gas chromatographic determination of plasma 2-chloroethyl derivatives and correction for dilution space in erythrocytes`. Acta Agric. Scand., Sect. A 50: 231-236.

Matsuzaki, M., S. Takizawa, and M. Ogawa. 1997. Plasma insulin, metabolite concentrations, and carcass characteristics of japanese black, japanese brown, and holstein steers. J. Anim. Sci. 75: 3287-3293.

McCann, M. A., J. M. Scheffler, S. P. Greiner, and D. E. Gerrard. Personnel communication.

Myers, S., D. Faulkner, F. Ireland, L. Berger, and D. Parrett. 1999a. Production systems comparing early weaning to normal weaning with or without creep feeding for beef steers. J. Anim. Sci. 77: 300 - 310.

Myers, S., D. Faulkner, T. Nash, L. Berger, D. Parrett, and F. McKeith. 1999b. 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.

Rius, A. G., H. A. Weeks, J. Cyriac, R. M. Akers, B. J. Bequette, and M. D. Hanigan. 2012. Protein and energy intakes affected amino acid concentrations in plasma, muscle, and liver, and cell signaling in the liver of growing dairy calves. J. Dairy Sci. 95: 1983-1991.

Saltiel, A. R., and C. R. Kahn. 2001. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414: 799-806.

Scheffler, J. M., M. A. McCann, S. P. Greiner, H. Jiang, M. D. Hanigan, G. A. Bridges, S. L. Lake, and D. E. Gerrard. Personnel communication. Early metabolic imprinting events increase marbling scores in fed cattle.

Schoonmaker, J. P., M. J. Cecava, D. B. Faulkner, F. L. Fluharty, H. N. Zerby, and S. C. Loerch. 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., M. J. Cecava, F. L. Fluharty, H. N. Zerby, and S. C. Loerch. 2004. Effect of source and amount of energy and rate of growth in the growing phase on performance and carcass characteristics of early- and normal-weaned steers. J. Anim. Sci. 82: 273-282.

Trenkle, A. 1978. Relation of hormonal variations to nutritional studies and metabolism of ruminants. J. Dairy Sci. 61: 281 - 293.

Young, J. W., S. B. Tove, and H. A. Ramsey. 1965. Metabolism of acetate, propionate, and n-butyrate in young milk-fed calves. J. Dairy Sci. 48: 1079-1087.

Table 3. 1. Ingredient composition of grain mixes fed to calves after early weaning and to all animals in the feedlot.

Ingredient	Grain mix composition, % (DM basis)					
	Early grain feeding[1]					Feedlot[2]
	Step 1	Step 2	Step 3	Step 4	Step 5	
Corn, cracked	35.0	40.0	40.0	41.0	41.0	50.0
Distillers grain	15.0	15.0	15.0	15.0	15.0	-
Soybean meal (48% CP)	14.0	9.5	5.0	1.5	-	-
Corn gluten feed	9.50	9.0	11.0	12.0	15.0	37.0
Wheat midds	10.0	10.0	11.0	12.0	10.5	10.0
Cottonseed hulls	8.00	8.0	9.5	10.0	10.0	-
Cane molasses	6.00	6.00	6.00	6.00	6.00	-
Limestone 38%	2.00	2.00	2.00	2.00	2.00	2.40
Salt	0.50	0.50	0.50	0.50	0.50	0.25
Vitamin ADE premix	0.02	0.02	0.02	0.02	0.02	0.02
Rumensin	-	-	-	-	-	0.02

[1]Initial ration fed to early weaned calves from weaning at 106 ± 4 d of age to 254 ± 4 d of age. Calves were transitioned from the initial to final diet over five phases. Balance weights for each steps: step 1 = 136 kg, step 2 = 159 kg, step 3 = 182 kg, step 4 = 204 kg and step 5 = 227 kg

[2]Feedlot ration provided to all steers from 395 ± 5 d of age to harvest

Table 3. 2. Least square means of carcass characteristics of normal (NW) and early weaned (EW) steers.

Variable[1]	Treatment		SEM	*P*-value
	NW[2]	EW[3]		
HCW, kg	313	335	2.80	>0.001
BF, cm	0.96	0.97	0.04	0.72
REA, sq. cm	87.66	87.32	0.90	0.82
KPH, %	2.59	2.30	0.08	0.01
Yield grade	2.38	2.29	0.04	0.11
Marbling scores	499	620	10.0	>0.001

[1]Sire was significant, thus included in the model

[2]NW = normal weaning at 251 ± 5 d of age

[3]EW = early weaning at 106 ± 4 d of age followed by grain feeding for 148 d

Table 3. 3. Least square means of phosphorylated (Phos.), total and ratio of phosphorylated:total forms (arbitrary units) of signaling proteins for steers normal (NW) and early weaned (EW).

Protein	Early grain feeding[1]		Grazing[2]		Feedlot[3]		SEM	*P*- value[6]
	NW[4]	EW[5]	NW[4]	EW[5]	NW[4]	EW[5]		
Phos. AMPK:Tubulin	0.99	0.74	1.43	1.60	1.19	1.17	0.36	0.83
Total AMPK:Tubulin	1.05	1.42	1.35	1.17	1.13	1.74	0.30	0.43
Phos:Total AMPK	1.13	0.56	1.06	1.15	1.25	1.05	0.26	0.41
Phos. ACC:Tubulin	1.96	1.50	1.04	1.86	1.01	0.94	0.42	0.21
Total ACC:Tubulin	1.88	0.97	1.29	1.83	1.24	1.36	0.49	0.31
Phos:Total ACC	1.26	1.64	1.24	1.21	1.72	1.72	0.46	0.91
Phos. Akt:Tubulin	1.74	1.30	1.43	1.02	1.15	1.53	0.40	0.52
Total Akt:Tubulin	1.48	1.02	1.23	1.07	1.06	0.91	0.27	0.76
Phos:Total Akt	1.27	1.40	1.40	1.05	1.05	1.98	0.31	0.14
Phos. S6K1:Tubulin	1.14	1.32	1.42	1.17	1.27	2.00	0.36	0.46
Total S6K1:Tubulin	1.17	1.28	1.40	1.54	1.29	1.71	0.34	0.89

Phos:Total S6K1	1.02[x]	1.29[y]	1.03	0.79	1.03	1.15	0.11	0.02
Phos. rpS6:Tubulin	1.14	1.37	1.32	1.11	1.17	1.34	0.24	0.60
Total rpS6:Tubulin	1.11	1.04	1.40	1.23	1.20	1.34	0.23	0.80
Phos:Total rpS6	1.02[a]	1.38[b]	1.02	0.87	1.03	1.22	0.12	0.05
Phos. 4EBP1:Tubulin	1.22	1.98	1.44	1.11	1.38	1.57	0.38	0.34
Total 4EBP1:Tubulin	1.22	2.18	1.26	1.20	1.24	1.08	0.31	0.17
Phos:Total 4EBP1	1.00	1.08	0.99	1.02	1.18	1.55	0.19	0.59
Phos. eEF2:Tubulin	1.06	0.98	1.22	1.20	1.25	2.26	0.49	0.48
Total eEF2:Tubulin	1.12	1.55	1.22	1.09	1.06	1.21	0.19	0.31
Phos:Total eEF2	1.35	0.94	1.18	1.25	1.38	1.50	0.27	0.48

[1]Biopsies were collected at 253 ± 5 of age representing the period that initial ration fed to early weaned calves from weaning at 106 ± 4 d of age to 254 ± 4 d of age.

[2]Biopsies were collected at 394 ± 5 of age representing the period that both treatment groups on pasture from 253 ± 5 d of age to 394 ± 5 d of age.

[3]Tissue samples were collected at 513 ± 10 of age representing the period that feedlot ration provided to all steers from 395 ± 5 d of age to harvest.

[4]NW = normal weaning at 251 ± 5 d of age

[5]EW = early weaning at 106 ± 4 d of age

[a,b] Treatment means differ within the period ($P < 0.05$), [x,y] Treatment means differ within the period ($P < 0.10$)

Table 3. 4. Relationship between average daily gain and phosphorylation of signaling proteins[1].

