INCREASING GLUCOSE OR GLUCOSE PRECURSORS TO INCREASE BEEF QUALITY AND JUICINESS

A Dissertation

by

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ABSTRACT

We hypothesized that abomasal infusion of glucose, which would provide more glucose for absorption from the small intestine, would promote intramuscular (IM) adipose tissue development to a greater extent than ruminal infusion of acetate, propionate, or glucose. At 22 months of age, Angus crossbred steers (n = 24) were fitted with ruminal cannulas and adapted to a standard, corn/sorghum finishing diet over a 2-week period. Subsequently, the steers were infused with isocaloric amounts (3.76 Mcal/d) of glucose, propionate, or acetate. Glucose was infused either into the rumen or into the abomasum, whereas propionate and acetate were infused into the rumen.

Abomasal and ruminal infusion of glucose resulted in greater dry matter, organic matter, digestible energy, and gross energy intake than ruminal acetate infusion (P = 0.02). Infusate had no effect on quality grade or marbling score (P= 0.18), though acetate-infused steers had numerically greater marbling scores. The rate of glucose incorporation into fatty acids was greater in IM adipose tissue of acetate and propionate-infused steers than in abomasal or ruminal glucose-infused steers ($P \leq 0.02$).

Steaks from the acetate, abomasal glucose-infused, and ruminal glucose-infused steers were higher in the flavor attributes brown/roasted, fat-like, and overall sweet, and lower in bitter basic taste, than steaks from the propionate-infused steers.

In a separate experiment, primals were taken from 4 USDA Select carcasses. Lean and fat trims were separated, and ground beef was formulated from each primal to contain 10, 20, or 30% total fat. Brisket patties contained higher proportions of

monounsaturated fatty acids and less saturated fatty acids than flank patties. There were no differences in n−6 or n−3 fatty acids across primal type or fat level. After cooking, brisket patties had higher bloody/serumy and fat-like flavor aromatics than flank patties. Plate patties generated higher amounts of lipid-derived volatiles than patties from the brisket or flank.

In summary, abomasal infusion of glucose did not promote higher marbling scores, whereas propionate infusion promoted the greatest rates of fatty acid synthesis from glucose in IM adipose tissue. Additionally, individual primals can be used to formulate ground beef with unique compositional and flavor characteristics.

DEDICATION

This work is dedicated to my family, friends, and colleagues who have helped to develop personal and professional skills to mold me into the woman I am today. Without their unwavering support and guidance, none of this work would have been possible.

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CHAPTER I

INTRODUCTION

Information pertaining to nutritional concepts and differences in biochemistry and metabolism among ruminants has successfully been used to address various health problems in humans such as obesity, atherosclerosis and diabetes (Palmquist et al., 2005; Khor et al., 2008). Ground beef available in retail outlets is relatively high in saturated fatty acids (SFA) and low in monounsaturated fatty acids (MUFA). Commercial ground beef in the U.S. contains fat with a MUFA:SFA ratio of 0.85–0.90, whereas ground beef from cattle fed high-grain diets has a MUFA:SFA greater than 1.10, and ground beef from grass-fed cattle has a MUFA:SFA ratio less than 0.75 (Gilmore et al., 2011; Turk and Smith, 2009).

We have demonstrated that fatty acid composition of ground beef can affect the concentration of high-density lipoprotein (HDL) cholesterol in men (Adams et al., 2010; Gilmore al., 2011) and women (Gilmore et al., 2013). Ground beef from grass-fed steers that was high in SFA and *trans*-fatty acids decreased HDL cholesterol in mildly hypercholesterolemic men, whereas ground beef from grain-fed cattle, naturally enriched with oleic acid (18:1n-9), returned HDL cholesterol concentrations to pre-intervention levels. Ground beef from grass-fed steers had no effect on HDL cholesterol concentrations in normocholesterolemic men, whereas high-oleic acid ground beef from grain-fed cattle increased HDL cholesterol (Gilmore et al., 2011), although ground beef from the grass-fed cattle contained three times as much α-linolenic acid (18:3n-3; ALA)

as ground beef from grain-fed cattle (90 vs 30 mg per patty). Our most recent study (Gilmore et al., 2013) provided further support for increasing MUFA in ground beef; ground beef from Akaushi cattle (MUFA:SFA ratio = 1.43) increased HDL cholesterol concentrations in postmenopausal women, while there was no effect of chub pack ground beef (MUFA:SFA ratio = 0.86) on HDL cholesterol. These studies demonstrated that practical increases in MUFA in ground beef have positive effects on risk factors for cardiovascular disease, and are consistent with previous research that demonstrated the health benefits of replacing SFA with MUFA in the diet (e.g., Allman-Farinelli et al., 2005).

In many species, the concentration of oleic acid in adipose tissue reflects the relative proportion of oleic acid in the diet (Go et al., 2012) but in ruminants, dietary oleic acid is hydrogenated to stearic acid by (18:0) ruminal microorganisms before reaching the small intestine (Ekeren et al., 1992). Any accumulation of oleic acid in tissues of ruminants is dependent on stearoyl-CoA desaturase (SCD) catalytic activity. Smith et al (1990) demonstrated that in cattle 12 mo of age, the concentration of stearic acid was lower, and oleic acid was concomitantly higher, in marbling adipose tissue of steers fed a corn-based diet for 4 mo than in steers that had grazed native pasture for 4 mo. Stearoyl-CoA desaturase gene expression was virtually undetectable in adipose tissue of both weaned calves and in pasture-fed steers, but was highly expressed in adipose tissue of corn-fed steers, so differences in SCD activity between corn-fed and pasture-fed steers certainly contributed to differences in beef fatty acid composition.

Though health concerns are important to consumers, they also want a product that is highly palatable. The concentration of oleic acid in beef is positively correlated with overall palatability (Waldman et al., 1968; Westerling and Hedrick, 1979). Baublits et al. (2009) reported that the concentration of MUFA (and specifically oleic acid) in beef was positively correlated with beef/brothy and beef fat flavors, indicating that as the concentration of oleic acid increases, so do beefy flavors. Therefore, increasing the MUFA:SFA ratio would increase the palatability as well as the healthfulness of beef and beef products. In cattle, it has not been practical to increase oleic acid in beef by feeding high-oleic oil or oil seeds (Chang, Lunt, and Smith, 1992; St. John et al., 1987), although fatty acid composition varies considerably across carcass fat depots and changes with animal age (Smith et al., 2012; Turk and Smith, 2009).

In the U.S., Australia, and many Asian countries, beef with higher degrees of intramuscular (IM) adipose tissue, the apparent portion also referred to as marbling, is associated with overall consumer acceptance. This is related to juiciness, flavor, tenderness and overall palatability of beef. Adipose tissue in beef cattle has historically been of scientific interest for the reason that beef carcass value is influenced by quantity and distribution of adipose tissue. Segments of the beef cattle industry are seeking effective and efficient methods to grow cattle to market weights that can increase economic returns by maximizing marbling scores and limit discounts due to increased carcass yield grade caused by excessive subcutaneous (SC) adipose tissue. Different management strategies have been proposed aiming to increase deposition of IM adipose

tissue without increasing SC adipose tissue and negatively affecting meat quality (Smith et al., 1984; Fluhartry et al., 2000).

An essential part of ruminant diets are carbohydrates, which provide energy for both rumen microorganisms and the animal. The primary carbohydrate sources in ruminants are fibrous feeds and grains rich in starch. Digested carbohydrates are fermented in the rumen to volatile fatty acids (VFA). These VFA serve as the main precursors for fatty acid and/or glucose biosynthesis in ruminants. Early research from our laboratory demonstrated that high concentrate diets nearly doubled glucose incorporation into fatty acids in SC adipose tissue as measured *in vitro*, but had no effect on acetate incorporation into fatty acids in IM adipose tissue (Smith and Crouse, 1984). Intramuscular adipose tissue uses glucose for a greater proportion of the carbon source for de novo fatty acid biosynthesis than SC adipose tissue (Smith and Crouse, 1984; Rhoades et al., 2007, 2009). However, it has been difficult to assess the actual effects of glucose on fat accumulation in growing beef cattle.

We hypothesized that glucose infusion into the abomasum would increase carcass adiposity and beef juiciness, relative to ruminal infusions of acetate, propionate, or glucose. Total IM lipid and fatty acid composition of IM and SC adipose tissues were measured to confirm results for carcass marbling scores, as increased marbling is associated with a higher concentration of MUFA in SC and IM adipose tissues (Brooks et al., 2011b). Moreover, we also hypothesized that unique ground beef products could be formulated from brisket, flank, and plate primals.

Therefore, the objectives of these research projects were to demonstrate that supplemental glucose in the abomasum increases beef quality and juiciness by:

-Increasing the delivery of glucose to the circulation to establish the relationship between supplemental glucose, marbling scores, fatty acid composition, and juiciness of beef;

-Infusing a gluconeogenic precursor (propionate) into the rumen to establish the relationship between supplemental propionate, marbling scores, fatty acid composition, and juiciness of beef;

-Infusing ruminal acetate to establish the relationship between supplemental acetate, marbling scores, fatty acid composition, and juiciness of beef;

-Using 50/50 lean/fat trimmings taken from beef brisket, flank and plate primals to alter the fatty acid composition, flavor profile, and sensory characteristics of ground beef with three levels of fat (10, 20, or 30%).

