INFLUENCE OF DIETARY ENERGY SOURCE ON IN VITRO SUBSTRATE UTILIZATION AND INSULIN SENSITIVITY OF MUSCLE AND ADIPOSE TISSUE OF BEEF CATTLE

A Dissertation

by

RYAN D. RHOADES

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2008

Major Subject: Animal Science

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Approved by:

Chair of Committee,	Jason E. Sawyer
Committee Members,	David P. Anderson
	David K. Lunt
	Stephen B. Smith
Head of Department,	Gary Acuff

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ABSTRACT

Influence of Dietary Energy Source on in vitro Substrate Utilization and Insulin Sensitivity of Muscle and Adipose Tissue of Beef Cattle. (August 2008)

Ryan D. Rhoades, B.S., Oklahoma State University;

M.S., Texas A&M University

Chair of Advisory Committee: Dr. Jason E. Sawyer

Beef carcass value is influenced by the quantity and distribution of adipose tissue. Elucidation of metabolic controls of caloric partitioning between adipose depots could lead to development of production solutions that enhance beef carcass value. Historical trends in Choice and Select beef supply and short-term demand structures for Choice and Select boxed beef were explored. Recent stabilization in slaughter mix may suggest an optimum is being approached. Evaluation of short-run demand supports this premise, and suggests that Choice and Select products may not be strong substitutes. Growth-based prediction equations relating carcass traits to growth traits using ultrasound measurements as the basis of projections under different growing systems were explored. Accuracy of carcass fat predictions from growth-based equations is influenced by weight gain between ultrasound and endpoint, breed, and gender; scans out to 120 d pre-harvest may be accurate. Angus steers were used to test effects of dietary energy source on muscle and adipose tissue metabolism and insulin sensitivity. Results suggest that feeding hay limited both glucose supply and tissue capacity to

increase glucose utilization in response to insulin without altering acetate conversion to fatty acids. Because subcutaneous (s.c.) adipose tissue consistently utilized more acetate and oxidized more glucose than intramuscular (i.m.), these results suggest that hay-based diets may alter i.m. adipose tissue metabolism with less impact on s.c. adipose tissue. Additionally, s.c. adipose tissue may become resistant to insulin in steers fed to an excessive s.c. fat thickness. A final experiment was designed to test the effects of dietary energy source during backgrounding and compositional endpoint on adipose tissue metabolism and insulin sensitivity. Feeding hay during backgrounding may have differential effects on tissue lipogenesis. Feeding hay increased both glucose oxidation and incorporation of acetate into fatty acids; in i.m. insulin failed to stimulate glucose conversion to lipid. As physiological maturity increases, glucose conversion to CO_2 and lactate increased, but the ability of insulin to stimulate lipid synthesis from glucose may be reduced. These data provide foundation for a hypothesis regarding diet-mediated regulation of differential adipose tissue metabolism. Validation of these hypotheses could generate nutritional strategies that alter the rate and site of adipose deposition.

DEDICATION

To my mother.

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I would like to thank my committee chair, Dr. Sawyer, and my committee members, Dr. Anderson, Dr. Lunt, and Dr. Smith for their guidance and support throughout the course of my time spent here at Texas A&M University. It has been a great pleasure to get to know and work so closely with each of you.

Thanks also go to my friends Flavio Ribeiro, Judson Vasconcelos, Christian Ponce, Dustin Dean, Andy King and other colleagues and the department faculty and staff for making my time at Texas A&M University a great experience.

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CHAPTER I

INTRODUCTION

Beef carcass value is influenced by the quantity and distribution of adipose tissue. Production systems utilizing high proportions of roughage typically result in lower value carcasses than those using high concentrate strategies (Schaake et al., 1993) and those that increase subcutaneous fat deposition excessively increase production costs while decreasing carcass value (Wertz et al., 2001). Recent reports have suggested that quality grade within the United States beef supply has declined dramatically over the last 20 years. Various production practices and management decisions have been implicated as causative for this decline. The continuing trend toward value-based marketing (e.g., grid pricing of carcasses) and relatively high Choice-Select price spreads, coupled with reports of declining quality grade, have caused beef producers to be concerned that value is being lost through production of lower quality product. National Beef Quality Audit (NBQA) data have been published (Lorenzen et al., 1993; Boleman et al., 1998; McKenna et al., 2002) or presented for years 1991, 1995, 2000, and 2005. These reports cited insufficient marbling as the largest source of economic loss in the beef industry. The most recent NBQA (2005) reported that a lack of premium beef production cost the industry $26.81 per head in lost opportunity as demand is strongest for high-quality beef.

Intramuscular (i.m.) fat or marbling is the most significant factor affecting the quality grade assigned to a carcass. An increase in i.m. fat deposition ultimately

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This dissertation follows the style and format of the Journal of Animal Science.

increases the value of carcasses. Consumers are willing to pay a premium for additional marbling, as marbling enhances beef tenderness, juiciness, and overall palatability (Feuz et al., 2004). Subcutaneous (s.c.) fat or external fat is the primary factor used to determine the yield grade of a carcass. An increase in s.c. fat deposition increases the yield grade and excessive waste of carcasses, thus decreasing carcass value. Pyatt et al. (2005) reported that marbling score and yield grade accounted for 10 and 8% of the variation in carcass value, while these traits also accounted for 18 and 12% of the variation in profit among steers used in this study. Current finishing strategies have been implemented to increase both weight and marbling score by feeding cattle for a longer period of time. This trend has resulted in a steady increase in annual hot carcass weight among our nations beef supply. However, these strategies can also elevate producer risk, as feeding cattle longer can increase yield grade resulting in increased discounts for excessive waste.

Strategies that increase quality grade without negatively impacting yield grade could increase carcass value while minimizing the risk of additional discounts. It has been well established that s.c. and i.m. adipose tissues differ in their preferred substrate for synthesis (Smith and Crouse 1984) and their rate of development (Bruns et al., 2004). These differences could potentially allow for the manipulation of accretion rates within specific fat depots. Smith and Crouse (1984) demonstrated that i.m. adipocytes utilize glucose while s.c. adipocytes utilize acetate as primary substrates for fatty acid synthesis. Therefore, it is plausible that an increase in the supply of dietary glucose might result in an increase in i.m. deposition relative to s.c. tissue. Elucidation of metabolic controls of

caloric partitioning between adipose depots could lead to development of production solutions that enhance beef carcass value.

CHAPTER II

REVIEW OF LITERATURE

Subcutaneous and Intramuscular Fat Accretion

Adipose tissue development occurs at specific sites throughout the body and form from an accumulation of adipocytes that are filled with triglyceride (Gerrard and Grant, 2003). Accretion is defined as the deposition of material between cells. Formation of an adipocyte begins with mesenchymal cells giving rise to adipoblasts. Adipoblasts collect giving rise to a larger lobe and as adipogenesis continues cells proliferate. During growth, fat accumulates as a result of hyperplasia (i.e., increase in cell number) via fat cell division and differentiation or recruitment of preadipocytes; and by hypertrophy (i.e., increase in cell size) via lipid filling increasing cell volume. Cells from different fat depots (i.m. and s.c.) differ dramatically in both cell size and cell number. Smith and Crouse (1984) reported that mean diameter of i.m. cells was smaller than s.c. cells, but i.m. tissue had a greater number of preadipocytes than s.c. tissue. It has also been reported that i.m. cells account for 45% of the total adipose tissue cells in a mature bovine but these i.m. cells only account for 15% of the total fat volume; therefore, total number of i.m. cells is more important in determining the quantity of lipid in bovine muscle.

Growth of adipose tissue by hypertrophy increases with age (Gerrard and Grant, 2003) and is affected by genetics and nutrition. Classically, it has been accepted that fat

depots develop chronologically, internal fat followed by subcutaneous, intermuscular, and intramuscular (Gerrard and Grant, 2003).

Trenkle et al. (1978) reported that intramuscular fat deposition is dependent upon both age and body weight. In this study, steers were fed either ad libitum or limit fed and serially slaughtered at various weights ranging from 110-500 kg in body weight. Results showed that extractable lipid increased in limit fed steers at 500 kg but not at 360 kg. Penthick et al. (2004) also reported that amount of i.m. is related to age of the animal since deposition occurs later in life. However, the age at which lipogenesis and adipocyte growth occurs is highly related to the age at which cattle are started on a high concentrate diet. Fluharty et al. (2000) reported that 85% of early weaned steers that were started on a high concentrate diet and harvested at 385 d of age graded Choice. These results suggest that age has less influence on i.m. deposition than the length of time cattle have been fed a high concentrate diet.

Dietary energy amount and source affect fat accretion depending on the depot evaluated. Studies conducted to evaluate the affect of restricted vs ad libitum feed intake on i.m. fat deposition (Yelich et al., 1995; Knoblich, 1997; and Loerch and Fluharty, 1998) concluded that feeding strategy had no impact on marbling score. Schoonmaker et al. (2004a) reported that s.c. fat increased when steers were fed a high-energy diet for a longer period of time. Additionally, when steers were fed a low-energy diet there is a greater decrease in s.c. fat deposition than i.m. fat deposition. Therefore, increases in the amount of energy fed may result in a greater increase in s.c. fat than i.m. fat. However, it is generally accepted that feeding a high-grain diet vs a high-forage will result in greater

i.m. fat deposition. Overall the effects of nutrition on fat accretion maybe inconclusive, as it was proposed that the greater i.m. fat deposition in cattle fed a high-grain diet was likely a result of greater net energy available for synthesis (Pethick, 2004).

Duckett et al. (1993) and May et al. (1992) expressed marbling accretion as a function of days on feed, which resulted in quadratic response, where marbling increased at a decreasing rate. In these studies, marbling accretion seemed to reach a plateau after approximately 110-112 d on feed within the feedlot phase. These results suggest that marbling accretion is quadratic or reaches a plateau toward the end of the feeding period. Conversely, Bruns et al. (2004) expressed marbling accretion as a function of change in hot carcass weight, which resulted in a linear response, where marbling increased linearly throughout the range of hot carcass weights reported (200-400 kg). This study provides evidence that marbling accretion should be considered a lifetime event, rather than simply a late maturing depot. Bruns et al. (2004) also reported that total carcass fatness increased in a quadratic fashion with increasing hot carcass weight, suggesting that the slope of marbling and total carcass fatness lines are different. Early in the growth period quality grade increases more rapidly than yield grade. Thus, these authors suggest that early management maybe the key to altering final quality grade. Evaluating fat accretion as a function of growth rather than time on feed could potentially lead to the development of nutritional strategies that manipulate fat deposition.

Tissue Substrate Utilization

Glucose is not an important precursor for lipogenesis in bovine s.c. adipose tissue (Smith, 1983). Acetate and lactate are the primary carbon sources for fatty acid synthesis

(Smith and Crouse, 1984). However, glucose is required for lipogenesis in ruminant adipose tissue as a precursor for glyceride-glycerol and the major source of NADPH required for fatty acid synthesis (Smith, 1983). Additionally, s.c. and i.m. differ in substrate preference. Smith and Crouse (1984) demonstrated that i.m. adipocytes utilize glucose while s.c. adipocytes utilize acetate as primary substrates for fatty acid synthesis. In this study, glucose provided 50-75% of the acetyl units for fatty acid synthesis in i.m. tissue, but only provided 1-10% of the acetyl units for fatty acid synthesis in s.c. tissue. Alternatively, acetate provided 70-80% of the acetyl units for fatty acid synthesis in s.c. tissue, but only 10-25% of the acetyl units in i.m. tissue (Smith and Crouse, 1984). These authors also concluded that different regulatory processes control fatty acid synthesis in i.m. and s.c. adipose tissue. Therefore, the enzymes responsible for fatty acid synthesis, and therefore lipogenesis and adipocyte hypertrophy, are regulated by the end products of ruminal fermentation, which are determined by diet. Hood and Allen (1978) previously demonstrated that acetate is incorporated into fatty acids at a greater rate in s.c. tissue than i.m. tissue. These differences may allow us to manipulate i.m. fat accretion relative to s.c. accretion by increasing the available glucose supplied by a diet. Adipose tissue synthesis requires a source of fatty acid and glycerol 3-phosphate, almost all of which comes from glucose. However for cattle that are grazing forages, acetate is the major fatty acid precursor for adipocyte synthesis. When animals are fed a high concentrate diet, the amount of propionate produced increases relative to acetate.

Dietary Sources of Tissue Substrate

Smith (1995) stated that the age of an animal dictated the timing of the onset of lipogenesis, but the diet influenced the rate of lipogenesis. However, Smith et al. (1984) reported that backfat thickness and the activities of several enzymes involved in lipogenesis were greater in steers fed a high concentrate, corn based diet vs steers fed a forage-based, alfalfa pellet diet, even though the energy intake was higher with the forage-based diet. Therefore, the end products of ruminal fermentation as well as net energy intake are interrelated with regard to adipocyte formation. Volatile fatty acids (VFA's) provide 70-80% of the energy requirements by a bovine animal. Different carbohydrate sources yield different VFA's when fermented in the rumen. High-concentrate feedstuffs produce a greater proportion of propionic acid than do forage diets (Orskov et al., 1991), and propionic acid is a preferred glucogenic substrate in ruminants. Diets higher in starch promote i.m. fat deposition relative to s.c. deposition (Choat et al., 2003). Choat et al. (2003) reported increased i.m. adipose tissue deposition in steers fed a concentrate diet that generated 39.3% greater propionate. Schaake et al. (1993) found that feeding grain or high-concentrate diets increased i.m. adipose tissue content relative to forage feeding. Propionate is both glucogenic and insulinogenic in ruminants (Sano et al., 1995), which may enhance i.m. deposition. Conversely, a roughage-based diet provides greater concentrations of acetate. Acetate is used less efficiently for tissue growth, since diets that contain high acetate: propionate ratio may lack an adequate supply of glucogenic precursors to provide a source of NADPH for fatty acid synthesis. Acetate loading may increase glucose demand (Cronje et al., 1991) or increase ketone

load. Ketone bodies may accumulate under acetate loading, particularly when glucose is limiting (Herdt et al., 1981). Recent studies (Schoonmaker et al., 2003; 2004a,b) reported no differences in performance due to changes in source and amount of energy, yet differences in carcass adiposity were present, suggesting that dietary source influenced partitioning of energy into different fat depots. Prolonged exposure to forage-based diets may decrease carcass value (quality) by promoting insulin insensitivity; weakening the ability to deposit i.m. fat (Sainz and Paganini, 2004). The major volatile fatty acids (VFA) produced by rumen microorganisms are acetate, propionate, and butyrate. These VFA are the main products of the digestion of feed by bacteria in the rumen, and serve as the main precursors for both glucose and fat in ruminants. On a forage based diet, the proportion of VFA would be approximately 65-70% acetate, 15-25% propionate, and 5-10% butyrate. Feeding diets high in readily fermentable carbohydrate (starch) increases the proportion of propionate produced through ruminal fermentation, and results in VFA proportions of approximately 50-60% acetate, 35-45% propionate, and 5-10% butyrate. Recent research by Johnson et al. (1982) and Bines and Hart (1984) found that increased peak insulin concentrations with increased propionate production will also lead to increased insulin secretion.

Insulin Sensitivity

Insulin stimulates peripheral tissue uptake of glucose, and increases lipogenesis or reduces lipolysis; also, plasma insulin concentration is positively correlated with carcass adiposity (Trenkle and Topel, 1978). Insulin regulates blood glucose, implying that the high-concentrate steers experienced greater glucose uptake, which is consistent

with the theory that propionate stimulates an insulin response (Sano et al., 1995). Schoonmaker et al. (2003) found greater levels of insulin in steers fed a high-concentrate diet than in steers fed a high-forage diet during the growing phase. However, negative correlations between plasma glucose concentrations and carcass adiposity have been reported (Matsuzaki et al., 1997; Schoonmaker et al., 2003). Variation in insulin sensitivity may impact caloric partitioning among tissues and tissue development. Gilbert et al. (2003) suggests that i.m. tissue is more sensitive to insulin than s.c. tissue. Ketone body accumulation (Tardif et al., 2001) and diet (Waterman et al., 2006) may impact insulin sensitivity and thus energy partitioning in cattle. A mechanism for this effect was provided by Tardif et al. (2001) who demonstrated that accumulation of ketones interrupted insulin signal transduction and reduced GLUT-4 migration to cell surfaces. This reduction in the insulin-sensitive glucose transporter translocation would reduce insulin-stimulated glucose uptake, and thus would limit the rate of glucose metabolism. Previous literature would also suggest that roughage feeding inhibits insulin action. When steers were fed an alfalfa diet, insulin failed to stimulate measures of lipogenesis (Smith et al., 1983). However, several examples with concentrate-fed cattle have demonstrated insulin effects on adipose metabolism, and that this activity increased with amount of concentrate in the diet (Baldwin et al., 1973; Miller et al., 1989; Miller et al., 1991).

CHAPTER III

HAS BEEF QUALITY GRADE DECLINED?

Overview

The objectives of this review were to quantify historical trends in Choice and Select beef supply based on annual output of federally inspected product and to explore short-term demand structures for Choice and Select boxed beef. Data (1938-2005) were acquired from USDA for pounds of federally graded Choice, Select and inspected beef. Data were analyzed as a time series using a regression model including linear, quadratic and cubic effects of time. Additionally, daily supply and demand data for boxed beef price were recorded, aggregated into weekly averages, and elasticity estimates calculated. There was a quadratic effect ($P < 0.01$) of time on amount of Choice beef produced over the entire time span; output increased at a decreasing rate. There was no effect ($P = 0.18$) of time on Choice output from 1997 to 2005; product output remained consistent. Over the entire time span there was a cubic effect ($P < 0.01$) of time on amount of Good/Select beef produced, resulting from relatively stable product volume from 1938 to 1986, followed by rapid escalation until 2000, followed by another period of stability. Short-run, Choice elasticity (-0.39) suggests limited change in weekly demand for Choice product at any price level. Price change has a relatively greater impact on Select beef demand (-0.90). Increases in Select beef output have resulted from increases in beef presented for grading. Recent stabilization in slaughter mix may

suggest an optimum is being approached. Evaluation of short-run demand supports this premise, and suggests that products may not be substitutes.

Introduction

The common interpretation of USDA quality grade data would suggest that quality grade within the United States beef supply has declined dramatically over the last twenty years. Specifically, the hypothesis is that the apparent decline in percentage of Choice grade beef has been the result of a significant increase in percentage of Select grade beef, and that this has represented a shift downward in overall beef quality. Various production practices and management decisions have been implicated as causative agents for this decline. The continuing trend toward value-based marketing and relatively high Choice-Select price spreads, coupled with reports of declining quality grade, have caused beef producers to be concerned that value is being lost through production of lower quality product.

National Beef Quality Audit (NBQA) data have been published or presented for years 1991, 1995, 2000, and 2005 (Lorenzen et al., 1993; Boleman et al., 1998; McKenna et al., 2002; NCBA, 2005). Over the 14-year time span of the NBQA data, there has been variation in the proportions of cattle grading Choice and Select, but an identifiable trend toward reduced quality grade has not been reported. This is in dramatic contrast to the common interpretation based on USDA grading proportion data, which would suggest that the proportion of Choice carcasses declined from 79 to 57.2% over the same time span. One explanation for this discrepancy is that historical USDA data are most often expressed as the proportion of carcasses in a particular grade versus

the number of carcasses graded, rather than as a proportion of total production. The primary objective of this review was to quantify historical trends in Choice and Select beef supply based on yearly pounds of federally inspected product rather than the standard method based on the percentage of carcasses grading Choice of carcasses graded. This approach allows for a more consistent comparison of historical trends with respect to increases in total number of carcasses graded and historical increases in carcass weight. A secondary objective of this review was to explore the short-term demand structures for Choice and Select products.

Materials and Methods

Historical Quality Grade Data

Annual data from 1938 to 2005 were acquired from USDA (USDA, 2006), and included pounds of federally graded Choice product, pounds of federally graded Select product, pounds of federally inspected beef and percent of carcasses grading Choice and Select.

Quality grade data (e.g., percent Choice) are typically expressed as a proportion of graded carcasses; however, there has been variation in the proportion of total carcasses graded over time. To resolve this distortion, the amount (pounds produced) of Choice product and the amount of Select product were expressed as proportions of the amount of all federally inspected beef, rather than only as proportions of graded product, to generate a stable basis for comparison.

The time span from 1938 to 1965 was considered representative of "historical" grading standards. Beginning in 1965, carcasses were required to have the face of the rib exposed for grading. Prior to 1965 quality grades were assigned on the basis of external fat cover (USDA, 1997). The second interval considered was 1965 to 1975. In 1975, muscle conformation was removed as a factor influencing quality grade because it was found to be unrelated to palatability (USDA, 1997). Additionally, quality grade and yield grade were separated as grading factors. The third time span considered was 1975 to 1987, because in 1987 the Select grade replaced the Good grade (USDA, 1997). The fourth time span considered was 1987 to 1997, because of the increased marbling requirement to achieve Choice grade for B maturity carcasses (USDA, 1997). Also, 1997 closely corresponded to the escalation in formula- and grid-based (i.e., carcass pricing) marketing (Cattle Fax, 2006). The final time span considered was 1997 to 2005, to capture recent changes in supply dynamics. The timeline in Figure 1 summarizes some of these key factors over time.

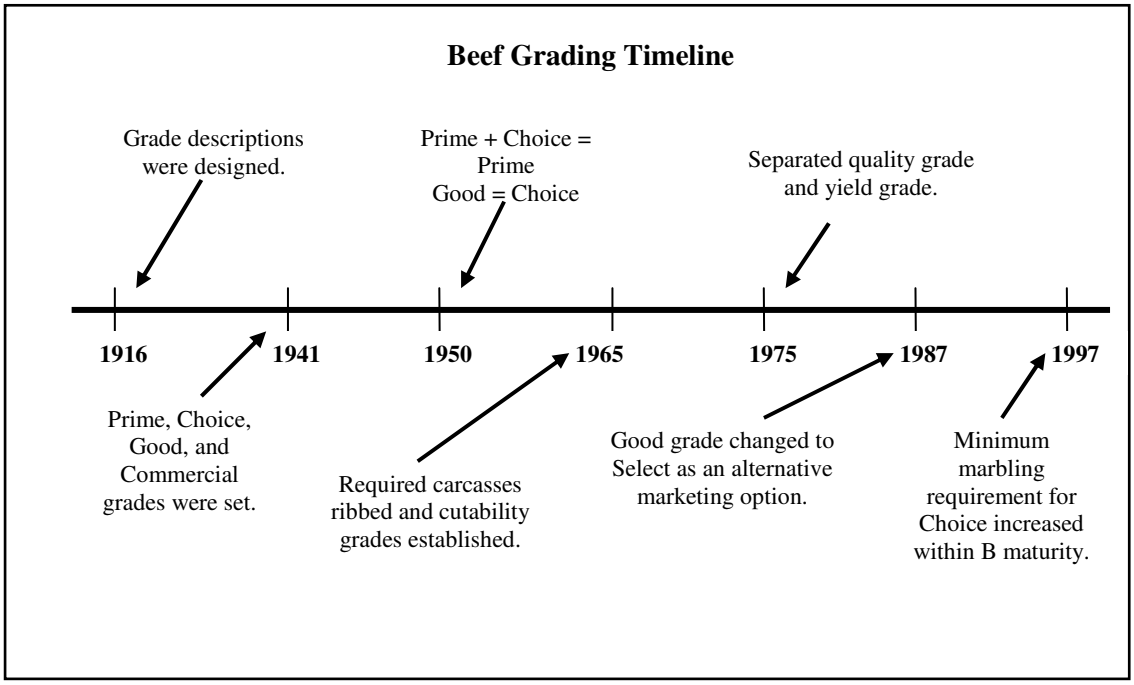

Figure 1. Timeline of key changes in grading standards or practices.

Demographic Data for Product Availability

Increases in total beef output over long time spans may be related to population increases, therefore total production of federally inspected and graded beef were coupled with historical data for U.S. population (United States Census Bureau, 2006) to express the quantity of Choice or Select product available per capita. This expression dampens the effects of both expanding total red meat production and expanding U.S. population over the time period studied, and provides an estimate of the amount of product available to a consumer.

Short Run Supply and Demand Data

Daily supply and demand data were recorded for a 52-week period (i.e., May, 25, 2006 to May, 26, 2007) to provide a short-run perspective of total product demand. Daily data were obtained from the USDA Agricultural Marketing Service Carcass Equivalent Index (Report # NW_LS410) and the National Daily Cattle and Beef Summary (Report # LM_XB403). Data collected from the carcass price equivalent index report included the daily price paid for live cattle grading Choice and Select (supply) and daily box beef price for Choice and Select product (demand). Data collected from the national cattle and beef summary report included Choice and Select daily load count (1 load = 40,000 lb. boxed beef equivalent). The daily boxed beef price for Choice and Select product was then multiplied by the daily Choice and Select load count to calculate revenue demand in total dollars. Daily data were aggregated into weekly averages for calculations.