Variable	Intercept	Regression coefficient		*P*-value[3]	R-square
		Signaling protein	Age[2]		
Phos. AMPK:Tubulin	0.48	-0.07	0.00180	0.15	0.16
Total AMPK:Tubulin	0.42	0.02	0.00169	0.68	0.14
Phos:Total AMPK	0.46	-0.04	0.00175	0.58	0.14
Phos. ACC:Tubulin	0.48	-0.01	0.00166	0.44	0.14
Total ACC:Tubulin	0.46	-0.008	0.00168	0.67	0.14
Phos:Total ACC	0.41	0.02	0.00168	0.42	0.14
Phos. Akt:Tubulin	0.41	0.02	0.00172	0.50	0.14
Total Akt:Tubulin	0.41	0.03	0.00171	0.77	0.14
Phos:Total Akt	0.40	0.04	0.00168	0.29	0.15
Phos. S6K1:Tubulin	0.43	0.01	0.00168	0.76	0.14
Total S6K1:Tubulin	0.47	-0.04	0.00175	0.46	0.14
Phos:Total S6K1	-0.09	0.45	0.00185	0.002	0.25
Phos. rpS6:Tubulin	0.42	0.02	0.00170	0.66	0.14
Total rpS6:Tubulin	0.47	-0.04	0.00174	0.43	0.14
Phos:Total rpS6	0.07	0.31	0.00179	0.001	0.22
Phos. 4EBP1:Tubulin	0.38	0.04	0.00172	0.21	0.15
Total 4EBP1:Tubulin	0.38	0.03	0.00177	0.31	0.14
Phos:Total 4EBP1	0.38	0.09	0.00158	0.11	0.16
Phos. eEF2:Tubulin	0.44	0.002	0.00170	0.93	0.14

Total eEF2:Tubulin	0.37	0.05	0.00174	0.42	0.14
Phos:Total eEF2	0.45	-0.02	0.00172	0.71	0.14

[1] Dependent variable = ADG and independent variables: age and phosphorylation status of signaling proteins

[2] *P*-value associated with regression coefficient of age is < 0.01

[3] *P*-value associated with regression coefficient of signaling proteins

Table 3. 5. Model parameter estimates for each period (mean values across animals), associated standard deviations and coefficient of variation.

Age, d	$K_{Clearance}$[1]		$K_{Synthesis}$[2]	
	Estimate	CV	Estimate	CV
89 ± 5	0.041 ± 0.002	6.20	0.009 ± 0.004	50.31
114 ± 5	0.037 ± 0.002	5.72	0.044 ± 0.008	15.41
141 ± 5	0.035 ± 0.001	3.18	0.085 ± 0.006	6.97
227 ± 5	0.069 ± 0.003	3.98	0.064 ± 0.006	8.77
348 ± 5	0.074 ± 0.006	7.71	0.078 ± 0.013	16.13
516 ± 5	0.071 ± 0.004	6.08	0.108 ± 0.012	13.56

[1]$K_{Clearance}$ in L x min^{-1}x MBW^{-1}

[2]$K_{Synthesis}$ in mmol x min^{-1}x MBW^{-1}

Table 3. 6. Errors associated with model predictions for blood acetate concentration.

Variable	Value[3]
RMSPE (%)[1]	22.28
Mean bias (%)[2]	1.06
Slope bias (%)[2]	27.64
Dispersion bias (%)[2]	71.30

[1]Root mean square prediction error (RMSPE) as a percentage of mean observed value

[2]Percentage of mean square prediction error (MSPE)

[3]Mean values across animals and periods

Table 3. 7. Least square means for acetate clearance and synthesis rate constants for normal (NW) and early weaned (EW) steers at different stages of growth.

Age, d	Treatment x Period[1]			Period[4]	SEM
	NW[2]	EW[3]	SEM		
	Acetate clearance rate constant, L x min^{-1}x MBW^{-1}				
114 ± 5	0.05*	0.02	0.009	0.04[a]	0.006
141 ± 5	0.03	0.04	0.009	0.03[a]	0.006
227 ± 5	0.08	0.06	0.009	0.07[b]	0.006
348 ± 5	0.07	0.07	0.009	0.07[b]	0.006
516 ± 5	0.09*	0.05	0.009	0.07[b]	0.006
	Acetate synthesis rate constant, mM x min^{-1}x MBW^{-1}				
114 ± 5	0.03	0.06	0.01	0.04[a]	0.009
141 ± 5	0.07	0.10	0.01	0.09[bc]	0.009
227 ± 5	0.06	0.07	0.01	0.06[ab]	0.009
348 ± 5	0.09	0.06	0.01	0.08[b]	0.009
516 ± 5	0.14 *	0.07	0.01	0.11[c]	0.009

[1]Values are expressed as Least square means (LSM) of acetate clearance and acetate synthesis rate constants with treatment x period effects. * Treatment means differ within the period ($P < 0.05$)

[2]NW = normal weaning at 251 ± 5 d of age

[3]EW = early weaning at 106 ± 4 d of age followed by grain feeding for 148 d

[4]Values are expressed as Least square means (LSM) of acetate clearance and acetate synthesis rate constants with period effect (across the treatments). [a, b, c]Means with different superscripts among the periods differ ($P < 0.05$)

Table 3. 8. Least square means for plasma glucose, insulin and β-hyroxybutyrate (BHBA) concentrations for normal (NW) and early weaned (EW) steers at different stages of growth.

Age, d	Treatment x Period[1]			Period[4]	SEM
	NW[2]	EW[3]	SEM		
			Glucose concentrations, mM		
114 ± 5	5.75 *	4.72	0.2	5.23[ab]	0.14
141 ± 5	5.10	5.59	0.2	5.35[ab]	0.14
227 ± 5	5.91	5.44	0.2	5.68[a]	0.14
348 ± 5	5.15	4.97	0.2	5.06[b]	0.14
516 ± 5	4.96	4.95	0.2	4.95[b]	0.14
			Insulin concentrations, pM		
114 ± 5	30.01	40.22	14.80	35.11[a]	10.28
141 ± 5	36.17	69.82	14.80	53.00[a]	10.28
227 ± 5	39.69	67.78	14.80	53.73[a]	10.28
348 ± 5	49.81	66.92	14.80	58.36[a]	10.28
516 ± 5	87.46	110.65	14,80	99.06[b]	10.28
			BHBA concentrations, mM		
114 ± 5	0.33*	0.96	0.06	0.65[a]	0.04
141 ± 5	0.36*	0.62	0.06	0.49[ab]	0.04
227 ± 5	0.52	0.54	0.06	0.53[ab]	0.04
348 ± 5	0.40	0.48	0.06	0.44[bc]	0.04
516 ± 5	0.33	0.26	0.06	0.29[c]	0.04

[1]Values are expressed as Least square means (LSM) of glucose, insulin and BHBA associated with treatment x period effects. *****Treatment means differ within the period ($P < 0.05$)

[2]NW = normal weaning at 251 ± 5 d of age

[3]EW = early weaning at 106 ± 4 d of age followed by grain feeding for 148 d

[4]Values are expressed as Least square means (LSM) of glucose, insulin and BHBA associated with period effect (across the treatments). [a, b, c]Means with different superscripts among the periods differ($P < 0.05$)

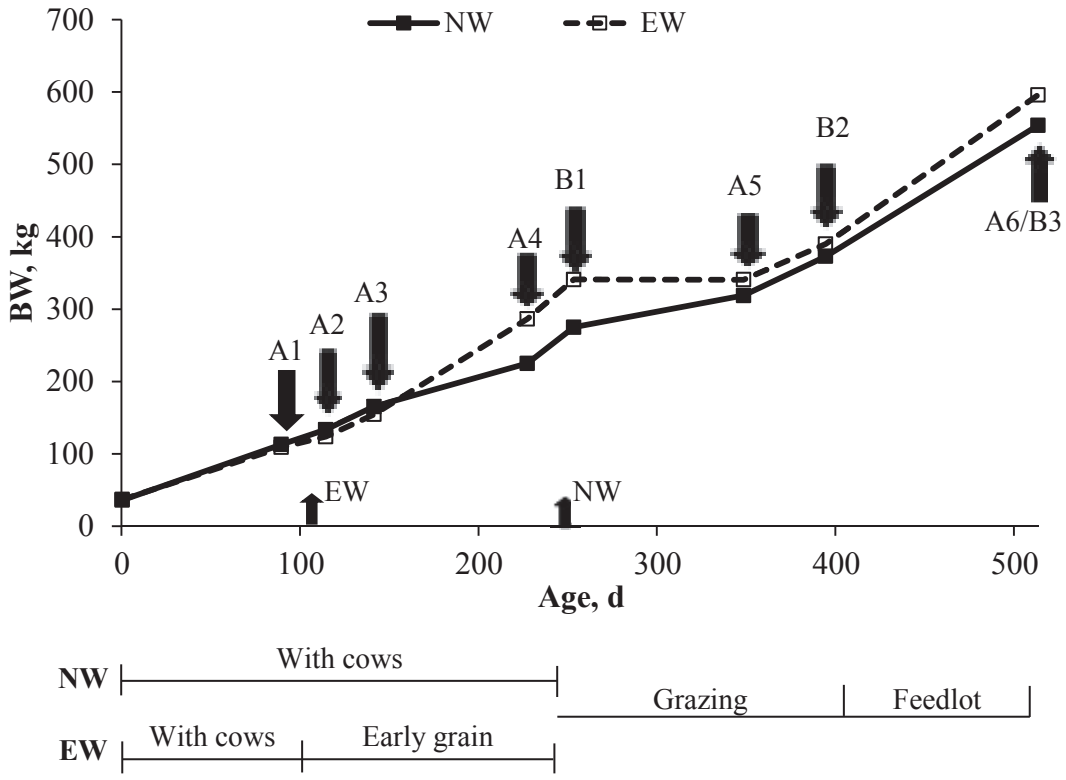

Figure 3. 1. Sampling schedule of the steers. NW = normal weaning at 251 ± 5 d of age, EW = early weaning at 106 ± 4 d of age, A1 to A6 represent acetate infusion time points, B1 to B3 represent tissue collection time points.