CHAPTER II

LITERATURE REVIEW

Carbohydrate Metabolism in the Rumen

The rumen is highly adapted to digest roughages and feeding highly digestible carbohydrate sources allow better energy consumption for cattle. The primary carbohydrate sources in ruminants are fibrous feeds containing cellulose and hemicellulose and grains rich in starch comprised of amylose and amylopectin. However, in ruminants limitations are placed on their capacity to digest and absorb carbohydrates. Grains contain about 57 to 77% of digestible dry matter; of these wheat has the highest starch content (77%), subsequent is corn and sorghum (72%), followed by barley and oats (57-58%) (Huntington, 1997). Ruminants digest 77% of starch and cellulose intake, fermented in the reticulorumen, which contains a range of microbial species and each species digests and metabolizes specific carbohydrates and produces specific end products (Huntington, 1997). The chemical structure of the starch and protein moieties within the kernel affect the extent of ruminal fermentation patterns and rate since the outer layers of grains are impermeable to bacterial degradation (Rooney and Pflugfelder, 1986; McAllister et al., 1994).

The major VFA produced by rumen microorganisms are acetate, propionate, and butyrate, and ruminal microorganisms also produce NH_3, CO, and CH_4. These VFA are the products of ruminal fermentation, and serve as the main precursors of fatty acid and/or glucose biosynthesis in ruminants. Only 5 to 20% of consumed dietary

carbohydrates are digested in the small intestines and feeding large amounts, especially highly processed grains, leads to increased amounts of starch escaping ruminal fermentation (Hurtaud et al., 1998; Knowlton et al., 1998; Meyer et al., 1965; Owens, 1998; Slyter, 1976). The site, extent, and kinetics of digestion of carbohydrates highly impact the amount and profile of nutrients delivered to peripheral tissues.

Microbes in the Rumen

Functionally, the rumen provides an excellence anaerobic environment, constant temperature, and pH for sustainability of microorganisms. Ruminal microbes include bacteria (most significant for starch digestion), protozoa, and fungi (which aids in bacterial attachment) (Huntington, 1997; McAllister et al., 1994). The fermentation processes involve transfer of molecular H and generate metabolic energy in the form of adenosine triphosphate (ATP) that subsequently is utilized by microorganisms for their maintenance and growth. The VFA composition, and the subsequent loss of C in gasses, are mainly determined by the composition of the microbial population. The development of fibrolytic microorganisms causes high levels of acetate, whereas the development of amylolytic microorganisms results in an increase in the proportion of propionate, allowing increased utilization of excess reducing power. Ruminal microorganisms ferment all carbohydrates and soluble carbohydrates are digested 100 times faster the storage carbohydrates (cereal grains), which are digested five times faster than structural carbohydrates (cellulose and hemicellulose) (Moren, 2005). However, only a few species of bacteria and fungi can produce amylases, cellulases, and hemicellulases, which

hydrolyze carbohydrates to monosaccharides. The diet of ruminants dictates the total and relative amount of each microorganism within the rumen.

Starch enters the rumen and microbial organisms attach to the outer surface of the starch molecule. After attachment, bacteria secrete amylases and additional digestive enzymes to hydrolyze the starch. Simultaneous with hydrolysis of the starch molecule, glucose molecules freed are absorbed by the bacterial cells and fermented to VFA. High starch diets are characterized by relatively higher propionate production and higher fiber diets are characterized by relatively higher acetate production (Bergman, 1990).

VFA are absorbed across the rumen wall or in the small intestine and are metabolized by the host as a major source of energy, and the proportions in which they are produced determine fat and protein synthesis in tissues. Ruminants depend on VFA for up to 80% of their maintenance energy requirements (Bergman, 1990). Cecum VFA provide, on average, 8.6% of metabolizable energy intake in ruminants (Siciliano-Jones and Murphy, 1989). Thus, the total and relative amounts of the VFA indicate dietary carbohydrates and subsequent rumen fermentation patterns.

Substrates for Fatty Acid Biosynthesis

VFA are absorbed across the ruminal wall rapidly and transferred to the liver via the blood stream. About 90% of butyrate is metabolized by the ruminal epithelium and converted to ketone bodies or CO_2. The remaining butyrate and most of the acetate and propionate are transported to the liver (Bergman, 1990). Almost all of the propionate and butyrate are removed by the liver and insignificant amounts appear in peripheral circulation. Therefore, acetate comprises more than 95% of the VFA present in the

peripheral circulation. Most of the propionate taken up from the portal blood by the liver is used to synthesize glucose. In addition to their involvement as the major source of energy, all animals can utilize glucose and acetate to some extent and lactate very effectively as substrates for lipid biosynthesis (Smith and Crouse, 1984). In ruminants, propionate is the major substrate of hepatic gluconeogenesis (Herdt, 1988). However, feeding ruminants high starch diets also increases direct glucose absorption from the gut (Huntington, 1997). Thus, effective absorption of VFA from the abomasum and large intestine is essential for ruminants. Altogether, acetate, propionate, and butyrate play important roles as substrates for glucose and lipid metabolism.

Adipose Tissue Growth and Development

Adipocytes are derived from multi-potential stem cells, which form adipoblasts. Adipoblasts differentiate further to preadipocytes, which subsequently differentiate into lipid-filled adipocytes. Hence, preadipocyte division increases the number of adipose cells. Adipose tissue mass can expand by formation of new adipocytes from precursor cells and cell enlargement through lipid filling. Adipocytes are found primarily in four major depots: IM adipose tissue, SC adipose tissue, visceral adipose tissue, and intermuscular (seam) adipose tissue.

Intramuscular adipose tissue results from accumulation of triacylglycerols (TAG) primarily located within adipocytes associated with the perimysium which surrounds bundles of muscle fibers in muscle tissue (Pethick et al., 2004). Intramuscular adipose tissue also is referred to as marbling, and is used as an indicator of beef quality. The amount and distribution of marbling in the longissimus dorsi cross section is an

important determinant of quality grade (USDA, 1997). Marbling contributes to tenderness, juiciness, flavor, and overall palatability of beef, and consumers are willing to pay a premium for additional marbling (Feuz et al., 2004). Thus, higher IM adipose tissue deposition increases market value of beef.

Subcutaneous adipose tissue is located under the skin. Yield grade is determined by SC adipose tissue measured at the 12th rib (Owens and Gardner, 2000), which provides an estimate of total carcass SC adipose tissue. Excess SC adipose tissue is usually trimmed, which consequently reduces profit margin. Production efficiencies ultimately are reduced due to feed consumed to synthesize SC adipose tissue. Further, the increase in health-concious consumers leads to high demand for lean beef (Vernon, 1980).

Subcutaneous adipocyte proliferation is complete around 8 mo of age in cattle, and further increases in adipose tissue mass occur by cell enlargement (Hood and Allen, 1975). In comparison to the IM adipose tissue, SC adipose tissue as a proportion of mature composition was approximately 60% at 11 to 19 mo age steers. Cianzio et al. (1985) reported that SC adipocyte diameter increased after 13 mo of age, whereas IM adipocyte diameter increased after 15 mo of age.

Lipogenesis

Lipogenesis is the process by which simple sugars such as glucose are converted to fatty acids, which are subsequently esterified with glycerol to form the TAG that are packaged in VLDL and secreted from the liver or stored as lipid droplets in tissues such as adipose tissue. Opposite to fatty acid β- oxidation, which occurs within the

mitochondria, *de novo* synthesis of fatty acids takes place within the cytosol. Exogenous fatty acids are also supplied by the diet.

Acetate is converted to acetyl-CoA, which is the precursor of fatty acid synthesis, catalyzed by acetyl-CoA synthetase in the cytoplasm. Acetyl-CoA is converted to malonyl-CoA by acetyl-CoA carboxylase, which is the first committed step to fatty acid biosynthesis. Fatty acid synthase then catalyzes seven different reactions within the fatty acid synthase enzyme complex, whereby two carbon units from malonyl-CoA are condensed, ultimately to form palmitic acid.

Glucose enters fatty acid synthesis via glycolysis, which produces pyruvate that is further transformed into acetyl-CoA within the mitochondria. Since the cell wall is impermeable to acetyl-CoA, two-carbon units have to be transported out of the mitochondria into the cytosol as citrate via a tricarboxylate translocase (citrate shuttle). In the cytosol, citrate is cleaved to oxaloacetate and acetyl-CoA. This reaction is catalyzed by ATP-citrate lyase (ATP-CL) and requires the hydrolysis of one ATP molecule.

Glucose is required for the generation of reducing equivalents (NADPH) in ruminants via the pentose phosphate pathway. The NADPH necessary for fatty acid synthesis produced by NADP-malate dehydrogenase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and NADP-dependent isocitrate dehydrogenase (Smith, 1983). Liver and adipose tissue can convert glucose into glycerol-3-phosphate (G3P) catalyzed by glycerol-3-phosphate dehydrogenase, which reduces dihydroxyacetone-phosphate from the glycolytic pathway. Glycerol-3-phosphate

combines with fatty acyl-CoA from fatty acid synthesis to yield 1-lysophosphotidic acid; two additional fatty acyl-CoA are used to generate a triacylglycerol.