The relative product demand ratio was calculated to evaluate the value of additional Choice and Select product and elasticity estimates were calculated to determine demand sensitivity in relation to price. The relative product demand ratio was calculated as the average weekly percentage change in the individual product category (i.e., Choice and Select) demand value divided by the average weekly percentage change in total demand value as follows:

Relative Demand Ratio = % Δ Product $ Value Demand / % Δ Total $ Demand

Demand elasticity was calculated as the percentage change in average weekly load count divided by the percentage change in average weekly load price, multiplied by average weekly load price divided by average weekly load count as follows:

Demand Elasticity = (%Δ Load Count / %Δ Load Price) * (Avg. Load Price / Avg. Load Count)

Statistical Analysis

Data were analyzed as time series data using a linear regression model including first- (linear), second- (quadratic) and third-order (cubic) effects of time. The linear regression model included annual pounds of graded Choice and Select product. Initial analyses were conducted across the entire time span of the data set, and subsequent analyses were conducted across shorter time spans in an attempt to resolve trends within more discrete time periods. A separate analysis to determine the correlation coefficient between the proportion of Choice product as a percentage of total graded product and pounds of total graded product from 1987 to 2005 was conducted.

Results and Discussion

Historical Trends in Quality Grade

Figure 2 depicts several expressions of the proportion of Choice product from 1938 to 2005. The solid (—) line is consistent with recent reports, and expresses the proportion of Choice product as a percentage of graded beef, not as a proportion of the total beef produced. This proportion reached a peak in 1986 at nearly 94%. From 1987 to 2005, the proportion of Choice product expressed as a percentage of graded product is inverse to the proportion of product actually graded ($r = -0.87$; $P < 0.01$). Also, during World War II, the large requirements for food products by the armed forces caused an escalation in the grading of beef, because specifications required that the beef be graded. During this time, the proportion of Choice carcasses declined precipitously. Correspondingly, in 1987 the proportion of the total slaughter that was actually graded also increased, presumably due to the reclassification of 'Good' as 'Select' and an increase in the marketability of this product. Again, a decline in the percentage Choice of carcasses graded was observed. This effect was most likely due to dilution by increasing the denominator in the calculation of proportion (number of carcasses graded), and was not necessarily indicative of a change in the numerator (the number of carcasses grading choice).

When the proportion of Choice product was expressed as a percentage of the total federally inspected slaughter, a more precise representation of the proportion of the population of beef cattle grading Choice, the apparent decline from 1986 to 1996 was

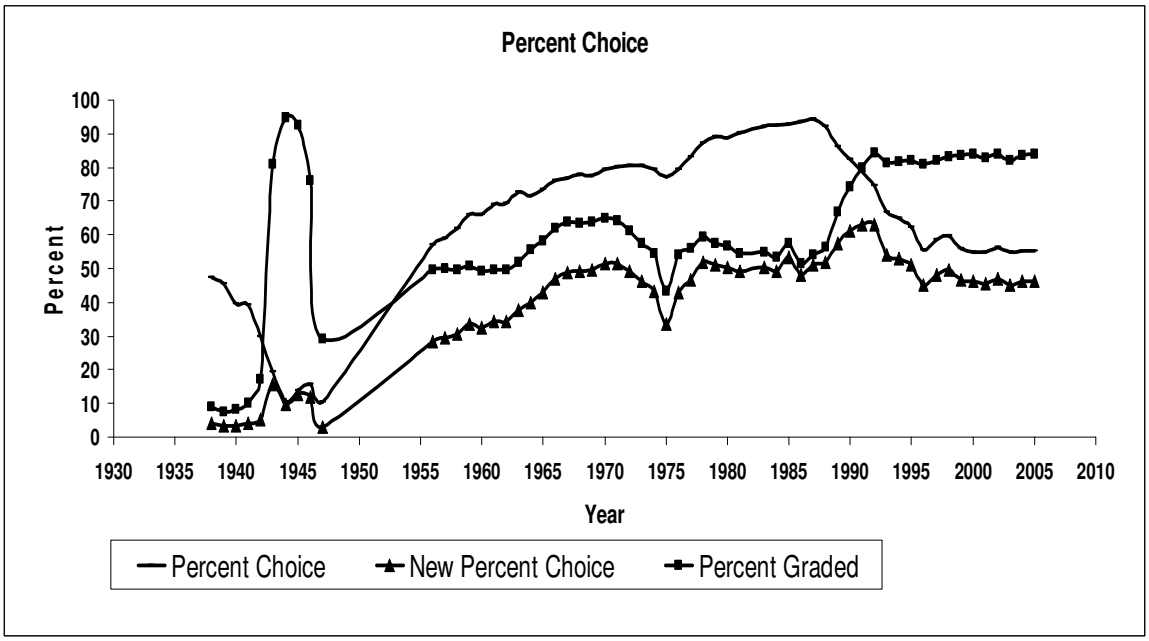

Figure 2. Yearly percent of pounds of beef that graded Choice from 1938 to 2005. The (▬) line represents the percentage pounds that graded Choice of the total pounds graded. The (■) line represents the percentage of pounds graded of the total pounds. The (▲) line represents the percentage pounds that graded Choice relative to the total percentage of pounds graded.

eliminated. Therefore, many of the dynamics in the proportion of Choice beef previously reported (i.e., apparent reductions in the "quality" of the US beef supply) are a function of the change in the amount of product graded. This is likely because of a change in the incentives of producers and processors to grade a larger number of cattle, rather than presenting for grading only those that were most likely to grade Choice. Expression as a percentage of the total beef supply provides a more consistent basis of evaluation when grading is a voluntary action. Figure 3 supports this premise. The proportion of Select ("Good" prior to 1987) beef was low and declining until 1987. This suggests that producers only elected to grade cattle that were likely to grade Choice, until an incentive (e.g., enhanced marketability of "Select"; increasing opportunities for value-based marketing) caused a greater proportion of the total supply to be presented for grading.

Trends in Choice and Select Beef Output

Annual Choice beef production (pounds of Choice beef produced) is shown in Figure 4. From 1938 to 2005 there were significant ($P < 0.01$) linear and quadratic effects of time on the amount of Choice beef produced. Over this time span, the amount of Choice product increased (positive linear coefficient) at a decreasing rate (negative quadratic coefficient). This is opposite to the prevailing public perception that quality grade has been in a steady decline for decades.

Annual Good/Select beef production is also shown in Figure 4. From 1938 to 2005 there were significant ($P < 0.01$) linear, quadratic, and cubic effects of time on the amount of Good/Select beef produced. The cubic effect was a result of a relatively stable

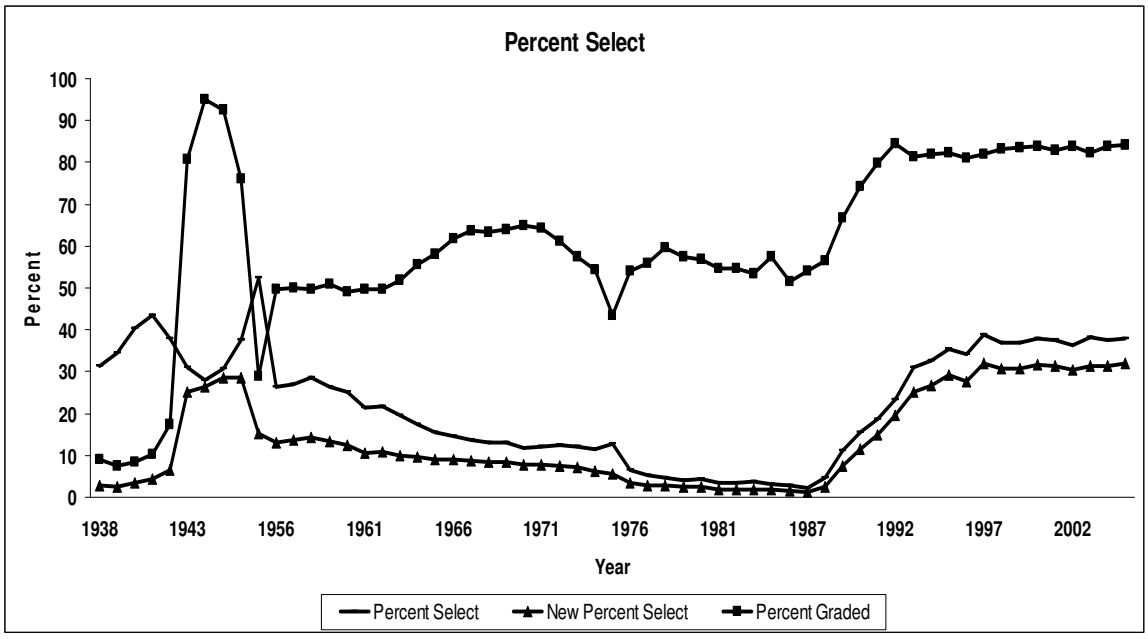

Figure 3. Yearly percent of pounds of beef that graded Select from 1938 to 2005. The (▬) line represents the percentage pounds that graded Select of the total pounds graded. The (■) line represents the percentage of pounds graded of the total pounds. The (▲) line represents the percentage pounds that graded Select relative to the total percentage of pounds graded.

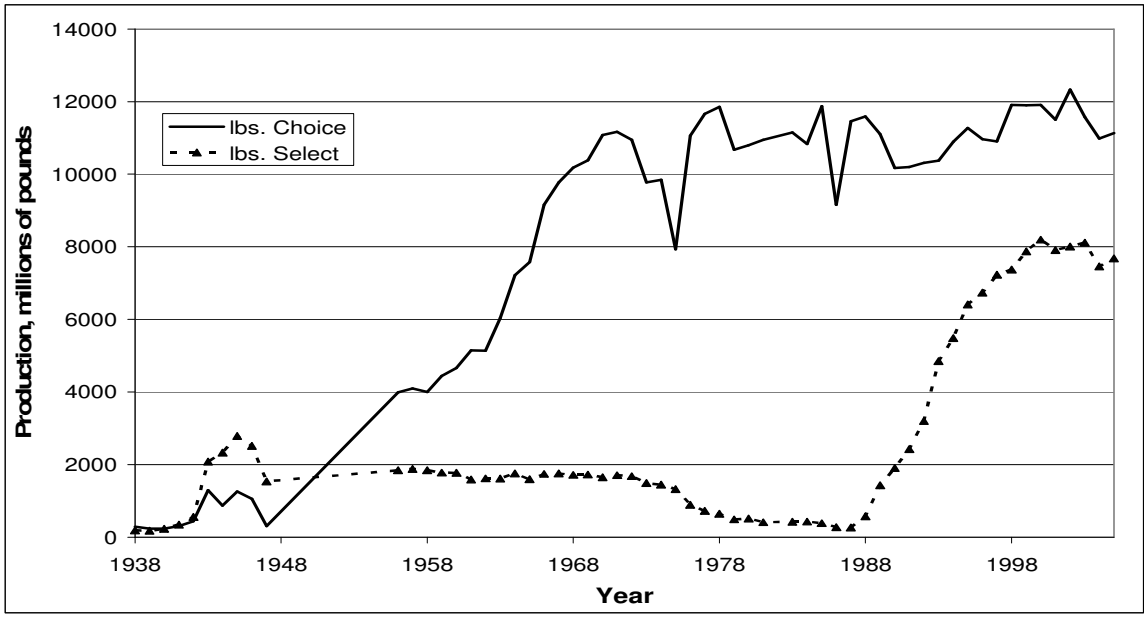

Figure 4. Production of Choice and Select graded product (millions of pounds) from 1938 to 2005.

product volume from 1938 to 1986, followed by a rapid escalation until 2000, followed by another period of apparent stability. It is important to note that the large increase in Select output during the late 1980s and 1990s does not appear to be offset by reductions in Choice production during the same time span, suggesting that the increase in Select graded product was a result of grading more cattle, not a result of declining overall marbling in the population.

Dynamics in Product Availability

The overall increases in production of both Choice and Select product over time have occurred at a time of population expansion in the United States. Therefore, the amounts of Choice and Select product produced per capita (scaled to the population) are indicators of consumer availability of these product categories over time (Figure 5). It is important to note that this is not equivalent to an expression of consumption, but rather an expression of the availability of product in the marketplace. Consumption would be the per capita production value adjusted for net beef exports and stocks or storage, and would vary from these values accordingly.

From 1938 to 2005 there were significant ($P < 0.01$) linear, quadratic, and cubic effects of time on the amount of Choice and Select beef available per capita. Over this time span, availability of both Choice and Select product increased, although the dynamics and timing of the increases are dissimilar. There were no statistically significant ($P = 0.19$) effects observed for Choice product availability from 1938 to 1965, although pounds of Choice product available to consumers increased from the period low of 1.74 lb (0.79 kg) per person in 1940 to the period high of 39.84 lb (18.11

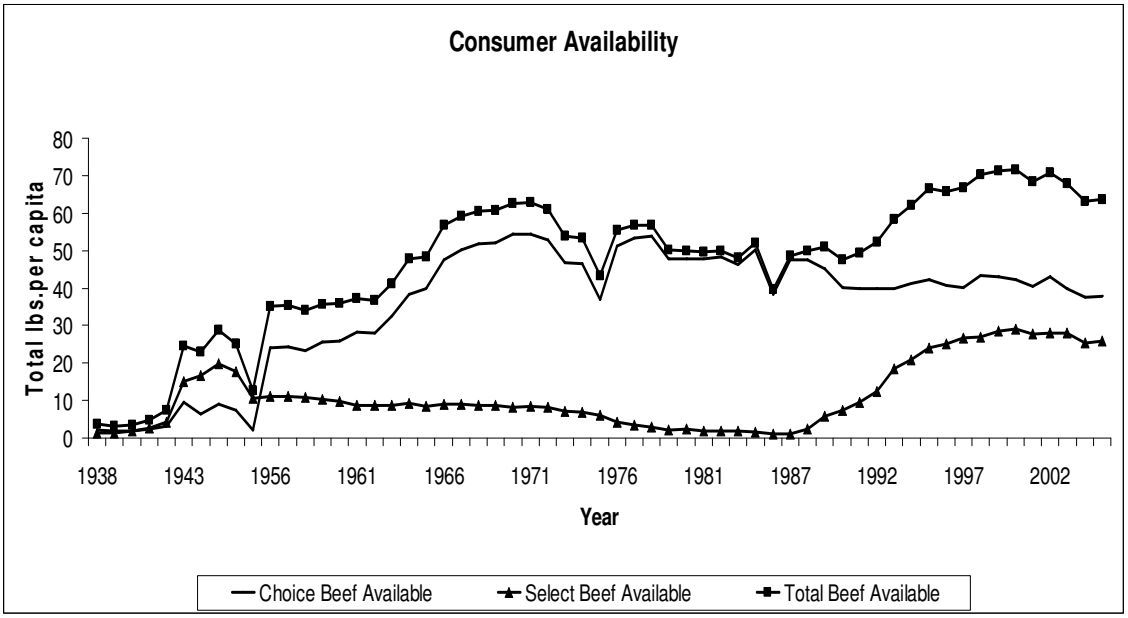

Figure 5. Per capita production (pounds per person) of Choice, Select, and total beef from 1938 to 2005.

kg) per person in 1965. The only exception to this annual increase was a decline in 1947 to 2.11 lb (0.96 kg) per person. The average amount of Choice beef available to consumers during this time span was 16.78 lb (7.63 kg) with a coefficient of variation (CV) of 79.8% (if the time trends are ignored). The lack of regression effects was a result of the variation in per capita Choice output in the 1940s and early 1950s. There were significant linear ($P < 0.01$), quadratic ($P = 0.01$), and cubic ($P = 0.04$) effects observed for Select product availability from 1938 to 1965, as the amount of Select product available to consumers increased from a period low of 1.35 lb (0.61 kg) in 1939 to a period high of 19.84 lb (9.02 kg) in 1945. From that point, Select product availability declined annually to 8.38 lb (3.81 kg) in 1965. The average amount of Select beef available during this period (9.42 lb per capita) was less than the amount of Choice beef available (16.78 lb per capita). This decline in Select availability from 1945 to 1965, in the face of expanding total beef availability over the same time frame, reflects the low incentive to present cattle for grading that were not highly likely to grade Choice.

Choice product availability increased linearly ($P = 0.03$) from 1965 to 1975. The amount of Choice product available per consumer steadily increased from 1965 to a period high of 54.51 lb (24.77 kg) in 1970, but then declined to a period low of 37.09 lb (16.86 kg) in 1975. The linear effect is positive; the last year observed in this period (1975) exhibited a sharp decline in product availability, but this decline lasted only one year. The average amount of Choice beef available during this period was almost three-fold greater than the previous period (48.49 lb annually) with a CV of 11.8%, a

substantial reduction in variability compared to the first period evaluated. There were no statistically significant ($P = 0.22$) effects observed for Select availability from 1965 to 1975, as the amount of Select product per person remained fairly consistent with a period high of 9.05 lb (4.11 kg) in 1966 to a period low of 6.18 lb (2.81 kg) in 1975. The average amount of available Select beef during this period was slightly less than the previous time span at 8.04 lb (3.65 kg) annually.

Between 1975 and 1987, there were significant linear ($P = 0.04$) and quadratic ($P = 0.05$) effects on Choice availability over time. The amount of available Choice product increased from a period low of 37.09 lb (16.86 kg) per capita in 1975 to a period high of 53.74 lb (24.23 kg) in 1978, but declined somewhat through 1987. The average amount of Choice beef available to the consumer during this period was similar to the previous period at 47.45 lb (21.57 kg) yearly, with a CV of 10.8%. It should be noted that the percentage decline in period mean Choice output (2.1%) across this period was much less than the intra-period variation. There were statistically significant linear, quadratic, and cubic ($P < 0.01$) effects observed for available Select beef from 1975 to 1987. Consistent with the amount of Select beef produced during this period, Select beef availability steadily declined from a period high of 6.18 lb (2.81 kg) in 1975 to a period low of 1.09 lb (0.49 kg) in 1987. The average amount of available Select beef during this period was also the lowest historically at 2.53 lb (1.15 kg) annually.

There was a significant linear ($P = 0.03$) and a tendency for a quadratic ($P = 0.09$) effect observed for available Choice beef from 1987 to 1997, as the amount of Choice beef available decreased slightly throughout this period. The statistical

significance of this low magnitude of change is a result of reduced variability in product availability, and thus a more sensitive test of the trend. Initially, there was a slight decline from the period high of 47.55 lb (21.61 kg) in 1988 to 39.88 lb (18.13 kg) in 1991, but availability subsequently stabilized with an average of 42.21 lb (19.19 kg) of available Choice beef during this time span. The dynamic in per capita output corresponds closely with the national cow herd inventory during this time period. A reduction in intra-period CV to 7.2% is evidence of some stabilization in availability compared to previous periods. There were statistically significant quadratic and cubic ($P = 0.02$) effects observed for available Select beef from 1987 to 1997. From the historical low of 1.09 lb (0.49 kg) in 1987, available Select product increased steadily to a period high of 26.61 lb (12.09 kg) in 1997. Over this period, the average amount of available Select product increased nearly seven-fold to 13.98 lb (6.35 kg) annually.

There were no significant ($P = 0.21$) effects observed for the amount of Choice beef available to consumers from 1997 to 2005. The available amount numerically declined from a period high of 43.42 lb (19.74 kg) in 1998 to the period low of 37.53 lb (17.06 kg) in 2004. The average amount of available Choice beef throughout this time span was 3.3% less than the previous period, at 40.82 lb (18.55 kg) annually with a CV of 5.5%. Similar to the results for Choice, there were no significant ($P = 0.15$) regression effects observed for the amount of Select beef available to consumers from 1997 to 2005. Also similar to Choice beef availability, available Select product declined numerically from a period high of 29.13 lb (13.24 kg) in 2000 to a low of 25.48 lb (11.58 kg) in 2004. Although numerical declines were observed within both product categories,

together (i.e., available Choice and available Select) this period represents the largest amount of total beef available to consumers. The intra-period decline of available product within each product category was likely a result of the reduction in cattle inventory witnessed during this period.

These data suggest that the addition of the Select grade dramatically expanded the availability of that product to consumers without negatively affecting the availability of Choice product. Effectively, a new category of graded beef was created, as was the intention of creating the Select quality grade. Additionally, these consumer availability data consistently reflect the results presented for the total amount of graded beef produced annually, suggesting that the production of graded beef has adjusted with changes in consumer demand.

Short Run Demand Structure for Choice and Select Beef

The relative product demand ratio shown in Table 1 suggests that for every one unit increase in total product demand, Choice product value increased by 1.62 units and Select product value increased by 0.16 units. Thus, weekly changes in total beef demand are more heavily influenced by changes in Choice product demand.

Demand elasticity measures the relationship between changes in quantity demanded and changes in its price, and is useful in predicting changes in quantity demanded given shifts in price (Nicholson, 2005). Values approaching 0 indicate inelastic demand while values greater than -1 indicate elastic demand (Nicholson, 2005). The Choice demand elasticity (-0.39) as shown in Table 1 suggests that there was limited change in the weekly quantity demanded for Choice product at any given price level. On

Table 1. Short-run (52-week, May 2006 to May 2007) measures of pricing and demand for Choice and Select beef

	Short-run Demand					
Item	**Average Price**	**Average Load Count**	**Average Total Demand**	**% Total**	**Load Elasticity**	**Relative Product Demand**
Choice	$138.27	671.1	$36,923,894	56%	-0.39	1.62
SD[a]	$7.23	121.7	$6,042,339			
Select	$125.18	576.2	$28,691,221	44%	-0.90	0.16
SD	$8.72	102.5	$4,691,606			
Total	$132.10	1247.3	$65,615,116	100%	-0.65	1.00
SD	$7.63	190.7	$9,182,145			

[a]Standard deviation.

the other hand, as weekly supply of Select product shifts, and subsequently new price points are discovered, there is a more dramatic impact on the quantity of Select beef demanded (elasticity of -0.90). Collectively, the overall (i.e., Choice and Select) product demand is intermediate at -0.65.

In the short term, demand for Choice product is relatively more inelastic while the demand for Select product is relatively more elastic in nature (Figure 6). As the weekly supply of total graded product varies, and pricing points are discovered for each product category, the amount of Choice product demanded remains relatively constant. As supply tightens, the price of either category would be expected to increase. This price increase would have minimum impact on the amount of Choice product sold, but might have a larger impact on the volume of Select product moved. It is important to note that when total product supply increases and price is reduced within both product segments, a greater relative reduction in the price of Choice beef compared to Select beef might be expected. This, coupled with a limited impact on the quantity of Choice product demanded (inelastic), could dampen increases in total expenditures for beef if the increase in supply is driven by a substantial increase in Choice product availability. Alternatively, a stable supply of Choice product provides for short term stability in overall beef price. Perhaps this is a possible explanation for the stability in Choice supply witnessed over the last two time periods discussed earlier. The discrepancy in elasticity among the two categories also suggests that Choice and Select are not necessarily strong substitutes. As supply tightens, the same amount of Choice is demanded, albeit at a much higher price. However, as prices increase, the quantity of

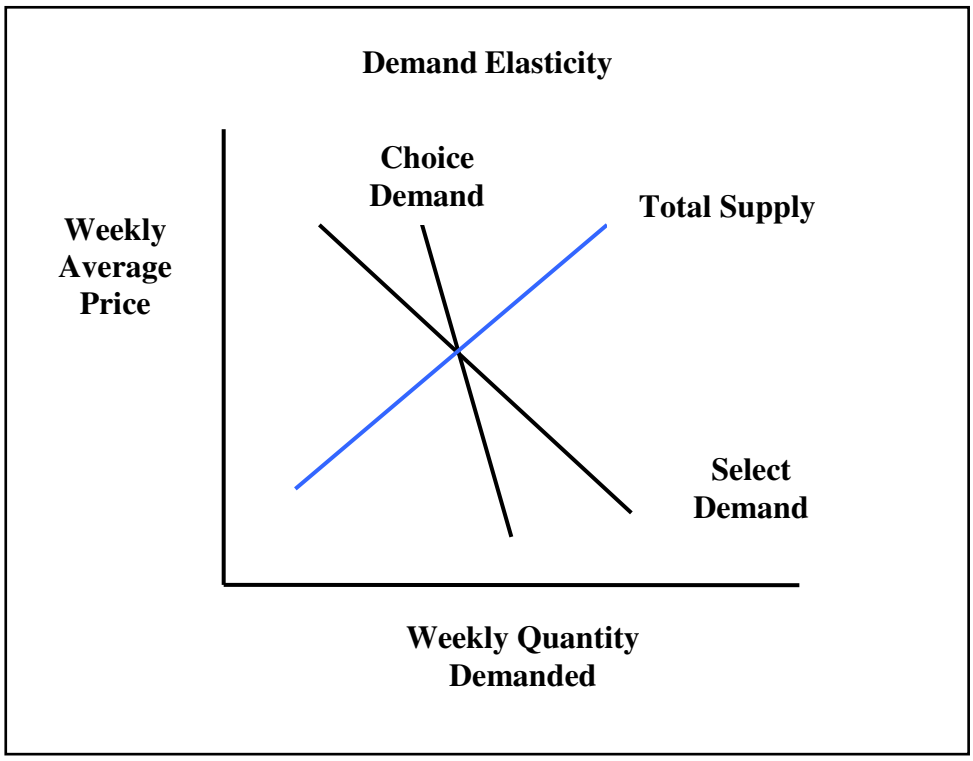

Figure 6. Illustration of the differential demand elasticity of Choice and Select beef calculated over the period May 2006 to May 2007.