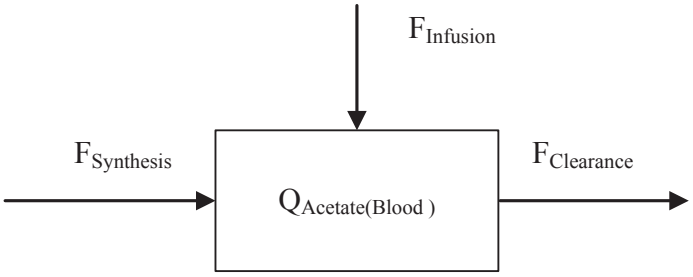

Figure 3. 2. A schematic representation of the model. The arrows represent fluxes. The box enclosed with a solid line represents the blood acetate pool.

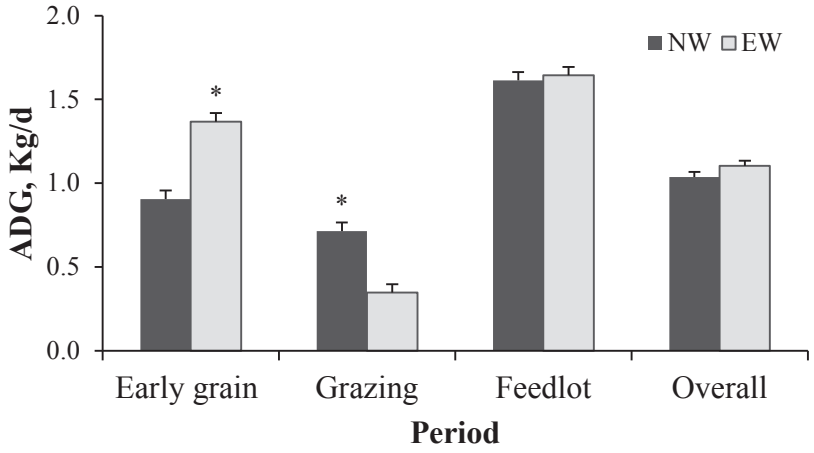

Figure 3. 3. Least square means of ADG of normal and early weaned steers during different feeding periods. NW = normal weaning at 251 ± 5 d of age, EW = early weaning at 106 ± 4 d of age, *Means within the period differ ($P < 0.05$).

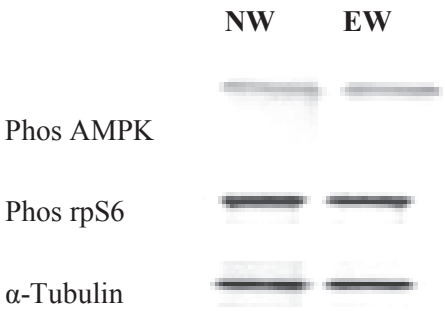

Figure 3. 4. Representative Western immunoblots for phosphorylated (Phos) AMPK, rpS6 and α-Tubulin signaling proteins from longissimus muscle tissues taken from normal weaned and early weaned steers at end of early grain feeding period (254 ± 4 d of age). NW = normal weaning at 251 ± 5 d of age, EW = early weaning at 106 ± 4 d of age.

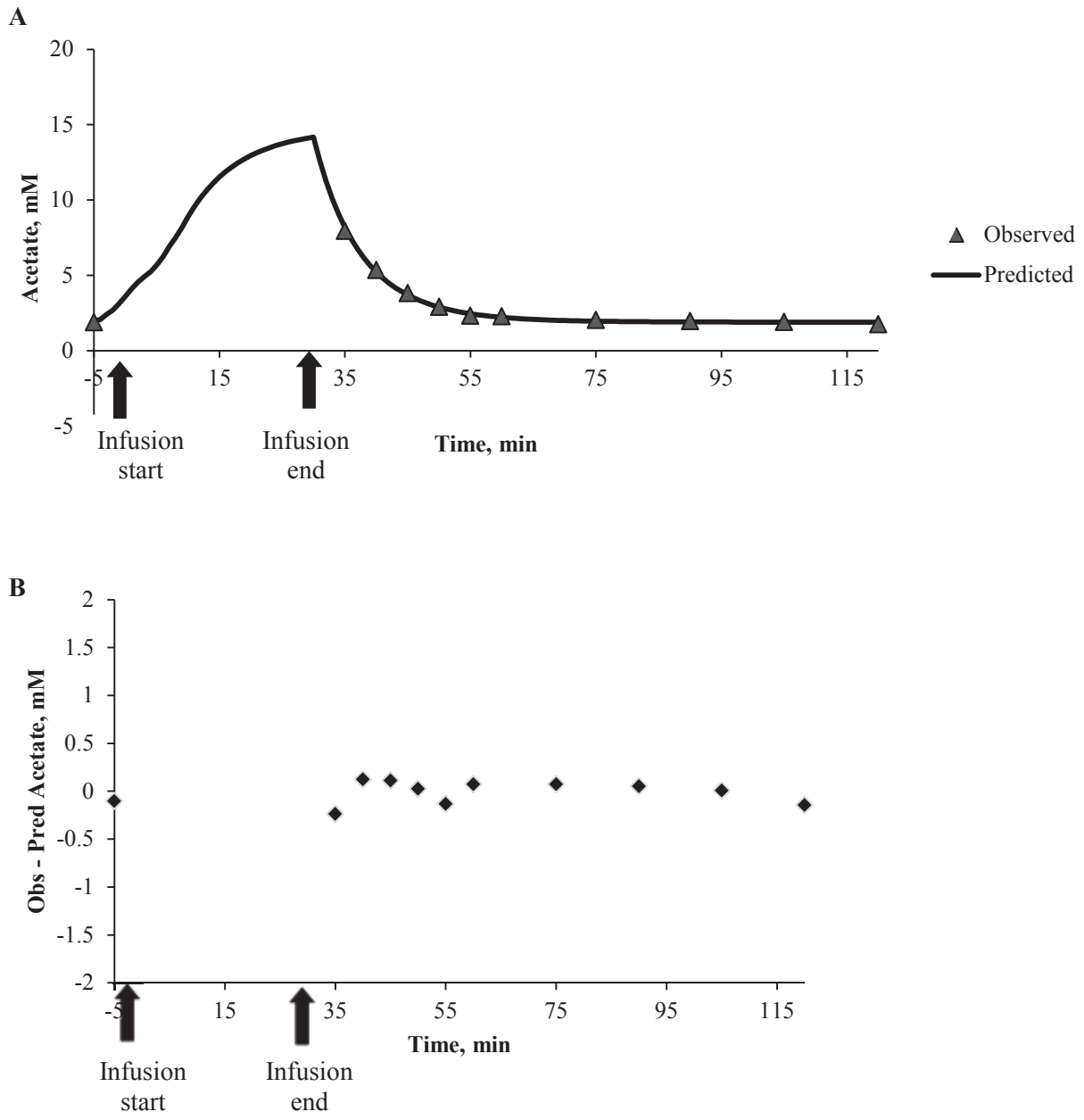

Figure 3. 5. Residuals (A), representation of predicted and observed blood acetate concentration vs. time (B), relationship between residuals and time (average across the animals in period A3).