High carbohydrate diets induce lipogenesis through direct and indirect liver nutrient-sensing mechanisms (Flowers and Ntambi, 2009). Glucose is transported to the bloodstream from the small intestine, pancreatic β-cells sense the glucose which results increase in insulin secretion. Liver sensing of glucose in conjunction with increased hepatic insulin signaling promotes the activation of various receptors and enzymes including stearoyl-CoA desaturase (SCD), acetyl-CoA carboxylase, and fatty acid synthase (Flowers and Ntambi, 2009).

Substrate Utilization by Adipose Tissues

Acetate is the major substrate utilized as a precursor for fatty acid biosynthesis, with a smaller contribution from glucose for some of the NADPH and all of the G-3-P in ruminant adipose (Smith and Crouse, 1984). Smith (1983) demonstrated that glucose is required as a precursor for G3P and a source of NADPH required for fatty acid synthesis in ruminant SC adipose tissue. Data from Smith (1983) indicated that without other exogenous substrates in the media, 12% of total glucose was metabolized in the pentose cycle, which increased to 22 - 37% when acetate and lactate were included in the media. With glucose in the media, 30% of NADPH was derived from the pentose cycle, and this increased to 72% when acetate and lactate were added to the media. The presence of glucose in the media stimulated acetate incorporation into fatty acids by 90% in SC adipose tissue slices (Smith, 1983).

Yang and Baldwin (1973) reported that glucose is an important stimulant of the incorporation of acetate into fatty acids in ruminant adipose tissues. When SC adipose tissue was incubated with acetate, the incorporation of acetate into fatty acids was increased by the presence of glucose and lactate in the media (Smith et al., 1984). Also, incubation of SC adipose tissue with glucose increased the glycolytic intermediates glucose-6-phosphate and fructose-6-phosphate, indicating that 6-phosphofructokinase limits the conversion of glucose to fatty acids in bovine adipose tissue (Smith, 1983)

Smith and Crouse (1984) observed that acetate and glucose utilization by IM and SC adipose tissues was different. Intramuscular adipose tissue prefers glucose as a substrate for fatty acid synthesis, whereas SC adipose tissue prefers acetate. Acetate contributed 70 to 80% of acetyl units for *in vitro* lipogenesis in SC adipose tissue, whereas it contributed only 10 to 25% of the acetyl units in IM adipose tissue. On the other hand, glucose provided 50 to 75% of acetyl units for in vitro lipogenesis in the IM adipose tissue, but only 1 to 10% in SC adipose tissue. The observed ratio for fatty acid synthesis from acetate:glucose was 0.28 in IM adipose tissue but 32.95 in SC adipose tissue (Smith and Crouse, 1984). Therefore, this early research suggested that *de novo* fatty acid synthesis in IM and SC adipose tissue is controlled by different regulatory mechanisms. Song et al. (2001) also examined lipogenic substrate utilization in different adipose tissues *in vitro* from Hanwoo steers. The ratio of acetate:glucose use for lipogenesis in for SC adipose tissue vs IM adipose tissue averaged 1.25 and 1.27 respectively. Ratios for incorporation of acetate and glucose into adipose tissue of Hanwoo steers were also studied by Lee et al. (2000), who reported acetate:glucose

incorporation ratios for SC adipose tissue and IM adipose tissue of 1.61 and 1.23, respectively.

Fatty Acids

Neural lipids (TAG) are the most abundant lipids in the body (Mottram, 1998). Their purpose is to act as sources of energy for the cell, contribute to cell membrane structure and function, or be involved in metabolic activity (Spector and Yorek, 1985). In animal adipose tissue, the SFA palmitic acid (16:0) and stearic acid are present in higher levels; lauric acid (12:0), myristic acid (14:0), or arachidonic acid (20:4n-6), are only present in small quantities (Wood et al., 2004). Palmitoleic (16:1n-7), oleic acid, linoleic acid (18:2n-6) and ALA are the predominant unsaturated fatty acids, with oleic acid being the most abundant fatty acid in the animal body (Wood et al., 2004).

Since lipids are organic compounds comprised of hydrogen, carbon, nitrogen, oxygen, and phosphorus, they are soluble in organic solvents such as dichloromethane, chloroform, hexane, and diethyl ether. Lipids are insoluble in aqueous solutions. When extracting lipids, the type of solution used depicts what portion of the lipid will be extracted. Phospholipids are extracted using chloroform-methanol (polar) and TAG are extracted using hexane. Fatty acid methyl esters (FAME) are generated by the method developed by Morrison and Smith (1964). To prepare FAME, fatty acids are first hydrolyzed from TAG, phospholipids, or any other lipid compound during methylation, to form free fatty acids. Once the free fatty acids are acetylated to a methane group, a FAME is created. The FAME are separated using gas chromatography. Lipids are then categorized by the number of carbons, and by the presence or absence of double bonds.

Beef Flavor

Beef flavor is not a single attribute, but rather multiple attributes and is, as a result, complex topic. Flavor research to understand what chemical compounds comprise positive and negative beef flavors is an ongoing process. Miller and Kerth (2012) identified positive and negative beef flavors from the beef lexicon (Adhikari et al., 2011). The positive beef flavors identified in the beef lexicon are beefy, brown/roasted, bloody/serumy, fat-like, sweet, salty, and umami (Miller and Kerth, 2012). Attributes that are generally considered negative are metallic, liver-like, sour, barnyard, musty-earthy/humus and bitter. Beefy, browned/roasted, bloody/serumy, sweet, salty and umami are associated with the lean portion of beef; fat like, liver-like, metallic and bitter are associated with the lipid portion (Miller and Kerth, 2012). Proteins, lipids, and carbohydrates play primary roles in flavor development, because they include numerous compounds that are capable of developing into important flavor precursors when heated (Mortem, 1998).

Mottram (1998) divided flavor precursors into two major categories: water-soluble components and lipids. Lipids and the volatiles produced during cooking greatly contribute to the odor and flavor of beef (Wood et al., 2004; Calkins and Hodgen, 2007). Adipose tissue acts as a solvent and traps the aromas that can be released during heating, therefore enhancing the intensity of the flavors present (Wasserman and Spinelli, 1972). Lipid-derived flavors have a higher odor threshold in comparison to water-soluble components (Shaidi, 1994). This makes lipid volatiles major precursors to the development of beef flavor (Mottram, 1998).

The interactions of the water- and lipid-soluble components interact to form lipid-derived aldehydes that play a vital role in the Maillard reaction and ultimately the overall aroma profiles of cooked meat. The Maillard reaction is a form of non-enzymatic browning that results from a chemical reaction between an amino acid and a reducing sugar, usually requiring heat. This reaction produces multiple hetercyclic compounds with long chain alkyl substituents, such as pyrazines, thiophenes, thiazoles, and thialzolines (Shahidi, 1994).

In addition, water-soluble compounds from live animal feed ingredients deposited in lipid influence the fatty acid composition and contribute to beef flavor. Baublits et al. (2009) showed a positive correlation between the positive sensory characteristics, beefy/brothy and beef fat, and fatty acids palmitic, palmitoleic and eladic acid (18:1trans-9), and a negative correlation with pentadecanoic acid (15:0), ALA, arachidonic acid, eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). Baublits et al. (2009) concluded that positive beef flavor attributes were enhanced by increased percentages of SFA and MUFA, while polyunsaturated fatty acids (PUFA) had a greater effect on the perceived negative aspects of beef flavor.

Benefits to Carcass Quality

Feeding grain-based diets for extended periods effectively increases oleic acid in beef (Huerta-Leidenz et al., 1996; Chung et al., 2006; Brooks et al., 2011b; Smith et al., 2012). Grain feeding increases oleic acid production by promoting increased glucose absorption from the small intestine (Huntington et al., 2006; Corrigan et al., 2009) and thereby stimulating SCD gene expression and catalytic activity. Thus, any production

method that increases marbling deposition also increases the concentration of oleic acid in beef (Chung et al., 2006; Brooks et al., 2011b).

Prior and Scott (1980) infused glucose, propionate, acetate, and lactate into the veins of beef cattle and demonstrated that only glucose promoted fatty acid synthesis in SC adipose tissue. Lemosquet et al. (2009) infused glucose into the abomasum and propionate into the rumen of dairy cows and documented that the whole body rate of glucose appearance was 24% greater in glucose-infused dairy cows. Thus, even though propionate is the primary gluconeogenic precursor in ruminants (Huntington et al., 2006), it is not as effective as glucose in increasing blood glucose and thereby promoting adipogenic gene expression in SC adipose tissue. Moreover, to date no one has documented the effects of supplemental glucose, infused into the circulatory system or into the abomasum, on marbling scores or juiciness.

Early research documented that a concentrate diet containing 78% ground corn strongly promoted carcass adiposity and ATP-CL and NADP-malate dehydrogenase (NADP-MD) activities in SC adipose tissue, relative to an alfalfa hay-based diet (Smith et al., 1984). The activities of ATP-CL and NADP-MD are essential for the incorporation of glucose carbon into fatty acids (Smith et al., 1981). Smith and Crouse (1984) demonstrated that a high concentrate diet nearly doubled glucose incorporation into fatty acids in IM adipose tissue, but had no effect on acetate incorporation into fatty acids in IM adipose tissue. More importantly, IM adipose tissue uses glucose as the primary carbon source for *de novo* fatty acid biosynthesis, whereas SC adipose tissue uses acetate as its carbon source for fatty acid synthesis (Smith and Crouse, 1984).