Select product demanded diminishes more rapidly, suggesting that consumers may substitute with non-beef products. Additionally, the relatively more inelastic demand schedule observed for Choice product may provide us with an alternative view of why dramatic changes in the Ch-Se spread are observed over a short period of time. The steeper or more vertical elasticity slope calculated for Choice product would suggest that a small change in the weekly supply of Choice beef translates into a larger change in the price of Choice product and thus a shift in the Ch-Se spread. For example, Figure 7 shows us that during the 52-week period used to calculate the demand elasticity's, the Ch-Se spread ranged from a weekly high of $23.08 (June 9, 2006) to a weekly low of $6.61(February 9, 2007). When the weekly Ch-Se high occurred (June 9, 2006) 62% of the total graded carcasses were Choice, while during the weekly low (February 9, 2007) 68% graded Choice. In the first quarter of 2008, the weight of carcasses did not adjust according to historical seasonal patterns, and a relatively high proportion of this product graded choice (i.e., the supply of Choice product escalated). During this same period, the Choice vs. Select price spread diminished to as low as $0.31 per cwt (45.4 kg) (USDA, 2008).

Implications

There is little evidence that the increase in Select beef output has been the result of declining amounts of Choice beef produced. Increases in amount of Select beef produced result from increases in proportion of beef presented for grading. An increase in the proportion of beef graded is likely due to enhanced marketing options available

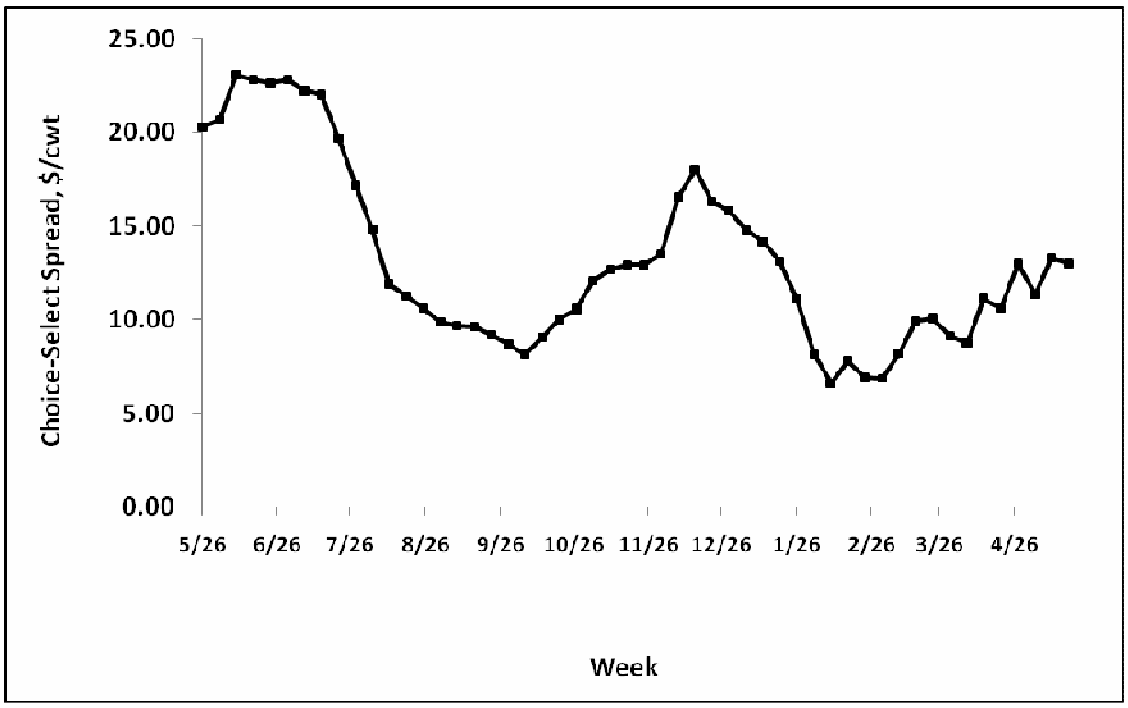

Figure 7. Choice-Select spread over the period of May 2006 to May 2007.

and increases in value-based pricing structures. Historical Choice beef production as a proportion of graded product is misleading, as it does not account for the expansion of total graded carcasses. Choice percentages previously reported reflect producers' ability to sort cattle, not overall quality of beef produced.

The recent stabilization in slaughter mix suggests an optimum is being approached. Evaluation of short-run demand structure supports this premise, and suggests Choice and Select products are not strong substitutes. It is difficult to support the hypothesis that beef quality has been declining, or that the mix of slaughter cattle is decidedly non-optimal. Evidence also exists that rapid escalation of the production of Choice beef removes market premiums for this product and may dampen total expenditures for beef.

CHAPTER IV

EFFICACY OF GROWTH BASED PREDICTIONS OF CARCASS FAT AND MARBLING AT HARVEST USING ULTRASOUND MEASUREMENTS

Overview

The objective of this study was to develop equations predicting carcass fat thickness (FAT) and marbling (MAR) deposition relative to HCW using carcass measurements from serially slaughtered steers fed different dietary energy sources during the backgrounding period. A subsequent objective was to apply these equations and evaluate the accuracy of growth-based prediction equations relating carcass traits to growth traits using ultrasound measurements as the basis of projections in beef cattle developed under different growing systems. Carcass data from serially slaughtered steers fed a different dietary energy source during backgrounding were used to formulate prediction equations based on actual FAT and MAR for steers fed different diets during the growing phase, but harvested at common endpoints relative to FAT and HCW. Carcass FAT and MAR were predicted as functions of carcass weight gain estimated from live weights. Projections were compared to carcass values and percentage differences were analyzed as responses with harvest group, breed, gender, treatment, and 2-way interactions in the model. Timing of scan influenced accuracy ($P < 0.05$). MAR was within 8% of actual values for scans taken within 123 d of slaughter, but decreased for more distant scans. FAT was within 10% at 158 or 180 d prior to slaughter but ranged from -88% to 35% during other spans. Breed type affected MAR accuracy from

both ultrasound sessions ($P = 0.04$). Accuracy was greater when US2 was used for projection and was within 6% for calves with Angus influence, but over 20% for Brahman influenced calves. Gender influenced MAR accuracy ($P > 0.01$) from US1 or US2. Gender impacted FAT accuracy from US2 ($P > 0.01$). Treatment influenced FAT accuracy from US1 ($P > 0.01$), underestimating PS cattle by 58.4%. Treatment did not affect FAT accuracy from US2, nor MAR accuracy from either session. Results suggest accuracy of FAT and MAR predictions from growth-based equations is influenced by weight gain between ultrasound and endpoint, breed, and gender although scans out to 120 d pre-harvest may be accurate. Results also suggest that accuracy of FAT predictions improves when using a linear-based prediction equation. Known sources of variation in accuracy could be used to scale predictions based on breed and gender to improve accuracy, and thus a growth-based, rather than time based, prediction system might be generated.

Introduction

Beef carcass value is influenced by the amount and distribution of adipose tissue. While increasing amounts of marbling (MAR) typically increases carcass value, excessive increases in carcass fat thickness (FAT) decreases value. Therefore, a certain amount of production risk is associated with feeding strategies designed to enhance intramuscular accretion by prolonging the finishing period. An accurate prediction of carcass fat deposition would allow a producer to mitigate losses in potential carcass value due to increased carcass waste while maximizing MAR and carcass weight. Body composition changes over a feeding period, and average daily gain values are reflective

of those changes (Owens et al., 1995). Brethour (2000) used ultrasound (US) measurements to predict body composition as a function of time on feed. However, because time on feed is related to average daily gain (ADG), and ADG varies with environmental conditions, diet composition, and other factors, a time-based model used to predict carcass endpoint may produce variable results if ADG or other conditions vary independently of time. Owens et al. (1995) established the relationship between empty body weight and body weight. This relationship provides estimates of hot carcass weight (HCW) gain from observed live weight gain or ADG. Bruns et al. (2004) quantified changes in fat thickness and marbling score relative to change in HWC in steers during a finishing program. It has been well documented that dietary energy source influences FAT and MAR deposition (Schoonmaker et al., 2004; Rhoades et al., 2007). The objective of this study was to develop equations predicting carcass FAT and MAR deposition relative to HCW using carcass measurements from serially slaughter steers fed different dietary energy sources during the backgrounding period. A subsequent objective was to apply these equations and evaluate the accuracy of growth-based prediction equations relating carcass traits to growth traits using ultrasound measurements as the basis of projections in beef cattle developed under different growing systems.

Materials and Methods

Experiment 1

Twenty-five Angus steers (223 +/- 25 kg) were randomly assigned to receive either a grain-based or hay-based diet after weaning (8 mo) for 120 d. Bermuda grass

(*Cynodon dactylon*) hay containing 9.5% crude protein was fed free choice for 8 d after the steers were transported to the Texas AgriLife Research-McGregor Center, McGregor, TX. Four steers were slaughtered immediately (d 0). Of the remaining 21 steers, eight were assigned to receive a high-energy, corn-based diet containing 48% ground corn, 20% ground milo, 15% cottonseed hulls, 6.5% molasses, 6 % cottonseed meal, 3% limestone, trace mineral salt, and vitamin premix, and 1.5% monensin (CORN) on a DM basis was fed for 120 d. Feeding this diet resulted in an average gain of 0.85 kg/d. The remaining steers (n = 13) were offered coastal bermuda grass hay *ad libitum*, supplemented daily with non-protein nitrogen in a cooked molasses carrier, and an amount of the corn-based diet to sustain a gain of 0.72 kg/d (HAY). The HAY treatment was offered for 120 d after weaning. Following the 120 d treatment period, all steers were fed the previously described corn-based diet until slaughter. Cattle were transported to the Rosenthal Meat Science and Technology Center, Texas A&M University. Four steers were slaughtered at weaning and randomly selected subsets of steers from each treatment group were serially slaughtered at 120, 240, and 300 d post-weaning to achieve common endpoints based on hot carcass weight and fat thickness. Post-harvest hot carcass weight, fat thickness, ribeye area, KPH percent, marbling score, lean maturity, and skeletal maturity were collected following a 48 h chill by Texas A&M University trained personal.

Data were analyzed using linear regression. The regression models contained HCW and HCW^2 (quadratic form) or only HCW (linear form) as independent variables, and marbling score or fat thickness as the dependent variable. Regression analyses were

conducted separately for the CORN-fed and HAY-fed treatment groups, and equations were also developed from data pooled across treatments. Marbling score at harvest from one steer from the CORN-fed treatment group was identified as an outlying observation based on residual analysis. This data point was removed from the subsequent analyses.

Experiment 2

Calves (n = 113) sired by Mashona bulls out of dams produced in a three-breed diallele mating system (Angus, Brahman, and Romosinuano), were early weaned (mean age = 74 d) preconditioned for 40 d, and transported from central Florida to the Texas AgriLife Research-McGregor Center, McGregor, TX for growing and finishing. Cattle ranged in age from 90 to 200 d at arrival and included both steers and heifers. Calves were stratified by breed type and gender and assigned to a confinement growing program in a feedlot (Feedlot) or to a forage-based growing program on pasture (Pasture) for an average of 141 d. The Feedlot-treated cattle were fed a grain-based diet containing 48% ground corn, 20% ground milo, 15% cottonseed hulls, 6.5% molasses, 6 % cottonseed meal, 3% limestone, trace mineral salt, and vitamin premix, and 1.5% monensin on a DM basis during the growing phase. The Pasture-treated cattle were grazed on a coastal Bermuda grass pasture and supplemented daily with cottonseed meal during the growing phase. Following the 141 d growing phase, all cattle received the previously described high-energy corn-based diet until harvest.

Ultrasound carcass fat thickness and marbling were measured twice for each animal. All ultrasound measurements were made by a certified ultrasound technician using an Aloka 500V ultrasound machine (Manufacturer and location) and a 17cm

3.5GHz probe. Fat thickness over the 12th rib (FAT), longissimus muscle area (REA), and intramuscular fat (MAR) percentage between the 12th and 13th rib location were measured at each ultrasonographic scanning session. Intramuscular fat percentage was converted to marbling score for use in prediction analysis (MAR score = 54.928 (IMF %) + 164.97). Initial ultrasound measurements (US1) were collected on d 142 of the trial, which corresponded to 158, 207, and 264 d prior to slaughter for harvest groups 1, 2, and 3, respectively. The second ultrasound scan was made on d 226 (US2), corresponding to 74, 123, and 180 d prior to harvest for Grp1, 2, and 3, respectively. Live weights were collected at 28 d intervals throughout the course of the study and at each ultrasound scan session.

Carcass FAT and MAR at slaughter were each predicted using two different equations. Equations were adapted (Model 1) from Bruns et al. (2004) or derived from experiment 1 (Model 2), as functions of carcass weight gain estimated from observed BW correspondent to the timing of ultrasound measurement and the observed HCW at slaughter. Bruns et al. (2004) results for FAT ($Y = 0.5442 – 0.0045(X) + 0.0000202(X^2)$) and MAR ($Y = 60.199 + 1.654(X)$) accretion were adapted and used as prediction equations in this study. For both the FAT and MAR equation (Model 1) used in this study, the first derivative of published FAT and MAR equations (Bruns et al., 2004) was used to remove the intercept from published results. Similar derivations were used for Model 2. Results from pooled data for FAT ($Y = -0.61 + 0.007(X)$) and MAR ($Y = 177.51 + 1.25(X)$) in experiment 1 (Model 2) of this study, were also reduced to the first

derivative form. The reduced equations used to predict carcass FAT and MAR for both Model 1 and Model 2 are as follows:

FAT Model 1= ((0.0000404*LW)-0.0045)*(HCW gain) + US FAT

MAR Model 1 = (1.654*HCW gain) + US MAR Score

FAT Model 2 = (0.007*HCW gain) + US FAT

MAR Model 2 = (1.25*HCW gain) + US MAR Score

Cattle were harvested after approximately 300 (Grp1), 345 (Grp2), or 405 (Grp3) time on feed. Cattle were sorted into respective outcome groups based on an ultrasound estimate of FAT and live body weight. Targeted FAT thickness at harvest for each group was 0.50 cm. Sorting was designed to place cattle into groups to maximize HCW while minimizing YG 4 carcasses. Cattle were transported and harvested at a commercial processor (Sam Kane Beef Processors Inc., Corpus Christi, TX). At slaughter, sequence numbers were collected, brisket tags were assigned to individual carcasses, and HCW was recorded. Following a 48-h chill period, fat thickness, ribeye area, KPH percent, marbling score, lean maturity, and skeletal maturity were collected by Texas A&M University personnel.

Carcass FAT and MAR projections based from both Model 1 and Model 2 equations were compared to the actual FAT and MAR carcass values taken at slaughter. Percentage differences (projected vs actual) were analyzed as response variable indicative of projection accuracy with harvest group, breed, gender, treatment, and 2-way interactions between harvest group, gender, and treatment as effects in the model.

Results and Discussion

Experiment 1

Performance and Carcass Traits

The production and carcass characteristics for serially slaughtered steers fed a different dietary energy source during backgrounding are shown in Table 2. This study was designed to formulate prediction equations based on actual FAT and MAR carcass data collected from serially slaughter steers fed different diets during the growing phase, but harvested at common endpoints relative to FAT and HCW. Steers used in this study ranged from 8 to 18 mo of age at harvest depending on treatment; Bruns et al. (2004) steers ranged in age from 12 to 18.5 mo of age. Difference in dietary energy source during backgrounding resulted in 1.5 kg ADG for those steers fed the corn-based diet at 12 mo of age, but when steers were fed a common diet ADG was 1.2 kg among treatment groups; Bruns et al. (2004) reported a cumulative range of 1.45 to 1.2 ADG over the course of the entire study. In this study HCW means ranged from 103 to 316 kg, whereas Bruns et al. (2004) reported an average HCW range of 208 to 380 kg. When steers were fed CORN, HCW was 49 kg greater at 12 mo of age, but was within 2 kg among treatment groups at final slaughter. Also CORN steers MAR score and FAT was 335% and 40% greater at 12 mo of age. At final slaughter, treatment group measurements were within 6% for MAR and identical for FAT. In our study MAR score means ranged from 300 to 592 and FAT from 0.2 to 1.7 cm; Bruns et al. (2004) reported a MAR range of means from 412 to 709 and FAT from 0.47 to 1.91 cm. Relative to

Table 2. Means for production and carcass characteristics of all steers fed CORN or HAY

	Diet							
Item	CORN			HAY				
Slaughter age, mo	8	12	16	8	12	16	18	SD[a]
ADG, kg	--	1.5	1.3	--	0.9	1.2	1.1	0.2
HCW, kg	103	222	314	103	173	292	316	92.2
Marbling score[b]	300	520	555	300	372	522	592	124.5
Fat thickness, cm	0.2	1.3	1.7	0.2	0.3	1.4	1.7	0.7

[a]Standard Deviation across all ages and across all diets.

[b]Marbling score, small = 500.

previous work, our study captures changes in FAT and MAR deposition at lighter HCW and from steers fed alternative energy sources during the growing phase.

Regression Analysis

The coefficients of parameters for regression analysis of FAT deposition from all serially slaughtered steers are shown in Table 3. For all regression analysis (FAT and MAR) reported in this study, the intercept values are only useful as a scaling variable but are not meaningful for prediction purposes. For FAT deposition, HCW accounted for approximately 82 and 89% of the variation for steers fed CORN or HAY-based diets, respectively. In the CORN-fed group, as FAT deposition increased linearly ($P < 0.01$) with increased HCW, but there was no significant quadratic effect ($P = 0.28$) of HCW^2 on FAT accretion. Similarly in the HAY-fed group, there was no significant quadratic effect ($P = 0.20$) of HCW^2 on FAT accretion, as FAT deposition increased linearly ($P < 0.01$) with increased HCW. When treatment groups were combined, analysis of pooled data also indicated no significant quadratic effect ($P = 0.93$) on FAT accretion, but again showed a linear relationship between FAT and HCW ($P < 0.01$). Bruns et al. (2004) reported that total carcass FAT increased in a quadratic fashion with increasing hot carcass weight, suggesting that the slope of total carcass FAT and MAR lines are different. Our linear relationship is in contrast with the quadratic relationship reported by Owens et al. (1995), Brethour (2000), and Bruns et al. (2004).

The coefficients of parameters for regression analysis of MAR deposition from serially slaughtered steers excluding the known outlier are shown in Table 4. For MAR deposition, HCW accounted for approximately 82 and 92% of the variation for steers fed

Table 3. Coefficients of parameters for regression analysis of FAT deposition on HCW as influenced by diet using all steers from Experiment 1

	FAT Coefficients					
Diet	β_0	β_1	β_2	R^2	SE	*P*-value[a]
Corn- Quadratic	-1.16	0.015	0.00	0.83	0.34	<0.01
Corn- Linear	-0.49	0.007**	--	0.81	0.34	<0.01
Hay- Quadratic	-0.14	0.001	0.00	0.90	0.26	<0.01
Hay- Linear	-0.73**	0.007**	--	0.88	0.26	<0.01
Pooled- Quadratic	-0.64	0.007	0.00	0.83	0.31	<0.01
Pooled- Linear	-0.61**	0.007**	--	0.83	0.31	<0.01

[a]P > F = Significance of regression model.

*Superscript = Significance < 0.05.

**Superscript = Significance < 0.01.

Table 4. Coefficients of parameters for regression analysis of MAR deposition on HCW as influenced by diet using all steers except the outlier from Experiment 1

	MAR Coefficients					
Diet	β_0	β_1	β_2	R^2	SE	*P*-value
Corn- Quadratic	13.29	3.40*	-0.005	0.84	57.81	<0.01
Corn- Linear	194.9**	1.21**	--	0.79	63.45	<0.01
Hay- Quadratic	218.4**	0.68	0.001	0.93	36.72	<0.01
Hay- Linear	163.1**	1.29**	--	0.92	36.41	<0.01
Pooled- Quadratic	133.9*	1.75*	-0.001	0.86	47.67	<0.01
Pooled- Linear	177.5**	1.25**	--	0.86	47.23	<0.01

*Superscript = Significance < 0.05.

**Superscript = Significance < 0.01.

CORN or HAY-based diets, respectively. When steers were fed the CORN-based diet MAR increased linearly ($P < 0.01$) with increased HCW, and the quadratic term was not significant ($P = 0.13$) for MAR deposition. In the HAY-fed steers, MAR increased linearly ($P < 0.01$) with increased HCW and the quadratic term was not significant ($P = 0.40$). When treatment groups were combined, analysis of pooled data also indicated a linear ($P < 0.01$) relationship between MAR and HCW.

Previous results suggest that MAR accretion is quadratic or plateaus toward the end of the feeding period. Duckett et al. (1993) and May et al. (1992) expressed MAR accretion as a function of time on feed, which resulted in quadratic response, where marbling increased at a decreasing rate. In these studies, MAR accretion seemed to reach a plateau after approximately 110 to 112 d on feed within the feedlot phase. The linear relationship between MAR and HCW found in this study is consistent with more recent data (Bruns et al., 2004). Our study also confirms the results from Bruns et al. (2004), which indicated MAR accretion should be considered a lifetime event, rather than simply a late maturing depot.

It has been well documented that dietary energy source influences FAT and MAR deposition (Schoonmaker et al., 2004; Rhoades et al., 2007). Differences in adipose tissue substrate preference and the interaction with energy source influence adipocyte metabolism and subsequently accretion rates (Rhoades et al., 2007). Schoonmaker et al. (2004) reported that FAT deposition increased when steers were fed a high-energy diet for a longer period of time. These authors also concluded that when steers were fed a low-energy diet there was a greater decrease in FAT deposition than

MAR deposition. Brethour (2000) predicted carcass FAT and MAR as a function of time on feed. Previous work would suggest that dietary energy source and time on feed dictate differences in adipose tissue accretion patterns. Overall, results from our experiment (FAT and MAR) suggest that accretion rates are independent of diet when FAT and MAR deposition is expressed as a function of changes in HCW; i.e., the impact of diet on MAR is a function of ADG. Also, due to high variation accounted for by HCW in our model, FAT and MAR could be predicted independently of dietary energy source in Angus cattle if a target weight were identified, or if ADG could be predicted base don dietary information.

Results from this experiment also suggest that a modest degree (500 +/- 47.2) of MAR was obtained at similar HCW independently of diet fed early in the growing phase. Further definition of relationship between FAT and HCW is required to generate optimization programs, between carcass quality and yield.

Experiment 2

The number of d between ultrasound session and harvest group are shown in Table 5. Cattle from all slaughter groups had similar fat thickness ($P = 0.58$) and marbling score ($P = 0.64$) at harvest (Table 6). Timing of ultrasound session relative to slaughter date influenced the accuracy ($P < 0.05$) of predictions. Previous studies have shown that FAT projection accuracy increases as days prior to slaughter decreases due to less change in overall fat thickness (Wall et al., 2004; Brethour, 2000). When using Model 1 FAT equation, results suggest the accuracy for FAT projection was within 10% at either 158 or 180 d prior to slaughter but ranged from -88 to 35% during other spans.

Table 5. Days from ultrasound measurement until slaughter

	Days from Ultrasound	
Item	US1	US2[a]
Group 1	158 d	74 d
Group 2	207 d	123 d
Group 3	264 d	180 d

[a]Ultrasound scan session 2 at d 242 of trial.

Table 6. Least squares means percentage differences for predicted vs actual FAT and MAR values due to harvest group using Model 1 and Model 2

	Harvest Group				
Item	Group 1	Group 2	Group 3	SE	*P*-value
FAT					
Actual[a]	0.58	0.55	0.54	0.035	0.58
Model 1					
US1	0.6%	-21.5%	-88.1%	11.95	<0.01
US2	20.6%	35.7 %	10.1%	6.81	0.02
Model 2					
US1	4.8%	19.5%	34.7%	8.18	0.02
US2	-10.6%	1.9%	13.2%	5.83	<0.01
MAR					
Actual[b]	524.8	530.4	509.4	17.65	0.64
Model 1					
US1	24.1%	26.9%	39.5%	4.93	0.04
US2	4.9%	7.8%	20.8%	3.82	<0.01
Model 2					
US1	24.2%	27.1%	39.7%	4.94	0.04
US2	5.1%	7.9%	20.9%	3.83	<0.01

[a] Carcass fat thickness, inches.