CHAPTER 4

Adipose Tissue Preferences for Acetate and Glucose by Finishing Steers

Abstract

The objectives of this study were to assess the effects of early grain feeding on acetate and glucose turnover rates, palmitate synthesis rates, and acetate and glucose preference by subcutaneous (SCF), intramuscular (IMF) and visceral fat (VF) in finishing steers. Sixteen Angus x Simmental steers were used in the study; 8 were EW and fed a high-grain diet immediately after weaning for 100 or 148 d and 8 remained with their dams on pasture until weaning at 202 ± 5 d or 253 ± 5 d of age. Groups were combined and grazed to 374 ± 5 d or 393 ± 5 d of age when they were placed on a corn silage based finishing ration until they achieved a SCF thickness of 1.0 to 1.2 cm (EW; 494 ± 17, 502 ± 12 d of age). Eight steers (4 from each treatment group) were infused continuously with [$^{2}H_3$] acetate (1.63 mmol/min), and the remainder (n = 8) were infused with [U-$^{13}C_6$] glucose (0.07 mmol/min) for 12 h immediately prior to harvest. Blood samples were collected prior to initiation of infusions and at the end of infusion from 8 animals. From the remaining 8 animals, blood samples were collected before infusion and at 1 h intervals for the first 11 h of infusion and at 15 min intervals for the last 1 h of infusion to confirm plateau enrichment. Adipose tissue samples from SCF, IMF and VF depots were collected at harvest, and lipids were extracted. Palmitate enrichment in lipids and acetate and glucose enrichment in blood were determined by GC-MS. DNA concentrations were determined. Plasma enrichment of acetate and glucose, and palmitate enrichment in each depot was used to calculate fractional synthesis rates (FSRs, % per h). Data were analyzed using the GLIMMIX procedure of SAS. DNA concentrations were significantly different ($P < 0.05$) by

depot. Turnover rates and FSR for EW and NW steers were similar. Acetate turnover and palmitate FSR from acetate were greater ($P < 0.05$) than the corresponding rates from glucose. However, FSR was not significantly different among the depots for either acetate or glucose. In conclusion, early grain feeding did not affect acetate or glucose turnover or fat synthesis at harvest. Acetate turnover was greater than glucose turnover. Acetate was used for lipid synthesis more than glucose. However, there were no differences in preference for acetate and glucose among the major fat depots. Thus diets leading to high glucose supply will not preferentially direct energy storage to intramuscular stores.

Keywords: acetate, fractional synthesis rate, glucose, intramuscular fat, subcutaneous fat

Introduction

Consumer demand for beef relies on beef quality and consistancy. Intramuscular fat (**IMF**) or marbling enhances tenderness, juiciness, flavor and overall palatability of beef and is one of the most important factors determining the quality grade of a carcass. Carcass yield grade is determined by subcutaneous fat (**SCF**) with greater SCF associated with reduced carcass yield and value (USDA, 1997).

Feeding practices are used to manage the yield and quality grade of a carcass. Early weaning (before 205 d of age) followed by high grain feeding until harvest increased weight gain, enhanced feed conversion efficiency and improved carcass quality grade (Fluharty et al., 2000; Myers et al., 1999). Hanley et al. (2010) reviewed data indicating that changes in nutrition and growth at early life development affect later physiological events, and processes referred to as metabolic imprinting. Barker (1995) showed that prepubertal metabolic imprinting affects

hormone profile, food intake, energy metabolism and adipocyte development in humans and rodents later in life. There is little research evidence for metabolic imprinting in beef production. Gotoh et al. (2010) showed that feeding steers high energy diets during the early growth phase while finishing them on grass influenced IMF content in Japanese black cattle. In previous chapter, we demonstrated that steers weaned early and fed a high energy diet in the early growing period from 100 to 250 d of age followed by a low input feeding period (grazing) before feedlot entry had higher marbling scores than conventionally weaned calves although, both groups had similar yield grades. This suggests early grain feeding could create metabolic imprinting partly by enhancing proliferation of preadipocytes and thereby establishing a large population of undifferentiated cells maintained during the forage phase and readily matured during the feedlot phase. Moreover, metabolic imprinting may be associated with modulation of nutrient partitioning and utilization efficiencies. In ruminants, acetate and glucose are the major precursors used in biosynthesis of fatty acids such as palmitate which is subsequently used in fat synthesis (Smith and Crouse, 1984) . Therefore, investigations on acetate and glucose turnover rates and palmitate synthesis rates in different adipose tissues could widen our understanding of proposed metabolic imprinting phenomenon.

Smith and Crouse (1984) concluded that intramuscular adipocytes prefer glucose as a substrate for fat synthesis while subcutaneous adipocytes prefer acetate when assessed *in vitro*. The observed ratio for fatty acid synthesis from acetate:glucose was 0.28 in IMF but 32.95 in SCF. Song et al. (2001) also examined the substrate utilization in lipogenesis in different adipose tissues (*in vitro*) from Hanwoo steers. They reported that the ratio of acetate:glucose use for lipogenesis in SCF vs. IMF averaged 1.25 and 1.27 respectively. Ratios for incorporation of acetate and glucose into adipose tissue of Hanwoo steers were also studied by Lee et al. (2000).

They reported ratios of incorporation for acetate:glucose into SCF and IMF of 1.61 and 1.23, respectively. If such differential precursor preference exists, it should be connected to differential fractional synthesis rates from precursors by different depots. Nonetheless, lack of consistency in the *in vitro* results and presence of numerous other physiological variables including metabolic imprinting warrant an investigation with an *in vivo* system.

We hypothesized that carry-over effects of early grain feeding period may affect acetate and glucose turnover rates and hence fat synthesis, but differential preference for acetate and glucose may not exist in different depots. Therefore, the objectives of this study were to assess the effects of early grain feeding on acetate and glucose turnover rates, palmitate synthesis, and on acetate and glucose preference by subcutaneous, intramuscular and visceral adipose tissues in finishing steers.

Materials and Methods

Experimental animals, diets and treatments

The Virginia Tech Animal Care and Use Committee (IACUC) approved all procedures. Eight fall born (2009) and 8 spring born (2010) Angus x Simmental steers were used in the study. Eight steers were weaned early (EW) at 105 ± 4 d of age from the fall group (n = 4) and 102 ± 5 d of age from the spring group (n = 4) and fed a high-grain diet (Table 4. 1) for 148 d and 100 d respectively. The remaining steers (NW; n = 8**)** stayed with their dams on pasture until weaning at 253 ± 5 d of age for the fall group and 202 ± 5 d of age for the spring group. Thereafter, groups were combined on grass, predominantly tall Fescue *(Festuca arundinacea),* until 393 ± 5 d of age for the fall group and 374 ± 5 d of age for the spring group. Then they

were placed in the feedlot and fed a corn silage plus supplement ration until harvest. The ration transitioned from 75% silage at entry to 25% silage at 441 ± 5 d of age for the fall group and 422 ± 5 d of age for the spring group (Table 4. 1).

Acetate and glucose infusions and blood sampling

Four animals were randomly selected from each treatment group (EW and NW) and infused with 100 g of [$^{2}H_3$] acetate (n = 8). The remaining 8 animals (4 from each treatment group) were infused with 10 g of [U-$^{13}C_6$] glucose (n = 8). During the infusion and sampling, steers were confined to holding pens adjacent to Virginia Tech meat science center and fed tall Fescue hay cubes. Labeled acetate and glucose solutions were prepared on the day of infusion. Saline bags (500 mL, 0.9%-NaCl, USP, Hospira Inc., Lake Forest, IL) were emptied and 100 g of [$^{2}H_3$] acetate or 10 g of [U-$^{13}C_6$] glucose (99% enriched, Cambridge isotope laboratories, Inc., Andover, MA) was dissolved in the solution from a single bag and adjusted to a pH of 7.0. The resulting solution was sterile filtered (0.22 μm; Millipore Corporation, Billerica, MA) back into the saline bags using a peristaltic pump (Harvard Apparatus Co. Inc., Millis, MA). On the same day, 2 sterile polyurethane catheters (o.d. = 2.03 mm, i.d. = 1.02 mm, Braintree Scientific Inc., Braintree, MA) were inserted into contralateral jugular veins of 4 steers from each treatment group. One catheter was used for isotope infusion and the other for frequent sampling. The remaining steers were fitted with a single catheter. Catheters were filled with heparinized saline (200 IU/mL) after insertion to prevent clotting.

Steers were continuously infused with [$^{2}H_3$] acetate or [U-$^{13}C_6$] glucose for 12 h immediately preceeding harvest at a rate of 1.63 mmol acetate/min or 0.07 mmol glucose/min in

a volume of 40 mL/h using clinical infusion pumps (Abbott laboratories, North Chicago, IL). Jugular blood samples (5 mL) were drawn using a heparinized syringe prior to initiation of infusions, at 1 h intervals for the first 11 h of infusion, and at 15 min intervals for the last 1 h of infusion, from the 8 animals with 2 catheters, and before and at the end of infusion for the remaining 8 animals with a single catheter. Catheters were filled with heparanized saline (20 IU/mL) between samplings. Blood samples were immediately transferred to tubes containing 125 IU of heparin, gently mixed by inversion, and placed on ice. Plasma were prepared by centrifugation at 1,200 x g for 10 min at 4 ^{0}C and stored at -80 ^{0}C until analysis.

Within 30 min of the end of the infusion animals were harvested in the Virginia Tech meat laboratory using normal slaughter procedures. Samples of longissimus muscle (with SCF) posterior to the last rib were obtained and SCF and IMF were separated. A sample of the VF surrounding the kidney was also obtained. Tissue samples were frozen in liquid nitrogen immediately after dissection and stored at -80 ^{o}C for subsequent analysis. In addition, hot carcass weights (**HCW**), rib eye area (**REA**), 12th rib fat thickness (**BF**), kidney, pelvic and heart (**KPH**) fat percentage, and marbling scores were recorded.