Although subsequent *in vitro* studies have confirmed these results (e.g., Rhoades et al., 2007, 2009), it has been difficult to firmly establish the importance of glucose for marbling development in the live animal. Consequently, understanding carbohydrate digestion, absorption, dietary glucose availability, and the involvement of gluconeogenesis in the regulation of glucose homeostasis is essential for the manipulation of marbling adipose tissue production.

Benefits to Health

At least 40% of per capita beef consumption in the U.S. (30 kg/y) is consumed as ground beef, and low-income households consume more ground beef per capita than do high-income households (USDA, 2011). Statistics from July 2009 to July 2010 indicate that 31.4% of ground beef consumed in the US contained 22-30% fat, while the next 34.8% of ground beef consumed contained 16-22% fat (Beef Retail, 2010). In contrast, ground beef containing 10% or less fat comprised about 18.8% ground beef consumption for that period (Beef Retail, 2010). Research has shown that ground beef naturally enriched with oleic acid may reduce risk factors for cardiovascular disease (Adams et al., 2010; Gilmore et al., 2011, 2013). Risk factors include, but are not limited to, increased blood cholesterol and high intakes of SFA and *trans*-fats. Oleic acid activates acyl-coenzyme A: cholesterol acyltransferase (ACAT); this decreases the free cholesterol (FC) pool by converting cholesterol to cholesterol esters. A decrease in FC is responsible for an increase in LDL uptake by the cell.

Benefits to Palatability

More importantly, higher concentrations of oleic acid are positively correlated with overall palatability (Waldman et al., 1968; Westerling and Hedrick, 1979), whereas stearic acid is the primary determinant of fat hardness (i.e., lipid melting point) (Smith et al., 1998; Wood et al., 2004; Chung et al., 2006; Turk and Smith, 2009). Baublits et al. (2009) reported that the concentration MUFA (and specifically oleic acid) in beef was positively correlated with beef/brothy ($r = 0.37$) and beef fat ($r = 0.44$) flavors, indicating that as the concentration of oleic acid increases, so do beefy flavors. Therefore, increasing the MUFA:SFA ratio would increase the fat softness and thereby improve palatability of beef and beef products. One goal of this research was to provide products to the consumers with greater quality (i.e., palatability) and consistency.

Benefits to Production

This laboratory demonstrated that pork and pork products from lean and fat trim from pigs fed high-oleic canola oil were highly palatable, as assessed by trained taste panels (St. John et al., 1987). Feeding oils to pigs to change fatty acid composition proved to be impractical due to the cost of the oil and the presence of relatively high concentrations of PUFA, which caused undesirably oily carcasses. In cattle, it has not been practical to increase oleic acid in beef by feeding high-oleic oil or oil seeds (St. John et al., 1987; Chang et al., 1992), although fatty acid composition varies considerably across carcass fat depots (Turk and Smith, 2009; Smith et al., 2012).

We have demonstrated that adipose tissue overlying the brisket is unusually high in MUFA, as indicated by the palmitoleic:stearic acid ratio, whereas the flank is low in

palmitoleic and high in stearic acid. The plate, which typically is used as raw materials for ground beef production, is intermediate in SFA content, whereas the flank typically contains more SFA (Turk and Smith, 2009). The fatty acid composition of brisket fat is virtually identical to that of fat depots in American Wagyu cattle, and MUFA concentration in brisket fat exceeds that which can be achieved in U.S. domestic cattle fed corn-based diets for over 16 mo (May et al., 1993; Chung et al., 2006). Feeding grain-based diets for extended periods (Huerta-Leidenz et al., 1996; Chung et al., 2006; Brooks et al., 2011; Smith et al., 2012) or using trim from exotic breed types (May et al., 1993; Chung et al., 2006) are effective strategies to increase oleic acid in beef, but these also may be impractical and/or not cost-effective. Research conducted by this laboratory has shown that the MUFA:SFA ratio of brisket is high and does not change significantly over animal age (Smith et al., 2012). For this reason, brisket lean and fat trim can be taken at virtually any production age to supply producers with a product with a consistent fatty acid composition. Thus, the raw materials for producing high-oleic acid, high-quality ground beef are readily available.

Summary and Hypotheses

We hypothesized that glucose infusion into the abomasum would increase carcass adiposity and beef juiciness, relative to ruminal infusions of acetate, propionate, or glucose. Total IM lipid and fatty acid composition of IM and SC adipose tissues were measured to confirm results for carcass marbling scores, as increased marbling is associated with a higher concentration of MUFA in SC and IM adipose tissues (Brooks

et al., 2011b). Moreover, we also hypothesized that unique ground beef products could be formulated from brisket, flank, and plate primals.

CHAPTER III

ABOMASAL INFUSION OF GLUCOSE INCREASES INTRAMUSCULAR LIPID CONTENT AND ACETATE INCORPORATION INTO FATTY ACIDS IN SUBCUTANEOUS ADIPOSE TISSUE RELATIVE TO RUMINAL ACETATE INFUSION

Introduction

High-concentrate diets are highly digestible and nearly double glucose incorporation into fatty acids in SC adipose tissue as measured *in vitro*, but had no effect on acetate incorporation into fatty acids in IM adipose tissue (Smith and Crouse, 1984). Intramuscular adipose tissue uses glucose for a greater proportion of the carbon source for *de novo* fatty acid biosynthesis than SC adipose tissue (Smith and Crouse, 1984; Rhoades et al., 2007, 2009). However, it has been difficult to assess the actual effects of glucose on fat accumulation in growing beef cattle.

Propiogenic diets (such as high-grain diets) promote greater marbling development than acetogenic diets (e.g., Rhoades et al., 2007, 2009). We predicted that "glucogenic" diets, which provide more glucose for absorption from the small intestine (e.g., dry-rolled corn; Huntington et al., 2006; Corrigan et al., 2009), would promote marbling development, hence juiciness, to a greater extent than propiogenic diets. This hypothesis was tested directly infusing isocaloric amount of glucose and propionate into the digestive tract of young, growing steers. Glucose was infused into the rumen (reflecting a high-starch diet) and into the abomasum, the latter treatment bypassing

ruminal metabolism of the infused glucose. We also infused acetate into the rumen to directly provide substrate for fatty acid biosynthesis in SC and IM adipose tissues. Total IM lipid and fatty acid composition of IM and SC adipose tissues were measured to confirm results for carcass marbling scores, as increased marbling is associated with a higher concentration of monounsaturated fatty acids in SC and IM adipose tissues (Brooks et al., 2011b). We also measured the *in vitro* incorporation of glucose and acetate into fatty acids to confirm the effects of acetate, propionate, and glucose infusions on SC and IM adipose tissue metabolism.

Materials and Methods

Animals and treatments

This experiment tested the hypothesis that absorption of equivalent energy amounts of acetate, propionate, and glucose would have different effects on marbling development and metabolism in growing steers. Specifically, abomasal infusion of glucose would more strongly promote marbling development, hence juiciness, than ruminal infusion acetate, propionate, or glucose. Also, we predicted that effects of infusing propionate into the rumen would provide results that were intermediate between glucose and acetate.

Twenty-four Angus crossbred steers were grown using conventional diets until approximately 22 mo of age. Steers were weighed at the start of the experiment and were housed in individual pens (2.1 by 1.5 m) in an enclosed barn with free access to water. The steers were fitted with ruminal cannulas as described previously (Bourg et al., 2012) at the Texas A&M University Animal Research and Teaching Center. Cattle were

adapted to a standard, corn/sorghum finishing diet containing ground milo, corn, cottonseed meal, cottonseed hulls, molasses, premix, ammonium chloride and R-1500 over a 2-wk period while recovering from the placement of the cannulas. After the adaptation period, steers were infused with 5 L of water for 3 d. The following treatments were continuously infused for the last 28 d of the finishing period:

1. Acetate, 1.077 kg/d (16.7 mol/d; 3.76 Mcal/d) ruminal infusion (n = 6)
2. Propionate, 0.758 kg/d (10.15 mol/d; 3.76 Mcal/d) ruminal infusion (n = 6)
3. Glucose, 1.0 kg/d (5.55 mol/d; 3.76 Mcal/d) ruminal infusion; (n = 6) (control group)
4. Glucose, 1.0 kg/d (5.55 mol/d; 3.76 Mcal/d) abomasal infusion (n = 6)

One steer from the acetate group and two steers from the propionate group were excluded from the study because they demonstrated symptoms of poor health.