[b] Carcass marbling score, small = 500.

This equation used for predicting FAT was based on the first derivative of a quadratic relationship between FAT and hot carcass weight (Bruns et al., 2004). Early observations (light BW) would result in estimation of a slower accretion rate of FAT due to the underlying equation. When using equations for FAT from Model 2, results suggest the accuracy for FAT projection was within 10% within 158 d and ranged from 13 to 35% from 180 to 264 d. The Model 2 equation used for predicting FAT was based on a linear relationship between FAT and hot carcass weight. Since projections were based on a linear relationship, prediction accuracy improved and variation was compressed over the entire time span.

Despite inaccuracies in projecting FAT, the MAR projection using either Model 1 or Model 2 equations was within 8% of the actual values for ultrasound measurements taken within 123 d of slaughter, but decreased as time between scan and harvest increased. For MAR projection, both Model 1 and Model 2 equations used to predict endpoint MAR were based on a linear relationship between MAR and hot carcass weight and therefore projection accuracies were similar. Wall et al. (2004) reported consistent projections for MAR at 65 or 100 d prior to slaughter, due to the linear nature of MAR deposition.

Breed type heavily influenced actual FAT ($P > 0.01$) and MAR score ($P > 0.01$) at slaughter (Table 7). Angus influenced cattle tended to have greater FAT pooled across all slaughter groups, compared to other breeds. Angus influenced cattle had higher MAR scores, while Brahman influenced had lower scores among various harvest groups. When

Table 7. Least squares means percentage differences for predicted vs actual FAT and MAR values due to breed type using Model 1 and Model 2

	Breed Effect							
Item	MAA	MAB	MBB	MBR	MRA	MRR	SE	*P*-value
FAT								
Actual[a]	0.74	0.57	0.59	0.48	0.51	0.45	0.075	<0.01
Model 1								
US1	-44.4 %	-27.4%	-52.5%	-26.4%	-26.1%	-41.2%	25.43	0.86
US2	7.8%	22.7%	2.8%	29.4%	32.1%	38.1%	14.48	0.18
Model 2								
US1	-4.1%	12.5%	16.9%	26.5%	28.7%	37.5%	17.42	0.22
US2	-12.9%	-0.8%	-14.2%	6.4%	8.3%	22.2%	12.41	0.06
MAR								
Actual[b]	607.5	535.7	474.2	452.6	550.7	508.6	37.55	<0.01
Model 1								
US1	16.6%	21.8%	43.4%	44.2%	20.3%	34.8%	10.49	<0.01
US2	2.6%	5.8%	21.8%	20.1%	4.5%	12.2%	8.13	0.04
Model 2								
US1	16.7%	22.0%	43.6%	44.3%	20.5%	34.9%	10.51	<0.01
US2	2.7%	5.9%	22.0%	20.2%	4.6%	12.3%	8.15	0.04

[a] Carcass fat thickness, inches.

[b] Carcass marbling score, small = 500.

using the Model 1 FAT equation, breed type did not affect projection accuracy of FAT from either ultrasound session ($P = 0.18$), suggesting that within breed accretion rates were similar. This implies that the effect of breed on FAT accretion is an intercept effect rather than a slope or accretion rate difference, thus breed type could be used a scaling factor or an initial starting point for predicting FAT. From the initial scan, results were similar ($P = 0.22$) when using the Model 2 FAT equation; there was however a tendency ($P = 0.06$) for a breed type effect for accuracy of projections based on US2, although a clear trend is difficult to identify. Overall, FAT prediction accuracies as it relates to breed type were improved using the linear-based equation (Model 2).

Breed type influenced projection accuracy for MAR from both ultrasound sessions ($P = 0.04$) when using either Model 1 or Model 2 MAR equations. Projection accuracy for MAR was greater when ultrasound data from US2 were used as basis of projection, yet relative separation among breeds was similar for US1 and US2. Projection accuracy from US2 was within 6% for calves with Angus influence, but over 20% for non-Angus, Brahman influenced calves. The greater accuracy for MAR prediction for Angus-influenced cattle may result from the use of prediction equations based on data generated from both serial slaughter experiments which included only Angus sired cattle. The over estimation of MAR for Brahman influenced calves suggests that accretion rate in these biotypes was lower than in the Angus based cattle. This difference, and the consistency of separation, may allow for a correction factor or breed coefficient to be added to the prediction equation to accommodate breed differences.

Gender impacted actual FAT ($P = 0.02$) and MAR score ($P > 0.01$) at harvest (Table 8). Heifers had greater FAT relative to steers and consistently produced carcasses with higher MAR scores. When fed a similar length of time, heifers would be expected to have greater carcass adiposity due to differences in composition of gain (Owens et al., 1993). Gender also impacted the accuracy of FAT projections ($P < 0.01$) when using the Model 1 FAT equation. There was a tendency ($P = 0.07$) to overestimate FAT in steers when using the Model 2 FAT equation. Gender also influenced projection accuracy of MAR using either Model 1 or Model 2 MAR equations from both US1 ($P < 0.01$) and US2 ($P > 0.04$). The over prediction of both FAT and MAR for steers is likely due to greater rates of carcass gain in steers coupled with slower rates of fat accretion. Both of these factors would result in over prediction of carcass FAT and MAR components using either Model 1 or Model 2 equations. Interestingly, heifers in this study were predicted more accurately even though the equations used in both models were developed using steer data. The reasonably consistent separation among predictions for steers and heifers could be used to create an adjustment factor to refine prediction accuracy further.

Dietary treatment during the growing period did not influence FAT ($P = 0.39$) but did impact MAR score ($P < 0.01$) at slaughter (Table 9). Interestingly, while FAT was similar, Pasture cattle had higher MAR scores following the finishing phase. Cattle were placed into slaughter groups based on an initial FAT measurement and body weight, so a lower initial FAT measurement or body weight would require an extended feeding period. The Pasture-fed cattle had lower FAT following initial treatment period,

Table 8. Least squares means percentage differences for predicted vs actual FAT and MAR values due to gender using Model 1 and Model 2

	Gender Effect			
Item	Heifers	Steers	SE	*P*-value
FAT				
Actual[a]	0.60	0.51	0.03	0.02
Model 1				
US1	-41.2%	-31.5%	9.23	0.40
US2	11.1%	32.7%	5.21	<0.01
Model 2				
US1	11.9%	27.4%	6.32	0.07
US2	-4.4%	7.4%	4.51	0.06
MAR				
Actual[b]	565	478	13.62	<0.01
Model 1				
US1	16.3%	43.9%	3.80	<0.01
US2	0.5%	21.9%	2.94	<0.01
Model 2				
US1	16.5%	44.1%	3.82	<0.01
US2	0.6%	22.0%	2.96	0.04

[a] Carcass fat thickness, inches.

[b] Carcass marbling score, small = 500.

Table 9. Least squares means percentage differences for predicted vs actual FAT and MAR values due to treatment using Model 1 and Model 2

	Treatment Effect			
Item	Feedlot	Pasture	SE	*P*-value
FAT				
Actual[a]	0.54	0.57	0.02	0.39
Model 1				
US1	-14.3%	-58.4%	9.02	<0.01
US2	26.3%	18.0%	5.13	0.21
Model 2				
US1	19.1%	20.3%	6.17	0.87
US2	3.2%	-0.3%	4.41	0.54
MAR				
Actual[b]	493	550	13.32	<0.01
Model 1				
US1	30.8%	29.6%	3.72	0.80
US2	14.6%	7.8%	2.88	0.07
Model 2				
US1	30.9%	29.7%	3.73	0.80
US2	14.7%	7.9%	2.90	0.07

[a] Carcass fat thickness, inches.

[b] Carcass marbling score, small = 500.

allowing for a longer finishing phase and greater MAR accretion. When using the Model 1 FAT equation, treatment only influenced FAT accuracy based on US1 ($P < 0.01$), underestimating Pasture-fed cattle by 58.4%. At the time when US1 measurement was taken, Pasture-fed cattle likely had less measurable FAT and resulted in the under prediction of FAT when forecasted out. Treatment did not affect FAT accuracy from US2 using the Model 1 FAT equation, or FAT accuracy using the Model 2 FAT equation from either session ($P > 0.21$). With respect to the Model 1 FAT equation, a common diet had been fed long enough by the time US2 was taken that accuracy was not altered due to treatment. FAT accuracy was not affected from either session using the Model 2 FAT equation, as serial slaughter carcass measurements used to calculate the FAT equation were based off of steers fed different dietary energy sources germane to the treatments in experiment 2. Prediction accuracy for MAR was not affected ($P > 0.07$) by dietary treatment from either scan session using either Model 1 or Model 2 MAR equations to project it. These results suggest that diet did not affect the rate of FAT or MAR accretion other than through dietary impacts on carcass gain and hot carcass weight.

Results suggest accuracy of FAT and MAR predictions from growth-based equations is influenced by weight gain between ultrasound and endpoint, breed, and gender; although scans out to 120 d pre-harvest may be accurate. Results also suggest that accuracy of FAT predictions improves when using linear-based prediction equation derived from objective 1. Equations derived from serial slaughter steers in objective 1 of this study are unique from previously published work as they capture FAT and MAR

accretion rates from a lighter hot carcass weight and include FAT and MAR accretion rates from steers fed different dietary energy sources. Known sources of variation in accuracy could be used to scale predictions based on breed and gender to improve accuracy, and thus a growth-based, rather than time based, prediction system might be generated.

Implications

Using growth-based prediction equations could further reduce the production risk associated with the variation in individual weight gain, which is inherent to time-based projections. Average daily gain values could be used to estimate carcass weight gain and potentially forecast an appropriate harvest weight relative to a desired carcass endpoint.

CHAPTER V

EFFECT OF DIETARY ENERGY SOURCE ON IN VITRO SUBSTRATE UTILIZATION AND INSULIN SENSITIVITY OF MUSCLE AND ADIPOSE TISSUES OF ANGUS AND WAGYU STEERS

Overview

Angus (n = 8; 210 kg) and 7/8 Wagyu (n = 8; 174 kg) steers were used to test effects of dietary energy source on muscle and adipose tissue metabolism and insulin sensitivity. Steers were assigned to either a grain-based (corn) or hay-based (hay) diet and fed to similar final BW. At slaughter, LM, and s.c., and i.m. adipose tissue samples were collected. Portions of the LM and adipose tissues were placed immediately in liquid nitrogen for later evaluation of glycolytic intermediate concentrations. Fresh LM and s.c. and i.m. adipose tissues were incubated with [U-^{14}C]glucose to assess the glucose metabolism in vitro. All in vitro measures were in the presence of 0 or 500 ng/mL insulin. Also, s.c. and i.m. adipose tissues were incubated with [1-^{14}C]acetate to quantify lipid synthesis in vitro. Glucose-6-phosphate and fructose-6-phosphate concentrations were 12.6 and 2.4-fold greater in muscle than in s.c. and i.m. adipose tissues, respectively. Diet did not affect acetate incorporation into fatty acids (P = 0.86). Insulin did not increase conversion of glucose to CO_2, lactate, or total lipid in steers fed hay, but caused a 116% increase in glucose conversion to CO_2, a 58% increase in glucose conversion to lactate, and a 100% increase in total lipid content in adipose tissue from steers fed corn. Subcutaneous adipose tissue had 37% greater glucose oxidation

than i.m. ($P = 0.04$), and 290% greater acetate incorporation into fatty acids than i.m. ($P = 0.04$) on a per cell basis. Glucose conversion to lactate was accelerated by at least 50% with insulin addition in tissues from steers fed corn, but was not affected by insulin additions in tissues from steers fed hay ($P < 0.001$). Insulin additions to s.c. adipose tissue from corn-fed steers also failed to stimulate increased glucose incorporation into fatty acids; however, exposing i.m. adipose tissue from corn-fed steers to insulin resulted in a 165% increase in glucose incorporation into fatty acids. These results suggest that feeding hay limited both glucose supply and tissue capacity to increase glucose utilization in response to insulin without altering acetate conversion to fatty acids. Because s.c. adipose tissue consistently utilized more acetate and oxidized more glucose than i.m., these results suggest that hay-based diets may alter i.m. adipose tissue metabolism with less impact on s.c. adipose tissue.

Introduction

Beef carcass value is influenced by the quantity and distribution of adipose tissue. Elucidation of metabolic controls of caloric partitioning between adipose depots could lead to development of production solutions that enhance beef carcass value.

Smith and Crouse (1984) demonstrated that i.m. adipocytes utilize glucose while s.c. adipocytes utilize acetate as primary substrates for fatty acid synthesis, and diets higher in starch promote i.m. fat deposition relative to s.c. deposition (Choat et al., 2003). Insulin stimulates peripheral tissue uptake of glucose, and increases lipogenesis or reduces lipolysis; also, plasma insulin concentration is positively correlated with carcass adiposity (Trenkle and Topel, 1978). However, negative correlations between plasma

glucose concentrations and carcass adiposity have been reported (Matsuzaki et al., 1997; Schoonmaker et al., 2003). Variation in insulin sensitivity may impact caloric partitioning among tissues and tissue development. Gilbert et al. (2003) suggests that i.m. tissue is more sensitive to insulin than s.c. tissue. Propionate is both glucogenic and insulinogenic in ruminants (Sano et al., 1995), which may enhance i.m. deposition. Acetate loading may increase glucose demand (Cronje et al., 1991) or increase ketone load. Ketone body accumulation (Tardif et al., 2002) and diet (Waterman et al., 2006) impact insulin sensitivity and thus energy partitioning in cattle. We hypothesized that dietary energy source may alter tissue sensitivity to insulin and the subsequent uptake of glucose. Due to the substrate preferences of adipose tissues, such alterations would result in differential accumulation of energy among fat depots, and may provide a mechanism for manipulating nutrient partitioning. Objectives of this study were to evaluate effects of energy source on in vitro metabolic function and insulin sensitivity in bovine muscle, and s.c. and i.m. adipose tissues.

Materials and Methods

Animals and Management

Animals from which tissues were collected for this experiment were handled according to guidelines established by the Institutional Animal Care and Use Committee at Texas A&M University (AUP# 2005-190AG). Eight Wagyu crossbred (7/8 Wagyu or higher) and 8 Angus steers were purchased as calves at weaning (approximately 8 mo of age). Coastal bermuda grass hay containing 9.5% crude protein was fed free choice for 8 d after the steers were transported to the Texas A&M University Research Center,

McGregor, TX. Four steers of each breed type (n = 8) were assigned to receive a high-energy, corn-based diet containing 48% ground corn, 20% ground milo, 15% cottonseed hulls, 7.5% molasses, 0.96% limestone, 0.56% trace mineral salt, and 0.08% vitamin premix (corn) fed for 480 d. Feeding this diet resulted in an average gain of 0.85 kg/d. The remaining 4 steers of each breed type (n = 8) were offered coastal bermuda grass hay *ad libitum*, supplemented daily with non-protein nitrogen in a cooked molasses carrier, and an amount of the corn-based diet to gain 0.72 kg/d (hay). The hay-fed steers were fed for 600 d after weaning. The mean initial weights for Wagyu and Angus steers were 174 kg and 210 kg, respectively. Targeted final body weights were 650 kg for steers fed for either 480 d on corn or 600 d on the hay-based diet. Although diet and time-on-feed were confounded in the production portion of the trial, steers were fed to BW-constant endpoints. Therefore, it is arguable that retained energy was similar among treatments, and thus differences in time-on-feed were required due to energy concentration differences between diets.

Sample Collection

At the conclusion of the feeding periods, steers from each group were slaughtered on two consecutive days at the Rosenthal Meat Science and Technology Center, Texas A&M University. A section of the LM between the 5th and 8th thoracic ribs was removed immediately following hide removal (approximately 20 min postmortem). The LM section and associated adipose tissues were immediately placed in oxygenated Krebs-Henseleit bicarbonate (KHB) buffer (pH = 7.4; 37^{o}C) with 5 m*M* glucose and transported to the laboratory. Within 20 min post collection, muscle, s.c., and i.m. tissue samples

were dissected from the LM section. Five grams of each tissue type were immersed in liquid nitrogen for analysis of glycolytic intermediate concentration, another 50 to 100 mg of each tissue type was used immediately for measurement of glucose metabolism in vitro, and another 50 to 100 mg of s.c. and i.m. was used for measurement of lipogenesis in vitro.

Substrate Concentrations

Glucose-6-phosphate (G-6-P), and fructose-6-phosphate (F-6-P) were measured from frozen LM, s.c., and i.m. samples from each animal using assay systems described by Bergmeyer (1974), with modifications as described by Rhoades et al. (2005). Briefly, a buffer system containing 0.9 m*M* $NADP^+$ and 1 m*M* ATP was added to each cuvette (total volume = 1.0 mL) along with 0.1 mL of extract for muscle tissue and 0.5 mL of extract for adipose tissue samples. Glucose-6-phosphate dehydrogenase was added to each cuvette catalyzing G-6-P to 6-phosphogluconate and the change in absorbance was measured using a Beckman DU-7400 Spectrophotometer (Palo Alto, CA) set at 339 nm. In the same cuvette, glucose was converted to G-6-P by the addition of hexokinase and the change in absorbance was measured. Subsequently, phosphoglucose isomerase was added to each cuvette to convert F-6-P to G-6-P and the change in absorbance was measured.

Glucose Metabolism in vitro

Glucose metabolism in vitro was measured using fresh muscle, s.c., and i.m. tissues from each animal as described by Espinal et al. (1983). Briefly, 50 to 100 mg of each tissue was incubated in 3 mL of 10 m*M* glucose, KHB and 20 m*M* HEPES buffer

and 1 μCi [U-^{14}C]glucose. Flasks were gassed for 1 min with 95% O_2:5% CO_2, capped with hanging center wells, and incubated in a shaking water bath for 2 hr at 37°C. Bovine insulin (0 or 500 ng/mL) was added only to these flasks receiving glucose and they were replicated within each tissue type, from each animal, at each level of insulin addition. Adding 3 mL of 5% trichloroacetic acid to incubation media stopped all reactions. Determination of [U-^{14}C]glucose carbon conversion to CO_2 trapped in hanging center wells was preformed according to Smith (1983), and the glucose carbon from incubation media was recovered as lactate and determined according to Smith and Freeland (1981). This method of lactate recovery also traps pyruvate and other carboxylic acids. The remaining glucose carbon in the form of fatty acids and glyceride-glycerol was extracted from the incubated tissue (adipose only) portion and the fatty acids synthesized from glucose were separated into the glyceride-fatty acids and glyceride-glycerol fractions by saponification methods described by Hood et al. (1972). All measures of radioactivity were counted using a Beckman liquid scintillation spectrometer (Beckman, Palo Alto, CA).

Lipogenesis in vitro

Lipogenesis from acetate was measured in fresh s.c. and i.m. tissue from each animal according to Page et al. (1997). Briefly, 50 to 100 mg of adipose tissue was incubated in 3 mL of a 10 m*M* sodium acetate, 10 m*M* glucose, KBH and 20 m*M* HEPES buffer (pH 7.40; 37°C), and 1 μCi [1-^{14}C]acetate for 2 h. Flasks were replicated within each tissue type, from each animal. Adding 3 mL of 5% trichloroacetic acid to incubation media stopped all reactions. Measurement of [1-^{14}C]acetate incorporation

into fatty acids was conducted as described by Page et al. (1997). Radioactivity was counted using a Beckman liquid scintillation spectrometer (Beckman, Palo Alto, CA).

Cellularity

Adipocyte number per gram of tissue was determined by method of Etherton et al. (1977), as modified by Smith et al. (1996). Adipose tissue samples were sliced into 1 mm-thick sections, placed into 20-mL scintillation vials and then processed as described by Smith et al. (1996). The fixed cells were filtered through 250-, 64-, and 20-µm mesh screens with 0.01% Triton X-100 in 0.154 *M* NaCl. Cell fractions from the 64- and 20-µm screens were used to determine the number of adipocytes per gram of s.c. and i.m. adipose tissue using a Coulter Counter (model ZM and Coulter Channelyzer 256, Beckman Coulter, Miami, FL).

Calculations involving glucose and acetate utilization measures among adipose tissue depots were expressed on a per cell basis. Diet had no effect on cellularity pooled across depots (corn = 2.12 x 10^5 cells vs. hay = 1.78 x 10^5 cells per 100 mg; $P = 0.44$). However, tissue type did influence adipocyte cellularity (s.c. = 1.67 x 10^5 cells vs. i.m. = 2.22 x 10^5 cells per 100 mg; $P = 0.03$) but no diet by tissue interaction was present.

Data Analysis

Data were analyzed as a split plot. Diet, breed, and their interaction served as main plot effects, and were tested using animal nested within breed x diet as the error term. For response variable relative to substrate concentration and acetate utilization (G-6-P, F-6-P, acetate incorporation into fatty acids), tissue type and diet x tissue served as subplot effects and were tested using the residual mean square as the error term. For

response variables relative to glucose utilization (glucose conversion into CO_2, lactate, and fatty acids), insulin level (0 or 500 ng/mL), tissue x insulin, diet x insulin, diet x tissue, and diet x insulin x tissue were subplot effects, and were tested with residual mean square as the error term. Breed was considered a whole plot blocking factor, and thus was not included in subplot interactions (Kuehl, 1994). Least square means and estimates of variability are consistent with respect to appropriate error terms. When overall F-tests were significant, means were separated using Fisher's Protected LSD. All analyses were performed using the General Linear Models procedures of SAS v.9 (SAS Institute, Cary, NC, USA).

Source of Chemicals

All chemicals and biochemicals were purchased from Fisher Scientific (Pittsburg, PA, USA) or Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). [U-^{14}C]glucose and [U-^{14}C]acetate were purchased from Amersham (Arlington Heights, IL, USA).

Results and Discussion

Concentrations of G-6-P were similar between i.m. and s.c. adipose tissues for cattle fed either diet, but a diet x tissue interaction was observed for G-6-P concentrations (Table 10; $P = 0.05$). The concentration of G-6-P was greater in LM than in adipose tissue depots and was greater in LM tissue of steers fed hay-based diet compared to steers fed corn-based diet. The F-6-P concentrations were similar among diets, but greater F-6-P concentrations were observed in LM than in i.m. or s.c. adipose tissues (Table 10; $P = 0.01$).

Table 10. Least squares means for glycolytic intermediate concentrations (µmol·g) in subcutaneous (s.c) or intramuscular (i.m.) adipose tissues and muscle tissue from steers fed hay- or corn-based diets

	Diet									
	Hay			Corn				*P*-value		
Tissue	s.c.	i.m.	LM	s.c..	i.m.	LM	SE[x]	Diet	Tissue	Diet X Tissue
G-6-P	0.04[c]	0.05[c]	0.80[a]	0.05[c]	0.03[c]	0.47[b]	0.07	0.04	0.01	0.05
F-6-P	0.10[b]	0.05[b]	0.25[a]	0.08[b]	0.04[b]	0.17[a]	0.03	0.26	0.01	0.40

[a,b,c,d]Least squares means without common superscript differ (P < 0.05).

[x]n = 8.

The rate of acetate incorporation into fatty acids was greater (Table 11; $P = 0.03$) in s.c. compared to i.m. adipose tissue, and was not influenced by diet ($P = 0.86$) or interactions between diet and tissue ($P = 0.41$).Glucose oxidation to CO_2 differed among tissues (Table 11; $P = 0.001$). Muscle converted 88% more glucose to CO_2 per 100 mg of tissue than adipose tissues, which were similar. Both dietary energy source ($P = 0.04$) and insulin addition to culture media ($P < 0.001$) influenced rate of glucose conversion to CO_2 per 100 mg of tissue (pooled across LM, s.c., and i.m. tissues). However, the impact of insulin addition on glucose conversion to CO_2 depended upon dietary energy source (Table 11; $P < 0.001$). When the hay-based diet was fed, insulin addition failed to stimulate an increase in glucose conversion to CO_2, however, when corn-based diet was fed, insulin additions resulted in a 116% increase in rate of conversion to CO_2 per 100 mg of tissue.

Consistent with CO_2 data, dietary energy source influenced (Table 11; $P < 0.01$) glucose conversion to lactate per 100 mg of tissue, with tissues from steers fed the corn-based diet converting 190% more glucose to lactate than tissues from steers fed the hay-based diet. Differences in lactate synthesis from glucose among tissues depended on diet (Table 11; diet x tissue, $P < 0.01$). This diet x tissue interaction was primarily due to the large increase in lactate production seen in adipose tissue depots when steers were fed the corn-based diet; the magnitude of difference in lactate yield from LM tissue was smaller among diets.