Sample analysis

Plasma samples

To determine plasma acetate enrichments**,** 2-chloroethyl esters of acetate were prepared as described by Kristensen (2000) with minor modifications. Plasma (450 μL) was gravimetrically transferred into 100 x 16 mm, screw-cap glass tubes. A volume of 750 μL 2-chloroethanol (Acros Organics, Fair Lawn, NJ) and 750 μL acetonitrile (Fisher Scientific, Fair

Lawn, NJ), were added, and the sample was vortexed at low speed and centrifuged at 2,550 x g for 30 min at 4°C. The 2-chloroethyl esters were extracted in 100 μL chloroform (Fisher Scientific, Fair Lawn, NJ). Eighty μL of the organic phase was transferred to an auto-sampler vial, and 1μL was injected onto a gas chromatograph mass spectrometer (**GC-MS**; Thermo PolarisQ mass spec in tandem with a Thermo electron Focus GC, Thermo Scientific, Waltham, MA). The GC was operated in split mode (split ratio 80) with an injection temperature of 225°C. The compounds were separated on a 30 m x 0.25 mm i.d. (with a 0.25 μm film) Factor FOUR VF-170ms column (Varian Inc., Palo Alto, CA) at a constant helium flow of 1.2 mL/min. The column temperature was initially set at 75°C, ramped at 5°C/min to 135°C, then ramped at 40°C/min to 225°C. The mass spectrometer was operated selective ion monitoring (**SIM**) mode with positive electron ionization. Molecular ions of mass to charge ratios (**m/z**) of 43 and 46 were monitored to determine normal and enriched ion abundance, respectively. Peak detection and area integration were conducted using the ICIS algorithm encoded in the XCalibur software (Ver. 1.4, Thermo Scientific, Waltham, MA). Background isotope ratios determined from the samples collected before infusion was subtracted from all sample isotope ratios. Isotope ratios were converted to enrichment using a gravimetric standard curve prepared from mixtures of pure labeled and unlabeled acetate. All enrichments were expressed as atom percent excess (**APE**) relative to background natural abundance.

Plasma glucose was derivatized using the method described by Tserng and Kalhan (1983). The resulting samples (1 μL) were analyzed by GC-MS (Thermo DSQ II in tandem with a Trace GC Ultra, Thermo Scientific, Waltham, MA). The GC was operated in splitless mode with an injection temperature of 250°C. The compounds were separated on a 30 m x 0.25 mm i.d. (with a 0.25 μm film) Rtx-5 capillary column (Restek, Bellefonte, PA) at a constant helium

flow of 1.2 mL/min. The column temperature was set at 80°C and held for 2 min, followed by a 15°C/min ramp to 320°C, and held at 320°C for 6 min. The mass spectrometer was operated in SIM mode with positive chemical ionization (**CI**) with a methane flow of 1.0 mL/min. Ions at m/z of 328 (m+0) and 334 (m+6) were monitored for abundance. Peak detection and area integration were conducted using the Genesis algorithm encoded in the XCalibur software (Ver. 2.1, Thermo Scientific, Waltham, MA). Isotope ratios were calculated and corrected for background and converted to APE as described for acetate.

Adipose tissue samples

Lipid was extracted from the adipose tissue samples according to the procedure of Hara and Radin (1978) using 3 ml of hexane:isopropanol (3:2, vol/vol) solution per 100 - 150 mg of adipose tissue sample plus 2 mL of 0.48 M Na_2SO_4. The lipid phase was separated and used to prepare fatty acid methyl esters (**FAME**) as described by Christie (1982) with the modifications described by Chouinard et al. (1999). The FAME were subjected to GC-MS analyses as described by Notter et al. (2008) with minor modifications. Briefly, 1 µL of FAME solution from acetate infused animals was injected into a GC-MS (Thermo DSQ II in tandem with a Trace GC Ultra; Thermo Scientific, Waltham, MA). The GC was operated in split mode (split ratio 50) with an injection temperature of 250 °C. Separation was achieved using a 30 m x 0.25 mm i.d. (0.25 µm film) Rtx-5 capillary column (Restek, Bellefonte, PA) with a constant helium flow of 1.0 mL/min. The initial column temperature was 120 °C and held for 1 min., followed by a 5 °C/min ramp to 240 °C, and held for 5 min. The mass spectrometer was configured for SIM in positive CI mode, with a methane flow of 2.5 mL/min. Ions of m/z 271 (m+0) and 272

(m+1) were monitored. Peak detection and area integration were as described above. The average of background isotope ratios for tissue samples collected from an uninfused animal was subtracted from sample enrichment to correct for background enrichment and obtain atom excess. Atom excess was expressed as a percentage (**APE**).

The FAME solutions (1 μL) from glucose infused animals were analyzed using a GC-combustion-isotope ratio mass spectrometer (GC-comb-**IRMS**; Trace GC Ultra coupled to a Delta V Advantage IRMS, Thermo Scientific, Waltham, MA). The GC was operated in split mode (split ratio of 100) with an injection temperature of 250 °C. Separation was achieved using a 30 m x 0.25 mm i.d. (0.25 μm film) DB-5 capillary column (Agilent technologies inc., Santa Clara, CA) with a constant helium flow of 1.0 mL/min. The initial column temperature was 120 °C which was held for 1 min followed by a 5 °C/min ramp to 240 °C, and held for 5 min. After separation, fatty acids were combusted at 1000 °C. The ionized CO_2 molecular ions of m/z 44 (m+0) and 45 (m+1) were monitored. The palmitate peak was identified using a pure unlabeled palmitate standard. Data were processed using the IsoDat software (Ver. 3.0, Thermo Scientific, Waltham, MA). The $\delta^{13}C$ ‰ value was calculated using the observed C13/C12 ratios for samples and the international standard Pee Dee Belemnite, $R_{PBD} = 0.0112372$. Atom percent excess was calculated as previously described by Schierbeek et al. (2012).

DNA was extracted from the adipose tissue samples in duplicate using the DNeasy blood and tissue kit (Cat # 69504, Qiagen, Valencia, CA) following the manufacturor's guidelines (Pasarica et al., 2008). DNA concentration and purity in each tissue sample was determined spectrophotometrically (NanoDrop 3300, Thermo Scientific, Waltham, MA) at absorbances of 260 and 280 nm. Recovery of DNA was calculated by adding known DNA concentrations to the

tissue samples and observed to be 83%. The observed recovery was used to adjust all observed values. The CV for duplicates was 12%.

Calculations and statistical analysis

Acetate and glucose turnover rates were calculated from the infusion rates and APE at steady state (Wolfe, 1984) and expressed per metabolic body weight (**MBW**).

$$Turnover\ (mmol\ xmin^{-1}x\ MBW^{-1}) = \frac{\text{Tracer infusion rate (mmol/min)}}{\text{Blood acetate or glucose APE x 0.01 x } MBW}$$

Fractional synthesis rates (FSR) of palmitate in each depot were calculated using the precursor – product method (Burd et al., 2011).

$$FSR, \%\ per\ h = \frac{\text{Lipid bound palmitate APE x 100}}{\text{Blood acetate or glucose APE x Infusion time (h)}}$$

Statistical analyses were conducted using the GLIMMIX procedure of SAS (Ver. 9.2, SAS Institute Inc., Cary, NC) and statistical significance declared at $P < 0.05$. Initially season and sire and their interactions with other main effects were tested. But as it was not significant, it was removed from models. The effect of treatment on HCW, BF, REA, KPH fat percentage, yield grade and marbling scores were analyzed using the following model. Rib eye area was adjusted for HCW.

$Y_{ij} = \mu + T_i + e_{ij}$

where Y_{ijk} = HCW, BF, REA, KPH, yield grade or marbling scores, μ = mean HCW, BF, REA, KPH, yield grade or marbling scores, T_i = fixed effect of treatment i (i =1, 2) and e_{ij} = random error.

Turnover rates of acetate and glucose, and DNA concentrations were analyzed according to 2 x 2, and 2 x 3 factorial arrangements of treatment and metabolite, and treatment and site, respectively. Animal was defined as a random effect. DNA concentrations were tested as a covariate for FSR analysis, but found to be non significant and thus excluded from the models. Fractional synthesis data were analyzed according to 2 x 2 x 3 factorial arrangement of treatment, metabolite and site. Animal was considered as a random effect. Acetate vs. glucose preference by different pairs of depots were analyzed by non orthogonal contrast statements and adjusted for Bonferroni *P* values.