Treatments were brought to a volume of 5 L with water and infused using a peristaltic pump. Ruminal infusions were accomplished by continuous infusion of substrate through infusion lines inserted through the cannulas. Abomasal infusion lines were accomplished by continuous infusion of substrate through infusion lines through the cannula bypassing the rumen and secured into the abomasum. Steers were fed the finishing diet free choice. Fecal samples were collected every 8 h, with the sampling time advanced by 2 h each day, so that samples were obtained at 2-h intervals after feeding in a 24-h period during the 4-d collection period. Samples for each steer in each treatment were thawed, composited and dried at 55°C. Fecal output was estimated by feeding 10 g/d of titanium oxide, hand mixed into the diet of each steer immediately

before feeding on d 15 through 25. Diet samples were composited by infusate, dried at 55°C, and ground to pass through a 1-mm screen. Dietary and fecal samples were dried at 105°C in a forced-air oven to determine dry matter (DM), and ashed at 450°C for 8 h to determine organic matter (OM). Determination of non-detergent fiber (NDF) was conducted using an ANKOM fiber analyzer (ANKOM Technology Corp., Fairport, NY). Calculations of intake and digestion were based on observations from d 21 through 25.

At the end of the infusion period, the steers were transported approximately 9 km to the Texas A&M University Rosenthal Meat Science & Technology Center, where the cattle were harvested by humane, industry standard procedures. Immediately post-exsanguination, a portion of the 5^{th}-8^{th} rib section of the longissimus thoracis muscle was removed and transported to the laboratory in oxygenated, 37°C Krebs Henseleit buffer (pH 7.35 – 7.40) containing 5 mM glucose and 5 mM acetate. Pieces of IM and SC adipose tissue were removed by dissection while still fresh, and incubated as described below. Other samples of adipose tissue, liver and longissimus dorsi tissue (obtained fresh in the abattoir) were stored at -80°C for subsequent analyses. Carcasses were graded after 48 h at 4°C, after which the strip loin was removed for sensory and fatty acid analyses.

Lipogenesis in vitro

At sample collection, lipogenesis *in vitro* was measured in IM and SC adipose tissue as described previously (May et al., 1995). Adipose tissue pieces (~100 mg) were incubated with 5 m*M* glucose, 5 m*M* acetate, 10 m*M* HEPES, 1 μCi [U-^{14}C]glucose or 1 μCi [1-^{14}C] sodium acetate (American Radiolabeled Chemicals, Inc.) in oxygenated

Krebs Henseleit buffer (pH 7.35-7.40). Adipose tissues were incubated in 3 mL of media for 2 h at 37.5°C in a shaking water bath. Neutral lipids in adipose tissues were extracted using the procedure of (Folch et al., 1957). The total lipids were saponified as described previously (Smith and Prior, 1982) and glyceride-glycerol and glyceride-fatty acids were isolated and resuspended in 10 mL of scintillation cocktail (Bio-safe2, Research Product international Corp., Mount Prospect, IL). Radioactivity of lipid extracts was counted with a scintillation counter (Packard 1600TR Liquid Scintillation Analyzer, Downers Grove, IL). Results are reported as nmol/(100 mg adipose tissue•2h).

Assay of enzyme activities

Previously frozen SC and IM adipose tissue samples (~100 mg) were homogenized in 3 volumes (wt/vol) of 0.1 M K_2HPO_4 (pH 7.4) for subsequent enzyme analyses. The homogenate was centrifuged at 14,000g for 5 min (4ºC) and the pellet as well as the fat cake were discarded. The infranate was centrifuged at 14,000g for 30 min (4ºC), and the pellet and any remaining lipid layer were discarded. The infranatant fractions were used to undiluted for all enzyme assays.

Glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) activities were determined by the procedure of Bernt and Bergmeyer (1974). Enzyme activities were measured as the reduction of NADP+ to NADPH + H+ using a Beckman DU-7400 Spectrophotometer (Palo Alto, CA). All enzyme activities are expressed as nmol of substrate converted to product per min per wet weight of tissue at 25ºC. Each reaction was determined to be linear over the period of incubation for the particular substrate concentrations tested.

Statistical analysis

Carcass data, lipogenesis, and fatty acid composition were analyzed using the General Linear Mixed Models (GLMM) of SPSS statistics 20 (IBM, Armonk, NY) as appropriate for completely randomized designs. The model tested main effects of infusate. Means were separated by the Fisher's protected LSD and considered different at $P < 0.05$.

Results

Nutrient intake and digestion

Abomasal and ruminal infusion of glucose resulted in greater DM, OM, GE, and DE intake than ruminal acetate infusion ($P = 0.02$; Table 1). DM, OM, GE, and DE were intermediate between acetate and glucose infusion in propionate-infused steers. Total tract digestion of DM, OM, NDF, ADF, and DE was not significantly affected by treatment**.**

Table 1. Effect of ruminal acetate infusion, abomasal glucose infusion, ruminal glucose infusion, or ruminal propionate infusion on nutrient intake and digestion

	Treatment[1]					
Item	A	P	GR	GA	SEM	*P*-value
No. of observations	5	4	6	6		
Intake, g/kg MBW						
DM	66.80[b]	79.38[ab]	92.66[a]	92.88[a]	8.93	0.02
OM	63.44[b]	75.18[ab]	87.70[a]	87.92[a]	8.42	0.02

Table 1. Continued

Item	Treatment[1]				SEM	*P*-value
	A	P	GR	GA		
GE, kcal/kg MBW[2]	265.96[b]	320.92[ab]	369.18[a]	370.72[a]	35.14	0.02
DE, kcal/kg MBW	226.33[b]	261.93[ab]	303.12[a]	313.43[a]	28.03	0.02
Total tract digestion, %						
DM	86.58	82.38	83.00	85.16	2.19	0.20
OM	87.53	83.38	84.16	86.17	2.12	0.21
NDF	76.34	72.55	75.10	75.51	3.49	0.41
ADF	68.47	63.72	67.78	66.61	5.01	0.47
GE	85.92	82.01	82.31	84.49	2.30	0.21

[1]A = acetate ruminal infusion; P = propionate ruminal infusion; GR = glucose ruminal infusion; GA = glucose abomasum infusion. [abc]Means in the same row with common superscripts are not different ($P > 0.05$).
[2]Gross energy intake is sum of energy from diet and treatment.

Carcass traits

There were no significant differences observed in carcass weight, fat thickness, adjusted fat thickness, ribeye area or KPH among treatment groups ($P \geq 0.73$) (Table 2). Yield grade values for acetate, propionate, abomasal and ruminal glucose-infused steers were 4.93, 4.06, 4.81 and 4.69, respectively (Table 2). Infusate had no effect on quality grade or marbling score ($P= 0.18$), though acetate-infused steers had numerically greater

marbling scores and consequently higher quality grade. There were no differences in percentage intramuscular lipid across treatment groups (Table 2).

Table 2. Effect of ruminal infusion of acetate, propionate, glucose, or abomasum infusion of glucose on carcass attributes

	Treatments[1]					
Item	A	P	GR	GA	SEM	*P*-value
Carcass weight, kg	327	333	342	353	11	0.27
Fat thickness, cm	2.59	1.91	2.26	2.18	0.24	0.22
Adjusted fat thickness, cm	2.67	2.03	2.56	2.43	0.24	0.25
Ribeye area, cm^2	68.9	73.7	70.3	71.8	2.3	0.46
Kidney, pelvic, heart fat, %	2.40	2.13	2.08	2.00	0.30	0.73
Yield grade	4.93	4.06	4.81	4.69	0.28	0.14
Quality grade[2]	341	324	317	321	9	0.18
Marbling score[3]	522	470	452	463	26	0.18
Intramuscular lipid, %	5.66	6.03	4.10	4.25	1.7	0.13

[1]A = acetate ruminal infusion; P = propionate ruminal infusion; GR = glucose ruminal infusion; GA = glucose abomasum infusion. [abc]Means in the same row with common superscripts are not different ($P > 0.05$).
[2]Quality Grade: 300 = USDA Choice[00]

Glyceride-fatty acid and glyceride-glycerol biosynthesis

Acetate incorporation into glyceride-fatty acids in IM adipose tissue was greater in glucose ruminal-infused steers (22.74 nmol/(100 mg adipose tissue•2 h)) than in acetate-infused or propionate-infused steers (10.48 and 10.28 nmol/(100 mg adipose tissue•2 h), respectively) (Table 3). Acetate carbon incorporation into glyceride-glycerol was significantly less in (P = 0.01) in IM adipose tissue of acetate-infused steers than in ruminal glucose-infused and abomasal-infused steers. Glucose incorporation into glyceride-fatty acids was higher in IM adipose tissue of acetate and propionate-infused steers than in abomasal and ruminal glucose-infused steers. Similarly, glucose incorporation into glyceride-glycerol was less in IM adipose tissue of abomasal and ruminal glucose-infused steers than in propionate-infused steers (P =0.02).

Acetate incorporation into glyceride fatty acids was less in SC adipose tissue of acetate-infused than in propionate-infused steers, ruminal glucose-infused and abomasal glucose infused steers than in acetate-infused steers in SC adipose tissue (P = 0.002) (Table 3). Acetate incorporation into glyceride-glycerol was less in SC adipose tissue of acetate-infused steers than in SC adipose tissue of propionate, abomasal and ruminal glucose-infused steers (P = 0.007). Glucose incorporation into glyceride-fatty acids was less in SC adipose tissue of acetate and propionate-infused steers than in abomasal and ruminal glucose-infused steers (P=0.03) (Table 3). Glucose incorporation into glyceride-glycerol was higher in SC adipose tissue of acetate and propionate-infused steers than in SC adipose tissue of abomasal and ruminal glucose-infused steers (P=0.005).