Table 11. Least squares means for rates of conversion of [U-^{14}C] acetate to fatty acids and conversion of [U-^{14}C] glucose to CO_2, lactate, total lipid, glyceride-fatty acids, and glyceride-glycerol (nmol·100 mg^{-1}·h^{-1}) in muscle (LM), subcutaneous (s.c.) and intramuscular (i.m.) adipose tissues from steers fed hay- or corn-based diets incubated with 0 or 500 ng/mL insulin

Diet	Hay						Corn												
Tissue	s.c.		i.m.		LM		s.c.		i.m.		LM			*P*-value					
Insulin	0	500	0	500	0	500	0	500	0	500	0	500	SE[x]	D	T	I	DxT	DxI	DxTxI[y]
Acetate																			
Fatty acids	15.9	--	7.6	--	--	--	21.4	--	4.2	--	--	--	5.1	0.86	0.03	--	0.41	--	--
Glucose																			
CO_2	6.9[c]	7.9[c]	5.7[c]	7.1[c]	16.1[b]	15.6[b]	8.3[c]	17.6[b]	8.4[c]	17.7[b]	13.4[b]	30.1[a]	2.3	0.04	0.01	0.01	0.94	0.01	0.28
Lactate	179[d]	161[d]	240[d]	171[d]	409[c]	392[c]	564[c]	838[b]	689[b]	1,093[a]	493[c]	819[b]	82.6	0.01	0.13	0.01	0.01	0.01	0.73
Total lipid	23.2[a]	31.5[a]	30.5[a]	23.3[a]	--	--	10.1[b]	16.2[b]	8.5[b]	21.1[a]	--	--	3.9	0.13	0.85	0.09	0.71	0.34	0.06
Glyceride-fatty acids	18.5[a]	27.9[a]	26.5[a]	20.2[a]	--	--	7.8[b]	11.7[b]	6.3[c]	16.8[b]	--	--	3.7	0.14	0.72	0.10	0.75	0.30	0.04
Glyceride-glycerol	4.7[a]	3.6[a]	4.0[a]	3.1[b]	--	--	2.3[b]	4.5[a]	2.1[b]	4.3[a]	--	--	0.4	0.12	0.17	0.05	0.50	0.01	0.79

[a,b,c,d]Least squares means without common superscript differ ($P < 0.05$).

[x]n = 8.

[y]Diet x tissue x insulin interaction effect.

Tissues from steers fed the hay-based diet tended to incorporate more glucose into fatty acids than those from steers fed the corn-based diet ($P = 0.14$), however, the rate of incorporation of labeled glucose into the glyceride-fatty acid fraction in response to insulin additions was dependent upon tissue type and diet when expressed per 100 mg of adipose tissue (Table 11; diet x tissue x insulin, $P = 0.04$). Insulin addition to either s.c. or i.m. adipose tissues from steers fed the hay-based diet had no effect on incorporation rates. Insulin additions to s.c. adipose tissue from steers fed the corn-based diet also failed to stimulate glucose incorporation into fatty acids; however, exposing i.m. adipose tissue from steers fed the corn-based diet to insulin resulted in a 165% increase in glucose incorporation into fatty acids.

The effect of insulin on the conversion of glucose to glyceride-glycerol depended on dietary energy source (Table 11; diet x insulin, $P < 0.01$). When expressed per 100 mg of adipose tissue, insulin decreased glucose incorporation into glyceride-glycerol in adipose tissues from steers fed the hay-based diet. However, adding insulin to tissues from steers fed the corn-based diet increased glyceride-glycerol synthesis from glucose, to the level similar to that observed in steers fed the hay-based diet without insulin.

When responses were analyzed only from adipose tissues, expressed on a per 10^5 cells basis, tissue type influenced the rate of acetate incorporation into fatty acids (Table 12; $P = 0.03$), with s.c. adipose tissue incorporating over 290% more acetate into fatty acids than i.m. adipose tissues.

Table 12. Least squares means for rates of conversion of [U-^{14}C] acetate to fatty acids and conversion of [U-^{14}C] glucose to CO_2, lactate, total lipid, glyceride-fatty acids, and glyceride-glycerol (nmol·10^5 cells^{-1}·h^{-1}) in subcutaneous (s.c.) and intramuscular (i.m.) adipose tissues from steers fed hay- or corn-based diets incubated with 0 or 500 ng/mL insulin

Diet	Hay				Corn										
Tissue	s.c.		i.m.		s.c.		i.m.			*P*-value					
Insulin	0	500	0	500	0	500	0	500	SE[x]	D	T	I	DxT	DxI	DxTxI[y]
Acetate															
Fatty acids	26.4	--	9.15	--	28.1	--	4.8	--	7.8	0.88	0.02	--	0.71	--	--
Glucose															
CO_2	10.8[b]	12.5[b]	6.5[b]	7.9[b]	11.3[b]	23.7[a]	9.5[b]	18.7[a]	2.6	0.04	0.04	0.01	0.76	0.02	0.71
Lactate	272[b]	251[b]	283[b]	205[b]	800[a]	1169[a]	788[a]	1215[a]	159.1	0.01	0.99	0.13	0.88	0.05	0.44
Total lipid	32.8[a]	46.3[a]	38.3[a]	28.5[a]	13.9[b]	22.9[b]	8.9[b]	23.1[b]	7.1	0.08	0.40	0.09	0.71	0.34	0.17
Glyceride-fatty acids	25.9[a]	40.8[a]	33.5[a]	24.8[a]	10.7[b]	16.8[b]	6.4[b]	18.4[b]	6.5	0.09	0.54	0.20	0.75	0.52	0.12
Glyceride-glycerol	6.9[a]	5.5[a]	4.8[a]	3.7[b]	3.2[b]	6.1[a]	2.4[c]	4.7[b]	0.80	0.14	0.01	0.24	0.50	0.01	0.66

[a,b,c]Least squares means without common superscript differ ($P < 0.05$).

[x]n = 8.

[y]Diet x tissue x insulin interaction effect.

Subcutaneous adipose tissue produced 37% more CO_2 than i.m. tissue (Table 12; P = 0.04). Again, when the hay-based diet was fed, insulin caused no significant increase in CO_2 production, but insulin increased glucose conversion to CO_2 by 103% when the corn-based diet was fed (diet X insulin, P = 0.02).

Glucose conversion to lactate per 10^5 cells was nearly four-fold greater in adipose tissues from steers fed the corn-based diet than in adipose tissues from steers fed the hay-based diet (Table 12; $P < 0.01$); because LM was not included in this evaluation, the interaction between diet and tissue was not significant (P = 0.88). Glucose conversion to lactate was accelerated by at least 50% with addition of insulin to tissues from steers fed the corn-based diet, but was not affected by insulin in adipose tissues from steers fed the hay-based diet.

When glucose incorporation into fatty acids was expressed on a cellular basis, the diet x tissue x insulin interaction approached significance (Table 12; P = 0.12). Although the magnitude and direction of separation was similar regardless of basis of expression, greater variation when expressed on a cellular basis resulted in a less sensitive test.

Diet x insulin interaction effects were observed (Table 12; $P < 0.01$) when glyceride-glycerol synthesis from glucose was expressed per 10^5 cells and the effect was similar in both magnitude and direction as when expressed per 100 mg of tissue.

An apparent accumulation of G-6-P and F-6-P would suggest decreased flux of glucose through pathways, and therefore reduced glucose utilization. The concentrations of G-6-P and F-6-P observed in this study were similar to those reported for bovine s.c.

adipose tissue (Smith, 1984), whereas higher concentrations of G-6-P and F-6-P in bovine muscle tissue were reported by Rhoades et al. (2005). Activities of hexokinase (HK) and phosphofructokinase (PFK) are low in bovine adipose tissue (Miller et al., 1991; Smith and Prior, 1981) and PFK limits glycolytic flux in bovine s.c. adipose tissue (Smith, 1984). Similar concentrations of F-6-P between adipose tissues in this study suggested that pathway flux was not constrained by PFK. Miller et al. (1991) reported that the activities of HK and PFK were twice as high in i.m. adipose tissue as in s.c. bovine adipose tissue. Similar metabolite concentrations in adipose tissues in this study suggest that pathway constraints were similar, and therefore, pathway flux is expected to be similar among tissues.

This was confirmed by the measurement of radiolabeled glucose utilization. Total glucose utilization was calculated as the amount of radiolabeled glucose carbon converted into CO_2, lactate, and total lipid. Total glucose utilization was similar across tissues, indicating that glucose uptake and metabolism was not greater in i.m. adipose tissue than in s.c. adipose tissue. Rhoades et al. (2005) reported similar glucose oxidation rates in bovine pars sternomandibularis muscle (14.7 nmol/h) as in the present study. Smith (1983) demonstrated that 55% of glucose was converted to lactate in s.c. adipose tissue when steers were fed a high-energy diet. In our study, at least 85% of glucose resulted in the production of lactate from either diet, but this was similar across tissues.

On a per cell basis, s.c. adipose tissue produced more CO_2 than i.m. adipose tissue. In adipose tissues, up to 25% of CO_2 from glucose is generated through the pentose cycle (Smith, 1983; Smith and Prior, 1986). The activities of glucose-6-

phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are higher in s.c. than in i.m. adipose tissue (Miller et al., 1991), and the greater rate of CO_2 production by s.c. adipose tissue is consistent with this earlier report. Others have demonstrated differences between adipose tissues in rates of CO_2 production. Baldwin et al. (1973) reported that s.c. adipose tissue was six times more active in converting glucose to CO_2 than perirenal adipose tissue and Smith and Crouse (1984) demonstrated that s.c. adipose tissue oxidized more glucose than i.m. adipose tissue in the presence of acetate in the incubation media. Also, early in vitro studies demonstrated that the oxidation of glucose was influenced by insulin. Yang and Baldwin (1973) showed that the addition of insulin to culture media increased glucose oxidation in s.c. adipose tissue from corn fed cattle by up to 45%. Similarly, Baldwin et al. (1973) showed that a progressive increase in insulin stimulation with increasing amounts of concentrate in the diet. In our study, these previous findings are synthesized, as s.c. tissue oxidized more glucose, and increasing concentrate level in our diets resulted in an increased response of glucose oxidation to insulin stimulation.

Utilization of glucose carbon for de novo lipid synthesis was quantified by the incorporation of labeled glucose into glyceride-glycerol and glyceride-fatty acid fractions. Similar results were reported by Gilbert et al. (2003), in which glucose incorporation into glyceride-glycerol was 50% lower in i.m. adipose tissue than in s.c. adipose tissue. Gilbert et al. (2003) also demonstrated that insulin stimulated glucose conversion to glyceride-glycerol in i.m. adipose tissue, but not s.c. adipose tissue. The cattle sampled by Gilbert et al. (2003) were grain fed, and the results of the current study

are similar in that, in the corn-fed steers, insulin stimulated glucose conversion to glyceride-fatty acid and glyceride-glycerol, but only in i.m. adipose tissue.

Glucose was not limiting in the culture media for tissues from steers fed either diet. Thus, it is unlikely that the greater rate of fatty acid synthesis from glucose observed in vitro in the steers fed the hay-based diet would be observed in vivo. This observation suggests that the capacity for fatty acid synthesis in tissue from steers fed the hay-based diet was not compromised, and thus, reduced adiposity in these steers was a function of reduced substrate availability and uptake. This is consistent with the lesser insulin sensitivity of adipose tissues from steers fed the hay-based diet. Our hypotheses were that diet would influence insulin sensitivity, and that i.m tissue would be more sensitive to insulin. These results confirm our hypothesis.

Collectively, these data provide substantial evidence that dietary energy source alters insulin sensitivity, as tissues became insulin resistant when cattle consumed a hay-based diet. A mechanism for this effect was provided by Tardif et al. (2001) who demonstrated that accumulation of ketones interrupted insulin signal transduction and reduced GLUT-4 migration to cell surfaces. This reduction in the insulin-sensitive glucose transporter translocation would reduce insulin-stimulated glucose uptake, and thus would limit the rate of glucose metabolism. Ketone bodies may accumulate under acetate loading, particularly when glucose is limiting (Herdt et al., 1981) and thus, the higher acetate loads anticipated with the hay-based diet may have affected this response. Schoonmaker et al. (2003) found greater levels of insulin in steers fed a high-concentrate diet than in steers fed a high-forage diet during the growing phase. This observation

coupled with our results suggests that LM and adipose tissues in steers fed high concentrate diets would not only be exposed to greater circulating insulin but would also be more sensitive to its effects on glucose uptake and subsequent utilization.

Because s.c. adipose tissue converted more glucose into CO_2 than in i.m. adipose tissue, most likely via the pentose shunt, greater amounts of reducing equivalents (NADPH) would be available to support fatty acid synthesis in this tissue. Past studies have suggested that rates of acetate incorporation into fatty acids are increased when glucose is added to the culture media, due to the additional NADPH available for fatty acid synthesis (Hanson and Ballard, 1967; Smith, 1983). Our results confirm previous findings in which s.c. adipose tissue exhibited greater utilization of acetate than i.m adipose tissue (Smith and Crouse, 1984). Due to the apparently low utilization by i.m. adipose tissue of acetate as a substrate for de novo fatty acid synthesis, and the similarities between tissues for glucose incorporation into lipids, the inference can be made that a limitation in glucose supply or uptake would have a more profound impact on rate of lipogenesis in i.m than in s.c. adipose tissue.

Overall, our results suggest that feeding the hay-based diet limited tissues capacity to increase glucose utilization in response to insulin, without altering acetate conversion to fatty acids. Recent studies (Schoonmaker et al., 2003; 2004a,b) reported no differences in performance due to changes in source and amount of energy, yet differences in carcass adiposity were present, suggesting that dietary source influenced partitioning of energy into different fat depots. Since s.c. adipose tissue consistently utilized more acetate and oxidized more glucose than i.m. adipose tissue, these results

suggest that feeding a hay-based diet may alter i.m. adipose tissue metabolism with less impact on s.c. adipose tissue accretion. These results also support the hypothesis that high-concentrate diets enhance glucose metabolism and increase insulin effects in muscle and adipose tissues. High-concentrate feedstuffs produce a greater proportion of propionic acid than do forage diets (Orskov et al., 1991), and propionic acid is a preferred glucogenic substrate. Conversely, a roughage-based diet provides greater concentrations of acetate. Results from this study confirm previous reports (Smith and Crouse, 1984) that acetate was much more effectively utilized for fatty acid synthesis by s.c. adipose tissue than in i.m. adipose tissue. Previous literature would also suggest that roughage feeding inhibits insulin action. When steers were fed an alfalfa diet, insulin failed to stimulate measures of lipogenesis (Smith et al., 1983). However, several examples with concentrate-fed cattle have demonstrated insulin effects on adipose metabolism, and that this activity increased with amount of concentrate in the diet (Baldwin et al., 1973; Miller et al., 1989; Miller et al., 1991). In this study, LM and adipose tissue from steers fed the hay-based diet was not responsive to additional insulin, while insulin had profound effects on muscle and adipose tissue glucose utilization rates from steers fed the corn-based diet. These differences could lead to a divergent partitioning of energetic substrate in adipose tissue depots from steers fed different diets, in which feeding a corn-based diet enhances glucose uptake in i.m. adipose tissue, whereas feeding a hay-based diet reduced insulin action without altering acetate incorporation in fatty acids. Because s.c. adipose tissue used acetate more effectively than i.m. adipose tissue, feeding a hay-based diet would promote s.c. adipose tissue

deposition over i.m. adipose tissue accretion. Reports exist that are consistent with this hypothesis. Choat et al. (2003) reported increased i.m. adipose tissue deposition in steers fed a concentrate diet that generated 39.3% greater propionate. Schaake et al. (1993) found that feeding grain or high-concentrate diets increased i.m. adipose tissue content relative to forage feeding. These findings, especially in light of our observation about diet effects on insulin sensitivity, correspond with increased accretion of i.m. adipose tissue lipid from glucose carbon (Smith and Crouse, 1984).

Implications

The results of this experiment demonstrate diet-mediated differences in insulin sensitivity of muscle and adipose tissues in steers. Apparent differences in s.c. vs. i.m. adipose tissue metabolism, and their interaction with diet, provide foundation for a hypothesis regarding diet-mediated regulation of differential adipose tissue metabolism. Validation of these hypotheses could generate nutritional strategies that alter the rate and site of adipose deposition.

CHAPTER VI

DIETARY ENERGY SOURCE IMPACT ON IN VITRO SUBSTRATE UTILIZATION AND DOSE RESPONSE TO INSULIN ADDITIONS OF SUBCUTANEOUS ADIPOSE TISSUE OF ANGUS STEERS

Overview

Angus (n = 12) steers were used to test effects of dietary energy source on subcutaneous adipose tissue metabolism and response to increasing levels of insulin. Steers were assigned to either a grain-based (CORN) or hay-based (HAY) diet and fed to common d on feed. Steers fed CORN had 2.44 cm fat thickness while HAY-fed steers had 1.04 cm fat thickness. At slaughter, s.c. adipose samples were collected. Portions of s.c. were cultured with [U-^{14}C]acetate to quantify fatty acid synthesis, or [U-^{14}C]glucose to assess glucose utilization in the presence of 0, 100 or 500 ng/ml insulin. Additional s.c. samples were used to evaluate glycolytic intermediate concentrations as indicators of glucose flux. Data were analyzed as a split-plot with diet in the main plot and insulin level and its interaction with diet in the sub-plot. Within diet linear and quadratic contrasts of insulin level were tested. Plasma and tissue glucose concentrations and glycolytic metabolite concentrations were similar ($P > 0.17$) among diets. Diet had minimal effect ($P > 0.31$) on glucose response variables or acetate utilization ($P = 0.32$). Insulin had no affect ($P > 0.21$) on glucose conversion to CO_2, lactate, and total lipids; nor did it affect ($P = 0.28$) acetate conversion to fatty acids. No diet by insulin interactions ($P > 0.36$) were observed for substrate responses. When steers were fed

CORN, there were no linear ($P > 0.22$) or quadratic ($P > 0.24$) effects of increasing insulin level. However, when steers were fed HAY, a positive linear ($P = 0.06$) effect for glucose oxidation and a tendency ($P = 0.13$) for a quadratic effect of insulin increasing acetate incorporation into fatty acids were observed. These results suggest that s.c. adipose tissue may become resistant to stimulation by insulin in steers fed to an excessive s.c. fat thickness.

Introduction

Smith and Crouse (1984) demonstrated that intramuscular (i.m.) adipocytes utilize glucose while subcutaneous (s.c.) adipocytes utilize acetate as primary substrates for fatty acid synthesis. In general, insulin stimulates peripheral tissue uptake of glucose, and increases lipogenesis or reduces lipolysis. Evidence exists to support the importance of insulin on ruminant adipose tissue metabolism (Yang and Baldwin, 1973; Etherton and Evock, 1986; Rhoades et al. 2007). However, numerous reports have indicated that fatty acid synthesis from glucose and acetate in bovine s.c. adipose tissue is not clearly affected by insulin in vitro (Prior and Smith, 1982; Vernon et al., 1985; Miller et al. 1991). Collectively, the affect of insulin on s.c. adipose tissue metabolism is inconclusive. Diet composition (Scott and Prior, 1980) and total energy intake (Prior, 1979) have also been shown to alter rates of several lipogenic enzymes. Propionate is both glucogenic and insulinogenic in ruminants (Sano et al., 1995), while ketone body accumulation (Tardif et al., 2001) and diet (Waterman et al., 2006) may also impact insulin sensitivity. Gilbert et al. (2003) suggests that i.m. tissue is more sensitive to insulin than s.c. tissue. Adipose tissue insulin sensitivity differences due to dietary

energy source were demonstrated by Rhoades et al. (2007). In this experiment, feeding HAY limited both glucose supply and tissue capacity to increase glucose utilization in response to insulin without altering acetate incorporation into fatty acids. However, feeding CORN increased s.c. glucose conversion to CO_2, lactate, and total lipids in response to insulin at a pharmacological dose. McCann and Reimers (1985) demonstrated that whole body insulin resistance was overcome when a maximum dose of insulin was administered to obese heifers. We hypothesized that differences in s.c. insulin sensitivity generated by dietary energy source in our previous experiment may have been due to insulin dose. Therefore, the objective of this study was to evaluate effects of dietary energy source on in vitro metabolic function and response to increasing levels of insulin in bovine in s.c. adipose tissue.

Materials and Methods

Animals and Management

Animals from which tissues were collected for this experiment were handled according to guidelines established by the Institutional Animal Care and Use Committee at Texas A&M University (AUP# 2006-221AG). Twelve Angus steers were purchased as calves at weaning (approximately 8 mo of age). Coastal bermuda grass hay containing 9.5% crude protein was fed *ad libitum* for 8 d after the steers were delivered to the Texas AgriLife Research-McGregor Center, McGregor, TX. Six steers were randomly assigned to receive a high-energy, corn-based diet containing 48% ground corn, 20% ground milo, 15% cottonseed hulls, 6.5% molasses, 6 % cottonseed meal, 3% limestone, trace mineral salt, and vitamin premix, and 1.5% monensin (CORN) on a DM

basis. Feeding this diet resulted in an average gain of 1.34 kg/d. The remaining 6 steers were offered coastal bermuda grass hay *ad libitum*, supplemented daily with non-protein nitrogen in a cooked molasses carrier, and an amount of the corn-based diet required to sustain a gain of 0.80 kg/d (HAY). All steers were fed for a common time on feed (228 d).

Sample Collection

At the conclusion of the feeding period, steers from each group were slaughtered at the Rosenthal Meat Science and Technology Center, Texas A&M University. At slaughter, blood samples were taken from the jugular vein and collected into a 10 mL tube. Blood tubes were immediately chilled in a cooled container at 7^{o}C. Plasma was harvested from chilled blood tubes by centrifugation at 2,000 x g at 4^{o}C for 20 min. Plasma was stored at -10^{o}C until analysis. A section of s.c. between the 5th and 8th thoracic ribs was removed immediately following hide removal (approximately 20 min postmortem). The s.c. tissue was immediately placed into oxygenated Krebs-Henseleit bicarbonate (KHB) buffer (pH = 7.4; 37^{o}C) with 5 m*M* glucose and transported to the laboratory, where 5 g of tissue were immediately excised and immersed in liquid nitrogen for analysis of glycolytic intermediate concentration. A 50 to 100 mg of tissue was sample was excised and used immediately for measurement of glucose metabolism in vitro, and an additional 50 to 100 mg sample of s.c. was used for measurement of lipogenesis in vitro.

Substrate Concentration

Plasma glucose concentrations were determined from blood samples collected at slaughter. Commercial kits (Glucose LIQUI-UV with Hexokinase, Stanbio Laboratory, Boerne, TX) were used for analysis of plasma glucose using 200 µL of provided reagent and 8 µL of sample for reaction and change in absorbance was measured using a KC4 v3.3 Synergy HT Multi-Detection Microplate Reader (Bio-Tek Instruments, Inc. Winooski, VT) at 340 nm. Glucose, glucose-6-phosphate (G-6-P), and fructose-6-phosphate (F-6-P) were analyzed from s.c. samples from each animal using assay systems described by Bergmeyer (1974), with modifications as described by Rhoades et al. (2005). Briefly, a buffer system containing 0.9 m*M* $NADP^+$ and 1 m*M* ATP was added to each cuvette (total volume = 1.0 mL) along with 0.5 mL of s.c. extract. Glucose-6-phosphate dehydrogenase was added to each cuvette catalyzing G-6-P to 6-phosphogluconate and the change in absorbance was measured using a Beckman DU-7400 Spectrophotometer (Palo Alto, CA) set at 339 nm. In the same cuvette, glucose was converted to G-6-P by the addition of hexokinase and the change in absorbance was measured at 339 nm. Subsequently, phosphoglucose isomerase was added to each cuvette to convert F-6-P to G-6-P and the change in absorbance was measured at 339 nm.

Glucose Metabolism in vitro

Glucose metabolism in vitro was measured using fresh s.c. tissue from each animal as described by Espinal et al. (1983). Briefly, 50 to 100 mg of s.c. tissue was incubated in 3 mL of 10 m*M* glucose, KHB and 20 m*M* HEPES buffer and 1 µCi [U-

^{14}C]glucose. Flasks were gassed for 1 min with 95% O_2:5% CO_2, capped with hanging center wells, and incubated in a shaking water bath for 2 hr at 37^{o}C. Bovine insulin (27 USP units/mg) from bovine pancreas (Sigma-Aldrich Corp. St. Louis, MO) was added to these flasks at 0, 100, or 500 ng/mL and replicated within each animal at each level of insulin addition. Adding 3 mL of 5% trichloroacetic acid to incubation media stopped all reactions. Determination of [U-^{14}C]glucose carbon conversion to CO_2 trapped in hanging center wells was preformed according to Smith (1983), and the glucose carbon from incubation media was recovered as lactate and determined according to Smith and Freeland (1981). This method of lactate recovery also traps pyruvate and other carboxylic acids. The remaining glucose carbon in the form of fatty acids and glyceride-glycerol was extracted from the incubated tissue. The fatty acids synthesized from glucose were separated into the glyceride-fatty acids and glyceride-glycerol fractions by saponification methods described by Hood et al. (1972). All measures of radioactivity were counted using a Beckman liquid scintillation spectrometer (Beckman, Palo Alto, CA).