Results

Early weaned steers had trend ($P = 0.06$) for greater marbling scores compared to NW steers (Table 4. 2). However, HCW, BF, REA, KPH and yield grades were similar between two treatments. The ^{2}H-enrichment of plasma acetate reached a steady state of approximately 25% APE in acetate infused steers whereas ^{13}C-enrichment of plasma glucose reached a steady state of 1% APE in glucose infused steers at 1 h post-infusion (Figure 4. 1 and Figure 4. 2). Treatment effect or interaction between treatment and metabolite on turnover rates was not significant ($P > 0.05$, Figure 4. 3). However, acetate turnover was greater than that of glucose turnover ($P = 0.04$).

There was no treatment x site interaction on DNA concentrations (Figure 4. 4). DNA concentrations were not significantly different between treatments. However, IMF had the greatest DNA concentrations (142.42 ± 4.84 ng/mg of tissue), and VF the least (77.85 ± 4.84 ng/mg of tissue).

There were no significant interactions among treatment, infusate and site for palmitate FSR (Table 4. 3). There was also no effect of treatment or site on palmitate FSR. However, FSR was significantly greater ($P = 0.0002$) from acetate than from glucose. Acetate vs. glucose preference by different pairs of depots was not significantly different. The ratio of palmitate FSR from acetate to that from glucose was 10.61, 13.80 and 15.79 in SCF, IMF and VF, respectively.

Discussion

The APE in plasma of both acetate and glucose infused animals came to a plateau within 1 hour of the infusion start as previously observed by Dunshea et al. (1992) and Greathead et al. (2001) in sheep and steers. However, APE for glucose varied during the last 2 h of infusion compared to other time points. This likely reflects some minor stress associated with the arrival of personnel to prepare for harvest. With compared to acetate turnover rates of 100 mmol x MBW^{-1}x d^{-1}in our study, Al-Mamun et al. (2010) observed acetate and glucose turnover rates of 151 and 38.4 mmol x MBW^{-1} x d^{-1} when using a primed continuous infusion in sheep kept in a thermo-neutral environment. Al-Mamun et al. (2010) fed high forage diets thus it is likely that increase in acetate from rumen fermentation led to more circulating acetate. This further explains the higher acetate turnover rate compared to ours. Therefore, observed acetate turnover herein is slightly less than those of Al-Mamun et al. (2010) which may reflect the feedlot diet being consumed at the time of slaughter by our steers. Further, observed numerical differences in acetate turnover rates between two treatment groups were consistent with the higher acetate synthesis rate we observed in previous study for NW steers at harvest. The groups were fed the

same diet for at least 120 d and feed intake was not significantly different. Therefore, the observed differences may be due to alterations of gut microflora because acetate primarily arises from ruminal fermentation. The observed glucose turnover rate of 60 mmol x MBW^{-1} x d^{-1} is fairly close to the glucose turnover rate of 50 mmol x MBW^{-1}x d^{-1} observed by Sano et al. (2007) in sheep fed high energy diets.

We observed a trend for higher marbling scores in EW steers as expected. However, early weaning followed by grain feeding did not affect palmitate FSR in IMF, and thus does not explain the carryover effect on marbling(Scheffler et al., Personnel communication). Therefore, the mechanism behind the higher marbling in metabolically imprinted group may be due to establishment of a large population of undifferentiated cells during the early grain feeding period. Our data showed that there were no differences in FSR across depots at harvest. However, Vernon (1980) reported that the development order for fat depots was visceral followed by intermuscular, SCF and IMF. It is possible that differences in fat synthesis existed earlier and dissipated as the animal reached the end of the grain feeding. Because tissues were obtained at harvest, and animals were at a stage where all three depots had filled, this might be the reason for similar FSR across the depots.

Our data clearly showed that FSR from acetate is much greater than that from glucose which demonstrates that the contribution of acetate to fatty acid synthesis is greater than that of glucose. Similarly, Lee et al. (2000) and Song et al. (2001) observed higher rates of acetate radioisotope incorporation in both subcutaneous and intramuscular adipose tissues than that of glucose *in vitro*. Hanson and Ballard (1967) also reported that more acetate than glucose was converted into lipids in ruminant adipose tissues. Moreover, as with the previous study, we did

not observe any differences in plasma acetate, glucose and insulin concentrations at harvest between two treatment groups.

Smith and Crouse (1984) concluded that intramuscular adipocytes prefer glucose as a substrate for fat synthesis while subcutaneous adipocytes prefer acetate. However, no differential preference for acetate over glucose by IMF or SCF was observed in the current study. They observed a ratio of acetate incorporation into fatty acids to glucose incorporation of 0.3 in IMF whereas it was 33 in SCF. Lee et al. (2000) reported *in vitro* incorporation ratios into subcutaneous and intramuscular adipose tissues of 1.61 and 1.23 respectively for Hanwoo steers, and Song et al. (2001) observed ratios of 1.25 and 1.27, respectively for the same breed. Smith and Crouse (1984) and Song et al. (2001) used 5 mM concentrations of acetate and glucose whereas Lee et al. (2000) used 2.5 mM and 5 mM acetate and glucose respectively in the media suggesting that culture differences likely do not explain the large range in observed ratios. There was some difference in age at slaughter between steers used by Smith and Crouse (1984) and those used in the other two studies. Miller et al. (1991) suggested lipid metabolism may be unique to each adipose tissue and differences could be observed with different breeds. This may be one of the reasons for various responses at different experimental stations. Our data showed that FSR from acetate:glucose were 10.61 and 13.80 in SCF and IMF, respectively which is not reflected in any of the *in vitro* studies suggesting the *in vitro* systems may not be valid. One challenge with the *in vitro* system is that tissues are in a net degradative state which may underestimate lipogenesis (Greathead et al., 2001).

In conclusion, early grain feeding does not affect acetate or glucose turnover and fat synthesis at harvest. Acetate turnover is greater than that of glucose turnover and FSR is greater from acetate than from glucose. However, there is no difference in preference for acetate over

glucose by IMF, SCF or VF suggesting that feeding by-products or low quality energy sources may not have negative impacts on the deposition of IMF in finishing steers while they are receiving the required energy intake.

References

Al-Mamun, M., M. Sawada, and H. Sano. 2010. Plasma acetate and glucose turnover rates in sheep exposed to cold. In: Energy and protein metabolism and nutrition, 3rd EAAP International Symposium on Energy and Protein Metabolism and Nutrition, Parma, Italy, 6-10 September, 2010 p127- 128.

Barker, D. J. P. 1995. The fetal and infant origins of disease. Eur. J. Clin. Invest. 25: 457-463.

Burd, N., D. West, T. Rerecich, T. Prior, S. Baker, and S. Phillips. 2011. Validation of a single biopsy approach and bolus protein feeding to determine myofibrillar protein synthesis in stable isotope tracer studies in humans. Nutr. Metab. 8: 15.

Chouinard, P. Y., L. Corneau, D. M. Barbano, L. E. Metzger, and D. E. Bauman. 1999. Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows. J. of Nutr. 129: 1579-1584.

Christie, W. W. 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesteryl esters J. Lipid Res. 23: 1072-1075.

Dunshea, F. R., D. M. Harris, D. E. Bauman, R. D. Boyd, and A. W. Bell. 1992. Effect of porcine somatotropin on in vivo glucose kinetics and lipogenesis in growing pigs. J. Anim. Sci. 70: 141-151.

Fluharty, F. L., S. C. Loerch, T. B. Turner, S. J. Moeller, and G. D. Lowe. 2000. Effects of weaning age and diet on growth and carcass characteristics in steers. J. Anim. Sci. 78: 1759-1767.

Gotoh, T., K. Etoh, K. Saitoh, K. Metoki, S. Kaneda, T. Abe, T. Etoh, Y. Shiotsuka, R. Fujino, K. Matsuda, H. Suzuki, H. Hasebe, F. Ebara, J. Wegner, and S. Tabata. 2010. Metabolic imprinting effect in beef production: Influence of nutrition manipulation during an early

growth stage on carcass characteristics and intramuscular fat content of longissimus muscle in wagyu (japanese black) Eaap publication no. 127. p 669-670. Wageningen Academic Publishers, Wageningen.

Greathead, H. M. R., J. M. Dawson, N. D. Scollan, and P. J. Buttery. 2001. In vivo measurement of lipogenesis in ruminants using [1-c]acetate. Br. J. Nutr. 86: 37-44.

Hanley, B., J. Dijane, M. Fewtrell, A. Grynberg, S. Hummel, C. Junien, B. Koletzko, S. Lewis, H. Renz, M. Symonds, M. Gros, L. Harthoorn, K. Mace, F. Samuels, and E. M. van Der Beek. 2010. Metabolic imprinting, programming and epigenetics – a review of present priorities and future opportunities. Br. J. Nutr. 104: S1-S25.

Hanson, R. W., and F. J. Ballard. 1967. The relative significance of acetate and glucose as precursors for lipid synthesis in liver and adipose tissue from ruminants. Biochem. J. 105: 529-536.

Hara, A., and N. S. Radin. 1978. Lipid extraction of tissues with a low-toxicity solvent. Anal. Biochem. 90: 420-426.