6PGDH activity in IM adipose tissue was greater in abomasal glucose- infused steers than in acetate- infused steers (P= 0.02) (Table 4). G6PDH activity was greater in i.m adipose tissue of abomasal glucose-infused steers than in acetate, propionate and ruminal glucose-infused steers (P = 0.002). There were no differences in 6PGDH and G6PGDH activity among treatments in SC adipose tissue ($P \geq 0.14$).

Table 3. Effect of ruminal infusion of acetate, propionate, or glucose, or abomasum infusion of glucose on glyceride-fatty acid and glyceride-glycerol synthesis from acetate and glucose in intramuscular and subcutaneous adipose tissues

	Treatment[1]					
Item	A	P	GR	GA	SEM	*P*-value
Intramuscular adipose tissue						
Acetate incorporation[2]						
Glyceride-fatty acids	10.48^{b}	10.28^{b}	22.74^{a}	19.14^{a}	1.80	0.02
Glyceride-glycerol	0.51^{c}	0.56^{bc}	0.71^{b}	0.92^{a}	0.05	0.01
Glucose incorporation						
Glyceride-fatty acids	1.94^{a}	2.44^{a}	1.46^{b}	1.17^{b}	0.17	0.01
Glyceride-glycerol	2.17^{ab}	2.40^{a}	2.01^{b}	1.75^{b}	0.33	0.02
Subcutaneous adipose tissue						
Acetate incorporation						
Glyceride-fatty acids	41.15^{b}	150.61^{a}	130.47^{a}	158.02^{a}	11.59	0.002
Glyceride-glycerol	0.51^{b}	0.91^{a}	0.75^{a}	0.80^{a}	0.05	0.007
Glucose incorporation						
Glyceride-fatty acids	1.51^{b}	1.94^{ab}	2.02^{a}	2.01^{a}	0.10	0.03
Glyceride-glycerol	5.95^{a}	6.48^{a}	4.46^{b}	4.72^{b}	0.30	0.005

[1]A = acetate ruminal infusion; P = propionate ruminal infusion; GR = glucose ruminal infusion; GA = glucose abomasum infusion. ^{abc}Means in the same row with common superscripts are not different ($P > 0.05$).
[2]Rates are expressed as nmol glucose or acetate incorporated into glyceride-fatty acids or glyceride-glycerol per 100 g adipose tissue per 2 h incubation.

Table 4. Effect of ruminal infusion of acetate, propionate, or glucose, or abomasum infusion of glucose on lipogenic enzyme activity in intramuscular and subcutaneous adipose tissues

	Treatment[1]					
Item	A	P	GR	GA	SEM	*P*-value
Intramuscular adipose tissue						
6PGDH	265.2[b]	273.0[ab]	359.5[ab]	532.5[a]	64.16	0.02
G6PDH	720.0[b]	1,251.0[b]	1,226.0[b]	2,575.5[a]	310.2	0.002
Subcutaneous adipose tissue						
6PGDH	322.8	477.7	402.5	457.0	75.40	0.51
G6PDH	1,299.0	1,831.0	1,728.5	2622.5	403.34	0.14

[1]A = acetate ruminal infusion; P = propionate ruminal infusion; GR = glucose ruminal infusion; GA = glucose abomasum infusion. [abc]Means in the same row with common superscripts are not different ($P > 0.05$).

Discussion

In previous studies, we demonstrated that the extent of carcass adiposity is associated with rates *de novo* fatty acid biosynthesis *in vitro* (Smith and Crouse, 1984; Smith et al., 1984; May et al., 1995). The acetate carbon incorporation into glyceride-fatty acids was less in then acetate- infused steers, though acetate-infused steers had numerically higher quality grades and marbling scores, which indicates that the increased availability of acetate for fatty acid synthesis was sufficient to promote greater carcass fatness than was seen with the other treatments. It also is possible that the greater fatness of the acetate-infused steers caused the depression in *de novo* fatty acid synthesis. In an early study (Smith et al., 1984), we demonstrated a sharp decline in fatty acid biosynthesis in SC adipose tissue in older, fatter cattle.

Contrary to our hypothesis, marbling scores were higher in the acetate-infused steers compared to glucose-infused groups of steers. However, the rate of acetate incorporation into fatty acids in SC adipose tissue of the acetate-infused steers was less than 25% of the rate in the other treatment groups. Also, propionate infusion, and not glucose infusion, caused the greatest rates of glyceride-fatty acid synthesis from in SC adipose tissue.

Prior and Scott (1980) infused glucose, propionate, acetate, and lactate into the veins of beef cattle and demonstrated that only glucose promoted fatty acid synthesis in SC adipose tissue. Lemosquet et al. (2009) infused glucose into the abomasum and propionate into the rumen of dairy cows and documented that the whole body rate of glucose appearance was 24% greater in glucose-infused dairy cows. This suggests that, even though propionate is the primary gluconeogenic precursor in ruminants (Huntington et al., 2006), propionate would not be as effective as glucose in increasing blood glucose and thereby promoting adipogenic gene expression in SC adipose tissue.

In early research, we demonstrated that the rate of acetate incorporation into fatty acids greatly exceeds the rate of glucose incorporation into fatty acids *in vitro* in SC adipose tissue (Smith and Prior, 1982; Smith, 1983; Smith and Crouse, 1984). We also demonstrated that glucose incorporation into fatty acids was higher in IM adipose tissue than in SC adipose tissue (Smith and Crouse, 1984; Rhoades et al., 2007), although other studies (Miller et al., 1991; the current study) have demonstrated greater rates of fatty acid synthesis from acetate than from glucose. Regardless, in all studies from this laboratory, glucose a greater proportion of the carbon for fatty acid biosynthesis in IM

adipose tissue than in SC adipose tissue. In the current study, less than 50% of the glucose carbon was recovered in glyceride-fatty acids, indicating that, in these cattle, there was a limitation in the ability of IM and SC adipose tissues to utilize glucose for fatty acid biosynthesis.

G6PDH and 6PGDH are enzymes in the pentose phosphate pathway and their anabolic activity generate nicotinamide adenine dinucleotide phosphate (NAPDH). The synthesis of long chain fatty acids is dependent on adequate amounts of NAPDH, the reducing equivalent for fatty acid synthesis (Flatt and Ball, 1964). Smith (1983) indicated that without other exogenous substrates in the media, 12% of total glucose was metabolized in the pentose cycle, which increased to 22 - 37% when acetate and lactate were included in the media. With glucose in the media, 30% of NADPH was derived from the pentose cycle, and this increased to 72% when acetate and lactate were added to the media. The lesser G6PDH activity in IM adipose tissue relative to SC adipose tissue was consistent to our early report (Smith and Crouse, 1984). Additionally, the IM adipose tissue pentose cycle activities were highest in steers receiving abomasal infusion of glucose, indicating that glucose availability was especially effective in promoting lipogenic enzyme activities in IM adipose tissue.

Conclusions

Contrary to our hypothesis, marbling scores were not different in acetate-infused steers and the glucose-infused steers. However, the rate of acetate incorporation into fatty acids in IM and SC adipose tissues of the acetate-infused steers was less than the

rate in the adipose tissues of the abomasal-infused steers. These data confirm that provide additional glucose for absorption promotes adipogenesis in beef cattle.

CHAPTER IV

INCREASING GLUCOSE OR GLUCOSE PRECURSORS TO INCREASE BEEF QUALITY AND JUICINESS

Introduction

In the U.S., Australia, and many Asian countries, beef with higher degrees of IM adipose tissue, the visible portion also referred to as marbling, is associated with overall consumer acceptance. Segments of the beef cattle industry are seeking effective and efficient methods to grow cattle to market weights that can increase economic returns by maximizing marbling scores and limit discounts due to increased carcass yield grade caused by excessive SC adipose tissue. Different management strategies have been proposed aiming to increase deposition of IM adipose tissue without increasing SC adipose tissue and negatively affecting meat quality (Smith et al., 1983; Fluharty et al., 2000). Due to the efforts of researchers to meet the beef quality demands of the American consumers, the 2012 National Beef Quality Audit reported that the percentage of USDA Prime plus Choice carcasses was at a 20-year high (61.1% for NBQA 2011 vs 54.5% for NBQA 2005; Gray et al., 2012).

Smith et al. (1983) reported that higher marbling scores lead to dramatic decreases in undesirable beef flavors. As marbling scores increased from Practically Devoid to Moderately Abundant, the undesirable ratings decreased from 55% to zero. The amount of marbling also has an impact on juiciness scores of meat. Miller (1994) proposed a mechanism whereby increased marbling causes salivary stimulation within

the mouth and gives the perception of increased juiciness of meat while chewing. This mechanism was described as the lubrication effect of marbling. Beef with higher quality grades will sustain salivary stimulation and perceived juiciness. Therefore, because species-specific flavors are located in the lipid portion of beef, i.m adipose tissue impacts beef flavor.