Lipogenesis in vitro

Lipogenesis from acetate was measured in fresh s.c. tissue from each animal according to Page et al. (1997). Briefly, 50 to 100 mg of s.c. adipose tissue was incubated in 3 mL of a 10 m*M* sodium acetate, 10 m*M* glucose, KBH and 20 m*M* HEPES buffer (pH 7.40; 37^{o}C), and 1 µCi [1-^{14}C]acetate for 2 h. Bovine insulin (0, 100, or 500 ng/mL) was added to these flasks and replicated within animal at each level of insulin addition. Adding 3 mL of 5% trichloroacetic acid to incubation media stopped all

reactions. Measurement of [1-^{14}C]acetate incorporation into fatty acids was conducted as described by Page et al. (1997). Radioactivity was counted using a Beckman liquid scintillation spectrometer (Beckman, Palo Alto, CA).

Cellularity

Adipocyte number per gram of tissue was determined by method of Etherton et al. (1977), as modified by Smith et al. (1996). Adipose tissue samples were sliced into 1 mm-thick sections, placed into 20-mL scintillation vials and then processed as described by Smith et al. (1996). The fixed cells (50 cells per sample) were sized using a microscope and recorded for further calculations. Mean cell diameter was calculated as the average diameter of counted and recorded cells from each sample. The mean cell volume was calculated based on mean cell size. The number of adipose cells per gram of tissue was then calculated based on mean cell volume. Calculations involving glucose and acetate utilization measures were expressed on a per cell basis.

Data Analysis

Data were analyzed as a split plot. Diet served as the whole plot effect, and was tested using animal nested within diet as the error term. For response variables related to substrate concentrations (plasma glucose, tissue glucose, G-6-P, F-6-P), diet served as the whole plot effect and was tested using animal nested within diet as the error term, as these samples represent the whole plot experimental unit. For response variables related to glucose utilization (glucose conversion into CO_2, lactate, and fatty acids) and acetate utilization (acetate incorporation into fatty acids), insulin level (0, 100, or 500 ng/mL) and diet x insulin were the subplot effects, and were tested with residual mean square as

the error term. When overall F-tests were significant, means were separated using Fisher's Protected LSD. In order to characterize the nature of the insulin dose response among diets, preplanned linear and quadratic contrasts of insulin level were tested and coefficients were adjusted for unequal spacing using IML procedures of SAS (SAS Institute, Cary, NC) to generate appropriate contrast coefficients. All analyses were performed using the Mixed Linear Models procedures of SAS v.9.

Source of Chemicals

All chemicals and biochemicals were purchased from Fisher Scientific (Pittsburg, PA, USA) or Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). [U-^{14}C]glucose and [U-^{14}C]acetate were purchased from Amersham (Arlington Heights, IL, USA).

Results and Discussion

Carcass Trait Measurements

The carcass characteristics for steers fed a HAY or CORN-based diet are shown in Table 13. Steers used in this study were fed a different dietary energy source throughout the entire finishing period and slaughtered at common time on feed. Therefore, by design, large differences in carcass characteristics were generated due to treatment. Difference in dietary energy source during finishing resulted in greater HCW for CORN-fed steers ($P < 0.01$). Additionally, carcass adjusted fat thickness and marbling scores were 123 and 48% greater for CORN-fed steers compared to HAY-fed steers at slaughter ($P < 0.01$). Differences in carcass weight were not sufficient to overcome the greater adiposity of CORN-fed, steers, such that numerical yield grade was greater ($P < 0.01$).

Table 13. Least squares means for carcass characteristics from steers fed HAY- or CORN-based diets

	Diet			*P*-value
Item	HAY	CORN	SE[x]	Diet
HCW, kg	284	364	14.6	<0.01
Adjusted fat thickness, cm	0.43	0.96	0.09	<0.01
Marbling score[y]	440	600	18.2	<0.01
Yield grade	3.03	4.82	0.36	<0.01

[x]n = 12.

[y]Marbling score, small = 500.

Cellularity

Adipocytes per gram of tissue was similar (Table 14; $P = 0.52$) in s.c. adipose tissue when steers were fed either diet. Additionally, diet did not impact ($P = 0.53$) mean cell diameter or mean cell volume in s.c. adipose tissue from steers used in this study. Differences in carcass fat thickness due to dietary treatment are difficult to explain based on similarity of adipocyte tissue traits among diets.

Glucose and Glycolytic Intermediate Concentrations

Plasma glucose concentrations tended to be greater when steers were fed CORN than for those steers fed HAY (Table 15; $P = 0.17$), with CORN-fed steers having concentrations 30% greater. Tissue glucose concentrations (Table 15; $P = 0.27$) were similar among CORN and HAY-fed steers, and were substantially lower than plasma concentrations. Similar concentrations (Table 15; $P = 0.31$) of G-6-P and F-6-P were present in s.c. tissue from steers fed CORN or HAY.

Substrate Utilization and Dose Response

Tissue from steers fed the HAY-based diet tended to incorporate more acetate into fatty acids than did tissue from steers fed the CORN-based diet (Table 16; $P = 0.11$). Pooled across insulin levels, HAY-fed steers incorporated 42% more acetate into fatty acids than CORN-fed steers when expressed per 100 mg of tissue. The rate of acetate incorporation into fatty acids was not influenced by insulin ($P = 0.20$) addition to culture media, nor the interaction between diet and insulin level ($P = 0.23$). However, there was tendency (Table 16; $P = 0.09$) for a quadratic effect of additional insulin on acetate incorporation into fatty acids when steers were fed the HAY diet. When steers were fed

Table 14. Least squares means for adipose tissue traits in subcutaneous (s.c.) adipose tissue from steers fed HAY- or CORN-based diets

	Diet			*P*-value
Item	HAY	CORN	SE[x]	Diet
Cells per g, 10^{-5}	9.71	8.35	1.45	0.52
Mean diameter, µm	59.9	62.8	3.25	0.53
Mean volume, pL	1,174	1,353	194	0.53

[x]n = 12.

Table 15. Least squares means for glycolytic intermediate concentrations (µmol·g) in subcutaneous (s.c) adipose tissue from steers fed HAY- or CORN-based diets

	Diet			*P*-value
Item[1]	HAY	CORN	SE[x]	Diet
Plasma glucose	112.2	146.3	16.39	0.17
Tissue glucose	1.329	0.595	0.443	0.27
G-6-P	0.064	0.098	0.035	0.51
F-6-P	0.276	0.131	0.096	0.31

[x]n = 12.

[1]G-6-P = glucose-6-phosphate, F-6-P = fructose-6-phosphate.

Table 16. Least squares means for rates of conversion of [U-^{14}C] acetate to fatty acids and conversion of [U-^{14}C] glucose to CO_2, lactate, total lipid, glyceride-fatty acids, and glyceride-glycerol (nmol·100 mg^{-1}·h^{-1}) in subcutaneous (s.c.) adipose tissue from steers fed HAY- or CORN-based diets incubated with 0, 100 or 500 ng/ml insulin

Diet	HAY			CORN				*P*-value						
											HAY		CORN	
Item	0	100	500	0	100	500	SE[x]	Diet	Insulin	Diet x Insulin	Linear	Quadratic	Linear	Quadratic
Acetate														
Fatty acids	109	151	118	68.5	73.1	79.0	23.9	0.11	0.20	0.23	0.80	0.09	0.27	0.76
Glucose														
CO_2	15.2	17.1	19.7	13.6	12.8	14.5	1.92	0.11	0.19	0.48	0.05	0.60	0.56	0.65
Lactate	348	409	385	404	422	476	33.3	0.24	0.47	0.35	0.82	0.50	0.12	0.93
Total lipid	12.3	11.7	13.2	11.9	10.3	10.3	1.05	0.18	0.46	0.37	0.33	0.51	0.36	0.29
Glyceride-fatty acids	3.16	3.33	3.33	3.51	3.28	3.34	0.34	0.76	0.99	0.77	0.74	0.71	0.78	0.63
Glyceride-glycerol	9.16	8.39	9.92	8.45	7.05	7.00	0.97	0.13	0.40	0.38	0.32	0.38	0.36	0.33

[x]n = 12.

CORN, there were no linear ($P = 0.27$) or quadratic ($P = 0.76$) effects of additional insulin on acetate incorporation into fatty acids. Similarly, s.c. tissue from steers fed the HAY-based diet tended to convert more glucose to CO_2 than did s.c. tissue from steers fed the CORN-based diet (Table 16; $P = 0.11$) when expressed per 100 mg of tissue. The rate of glucose conversion to CO_2 was not influenced by insulin ($P = 0.19$), nor the interaction between diet and insulin ($P = 0.48$). There was a positive linear (Table 16; $P = 0.05$) effect of increasing insulin levels on CO_2 production when steers were fed HAY, but no linear ($P = 0.56$) or quadratic ($P = 0.65$) effects of additional insulin on CO_2 production when steers were fed CORN.

Glucose conversion to lactate when expressed per 100 mg of tissue was not influenced by dietary energy source (Table 16; $P = 0.24$), insulin ($P = 0.47$), or the interaction between diet and insulin ($P = 0.35$). Additionally, there were no linear (Table 16; $P = 0.82$) or quadratic ($P = 0.50$) effects of additional insulin on lactate production when steers were fed HAY. When steers were fed the CORN-based diet there was a tendency ($P = 0.12$) for a linear effect of additional insulin on lactate production.

Incorporation of glucose into fatty acids when expressed per 100 mg of tissue was not influenced by diet (Table 16; $P = 0.76$), insulin ($P = 0.99$), or the interaction between diet and insulin ($P = 0.77$). Similarly, there were no linear (Table 16; $P = 0.74$) or quadratic ($P = 0.63$) effects of additional insulin on glucose conversion to the fatty acid portion of total lipids when steers were fed either diet.

Tissue from steers fed the HAY-based diet tended to incorporate more glucose into the glyceride-glycerol fraction than did tissue from steers fed the CORN-based diet

(Table 16; $P = 0.13$) when expressed per 100 mg of tissue. However, incorporation of glucose into glyceride-glycerol was not influenced by insulin ($P = 0.40$), or the interaction between diet and insulin ($P = 0.38$). No linear (Table 16; $P = 0.32$) or quadratic ($P = 0.33$) effects of additional insulin on glucose conversion to the glycerol-glyceride fraction of total lipid were observed when steers were fed either diet.

When responses were analyzed on a per 10^5 cells basis, all tendency differences due to dietary treatment were eliminated for all glucose and acetate response variables. Neither diet, insulin, nor their interaction impacted the rate of acetate incorporation into fatty acids per cell ($P = 0.28$; Table 17). However, there was still a tendency ($P = 0.13$) for a quadratic effect of additional insulin level on acetate incorporation into fatty acids when steers were fed HAY. The addition of 100 ng/ml of insulin to HAY-fed s.c. tissue resulted in a 38% increase in fatty acid synthesis from acetate, but then declined to an intermediate level with the addition of 500 ng/ml insulin.

When the rate of glucose incorporation into CO_2 was expressed on a per cell basis, dietary treatment had no effect on CO_2 production ($P = 0.63$; Table 17). A linear effect of insulin addition on CO_2 production was still observed in s.c. tissue from steers fed the HAY-based diet. Subcutaneous adipose tissue from HAY-fed steers produced 11 and 15% more CO_2 with increasing insulin levels ($P = 0.06$) when expressed on a per cell basis. No linear or quadratic trends in glucose conversion to CO2 were observed in s.c. tissue from CORN-fed steers.

Table 17. Least squares means for rates of conversion of [U-^{14}C] acetate to fatty acids and conversion of [U-^{14}C] glucose to CO_2, lactate, total lipid, glyceride-fatty acids, and glyceride-glycerol (nmol·10^5 cells^{-1}·h^{-1}) in subcutaneous (s.c.) adipose tissue from steers fed HAY- or CORN-based diets incubated with 0, 100 or 500 ng/ml insulin

Diet	HAY			CORN				*P*-value						
											HAY		CORN	
Item	0	100	500	0	100	500	SE[x]	Diet	Insulin	Diet x Insulin	Linear	Quadratic	Linear	Quadratic
Acetate														
Fatty acids	128	177	151	98.8	101	106	35.9	0.32	0.28	0.36	0.78	0.13	0.60	0.91
Glucose														
CO_2	18.1	20.3	23.2	17.8	16.5	20.2	3.75	0.63	0.21	0.70	0.06	0.57	0.42	0.61
Lactate	448	475	450	575	571	651	100	0.31	0.62	0.42	0.86	0.54	0.22	0.77
Total lipid	15.1	14.1	16.2	15.9	13.8	14.2	3.09	0.91	0.41	0.52	0.40	0.48	0.49	0.24
Glyceride-fatty acids	3.65	3.97	3.94	4.82	4.36	4.54	0.76	0.47	0.98	0.66	0.71	0.60	0.84	0.54
Glyceride-glycerol	11.5	10.1	12.2	11.1	9.48	9.72	2.52	0.73	0.37	0.55	0.41	0.35	0.50	0.31

[x]n = 12.

Glucose conversion to lactate per 10^5 cells was not influenced by diet, insulin, or their interaction ($P = 0.31$; Table 17). The tendency toward a linear effect of insulin addition on lactate production in s.c. tissue from CORN-fed steers when expressed per unit of tissue weight was obviated when expressed on the basis of cellularity ($P = 0.22$). Although the direction of separation was similar regardless of basis of expression, greater variation when expressed on a cellular basis resulted in a less sensitive test.

When glucose incorporation into fatty acids was expressed on a cellular basis, the results were similar to those reported on unit weight of tissue basis, as neither diet, insulin, nor their interaction influenced incorporation rate ($P = 0.47$; Table 17). Hay-fed steers tended to convert more glucose into glyceride-glycerol when expressed per gram of tissue, however when rate were calculated on a per cell basis, diet did not influence incorporation rate ($P = 0.73$).

The numerically greater concentration of circulating glucose in CORN-fed steers is expected due to the higher production of propionate from starch fermentation associated with the CORN-based diet (Orskov et al., 1991), and the preferential use of propionate as a glucogenic substrate by ruminants (Danfaer et al., 1995). Tissue glucose concentration reflects the balance between glucose uptake and subsequent tissue utilization of glucose. The apparent accumulation of free glucose in s.c. tissue from HAY-fed steers maybe due to low hexokinase activity. An accumulation of G-6-P and F-6-P would suggest decreased flux of glucose through pathways, and therefore reduced glucose utilization. The concentrations of G-6-P and F-6-P observed in this study were higher than those previously reported for bovine s.c. adipose tissue (Smith, 1984;

Rhoades et al., 2007). Activities of hexokinase (HK) and phosphofructokinase (PFK) are low in bovine adipose tissue (Miller et al., 1991; Smith and Prior, 1981) and PFK limits glycolytic flux in bovine s.c. adipose tissue (Smith, 1984). A similar concentration of G-6-P and F-6-P in adipose tissue in this study suggests that pathway flux was not constrained by PFK. Therefore, the apparent accumulation of free glucose in s.c. tissue from HAY-fed steers observed in this study is less likely the result of reduced pathway flux and more likely the result of low hexokinase activity. Thus, since CORN-fed steers were exposed to a greater amount of circulating glucose, yet had lower tissue glucose concentrations, suggests that the CORN-fed steers may have experienced a reduction in initial glucose uptake compared to HAY-fed steers.

Total glucose utilization could be calculated as the amount of radiolabeled glucose carbon converted into CO_2, lactate, and total lipid. Since glucose response variables were similar across diet, total glucose utilization was similar among diets. This indicates that glucose uptake and metabolism was not greater in HAY-fed adipose tissue than in CORN-fed adipose tissue. In steers fed similar diets, total glucose utilization was similar although the partioning among metabolic fates was different (Rhoades et al., 2007). Robertson et al. (1982) reported that a large portion of the glucose taken up by adipose tissue can be recycled as lactate. Smith (1983) demonstrated that 55% of glucose was converted to lactate in s.c. adipose tissue when steers were fed a high-energy diet. Results in this study are similar to those reported by Rhoades et al. (2007), that at least 85% of glucose resulted in the production of lactate from either diet.

On a per cell basis, there was a tendency for the quadratic effect of additional insulin on fatty acid synthesis from acetate in s.c. tissue from HAY-fed steers, such that insulin increased acetate incorporation into fatty acids at a decreasing rate. Similarly, on a per cell basis, a positive linear effect of increasing insulin level on CO_2 production was observed when steers were fed the HAY-based diet. Previous studies have demonstrated that rates of acetate incorporation into fatty acids are increased when glucose is added to the culture media, due to the additional NADPH available for fatty acid synthesis (Hanson and Ballard, 1967; Smith, 1983). Early work demonstrated that a change in NADPH generation was in response to an increase in fatty acid synthesis (Ingle et al., 1972; Martin et al., 1973). Thus, in the current study it is likely the greater oxidation rate and subsequent yield of reducing equivalents (NADPH) through the pentose shunt was observed in s.c. tissue from HAY-fed steers in response to the greater rate of acetate incorporation into fatty acids observed in HAY-fed s.c. adipose tissue. Smith (1983) demonstrated that 33 to 43% of the NADPH required for lipogenesis from acetate can be derived from the pentose cycle.

In part, our original hypothesis was that feeding HAY would limit the ability of s.c. adipose tissue to utilize substrate in response to insulin. Previous literature would also suggest that roughage feeding inhibits insulin action. When steers were fed an alfalfa diet, insulin failed to stimulate measures of lipogenesis (Smith et al., 1983). Few reports exits that demonstrate a positive effect of insulin on acetate or glucose utilization in s.c. adipose tissue; particularly when cattle are fed a HAY-based diet. However, Smith et al. (1983) reported that injections of insulin tended to increase acetate

incorporation and lipogenic enzyme activity, when simultaneously infused with glucose when steers were fed a roughage-based diet. Glucose is required for lipogenesis in ruminant adipose tissue as a precursor for glyceride-glycerol and the major source of NADPH required for fatty acid synthesis (Smith, 1983). In our study, glucose was not limiting in the culture media for tissue from steers fed either diet. Thus, it is unlikely that insulin would impact the rate of fatty acid synthesis from acetate in vivo in the steers fed the HAY-based diet when glucose was limiting. If rate of fatty acid synthesis in vivo cannot be increased due to the lack of available glucose than it is likely that glucose oxidation in s.c. tissue from HAY-fed steers would not be sensitive to insulin either.

Utilization of glucose carbon for de novo lipid synthesis was quantified by the incorporation of labeled glucose into glyceride-glycerol and glyceride-fatty acid fractions. Glucose incorporation into glyceride-glycerol and glyceride-fatty acids was not affected by diet, insulin, or their interaction. For glucose conversion to glyceride-glycerol, previous studies have demonstrated that intramuscular adipose tissue not s.c. tissue was sensitive to insulin additions when steers are fed CORN (Gilbert et al., 2003; Rhoades et al., 2007). The lack of insulin sensitivity with regard to glucose incorporation into total lipid in s.c. adipose tissue from steers fed either diet in this study is consistent with our previous results.

Our other hypothesis was that diet would influence insulin sensitivity, such that s.c tissue from CORN-fed steers would be more sensitive to insulin. High-concentrate feedstuffs produce a greater proportion of propionic acid than do forage diets (Orskov et al., 1991), and propionic acid is a preferred glucogenic substrate. Schoonmaker et al.

(2003) found greater levels of insulin in steers fed a high-concentrate diet than in steers fed a high-forage diet during the growing phase. Several examples with concentrate-fed cattle have demonstrated insulin effects on adipose metabolism, and that this activity increased with amount of concentrate in the diet (Baldwin et al., 1973; Miller et al., 1989; Miller et al., 1991). Most recently Rhoades et al. (2007) reported that when steers were fed a high-concentrate diet, glucose metabolism was stimulated by insulin in adipose tissue samples. However, in our previous study the effect of diet on insulin sensitivity was most noticeable in intramuscular adipose tissue. Additionally, in Rhoades et al. (2007) cattle from either diet were fed to a common compositional endpoint (time on feed varied); as a result adjusted fat thickness was similar among dietary treatment groups. The overall adjusted fat thickness from cattle used in Rhoades et al. (2007) was 45% less than the adjusted fat thickness from cattle fed the CORN diet in the current study. Thus, it is possible that the insulin resistance observed in s.c. adipose tissue from those steers fed CORN in the current study is a result of these cattle being fed to an excessive fat thickness. McCann and Reimers (1985) demonstrated that obese heifers were resistant to the glucoregulatory effects of insulin. However, these authors reported that whole body insulin resistance was overcome when a maximum dose of insulin was administered in vivo to obese heifers; concluding that in obese heifers the decreased insulin action to a low dose administration was a result of a decrease in the number of receptors. In the current study, insulin dose had no real effect on s.c. adipose tissue sensitivity and subsequent uptake of substrate. Schoonmaker et al. (2004b) established that greater lipid filling or complete maturity of an adipocyte occurs

sooner when cattle are fed CORN vs. HAY. Production data from steers used in the current study indicate that CORN fed cattle had a greater amount of carcass fat thickness, while cellularity data suggests that a similar number of adipocytes were present. These observations suggest a greater level of maturity may have reduced the incorporation of acetate and glucose into lipid within CORN-fed adipocytes. When the tissues ability to synthesize acetate and glucose into lipid is not compromised; it becomes a matter of how much glucose can enter the cell due to limited insulin sensitivity.

Collectively, these data provide evidence that glucose and acetate metabolism in s.c. adipose tissue was minimally impacted by dietary energy source. These data also suggest that insulin resistance in s.c. adipose tissue is not dose driven, but that s.c. adipose tissue is inherently resistant to the effects of insulin. This is consistent with numerous other reports that suggest insulin has little to no effect on ruminant s.c. adipose tissue metabolism (Prior and Smith, 1982; Vernon et al., 1985; Miller et al., 1991). It was anticipated that s.c. adipose tissue from those steers fed CORN would be more sensitive to the action of insulin based off of previous results generated in our lab. However, in our previous study (Rhoades et. al., 2007) insulin was only added to culture media at a pharmacological dose of 500 ng/mL, so an increase in substrate utilization in response to insulin would suggest that s.c. tissue was responsive not necessarily sensitive to the action of insulin. Feeding HAY for a long period of time in our previous study blunted any response to insulin by either tissue, so the minimal impact that insulin had on substrate utilization when steers were fed HAY in this study is consistent with our hypothesis. Overall, our results suggest that feeding cattle to an excessive fat thickness

limited s.c. tissues capacity to increase glucose and acetate utilization in response to insulin.

Implications

The results of this experiment demonstrate that dietary energy source has minimal impact on glucose and acetate metabolism in s.c. adipose tissue in steers. Results also suggest that s.c. adipose tissue is not sensitive to the action of insulin. More work is needed to validate the role that carcass adiposity plays on adipose tissue metabolism and insulin sensitivity of fat depots.

CHAPTER VII

EFFECT OF DIETARY ENERGY SOURCE DURING BACKGROUNDING ON IN VITRO SUBSTRATE UTILIZATION AND INSULIN SENSITIVITY OF MUSCLE AND ADIPOSE TISSUES OF ANGUS STEERS

Overview

An experiment was designed to test effects of dietary energy source and compositional endpoint on adipose tissue metabolism and insulin sensitivity. Angus steers (n = 17; 223 kg) were randomly assigned to either a grain-based (CORN) or hay-based (HAY) diet after weaning (8 mo) for 120 d, after which all steers were fed a CORN diet. Steers were serially slaughtered at 120, 240 and 300 d to achieve two common endpoints (A and B) based on similar fat thickness. At slaughter, muscle (LM), s.c., and i.m. samples were collected. Portions of LM, s.c., and i.m. were incubated with [U-^{14}C]glucose and adipose tissues only with [U-^{14}C]acetate to quantify utilization in vitro with 0 or 500 ng/mL insulin. Data were analyzed as a split-split plot with diet, endpoint, and their interaction in the main plot; tissue and its interaction with main plot effects in the sub-plot, and insulin and its interaction with sub-plot effects in the sub-sub plot. There was greater ($P < 0.01$) glucose oxidation in i.m. from HAY-fed steers. Endpoint B HAY-fed steers converted 28 and 50% more ($P < 0.04$) glucose to CO_2 and lactate than CORN-fed steers. Insulin increased ($P = 0.06$) glucose conversion to lipid in endpoint A steers, but had minimal impact in endpoint B steers. Insulin increased ($P = 0.06$) glucose conversion to lipid in i.m. from CORN-fed steers, but had limited impact

on i.m. from HAY-fed steers or s.c. from steers fed either diet. HAY-fed steers incorporated 204% more ($P = 0.05$) acetate into fatty acids. Insulin increased ($P = 0.02$) acetate incorporation into fatty acids in s.c., but not i.m. adipose tissue. Results suggest that feeding HAY during backgrounding may have differential effects on tissue lipogenesis. Feeding HAY increased both glucose oxidation and incorporation of acetate into fatty acids; in i.m. insulin failed to stimulate glucose conversion to lipid. Additionally, as physiological maturity increases, glucose conversion to CO_2 and lactate increased, but the ability of insulin to stimulate lipid synthesis from glucose maybe reduced.