Kristensen, N. B. 2000. Quantification of whole blood short chain fatty acids by gas chromatographic determination of plasma 2-chloroethyl derivatives and correction for dilution space in erythrocytes`. Acta Agric. Scand., Sect. A 50: 231-236.

Lee, S. C., H. J. Lee, D. W. Kim, J. G. Park, and I. K. Han. 2000. Effects of carbon precursors and hormones on the lipogenesis and lipolysis of hanwoo cattle adipose tissues. Asian-Australasian J. Anim. Sci. 13: 300-306.

Miller, M. F., H. R. Cross, D. K. Lunt, and S. B. Smith. 1991. Lipogenesis in acute and 48-hour cultures of bovine intramuscular and subcutaneous adipose tissue explants. J. Anim. Sci. 69: 162-170.

Myers, S. E., D. B. Faulkner, F. A. Ireland, and D. F. Parrett. 1999. Comparison of three weaning ages on cow-calf performance and steer carcass traits. J. Anim. Sci. 77: 323-329.

Notter, S. J., B. H. Stuart, B. B. Dent, and J. Keegan. 2008. Solid-phase extraction in combination with gc/ms for the quantification of free fatty acids in adipocere. Eur. J. Lipid Sci. and Technol. 110: 73-80.

Pasarica, M., S. Loiler, and N. Dhurandhar. 2008. Acute effect of infection by adipogenic human adenovirus ad36. Arch. Virol. 153: 2097-2102.

Sano, H., H. Sawada, A. Takenami, S. Oda, and M. Al-Mamun. 2007. Effects of dietary energy intake and cold exposure on kinetics of plasma glucose metabolism in sheep. J. Anim. Physiol. and Anim. Nutr. 91: 1-5.

Scheffler, J. M., M. A. McCann, S. P. Greiner, H. Jiang, M. D. Hanigan, G. A. Bridges, S. L. Lake, and D. E. Gerrard. Personnel communication. Early metabolic imprinting events increase marbling scores in fed cattle.

Schierbeek, H., C. H. P. van den Akker, L. B. Fay, and J. B. van Goudoever. 2012. High-precision mass spectrometric analysis using stable isotopes in studies of children. Mass Spectrom. Rev. 31: 312-330.

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.

Song, M. K., H. J. Sohn, S. K. Hong, and H. C. Kim. 2001. Utilization of substrate for in vitro lipid synthesis in adipose tissue of hanwoo steers. Asian-Aust. J. Anim. Sci. 14: 1560-1563.

Tserng, K. Y., and S. C. Kalhan. 1983. Estimation of glucose carbon recycling and glucose turnover with [u-13c] glucose. Am. J. of Physiol. 245: E476-E482.

USDA. 1997. United states standards for grades of carcass beef, AMS, USDA, Washington, DC.

Vernon, R. G. 1980. Lipid metabolism in the adipose tissue of ruminant animals. Prog. Lipid Res. 19: 23-106.

Wolfe, R. R. 1984. Tracers in metabolic research, radioisotope and stable isitope/mass spectrometry methods. Alan R. Liss, Inc., New York.

Table 4. 1. Ingredient composition of the grain mixes fed to calves after early weaning and to all animals in the feedlot.

Ingredient	Grain mix composition, % (DM basis)					
	Early grain feeding[1]					Feedlot[2]
	Step 1	Step 2	Step 3	Step 4	Step 5	
Corn, cracked	35.0	40.0	40.0	41.0	41.0	50.0
Distillers grain	15.0	15.0	15.0	15.0	15.0	-
Soybean meal (48% CP)	14.0	9.5	5.0	1.5	-	-
Corn gluten feed	9.50	9.0	11.0	12.0	15.0	37.0
Wheat midds	10.0	10.0	11.0	12.0	10.5	10.0
Cottonseed hulls	8.00	8.0	9.5	10.0	10.0	-
Cane molasses	6.00	6.00	6.00	6.00	6.00	-
Limestone 38%	2.00	2.00	2.00	2.00	2.00	2.40
Salt	0.50	0.50	0.50	0.50	0.50	0.25
Vitamin ADE premix	0.02	0.02	0.02	0.02	0.02	0.02
Rumensin	-	-	-	-	-	0.02

[1]Initial ration fed to early weaned calves from weaning at 105 ± 4 and 102 ± 5 d of age to 253 ± 5 and 202 ± 5 d of age for fall and spring groups respectively. Calves were transitioned from the initial to final diet over five phases. Balance weights for each steps: step 1 = 136 kg, step 2 = 159 kg, step 3 = 182 kg, step 4 = 204 kg and step 5 = 227 kg

[2]Feedlot ration provided to all steers from 393 ± 5 and 374 ± 5 d of age for fall and spring groups, respectively to end of feedlot period

Table 4. 2. Least square means of carcass characteristics of normal (NW) and early weaned (EW) steers.

Variable	Treatment		SEM	*P*-value
	NW	EW		
HCW, kg	315	321	7.00	0.56
BF, cm	0.90	1.15	0.09	0.07
REA, sq. cm	78.95	80.12	2.18	0.71
KPH, %	2.66	2.44	0.18	0.35
Yield grade	2.69	2.78	0.19	0.73
Marbling scores*	491	568	22.0	0.06

*Sire was significant, thus included in the model.

Table 4. 3. Least square means of palmitate fractional synthesis rates (FSR) from acetate and glucose in normal (NW) and early weaned steers (EW) continuously infused with [$^{2}H_3$] acetate or [U-$^{13}C_6$] glucose for 12 h.

Isotope	Site[1]	FSR, %/h		SEM	*P* value
		NW	EW		
Acetate	IMF	0.29	0.31	0.06	1.00
Acetate	SCF	0.21	0.33	0.06	0.97
Acetate	VF	0.28	0.22	0.06	1.00
Glucose	IMF	0.02	0.02	0.06	1.00
Glucose	SCF	0.02	0.03	0.06	0.99
Glucose	VF	0.01	0.02	0.06	1.00
Acetate vs. Glucose	IMF vs. SCF			0.05	0.94
Acetate vs. Glucose	IMF vs. VFA			0.05	0.83
Acetate vs. Glucose	SCF vs. VF			0.05	0.94
Acetate vs. Glucose	Average (IMF and SCF) vs. VF			0.06	0.99

[1] IMF = intramuscular fat, SCF = subcutaneous fat and VF = visceral fat

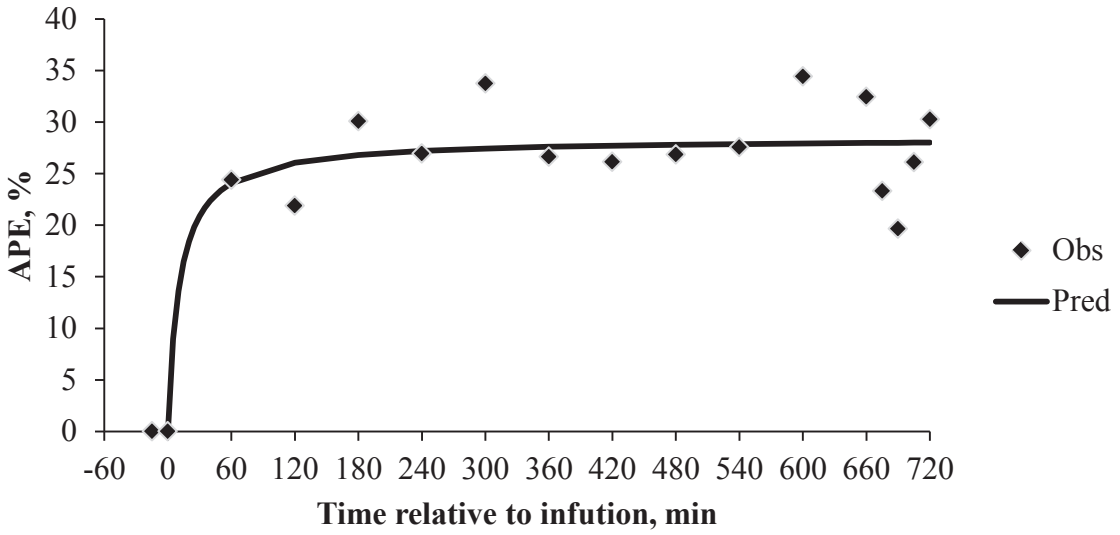

Figure 4. 1. Plasma acetate enrichment expressed in atom percent excess (APE) during a 12 h infusion, Predicted APE = 28 / (1+(11/Time)).