Propiogenic diets (such as high-grain diets) promote greater marbling development than acetogenic diets (Rhoades et al., 2007, 2009). In ruminants, the major VFA produced by rumen microorganisms are acetate, propionate, and butyrate. These VFA are the products of ruminal fermentation, and serve as the main precursors of glucose and fatty acid biosynthesis in ruminants. In animals on grain-based diets, some starch escapes fermentation and is digested to and absorbed as glucose from the small intestine. Little glucose is absorbed from the small intestine in ruminants, so gluconeogenesis provides the glucose needed to support essential functions. Intramuscular adipose tissue uses glucose for a greater proportion of the carbon source for *de novo* fatty acid biosynthesis than SC adipose tissue (Smith and Crouse, 1984; Rhoades et al., 2007, 2009b). However, the impact of directly providing glucose for absorption from the small intestine on carcass quality and lipogenesis in vitro has not been documented.

We hypothesized that "glucogenic" diets, which provide more glucose for absorption from the small intestine (e.g., dry-rolled corn; Huntington et al., 2006; Corrigan et al., 2009), would promote marbling development, hence juiciness, to a greater extent than propiogenic diets. This hypothesis was tested directly infusing

isocaloric amount of glucose and propionate into the digestive tract of young, growing steers. Glucose was infused into the rumen (reflecting a high-starch diet) and into the abomasum, the latter treatment bypassing ruminal metabolism of the infused glucose. We also infused acetate into the rumen to directly provide substrate for fatty acid biosynthesis in SC and IM adipose tissues.

We predicted that glucose infusion into the abomasum would increase carcass adiposity and beef juiciness, relative to ruminal infusions of acetate, propionate, or glucose. Total IM lipid and fatty acid composition of IM and SC adipose tissues were measured to confirm results for carcass marbling scores, as increased marbling is associated with a higher concentration of MUFA in SC and IM adipose tissues (Brooks et al., 2011b).

Materials and Methods

Animals and treatments

This experiment tested the hypothesis that absorption of equivalent energy amounts of acetate, propionate, and glucose would have different effects on marbling development and metabolism in growing steers. Specifically, abomasal infusion of glucose would more strongly promote marbling development, hence juiciness, than ruminal infusion acetate, propionate, or glucose. Also, we predicted that effects of infusing propionate into the rumen would provide results that were intermediate between glucose and acetate.

Twenty-four Angus crossbred steers were grown using conventional diets until approximately 22 mo of age. Steers were weighed at the start of the experiment and

were housed in individual pens (2.1 by 1.5 m) in an enclosed barn with free access to water. The steers were fitted with ruminal cannulas as described previously (Bourg et al., 2012) at the Texas A&M University Animal Research and Teaching Center. Cattle were adapted to a standard, corn/sorghum finishing diet containing ground milo, corn, cottonseed meal, cottonseed hulls, molasses, premix, ammonium chloride and R-1500 over a 2-wk period while recovering from the placement of the cannulas. After the adaptation period, steers were infused with 5 L of water for 3 d. The following treatments were continuously infused for the last 28 d of the finishing period:

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2. Propionate, 0.758 kg/d (10.15 mol/d; 3.76 Mcal/d) ruminal infusion (n = 6)
3. Glucose, 1.0 kg/d (5.55 mol/d; 3.76 Mcal/d) ruminal infusion; (n = 6) (control group)
4. Glucose, 1.0 kg/d (5.55 mol/d; 3.76 Mcal/d) abomasal infusion (n = 6)

One steer from the acetate group and two steers from the propionate group were excluded from the study because they demonstrated symptoms of poor health.

Treatments were brought to a volume of 5 L with water and infused using a peristaltic pump. Ruminal infusions were accomplished by continuous infusion of substrate through infusion lines inserted through the cannulas. Abomasal infusion lines were accomplished by continuous infusion of substrate through infusion lines through the cannula bypassing the rumen and secured into the abomasum. Steers were fed the finishing diet free choice.

At the end of the infusion period, the steers were transported approximately 9 km to the Texas A&M University Rosenthal Meat Science & Technology Center, where the cattle were harvested by humane, industry standard procedures.

Fatty acid composition

Total lipids of IM and SC adipose tissues were extracted by a modification of the method of Folch et al. (1957). One hundred milligrams of tissue were homogenized and extracted in chloroform:methanol (2:1, vol/vol) and lipid content was measured gravimetrically. Fatty acid methyl esters (FAME) were prepared as described by Morrison and Smith (1964), modified to include an additional saponification step (Archibeque et al., 2005). The FAME were analyzed using a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 auto sampler, Varian Inc., Walnut Creek, CA). Separation of FAME was accomplished on a fused silica capillary column CP-Sil88 [100 m × 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands), with hydrogen as the carrier gas (flow rate = 1.2 mL/min). Initial oven temperature was set at 150°C and increased at 1°C/min to 160°C. After temperature reached 160°C, oven temperature was increased at 5°C/min to 220°C and held for 30 min. Total run time was 52 min. Injector and detector temperatures were at 270°C. Individual fatty acids were identified using genuine external standards (Nu-Chek Prep, Inc., Elysian, MN).

Slip points

Melting points of the adipose tissue lipids were approximated by determining slip points (Smith et al., 1998). After heating to approximately 45°C, extracted lipids were drawn 1 cm into glass capillary tubes and frozen at -20°C. After freezing, the capillary

tubes were suspended vertically in a chilled water bath with the portion of the tube containing the lipid submerged. The water bath was heated gradually and the temperature of the water was monitored with a glass thermometer (model 9095-564, VWR International, Radnor, PA). Slip point is defined as the temperature at which the lipid began to move up the capillary tube.

Tenderness and sensory analyses

Warner-Bratzler shear force determination. Steaks designated for shear force evaluation were cooked in electric skillets (Rival, Inc., Boca Raton, FL., Model CKRVSK11 Skillet). Steaks were turned once at 40ºC and brought to an internal temperature of 71ºC. Internal temperatures were monitored by iron-constantan thermocouples (Omega Engineering, Stanford, CT) inserted into the geometric center of each patty and monitored using a digital thermometer (Omega Engineering, model HH501BT type T, Stamford, CT) with a type T thermocouple (Omega Engineering, model TMQSS). Once internal temperature was reached, steaks were allowed to cool to room temperature. Six, 1.27-cm-diameter cores were taken from each steak. Cores were removed parallel to the muscle fibers. Each core was sheared once perpendicular to the grain of the muscle fiber using a Warner-Bratzler shearing machine.

Sensory evaluation. Sensory evaluation was conducted at the Texas A&M University Meat Products Evaluation Laboratory. Descriptive flavor analysis was conducted by a trained, 5-member descriptive panel for 2 consecutive days after 5 d of training. Panelist evaluated samples using a descriptive flavor analysis, using the Spectrum Universal intensity scale where 0 = none and 15 = extremely intense for each

attribute (Adhikari et al., 2011; AMSA, 1995). Each panelist evaluated 24 different samples, each coming from a different source of infusate. Samples were assigned randomly to a different order for each day of sensory evaluation. Each sample was presented with a three-digit random code and placed in a small clear plastic serving cup. Panelists were seated in individual booths separated from the sample preparation area. Panelists were also given salt-free saltine crackers and double-distilled, deionized water as palate cleansers to be used prior to evaluation of each sample. The steaks were thawed for 4 h in a cooler at 4°C on each day of the evaluation. Steaks were cooked in electric skillets (Rival, Inc., Boca Raton, FL., Model CKRVSK11 Skillet) set at 204°C to an average internal temperature of 71ºC. Internal temperatures were monitored by iron-constantan thermocouples (Omega Engineering, Stanford, CT) inserted into the geometric center of each steak and monitored using a digital thermometer (Omega Engineering, model HH501BT type T, Stamford, CT) with a type T thermocouple (Omega Engineering, model TMQSS). Once steaks reached 71°C, they were cut into eight individual pieces, providing two samples for each panelist. Samples were served a minimum of 4 min apart. A 5-member, trained sensory panel was used to evaluate a second steak for juiciness, muscle fiber tenderness, overall tenderness, and connective tissue amount, and overall flavor using a 15-point scale, as described above

Statistical analysis

Data were analyzed using the Proc GLM procedure of SAS (v9.3, SAS Institute Inc., Cary, NC) with an alpha < 0.05. An initial analyses was conducted to examine the effects of panelist and panelist by infused metabolite to validate the sensory panel data. Panelist x infusate interactions were not significant (P > 0.05). Therefore, sensory data were averaged across panelists within a sample. In the final analysis, the main effect was infusate; this was analyzed as a fixed effect. Sensory day and order served were defined as random effects. Least squares means were calculated and when significance was defined in the Analysis of Variance table, least squares means were separated using the pdiff function of SAS.

Results

Fatty acid composition and slip points

There no differences in fatty acid composition among treatment groups ($P \geq 0.12$) (Table 5). Correspondingly, there were no differences in lipid melting points (slip points) among treatment groups ($P = 0.26$).