Introduction

Smith and Crouse (1984) demonstrated that intramuscular (i.m.) adipocytes utilize glucose while subcutaneous (s.c.) adipocytes utilize acetate as primary substrates for fatty acid synthesis. In general, insulin stimulates peripheral tissue uptake of glucose, and increases lipogenesis or reduces lipolysis. Gilbert et al. (2003) suggests that i.m. tissue is more sensitive to insulin than s.c. tissue. Propionate is both glucogenic and insulinogenic in ruminants (Sano et al., 1995), while ketone body accumulation (Tardif et al., 2001) and diet (Waterman et al., 2006) may also impact insulin sensitivity. Adipose tissue insulin sensitivity differences due to dietary energy source were demonstrated by Rhoades et al. (2007). However, our previous study only tested the effects of dietary energy source on substrate utilization and insulin sensitivity when cattle were fed a single energy source from weaning to harvest. A typical beef production system would background cattle on a hay-based diet previous to feeding a high-energy

diet in a finishing program. Sainz and Paganini (2004) suggested that the decrease in i.m. deposition when cattle were grazed for a prolonged period of time maybe the result of the animal's inability to deposit i.m. fat. Other work (Rhoades et al., 2008*) would suggest that compositional endpoint (i.e. excessive fat thickness) may limit the ability of adipose tissue to utilize substrate in response to insulin. We hypothesized that feeding a hay-based diet during the growing phase may limit the ability of i.m. tissue to utilize glucose in response to insulin. Additionally, there is reason to believe that in adipose tissues, substrate incorporation into fatty acids in response to insulin maybe compromised with advancing physiological maturity. Therefore, the objective of this study was to evaluate effects of dietary energy source during backgrounding and compositional endpoint on in vitro metabolic function and insulin sensitivity in bovine muscle and i.m. and s.c. adipose tissue.

Materials and Methods

Animals and Management

Animals from which tissues were collected for this experiment were handled according to guidelines established by the Institutional Animal Care and Use Committee

at Texas A&M University (AUP# 2006-221AG). Seventeen Angus steers were purchased as calves at weaning (approximately 8 mo of age). Coastal bermuda grass hay containing 9.5% crude protein was fed free choice for 8 d after the steers were transported to the Texas AgriLife Research-McGregor Center, McGregor, TX. Eight steers were assigned to receive a high-energy, corn-based diet containing 48% ground corn, 20% ground milo, 15% cottonseed hulls, 6.5% molasses, 6 % cottonseed meal, 3% limestone, trace mineral salt, and vitamin premix, and 1.5% monensin (CORN) on a DM basis and fed for 120 d. Feeding this diet resulted in an average gain of 0.85 kg/d. The remaining nine steers were offered coastal bermuda grass hay *ad libitum*, supplemented daily with non-protein nitrogen in a cooked molasses carrier, and an amount of the corn-based diet to gain 0.72 kg/d (HAY). The hay-fed steers were fed for 120 d after weaning. Following the 120 d treatment period, all steers were fed the corn-based diet until slaughter. Steers were serially slaughtered at 120, 240, and 300 d post-weaning to achieve common endpoints based on adjusted fat thickness. A more detailed description of treatment structure is in Figure 8.

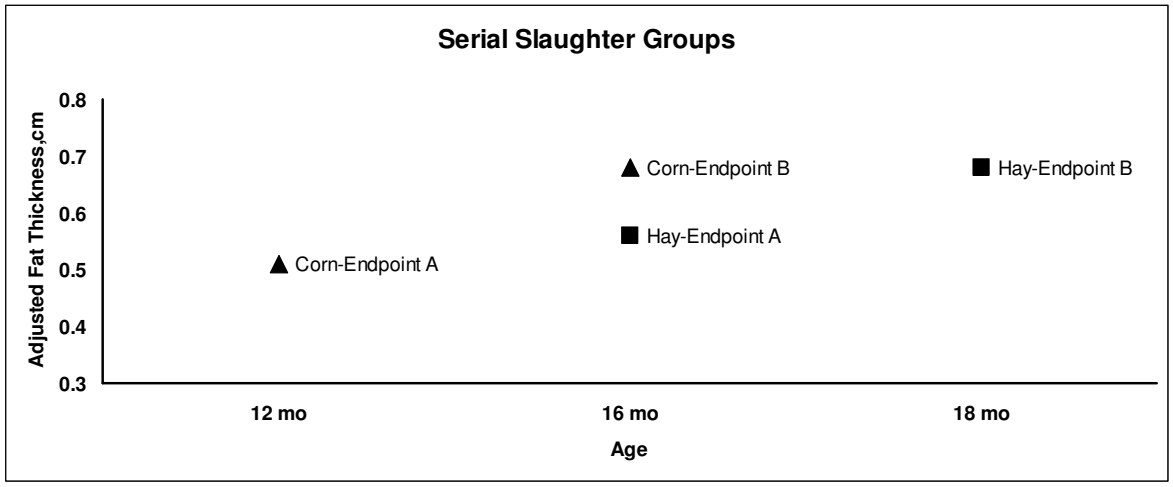

Figure 8. Treatment structure for steers fed Hay or Corn harvested at endpoint A and B

Sample Collection

At the conclusion of the feeding periods, steers from each group were slaughtered at the Rosenthal Meat Science and Technology Center, Texas A&M University. At slaughter, blood samples were taken from the jugular vein and collected into a 10 mL tube. Blood tubes were immediately chilled in a cooled container at 7^{o}C. Plasma was harvested from chilled blood tubes by centrifugation at 2,000 x g at 4^{o}C for 20 min. The plasma was stored at -10^{o}C until further analysis. A section of the LM between the 5th and 8th thoracic ribs was removed immediately following hide removal (approximately 20 min postmortem). The LM section and associated adipose tissues was immediately placed in oxygenated Krebs-Henseleit bicarbonate (KHB) buffer (pH = 7.4; 37^{o}C) with 5 m*M* glucose and transported to the laboratory. Within 20 min post collection, LM, s.c. and i.m. tissue samples were dissected from the LM section. Five grams of each tissue type were immersed in liquid nitrogen for analysis of glycolytic intermediate concentration, another 50 to 100 mg of each tissue type were used immediately for measurement of glucose metabolism in vitro, and another 50 to 100 mg of each tissue was used for measurement of lipogenesis in vitro.

Substrate Concentrations

Plasma glucose concentrations were determined from blood samples collected at slaughter. Commercial kits (Glucose LIQUI-UV with Hexokinase, Stanbio Laboratory Boerne, TX) were used for analysis of plasma glucose using 200 µL of provided reagent and 8 µL of sample and change in absorbance was measured using a KC4 v3.3 Synergy HT Multi-Detection Microplate Reader (Bio-Tek Instruments, INC. Winooski, VT) at

340 nm. Also glucose, glucose-6-phosphate (G-6-P), and fructose-6-phosphate (F-6-P) was measured from frozen LM, s.c. and i.m. samples from each animal using assay systems described by Bergmeyer (1974), with modifications as described by Rhoades et al. (2005). Briefly, a buffer system containing 0.9 m*M* $NADP^+$ and 1 m*M* ATP was added to each cuvette (total volume = 1.0 mL) along with 0.1 mL of LM and 0.5 mL of extract from adipose tissue samples. Glucose-6-phosphate dehydrogenase was added to each cuvette catalyzing G-6-P to 6-phosphogluconate and the change in absorbance was measured using a Beckman DU-7400 Spectrophotometer (Palo Alto, CA) set at 339 nm. In the same cuvette, glucose was converted to G-6-P by the addition of hexokinase and the change in absorbance was measured. Subsequently, phosphoglucose isomerase was added to each cuvette to convert F-6-P to G-6-P and the change in absorbance was measured.

Glucose Metabolism in vitro

Glucose metabolism in vitro was measured using fresh LM, s.c. and i.m. tissues from each animal as described by Espinal et al. (1983). Briefly, 50 to 100 mg of each tissue was incubated in 3 mL of 10 m*M* glucose, KHB and 20 m*M* HEPES buffer and 1 μCi [U-^{14}C]glucose. Flasks were gassed for 1 min with 95% O_2:5% CO_2, capped with hanging center wells, and incubated in a shaking water bath for 2 hr at 37^{o}C. Bovine insulin (0 or 500 ng/mL) was added to these flasks and they were replicated within each tissue type, from each animal, at each level of insulin addition. Insulin (27 USP units/mg powder) from bovine pancreas was used (Sigma-Aldrich Corp. St. Louis, MO). Adding 3 mL of 5% trichloroacetic acid to incubation media stopped all reactions. Determination

of [U-^{14}C]glucose carbon conversion to CO_2 trapped in hanging center wells was preformed according to Smith (1983), and the glucose carbon from incubation media was recovered as lactate and determined according to Smith and Freeland (1981). This method of lactate recovery also traps pyruvate and other carboxylic acids. The remaining glucose carbon in the form of fatty acids and glyceride-glycerol was extracted (adipose tissue only) from the incubated tissue portion and the fatty acids synthesized from glucose was separated into the glyceride-fatty acids and glyceride-glycerol fractions by saponification methods described by Hood et al. (1972). All measures of radioactivity were counted using a Beckman liquid scintillation spectrometer (Beckman, Palo Alto, CA).

Lipogenesis in vitro

Lipogenesis from acetate was measured in fresh s.c. and i.m. tissue from each animal according to Page et al. (1997). Briefly, 50 to 100 mg of adipose tissue was incubated in 3 mL of a 10 m*M* sodium acetate, 10 m*M* glucose, KBH and 20 m*M* HEPES buffer (pH 7.40; 37^{o}C), and 1 µCi [1-^{14}C]acetate for 2 h. Bovine insulin (0 or 500 ng/mL) was added to these flasks and was replicated within each tissue type, from each animal, at each level of insulin addition. Adding 3 mL of 5% trichloroacetic acid to incubation media stopped all reactions. Measurement of [1-^{14}C]acetate incorporation into fatty acids was conducted as described by Page et al. (1997). Radioactivity was counted using a Beckman liquid scintillation spectrometer (Beckman, Palo Alto, CA).

Cellularity

Adipocyte number per gram of tissue was determined by method of Etherton et al. (1977), as modified by Smith et al. (1996). Adipose tissue samples were sliced into 1 mm-thick sections, placed into 20-mL scintillation vials and then processed as described by Smith et al. (1996). The fixed cells (50 cells per sample) were sized using a microscope and recorded for further calculations. Mean cell diameter was calculated as the average diameter of counted and recorded cells from each sample. The mean cell volume was calculated based on mean cell size. The number of adipose cells per gram of tissue was then calculated based on mean cell volume. Calculations involving glucose and acetate utilization measures among adipose tissue depots were expressed on both a per gram of tissue and a per cell basis.

Data Analysis

Data were analyzed as a split-split plot. Diet, endpoint, and their interaction served as main plot effects, and were tested using animal nested within diet as the error term. For response variable related to substrate concentration (glucose, G-6-P, F-6-P), tissue type and diet x tissue served as subplot effects and were tested using the residual mean square as the error term. For response variables related to glucose utilization (glucose conversion into CO_2, lactate, and fatty acids) and acetate utilization (acetate incorporation into fatty acids), tissue and its interaction with the main plot effects served as the sub-plot, insulin level (0 or 500 ng/mL) and their interaction with sub-plot effects served as the sub-sub-plot, and were tested with residual mean square as the error term. When overall F-tests were significant, means were separated using Fisher's Protected

LSD. All analyses were performed using the Mixed Linear Models procedures of SAS v.9 (SAS Institute, Cary, NC, USA).

Source of Chemicals

All chemicals and biochemicals were purchased from Fisher Scientific (Pittsburg, PA, USA) or Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). [U-^{14}C]glucose and [U-^{14}C]acetate were purchased from Amersham (Arlington Heights, IL, USA).

Results and Discussion

Carcass Trait Measurements

Least squares means for carcass characteristics from steers fed either a hay or corn-based diet during backgrounding and harvested at either compositional endpoint A or B are shown in Table 18. Diet influenced HCW but was dependent upon endpoint (diet x endpoint; $P < 0.01$). Steers fed the corn-based diet and harvest at endpoint A had a lighter HCW than all other combinations, which were similar. Diet did not influence ($P = 0.69$) adjusted fat thickness, but steers harvest at endpoint B tended to have greater ($P = 0.06$) fat thickness. Within endpoint fat thickness was similar for steers fed either diet. Marbling score was not impacted by diet, endpoint, or their interaction ($P > 0.23$). Interestingly, marbling score did not increase with an additional 4 mo on feed (i.e. from endpoint A to B) when steers were fed only the Corn-based diet, yet fat thickness increased significantly over the same time period. Similar to HCW, carcass REA was influenced by diet but was dependent upon endpoint (diet x endpoint; $P = 0.04$). Steers fed the corn-based diet and harvested at endpoint A had a smaller REA than all other combinations, which were similar.

Table 18. Least squares means for carcass characteristics from steers fed Hay- or Corn-based diets at A and B endpoints

	Diet/Endpoint							
	Hay		Corn			*P*-value		
Item	A	B	A	B	SE[x]	Diet	Endpoint	Diet x Endpoint
HCW, kg	293[a]	316[a]	221[b]	315[a]	11.28	<0.01	<0.01	<0.01
Adjusted fat thickness, cm	0.56[a]	0.68[a]	0.51[a]	0.68[a]	0.07	0.69	0.06	0.74
Marbling score[y]	523[a]	592[a]	553[a]	555[a]	29.39	0.91	0.23	0.26
REA	11.8[a]	11.8[a]	10.3[b]	11.8[a]	0.34	0.05	0.06	0.04

[a,b]Within a row, least squares means without common superscript differ ($P < 0.05$).

[x]n = 17.

[y]Carcass marbling score, small = 500

Cellularity

Neither diet (Table 19; $P > 0.39$), or endpoint ($P > 0.18$) had an impact on adipose tissue traits. Differences in cells per gram of tissue, cell size, and cell volume among tissue was dependent upon endpoint (endpoint x tissue; $P < 0.01$). Mean cell size was greater in s.c. adipose tissue from cattle harvested at endpoint A, resulting in greater volume and less cells per gram of tissue.

Glucose and Glycolytic Intermediate Concentrations

Plasma glucose concentration was 28% greater from steers fed corn than those steers fed the hay-based diet (Figure 9; $P <0.01$). Differences in plasma glucose concentrations among diets were dependent upon endpoint (diet x endpoint; $P = 0.02$). The diet x endpoint interaction was primarily due to the large increase in circulating glucose seen in hay-fed steers from endpoint A to B, when steers were fed corn glucose concentrations were similar across endpoint.

Neither diet (Table 20; $P = 0.63$) or endpoint ($P = 0.95$) had significant main effects on tissue glucose concentration. Tissue type ($P = 0.02$) impacted glucose concentration, but differences among tissue type were dependent upon diet and endpoint (diet X endpoint X tissue; $P = 0.02$). An accumulation of glucose was observed in LM and i.m. tissues from steers fed both diets but at different endpoints. Greater glucose concentrations in LM and i.m. tissues from steers fed hay was observed at endpoint A, while greater concentrations for these tissues were observed at endpoint B when steers were fed the corn-based diet. Conversely, glucose concentrations were similar in s.c. adipose tissue across both diet and endpoint. Concentrations of G-6-P were similar

Table 19. Least squares means for intramuscular (i.m.) and subcutaneous (s.c.) adipose tissue traits of steers harvested at endpoints A and B

	Endpoint/Tissue							
	A		B			*P*-value		
Item	i.m.	s.c.	i.m.	s.c.	SE[x]	Endpoint	Tissue	Endpoint x Tissue
Cellularity								
Cells per g, 10^{-5}	57.3[a]	11.7[c]	42.9[b]	16.9[c]	3.11	0.20	<0.01	<0.01
Mean diameter, µm	32.5[c]	55.9[a]	35.9[c]	50.2[b]	2.34	0.55	<0.01	<0.01
Mean volume, pL	180[c]	942[a]	249[c]	682[b]	63.3	0.18	<0.01	0.01

[x]n = 17.

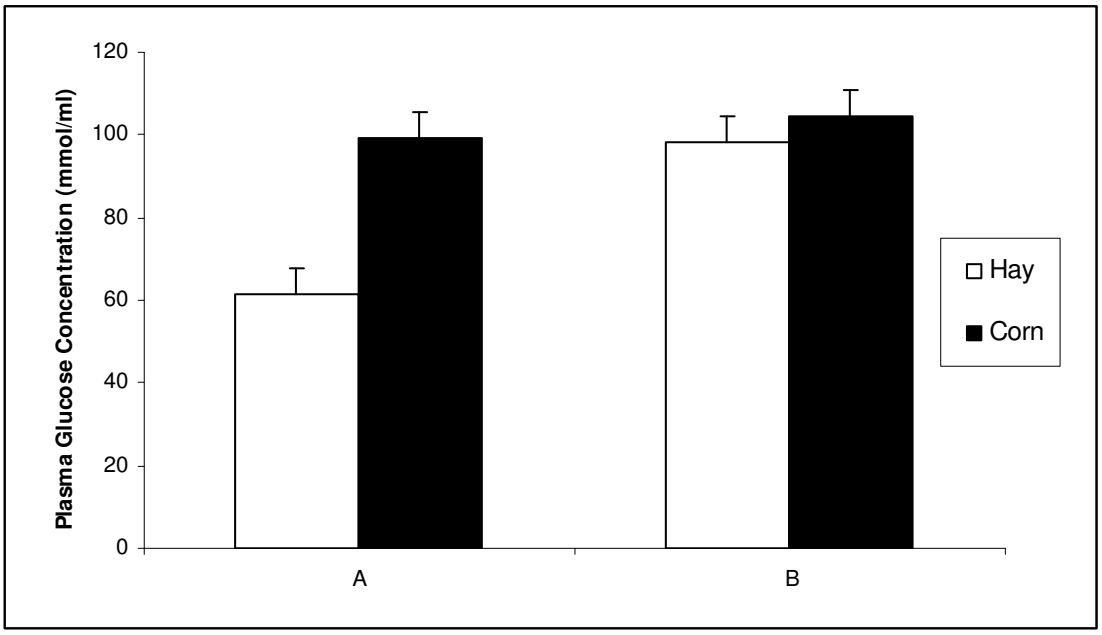

Figure 9. Least squares means for plasma glucose concentration (mmol/ml) for steers fed Hay- or Corn-based diets at A and B endpoints

Table 20. Least squares means for glycolytic intermediate concentrations (μmol·g) in muscle (LM), and subcutaneous (s.c) and intramuscular (i.m.) adipose tissue from steers fed Hay- or Corn-based diets at A and B endpoints

Diet	Hay						Corn													
Endpoint	A			B			A			B				*P*-value						
Item[1]	LM	i.m.	s.c.	LM	i.m.	s.c.	LM	i.m.	s.c.	LM	i.m.	s.c.	SE[2]	D	E	T	DxE	DxT	ExT	DxExT[3]
Glucose	1.58[a]	1.79[a]	0.29[b]	0.25[b]	0.36[b]	0.21[b]	0.63[b]	0.12[b]	0.53[b]	2.19[a]	1.41[a]	0.41[b]	0.43	0.63	0.95	0.02	<0.01	0.35	0.91	0.02
G-6-P	3.16[a]	0.84[b]	0.81[b]	2.32[a]	0.56[b]	0.26[b]	1.98[a]	0.51[b]	0.67[b]	3.24[a]	1.11[b]	0.81[b]	0.46	0.81	0.84	<0.01	0.03	0.86	0.78	0.50
F-6-P	0.13[a]	0.38[a]	0.13[a]	0.25[a]	0.59[a]	0.20[a]	0.11[a]	0.25[a]	0.19[a]	0.30[a]	0.08[a]	0.07[a]	0.23	0.37	0.70	0.49	0.51	0.48	0.82	0.75

[a,b]Within a row, least squares means without common superscript differ ($P < 0.05$).

[1]G-6-P = glucose-6-phosphate; F-6-P = fructose-6-phosphate.

[2]n = 17.

[3]Diet x endpoint x tissue interaction.

among diet (Table 20; $P = 0.81$) and endpoint ($P = 0.84$). Greater G-6-P concentrations were observed in LM tissue ($P < 0.01$). Also, when steers were fed hay G-6-P concentrations were greater at endpoint A and decreased 55% at endpoint B (1.61 vs. 1.04, respectively), alternatively when steers were fed corn G-6-P concentrations were greater at endpoint B and decreased 60% at endpoint A (diet x endpoint; $P = 0.03$). Concentrations of F-6-P were similar among diet (Table 20; $P = 0.37$), endpoint ($P = 0.70$), and tissue type ($P = 0.49$).

Substrate Utilization and Insulin Sensitivity

Tissue from hay-fed steers converted more glucose to CO_2 (Table 21; $P = 0.02$) than tissue from corn-fed steers. Neither endpoint ($P = 0.19$) nor insulin ($P = 0.45$) had an effect on overall CO_2 production. Tissue type impacted glucose conversion to CO_2 ($P < 0.01$), as LM and i.m. adipose tissue converted more than s.c. adipose tissue. Tissue from hay-fed steers converted more glucose to lactate (Table 21; $P < 0.01$) than tissue from corn-fed steers. Greater glucose incorporation into lactate was also observed by tissue from steers harvested at endpoint B ($P < 0.01$) than at endpoint A. Additionally, i.m. adipose tissue converted more glucose to lactate than LM or s.c. adipose tissue ($P < 0.01$). Insulin had a significant negative effect on glucose conversion to lactate ($P < 0.01$).

For glucose conversion to CO_2, when the hay diet was fed 27% greater glucose oxidation was observed at endpoint B compared to endpoint A, but when the corn diet was fed glucose oxidation was similar at both endpoints (Table 22; diet x endpoint; $P = 0.04$). Differences due to endpoint for glucose conversion to lactate were dependent

Table 21. Least squares means for rates of conversion of [U-^{14}C]glucose to CO_2 and lactate (nmol·h^{-1}·100 mg tissue) in muscle (LM), intramuscular (i.m.) and subcutaneous (s.c.) adipose tissue from steers fed Hay- or Corn-based diets at A and B endpoints incubated with 0 or 500 ng/mL insulin

	Diet			Endpoint			Tissue				Insulin			*P*-value			
Item	Hay	Corn	SE[x]	A	B	SE	LM	i.m.	s.c.	SE	0	500	SE	Diet	Endpoint	Tissue	Insulin
CO_2	14.2	11.8	0.66	12.4	13.6	0.66	14.1	14.8	10.1	0.71	12.8	13.2	0.53	0.02	0.19	<0.01	0.45
Lactate	382	321	14.3	317	387	14.3	342	431	282	17.1	377	327	12.1	<0.01	<0.01	<0.01	<0.01

[x]n = 17.

Table 22. Least squares means for rates of conversion of [U-^{14}C]glucose to CO_2 and lactate (nmol·h^{-1}·100 mg tissue) from tissue from steers fed Hay- or Corn-based diets at A and B endpoints

	Diet/Endpoint							
	Hay		Corn			*P*-value		
Item	A	B	A	B	SE[x]	Diet	Endpoint	Diet x Endpoint
CO_2	12.57[b]	15.85[a]	12.16[b]	11.37[b]	0.93	0.02	0.19	0.04
Lactate	302[b]	463[a]	332[b]	310[b]	20.2	<0.01	<0.01	<0.01

[x]n = 17.

upon diet (Table 22; diet x endpoint; $P < 0.01$), as tissue from hay-fed steers harvested at endpoint B converted 40% more glucose to lactate than at endpoint A and when steers were fed corn rates were similar to endpoint A hay-fed steers.

Tissue differences for CO_2 production were dependent upon diet (Table 23; diet x tissue; $P < 0.01$), as LM and i.m. tissue from hay-fed steers converted more glucose to CO_2 than when steers were fed corn. Glucose oxidation was similar among diets for s.c. adipose tissue.