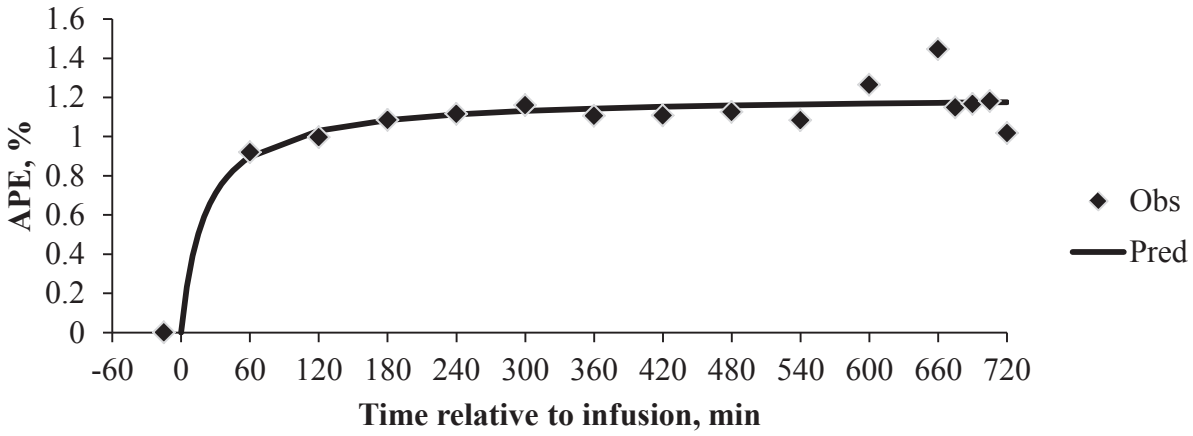

Figure 4. 2. Plasma glucose enrichment expressed in atom percent excess (APE) during a 12 h infusion, Predicted APE = 1.3 / (1+(21/Time)).

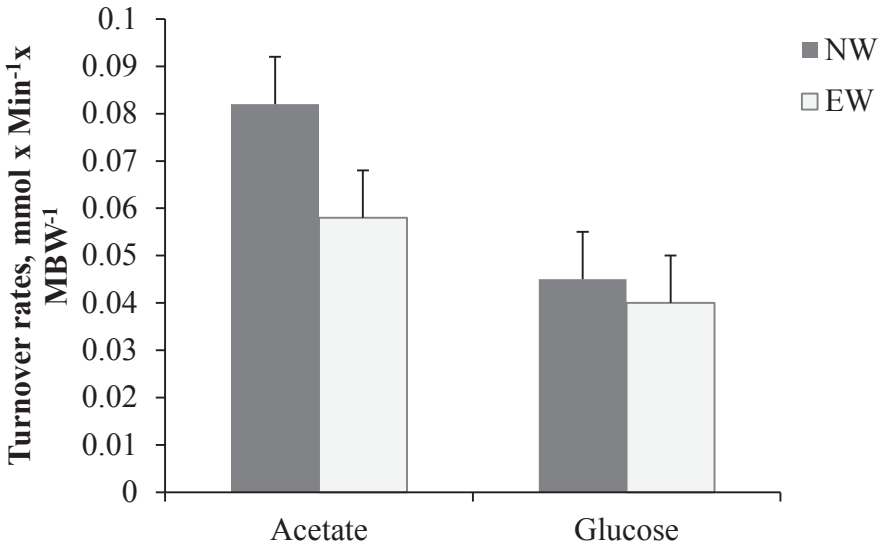

Figure 4. 3. Turnover rates of acetate and glucose in normal weaned (NW) and early weaned (EW) steers infused with [$^{2}H_3$] acetate and U-$^{13}C_6$] glucose isotope. Means across metabolite differ ($P < 0.05$).

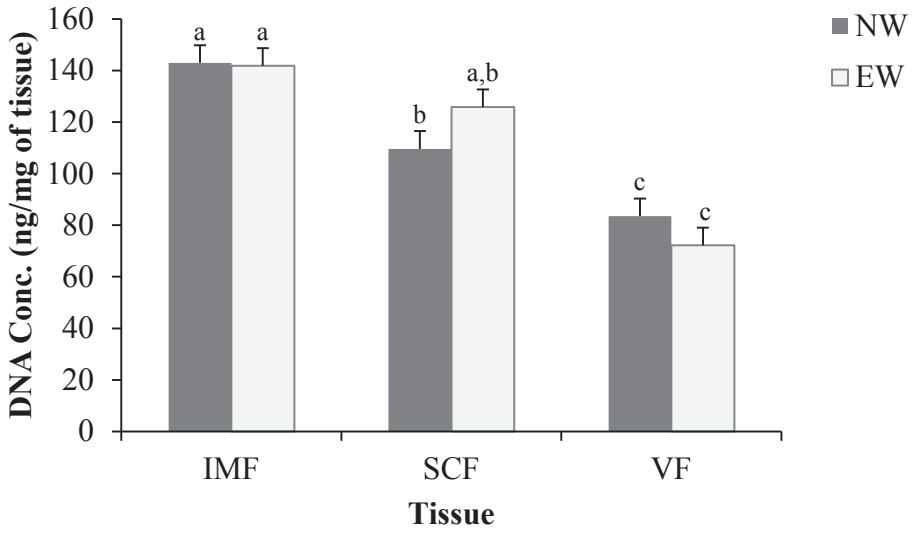

Figure 4. 4. DNA concentrations in adipose tissue obtained from normal weaned (NW) and early weaned (EW) steers at the time of slaughter. IMF = intramuscular fat, SCF = subcutaneous fat and VF = visceral fat dissected out after conclusion of infusion at harvest. [a, b, c] Means across treatment x depot differ ($P < 0.05$).

CHAPTER 5

Summary and Conclusions

In the first study, we hypothesized that signaling protein phosphorylation and acetate clearance rates would be affected by a period of high-grain feeding after early weaning (EW) compared to normal weaning (NW). The aims of first study were to determine the effects of early weaning followed by a period of high-grain feeding on signaling protein phosphorylation in muscle and on acetate clearance in growing steers. Specific findings of this study were:

- There were no treatment differences in signaling protein phosphorylation ratios for any sampling times except ribosomal protein S6 and ribosomal protein S6 kinase 1.
- Phosphorylated:total ratios of S6K1, rpS6 and 4EBP1 were positively correlated with ADG.
- Early weaned calves had greater ADG during the early grain feeding period while NW calves had greater ADG during the subsequent grazing period.
- Early weaned steers had greater marbling scores and carcass weight compared to NW steers.
- Acetate clearance rates were less and synthesis rates were greater in EW steers during early grain feeding period.
- Acetate clearance increased when steers were heavier and older.
- Acetate synthesis and clearance were greater in NW steers at harvest.
- Plasma glucose and insulin concentrations were not affected by acetate infusion.
- Plasma β-hydroxy butyrate concentrations were increased with acetate infusion.

In conclusion, phosphorylation ratios of signaling proteins were not affected treatment. Results of the study indicated that early grain feeding increased rates of acetate appearance and reduced acetate clearance during early grain feeding period in growing steers. Acetate clearance increased when steers were heavier and older. Thus older calves are able to clear more acetate per unit of time and body mass than younger calves reflective of an enhanced ability to utilize the substrate.

In the second study, we hypothesized that carry over effects of early grain feeding period may affect acetate and glucose turnover rates and hence fat synthesis but differential preference for acetate and glucose may not exist in different depots. Therefore, the objectives of this study were to assess the effects of early grain feeding on acetate and glucose turnover rates, palmitate synthesis, and on acetate and glucose preference by subcutaneous, intramuscular and visceral adipose tissues in finishing steers. Specific findings of this study were:

- DNA concentrations were significantly different by depot.
- Acetate and glucose turnover rates and palmitate fractional synthesis rates (FSRs) were similar between EW vs. NW steers.
- Acetate turnover and palmitate FSR from acetate were greater than that of glucose turnover and FSR from glucose, respectively.
- There were no significant differences for palmitate FSRs among depots.

In conclusion, early grain feeding did not affect acetate or glucose turnover and fat synthesis. Acetate turnover was greater than that of glucose turnover and FSR was greater from acetate compared to the glucose. Differences in acetate and glucose preference by major fat depots did not exist in finishing feedlot steers. Since intramuscular fat does not have a

preference for glucose, finishing programs do not require high starch diets to achieve acceptable levels of marbling. Therefore, high energy diets can be constructed from a combination of low starch by-products, dietary fat, and forage to achieve the energy content needed to finish the animal. This will reduce the grain use and increase usage of by-products.

Early weaned steers synthesized more acetate during early grain feeding period. Feeding excess energy causes storage of fat in existing adipocytes and development of new adipocytes. With our findings, it seems intramuscular fat cells formed during the early grain feeding period have not responded differently to nutrient signals in the feedlot. It is likely that the early grain feeding period induced an expansion of the adipocyte population. These newly formed cells may be retained through the grass feeding period, and it may require less energy excess to refill these existing adipocytes than to establish new adipocytes and subsequently fill those cells. Future studies are necessary to clarify this mechanism.