Diet ($P = 0.05$), tissue type ($P < 0.01$), and insulin ($P < 0.01$) were significant main effects for acetate incorporation into fatty acids when rates were expressed per gram of tissue. When steers were fed hay adipose tissue incorporated more acetate into fatty acids than tissue from those steers fed corn (Table 24; $P = 0.050$ when rate were expressed per gram of tissue. Additionally, greater fatty acid synthesis from acetate was observed in s.c. adipose tissue ($P < 0.01$) and insulin stimulated fatty acid synthesis from acetate in s.c. adipose tissue (tissue x insulin; $P = 0.01$). Insulin failed to stimulate fatty acid synthesis from acetate in i.m. tissue from steers fed either diet. Diet had a minimal impact on glucose incorporation into glyceride-glycerol (Table 24; $P = 0.23$). Insulin increased glucose conversion to glyceride-glycerol ($P = 0.02$), however, insulin only significantly increased glucose conversion to glyceride-glycerol in i.m. adipose tissue from steers fed the corn-based diet (diet x tissue x insulin; $P = 0.06$). Insulin failed to stimulate incorporation of glucose into glyceride-glycerol in s.c. adipose tissue from steers fed either diet and in i.m. tissue from steers fed the hay-based diet. Diet ($P = 0.59$) and insulin ($P = 0.26$) had no impact on glucose conversion to fatty acids. However,

Table 23. Least squares means for rates of conversion of [U-^{14}C]glucose to CO_2 and lactate (nmol·h^{-1}·100 mg tissue) in muscle (LM), intramuscular (i.m.), and subcutaneous adipose tissue from steers fed Hay- or Corn-based diets

	Diet/Tissue									
	Hay			Corn				*P*-value		
Item	LM	i.m.	s.c.	LM	i.m.	s.c.	SE[x]	Diet	Tissue	Diet x Tissue
CO_2	17.2[a]	16.1[a]	9.41[c]	11.0[c]	13.6[b]	10.7[c]	0.71	0.02	<0.01	<0.01
Lactate	405[a]	455[a]	288[b]	280[b]	407[a]	276[b]	24.8	<0.01	<0.01	0.07

[x]n = 17.

Table 24. Least squares means for rates of conversion of [U-^{14}C]acetate to fatty acids and conversion of [U-^{14}C]glucose to total lipid, glyceride-glycerol, and glyceride-fatty acids (nmol·h^{-1}·100 mg tissue) in intramuscular (i.m.) and subcutaneous (s.c.) adipose tissue from steers fed HAY- or CORN-based diets incubated with 0 or 500 ng/mL insulin

Diet	Hay				Corn											
Tissue	i.m.		s.c.		i.m.		s.c.			*P*-value						
Item	0	500	0	500	0	500	0	500	SE[x]	Diet	Tissue	Insulin	Diet x Tissue	Diet x Insulin	Tissue x Insulin	Diet x Tissue x Insulin
Acetate																
Fatty acids	4.01^{c}	4.39^{c}	20.23^{b}	27.46^{a}	1.19^{c}	1.36^{c}	6.58^{c}	9.49^{c}	4.44	0.05	<0.01	<0.01	0.11	0.26	0.01	0.31
Glucose																
Total lipid	20.87^{b}	20.65^{b}	15.19^{b}	19.74^{b}	23.38^{b}	32.28^{a}	17.90^{b}	19.10^{b}	3.95	0.29	0.08	0.03	0.38	0.37	0.65	0.05
Glyceride-glycerol	10.32^{b}	10.60^{b}	9.61^{b}	12.66^{b}	13.08^{b}	20.25^{a}	11.08^{b}	12.45^{b}	3.02	0.23	0.45	0.02	0.32	0.27	0.51	0.06
Glyceride-fatty acids	10.55^{a}	10.05^{a}	5.58^{b}	7.08^{b}	10.30^{a}	12.03^{a}	6.82^{b}	6.65^{b}	1.14	0.59	<0.01	0.26	0.76	0.80	0.96	0.09

[x]n = 17.

glucose incorporation into fatty acids was greater ($P < 0.01$) in i.m. adipose tissue than in s.c. adipose tissue.

Compositional endpoint (Table 25; $P = 0.48$) and its interaction with insulin had minimal impact on lipogenesis from acetate (endpoint x insulin; $P = 0.14$). The main effect of insulin on glucose conversion to glyceride-glycerol was dependent upon endpoint, as glucose conversion to glyceride-glycerol was greater in tissue from steers harvested at endpoint A (Table 25; endpoint x insulin; $P = 0.04$). Compositional endpoint (Table 25; $P = 0.13$) and its interaction with insulin had minimal impact on glucose incorporation into fatty acids (endpoint x insulin; $P = 0.24$).

When responses were analyzed on a per 10^5 cells basis, all differences due to dietary treatment were eliminated for glucose and acetate response variables and tissue differences were magnified. Mean cell size was greater in s.c. adipose tissue than i.m. adipose tissue. As a result the number of cells per gram was significantly lower in s.c. adipose tissue. This causes rates of substrate incorporation to elevate for s.c. tissue while rates are depressed in i.m. adipose tissue. For acetate incorporation into fatty acids neither diet (Table 26; $P = 0.09$) or endpoint ($P = 0.53$) impacted synthesis rate. Greater fatty acid synthesis from acetate was observed in s.c. adipose tissue ($P < 0.01$) and insulin stimulated synthesis rate ($P < 0.01$) when expressed on a per cell basis. Glucose oxidation and glucose conversion to lactate were greater in s.c. adipose tissue compared to i.m. adipose tissue (Table 26; $P < 0.01$). Insulin had a negative impact on glucose conversion to lactate ($P < 0.01$). Tissue from those steers harvested at endpoint A had greater glucose incorporation into glyceride-glycerol than steers harvested at endpoint B

Table 25. Least squares means for rates of conversion of [U-^{14}C]acetate to fatty acids and conversion of [U-^{14}C]glucose to total lipid, glyceride-glycerol, and glyceride-fatty acids (nmol·h^{-1}·100 mg tissue) in intramuscular (i.m.) and subcutaneous (s.c.) adipose tissue from steers fed Hay- or Corn-based diets incubated with 0 or 500 ng/mL insulin at A and B endpoints

	Endpoint/insulin							
	A		B			*P*-value		
Item	0	500	0	500	SE[x]	Endpoint	Insulin	Endpoint x Insulin
Acetate								
Fatty acids	7.14[b]	8.32[a]	8.86[a]	13.03[a]	3.39	0.48	<0.01	0.14
Glucose								
Total lipid	21.88[b]	28.59[a]	16.79[b]	17.30[b]	2.91	0.04	0.03	0.05
Glyceride-glycerol	12.96[b]	18.37[a]	9.08[b]	9.62[b]	2.14	0.04	0.02	0.04
Glyceride-fatty acids	8.92[a]	10.22[a]	7.71[a]	7.68[a]	0.93	0.13	0.26	0.24

[x]n = 17.

Table 26. Least squares means for rates of conversion of [U-^{14}C]acetate to fatty acids and conversion of [U-^{14}C]glucose to CO_2, lactate, total lipid, glyceride-glycerol, and glyceride-fatty acids (nmol·10^5 cells^{-1}·h^{-1}) in intramuscular (i.m.) and subcutaneous (s.c.) adipose tissue from steers fed Hay- or Corn-based diets at A and B endpoints incubated with 0 or 500 ng/mL insulin

	Diet			Endpoint			Tissue			Insulin			*P*-value			
Item	Hay	Corn	SE[x]	A	B	SE	i.m.	s.c.	SE	0	500	SE	Diet	Endpoint	Tissue	Insulin
Acetate																
Fatty acids	9.05	3.89	2.01	7.38	5.56	2.01	0.64	12.3	1.94	5.55	7.39	1.14	0.09	0.53	<0.01	<0.01
Glucose																
CO_2	5.26	6.32	0.75	6.36	5.22	0.75	3.26	8.32	0.68	5.49	6.09	0.55	0.32	0.29	<0.01	0.09
Lactate	160	164	20.5	165	159	20.5	96.3	228	17.9	172	151	14.8	0.87	0.84	<0.01	0.03
Total lipid	9.08	11.5	1.69	13.1	7.49	1.69	5.23	15.4	1.51	9.39	11.2	1.22	0.31	0.03	<0.01	0.02
Glyceride-glycerol	5.50	7.22	1.19	8.44	4.28	1.19	2.91	9.81	1.15	5.65	7.06	0.87	0.32	0.03	<0.01	0.02
Glyceride-fatty acids	3.58	4.31	0.60	4.68	3.21	0.60	2.32	5.57	0.49	3.74	4.15	0.44	0.39	0.10	<0.01	0.17

[x]n = 17.

(Table 26; $P = 0.03$) when expressed on a per cell basis. Greater glucose incorporation into glyceride-glycerol and fatty acids were observed in s.c. adipose tissue ($P < 0.01$) and insulin stimulated glucose conversion to glyceride-glycerol ($P = 0.02$).

Tissue differences for glucose conversion to CO_s and glyceride-fatty acids when expressed on a per gram basis were dependent upon endpoint (Table 27; endpoint x tissue; $P = 0.04$). Similar rates of glucose oxidation and glucose conversion to glyceride-fatty acids were observed in i.m. tissue at either endpoint, but greater glucose oxidation and incorporation of glucose into glyceride-fatty acids occurred at endpoint A than at endpoint B in s.c. adipose tissue.

Insulin differences for acetate incorporation into fatty acids when expressed on a per cell basis were dependent upon tissue type (Table 28; tissue x insulin; $P < 0.01$). Insulin failed to stimulate fatty acid synthesis from acetate in i.m. adipose tissue, but resulted in a 35% increase in acetate incorporation into fatty acids in s.c. adipose tissue when insulin was added. Likewise insulin increased glucose oxidation in s.c. tissue, but failed to stimulate glucose oxidation rate in i.m. adipose tissue (Table 28; tissue x insulin; $P = 0.04$).

This study was designed to test the effects of diet and compositional endpoint on in vitro substrate utilization and insulin sensitivity in muscle and adipose tissues from steers fed a different diet during backgrounding but harvested at common compositional endpoints based on adjusted fat thickness. It was designed so that a group of steers (n = 4 or 5) from each dietary treatment group (Hay and Corn) would achieve two different levels of adjusted fat thickness (A and B). Steers fed corn during backgrounding and

Table 27. Least squares means for rates of conversion of [U-^{14}C]acetate to fatty acids and conversion of [U-^{14}C]glucose to CO_2, lactate, total lipid, glyceride-glycerol, and glyceride-fatty acids (nmol·10^5 cells^{-1}·h^{-1}) in intramuscular (i.m.) and subcutaneous (s.c.) adipose tissue at A and B endpoints

	Endpoint/tissue							
	A		B			*P*-value		
Item	i.m.	s.c.	i.m.	s.c.	SE[x]	Endpoint	Tissue	Endpoint X Tissue
Acetate								
Fatty acids	0.30[c]	14.46[a]	0.99[c]	10.14[b]	2.82	0.53	<0.01	0.37
Glucose								
CO_2	2.82[c]	9.91[a]	3.71[c]	6.73[b]	0.99	0.29	<0.01	0.04
Lactate	76.5[d]	253[a]	116[c]	202[b]	25.9	0.84	<0.01	0.07
Total lipid	5.78[c]	20.46[a]	4.68[c]	10.29[b]	2.19	0.03	<0.01	0.04
Glyceride-glycerol	3.52[c]	13.37[a]	2.30[c]	6.26[b]	1.67	0.03	<0.01	0.09
Glyceride-fatty acids	2.26[c]	7.09[a]	2.38[c]	4.03[b]	0.71	0.10	<0.01	<0.01

[x]n = 17.

Table 28. Least squares means for rates of conversion of [U-^{14}C]acetate to fatty acids and conversion of [U-^{14}C]glucose to CO_2, lactate, total lipid, glyceride-glycerol, and glyceride-fatty acids (nmol·10^5 cells^{-1}·h^{-1}) in intramuscular (i.m.) and subcutaneous (s.c.) adipose tissue from steers fed Hay- or Corn-based diets incubated with 0 or 500 ng/mL insulin

	Tissue/insulin					*P*-value		
	i.m.		s.c.					
Item	0	500	0	500	SE[x]	Tissue	Insulin	Tissue x Insulin
Acetate								
Fatty acids	0.61[c]	0.67[c]	10.49[b]	14.11[a]	1.98	<0.01	<0.01	<0.01
Glucose								
CO_2	3.33[c]	3.19[c]	7.65[b]	8.98[a]	0.72	<0.01	0.09	0.04
Lactate	104[b]	88.4[b]	240[a]	214[a]	18.9	<0.01	0.03	0.55
Total lipid	4.73[b]	5.74[b]	14.1[a]	16.7[a]	1.59	<0.01	0.02	0.28
Glyceride-glycerol	2.51[b]	3.32[b]	8.81[a]	10.8[a]	1.22	<0.01	0.02	0.29
Glyceride-fatty acids	2.22[b]	2.42[b]	5.25[a]	5.88[a]	0.53	<0.01	0.17	0.45

[x]n = 17.

harvested at endpoint A (Corn A) accumulated carcass fat at a greater rate during the initial phase than those steers fed hay during backgrounding and harvested at endpoint A (Hay A). Therefore, since steers were fed different diets during the 120 d growing period, those steers fed the hay diet required additional time on feed to achieve a common endpoint based on adjusted fat thickness. The additional time on feed needed by the hay group led to differences in HCW when steers from both dietary treatment groups were harvested but only at endpoint A. After the 120 d growing period, all cattle were placed on the high-energy diet; as a result it took less time for the remaining hay group (Hay B) to achieve a similar adjusted fat thickness as the remaining corn group (Corn B). Thus HCW was similar between those steers harvested at compositional endpoint B, regardless of dietary treatment during the initial growing phase. By design steers from both diets harvested at endpoint B had greater adjusted fat thickness than those harvested at endpoint A.

Plasma glucose concentration was measured to access the amount of substrate available for tissue uptake. An increase in plasma glucose concentration would allow for greater glucose extraction from peripheral blood into the tissue for subsequent utilization. Plasma insulin level has been shown to increase when cattle were fed a high-energy diet (Trenkle, 1970). An increase in plasma insulin may promote an increase in glucose uptake and subsequent metabolism (Bell and Bauman, 1997), if the target tissue is insulin sensitive. Plasma glucose concentrations are expected to be higher when cattle are fed a high-energy, corn-based diet. This effect is expected due to the higher production of propionate from starch fermentation associated with the corn diet (Orskov

et al., 1991). In this study, corn-fed steers had greater plasma glucose concentration and the interaction between diet and compositional endpoint was due to the large increase in circulating glucose when hay-fed steers were harvested at endpoint B vs. endpoint A. It is likely that the substantial increase in plasma glucose concentration observed by hay-fed steers at endpoint B was due to adaptation to the same high-energy diet as the corn-fed steers. Since the hay-fed steers harvested at endpoint A were the only group to consume the hay treatment these cattle had a significantly lower level of plasma glucose, thus we would expect glucose availability and plasma insulin concentration to be reduced.

Tissue glucose concentration reflects the balance between glucose uptake and subsequent tissue utilization of glucose. Glucose concentrations were greater in LM and i.m. adipose tissue than s.c. adipose tissue. Rhoades et al. (2005) reported free glucose of 0.84 µmol/g concentrations in muscle tissue from finished steers. Glucose values have been reported to range from 0.38µmol/g in bovine s.c. adipose tissue (Smith et al., 1981) to 0.55 µmol/g in rat adipose tissue (Ballard and Hanson, 1969). Fewer published reports of glucose concentration specifically include i.m. tissue results; however, observations from s.c. and muscle tissue in this study are within a reasonable range with other reports. Increased glucose concentration in LM and i.m. tissues might be the result of either increased absorption or decreased utilization of glucose relative to s.c adipose tissue. Increased absorption would be consistent with the idea that i.m. adipose tissue prefers glucose as a substrate for fatty acid synthesis (Smith and Crouse, 1984). There was also an interaction between diet and endpoint for tissue glucose concentration.

Greater tissue glucose was observed in hay-fed steers harvested at endpoint A, while greater glucose levels were observed in corn-fed steers at endpoint B. The elevated glucose concentration in tissue from hay steers at endpoint A is likely a result of decreased utilization of glucose by the tissue. Conversely, greater glucose concentrations in tissue from the corn-fed steers are expected due to preferential use of propionate as a glucogenic substrate by ruminants (Danfaer et al., 1995).

Glycolytic intermediate concentrations were measured to assess the balance between glucose concentration in the tissue and total glucose utilization. An apparent accumulation of intermediates would suggest decreased flux of glucose through pathways, and therefore reduced glucose utilization. The G-6-P concentrations in LM tissue were greater than in adipose tissue. Higher concentrations of G-6-P in bovine muscle tissue have been reported (Rhoades et al., 2005). Concentrations of both glycolytic metabolites are much higher than those previously reported for bovine adipose tissue (Smith, 1984; Rhoades et al., 2007). Previous work (Smith and Prior, 1981; Miller et al., 1991) has reported low activity of key regulatory enzymes (hexokinase and phosphofructokinase) in bovine adipose tissue. The relative increase of G-6-P in muscle tissue is expected due to the higher activity of these enzymes in muscle tissue. Similar to the interaction between diet and endpoint observed for tissue glucose concentration, greater G-6-P concentrations were found in tissue from hay-fed steers harvested at endpoint A and from corn-fed steers harvested at endpoint B. Similar concentrations of F-6-P among adipose tissues in this study suggest that pathway flux was not constrained by phosphofructokinase. Low hexokinase activity may result in an accumulation of free

glucose; however, Miller et al. (1991) reported higher activity of both enzymes in i.m. tissue vs. s.c. bovine adipose tissue. Therefore, the accumulation of glucose in i.m. tissue observed in this study is less likely to be a result of reduced pathway flux, and more likely the result of increased uptake by i.m (tissue effect). The accumulation of glucose in tissue from hay-fed steers harvested at endpoint A and corn-fed steers harvested at endpoint B is likely a result of decreased utilization due to decreased pathway flux caused by an accumulation of glycolytic intermediate.

On a per gram of tissue basis, tissue from hay-fed steers harvested at endpoint B converted more glucose to CO_2 than other groups. Schoonmaker et al. (2004b) reported that activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate are greater when steers are fed a high-concentrate diet. When steers were fed only a hay diet for 12 mo or corn diet for 8 mo, Rhoades et al. (2007) provides additional evidence that feeding a grain-based diet stimulates greater glucose oxidation in adipose tissue depots. In this study, steers backgrounded on a hay-based diet but were subsequently fed the high-concentrate diet for a longer period of time relative to the hay diet, by harvest at endpoint B. This result suggests that feeding hay during backgrounding does not limit the capacity of tissue to oxidize glucose. This result also suggest that greater glucose oxidation in tissue from hay-fed steers harvested at endpoint B would provide greater reducing equivalents (NADPH) for use in fatty acid synthesis. Early work demonstrated that a change in NADPH generation was in response to an increase in fatty acid synthesis (Ingle et al., 1972; Martin et al., 1973). Consistent with CO_2 results, when steers were fed hay adipose tissue incorporated more acetate into fatty acids than tissue from those

steers fed corn. Hood and Thornton (1980) reported that larger adipocytes incorporated more acetate into fatty acids than smaller adipocytes from the same tissue. In this study, hay-fed steers at endpoint B had larger s.c. adipocytes than steers fed corn. Rhoades et al. (2007) reported acetate incorporation into fatty acids was not influenced by diet, however steers were only fed to one compositional endpoint and adipocyte size was similar among diet. Additionally, greater fatty acid synthesis from acetate was observed in s.c. adipose tissue compared to i.m. adipose tissue. This result coupled with similar findings (Rhoades et al., 2007) continues to confirm the idea that s.c. adipose tissue prefers acetate as a lipogenic substrate. Collectively, this findings indicate that feeding hay during backgrounding increased glucose oxidation and rate of fatty acid synthesis from acetate; perhaps this is because all steers were fed a high-concentrate diet for at least 120 d.

Our hypotheses were that feeding hay during the growing phase would limit i.m. adipose tissue sensitivity to insulin and that advancing compositional maturity would cause adipose tissue to be come insulin resistant. Similar results were reported (Rhoades et al., 2007), in which insulin stimulated glucose conversion to glyceride-glycerol in i.m. adipose tissue when steers were fed a high-concentrate diet but not in i.m. tissue when fed a hay diet. Rhoades et al. (2008*) also demonstrated that when cattle are fed to an excessive fat thickness adipose tissue maybe come resistant to the action of insulin. In this study, results are consistent with our hypothesis, such that insulin failed to stimulate glucose conversion to glyceride-glycerol in i.m. adipose tissue when steers were fed hay during backgrounding, but insulin had a dramatic effect on i.m. tissue when steers were

fed corn. Also consistent with our hypothesis, insulin failed to stimulate glucose conversion to glyceride-glycerol when steers from either diet were fed to an advancing physiological maturity (i.e. compositional endpoint). These findings suggest that feeding hay during backgrounding could limit glucose uptake by i.m. adipocytes in response to insulin even when steers are finished on a high-concentrate diet. These data also suggest that insulin may have a more profound effect on glucose incorporation into lipid in i.m. tissue at earlier stages of compositional maturity.

Overall, results suggest that feeding hay during backgrounding may have differential effects on adipose tissue lipogenesis. When steers were fed hay they consistently oxidized more glucose and incorporated more acetate into fatty acids, but insulin failed to stimulate glucose conversion into glyceride-glycerol in i.m. tissue. These results confirm our hypothesis that feeding hay during backgrounding may impair the ability of i.m. tissue to increase glucose utilization when insulin is added to culture media. However, production data from this study suggests that feeding hay during backgrounding did not limit marbling score when steers from both diets were fed to a common adjusted fat thickness.

Overall results also suggest that as physiological maturity the ability of insulin to stimulate lipid synthesis from glucose maybe reduced. Advancing maturity did increase glucose conversion to CO_2 and lactate, suggesting that adipose tissue is burning more glucose than incorporating into lipid synthesis. It has been demonstrated that greater rates of glucose oxidation are in response to greater rates of fatty acid synthesis in adipose tissue. This finding coupled with our results suggest that as physiological

maturity increases lipid synthesis is not constrained by the availability of reducing equivalents (NADPH). These results confirm our hypothesis that advancing maturity may limit the ability of insulin to stimulate lipid synthesis from glucose and are consistent with previous results generated in our lab.

Implications

The results of this experiment demonstrate that feeding hay during backgrounding may have differential effects on adipose tissue lipogenesis. As physiological maturity increases the ability of insulin to stimulate lipid synthesis from glucose maybe reduced. Differences in adipose tissue metabolism and their interaction with diet provide a foundation for generating nutritional strategies that alter rate and site of deposition.

CHAPTER VIII

CONCLUSIONS

There is little evidence that the increase in Select beef output has been the result of declining amounts of Choice beef produced. Increases in amount of Select beef produced result from increases in proportion of beef presented for grading. An increase in the proportion of beef graded is likely due to enhanced marketing options available and increases in value-based pricing structures. Historical Choice beef production as a proportion of graded product is misleading, as it does not account for the expansion of total graded carcasses. Choice percentages previously reported reflect producers' ability to sort cattle, not overall quality of beef produced. The recent stabilization in slaughter mix suggests an optimum is being approached. Evaluation of short-run demand structure supports this premise, and suggests Choice and Select products are not substitutes. It is difficult to support the hypothesis that beef quality has been declining, or that the mix of slaughter cattle is decidedly non-optimal. Evidence also exists that rapid escalation of the production of Choice beef removes market premiums for this product and may dampen total expenditures for beef.

Depending on current market conditions, using growth-based prediction equations could further reduce the production risk associated with the variation in individual weight gain, which is inherent to time-based projections. Results suggest accuracy of FAT and MAR predictions from growth-based equations is influenced by weight gain between ultrasound, endpoint, breed, and gender; although scans out to 120

d pre-harvest may be accurate. Results also suggest that accuracy of FAT predictions improves when using a linear-based prediction equation. Average daily gain values could be used to estimate carcass weight gain and potentially forecast an appropriate harvest weight relative to a desired carcass endpoint. Known sources of variation in accuracy could be used to scale predictions based on breed and gender to improve accuracy, and thus a growth-based, rather than time based, prediction system might be generated.

The potential to manipulate carcass endpoint by improving quality grade or reducing yield grade was also demonstrated. Results from these experiments demonstrate diet-mediated differences in insulin sensitivity of muscle and adipose tissues in steers. In these studies, LM and adipose tissue from steers fed the hay-based diet was not responsive to additional insulin, while insulin had profound effects on muscle and adipose tissue glucose utilization rates from steers fed the corn-based diet. These differences could lead to a divergent partitioning of energetic substrate in adipose tissue depots from steers fed different diets, in which feeding a corn-based diet enhances glucose uptake in i.m. adipose tissue, whereas feeding a hay-based diet reduced insulin action without altering acetate incorporation in fatty acids. Because s.c. adipose tissue used acetate more effectively than i.m. adipose tissue, feeding a hay-based diet would promote s.c. adipose tissue deposition over i.m. adipose tissue accretion.

Results of in vitro metabolism studies also demonstrate that under certain conditions s.c. adipose tissue is not sensitive to the action of insulin. Feeding hay during backgrounding may have differential effects on adipose tissue lipogenesis. Additionally, as physiological maturity (i.e., carcass fat thickness) increases the ability of insulin to

stimulate lipid synthesis from glucose may be reduced. Apparent differences in s.c. vs. i.m. adipose tissue metabolism, and their interaction with diet, provide foundation for a hypothesis regarding diet-mediated regulation of differential adipose tissue metabolism. Validation of these hypotheses could generate nutritional strategies that alter the rate and site of adipose deposition.

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