Table 5. Effect of ruminal infusion of acetate, propionate, or glucose, or abomasum infusion of glucose on the fatty acid composition and subcutaneous adipose tissue

	Treatment[1]					
Item	A	GA	GR	P	SEM	*P*-value
Intramuscular adipose tissue						
Myristic (14:0)	5.82	4.79	5.33	5.29	0.84	0.86
Palmitic (16:0)	33.9	33.6	33.5	34.1	1.82	0.99
Palmitoleic (16:1n-7)	2.96	2.67	2.62	2.66	0.29	0.86
Stearic (18:0)	16.9	17.0	17.0	15.4	2.00	0.94
trans-18:1	2.52	2.33	2.50	2.53	0.20	0.86

Table 5. Continued

	Treatment[1]					
Item	A	GA	GR	P	SEM	*P*-value
Oleic (18:1n-9)	30.1	31.2	29.2	31.2	2.08	0.86
cis-Vaccenic (18:1n-7)	0.53	0.99	0.89	0.80	0.15	0.89
Linoleic (18:2n-6)	2.12	1.93	2.09	2.32	0.18	0.54
α-Linolenic (18:3n-3)	0.32	0.32	0.31	0.30	0.02	0.87
Slip point, °C	42.0	41.8	43.6	43.5	0.82	0.26
Subcutaneous adipose tissue						
Myristic (14:0)	6.35	5.49	4.14	5.51	0.83	0.31
Palmitic (16:0)	33.1	32.0	28.6	32.4	1.53	0.16
Palmitoleic (16:1n-7)	2.94	2.04	1.8	2.22	0.32	0.12
Stearic (18:0)	16.3	18.7	21.4	19.1	1.66	0.20
trans-18:1	2.70	2.47	3.1	2.79	0.19	0.13
Oleic (18:1n-9)	30.1	29.5	32.3	28.7	2.11	0.65
cis-Vaccenic (18:1n-7)	0.69	0.81	0.71	0.98	0.15	0.59
Linoleic (18:2n-6)	2.06	1.77	2.33	2.17	0.25	0.44
α-Linolenic (18:3n-3)	0.22	0.76	0.28	0.25	0.30	0.53
Slip point, °C	42.5	41.8	43.7	43.3	0.82	0.26

[1]A = acetate ruminal infusion; GA = glucose abomasum infusion; GR = glucose ruminal infusion; P = propionate ruminal infusion There were no treatment effects for any fatty acids ($P > 0.05$)

Tenderness and sensory analyses

Table 6 contains the definitions and reference standards for meat descriptive flavor aromatics and basic taste sensory attributes and their intensities. Ribeye steaks from abomasal glucose-infused steers had higher shear force values (2.62 kg) than steaks from ruminal glucose-infused or propionate-infused steers (2.15 and 2.33 kg, respectively; $P \leq 0.05$) (Table 7). Ribeye steaks from the acetate, abomasal glucose-infused, and ruminal glucose-infused steers were higher in the flavor attributes brown/roasted, fat-like, and overall sweet, and lower in bitter off-flavor, than steaks from propionate infused steers ($P \leq 0.05$). Steaks from acetate-infused steers were higher in the flavor attribute bloody/serumy than steaks from abomasal and ruminal glucose-infused steers ($P \leq 0.07$). Steaks from propionate-infused steers were higher in metallic off-flavor than steaks from ruminal glucose-infused steers ($P = 0.03$), and were higher in sour off-flavor than abomasal and ruminal glucose-infused steers ($P \leq 0.02$). For taste panel texture analysis, ribeye steaks from acetate infused steers had higher juiciness scores than steaks from all other infusion groups ($P \leq 0.04$), and had higher myofibrillar and overall tenderness scores than steaks from abomasal glucose-infused steers ($P \leq 0.08$). Steaks from propionate-infused steers had higher myofibrillar and overall tenderness scores than steaks from abomasal glucose infused steers ($P \leq 0.006$), whereas steaks from acetate and propionate-infused steers had higher connective tissue scores than steaks from ruminal glucose-infused steers ($P \leq 0.06$) (Table 7).

Table 6. Definition and reference standards for meat descriptive flavor aromatics and basic taste sensory attributes and their intensities where 1 = none and 15 = extremely intense (Adhikari et al., 2011)

Sensory Attributes	Definition	Reference, standard flavor scale value unless otherwise defined
Beef identity	Amount of beef flavor identity in the sample.	Swanson's beef broth = 5.0 80% lean ground beef = 7.0 Beef brisket = 11.0
Brown/roasted	A round, full aromatic generally associated with beef suet that has been boiled.	Beef suet = 8.0 80% lean ground beef = 10.0
Bloody/serumy	The aromatics associated with blood on cooked meat products. USDA choice strip steak = 5.5 Closely related to metallic aromatic.	USDA choice strip steak = 5.5 Beef brisket = 6.0
Fat-like	The aromatics associated with cooked animal fat.	Hillshire farms Lit'l beef smokies = 7.0 Beef suet = 12.0
Metallic	The impression of slightly oxidized metal, such as iron, copper and silver spoons.	0.10% potassium chloride solution = 1.5 USDA choice strip steak = 4.0
Umami	Flat, salty, somewhat brothy. The taste of glutamate, salts of amino acids and other molecules called nucleotides.	0.035% accent flavor enhancer solution = 7.5
Overall sweet	A combination of sweet taste and sweet aromatics. The aromatics associated with the impression of sweet.	Hillshire farms Lit'l beef smokies = 3.0 SAFC ethyl maltol 99% = 4.5 (aroma) Post-shredded wheat spoon size = 1.5
Sweet	The fundamental taste factor associated with sucrose.	2.0% sucrose solution = 2.0
Bitter	The fundamental taste factor associated with a caffeine solution.	0.01% caffeine solution = 2.0 0.02% caffeine solution = 3.5

Table 6. Continued

Sensory Attributes	Definition	Reference, standard flavor scale value unless otherwise defined
Sour	The fundamental taste factor associated with citric acid.	0.015% citric acid solution = 1.5 0.050% citric acid solution = 3.5
Salty	The fundamental taste factor of which sodium chloride is typical.	0.15% sodium chloride solution = 1.5 0.25% sodium chloride solution = 3.5
Tenderness	The opposite of the force required to bite through the sample with the molars	
Juiciness	The amount of moisture released by the sample after the first two chews	Carrot = 8.5; Mushroom = 10.0; Cucumber = 12.0; Apple=13.5; Watermelon=15.0
Connective tissue amount	The amount of connective tissue perceived before swallowing	

Table 7. Effect of ruminal infusion of acetate, propionate, or glucose, or abomasum infusion of glucose on sensory panel attributes, texture analysis, and Warner-Bratzler shear force of cooked ribeye steaks

	Treatment[1]				
Item	A	GA	GR	P	SEM
Warner-Bratzler shear force, kg	2.37	2.62	2.15	2.33	0.07
Flavor descriptors and off-flavors[2]					
Beef identity	5.10	5.10	5.13	5.00	0.06
Brown/roasted	1.07	1.10	1.23	0.47	0.08
Bloody/serumy	1.57	1.23	1.23	1.50	0.08
Fat-like	1.10	0.87	1.30	0.80	0.07
Metallic	1.87	1.83	1.70	2.03	0.06
Umami	0.53	0.57	0.37	0.40	0.07
Overall sweet	0.60	0.30	0.50	0.17	0.05
Sweet	0.23	0.13	0.23	0.13	0.04
Sour	2.17	2.00	1.87	2.40	0.07
Salty	1.43	1.23	1.47	1.40	0.05
Bitter	1.73	1.87	1.80	2.13	0.06
Texture analysis[3]					
Juiciness	10.97	10.12	10.05	10.83	0.15
Myofibrillar tenderness	11.93	11.20	11.70	12.10	0.14
Overall tenderness	11.83	11.32	11.65	12.10	0.14
Connective tissue	11.93	11.72	11.05	11.90	0.16

[1]A = acetate ruminal infusion; GA = glucose abomasum infusion; GR = glucose ruminal infusion; P = propionate ruminal infusion [abc]Means in the same row with common superscripts are not different ($P > 0.05$).

[2]0 = absent and 15 = extremely intense.

[3]1 = extremely dry, tough, tough, abundant; and 15 = extremely juicy, tender, tender, none.

Discussion

The effect of IML, i.e. quality grade, on fatty acid concentrations has been reported previously (Wood et al., 2008; Scollan et al., 2006), which revealed that as IML increases, concentrations of all fatty acids increase accordingly. However, MUFA and SFA increase to a much greater extent than PUFA because of a direct result of TAG accumulation during animal fattening. Feeding grain-based diets for extended periods effectively increases oleic acid in beef (Huerta-Leidenz et al., 1996; Chung et al., 2006; Brooks et al., 2011b; Smith et al., 2012)by promoting increase glucose absorption from the small intestine (Huntington et al., 2006; Corrigan et al., 2009) and thereby stimulating SCD gene expression and catalytic activity (Brooks et al., 2011b). Stearoyl-CoA desaturase encodes the Δ^9- desaturase responsible for the synthesis of oleic acid and other MUFA. In general, production methods that increase marbling deposition also increase the concentration of oleic acid in beef. However, the relationship between fatty acid composition and marbling scores can be demonstrated only across large variations in marbling scores (Chung et al., 2006; Brooks et al., 2011b). In the current study, there were no significant differences in marbling scores across treatment groups; similarly, there were no differences across treatments for MUFA, either in IM or SC adipose tissue.

Higher concentrations of oleic acid are positively correlated with overall palatability (Waldman et al., 1968; Westerling and Hedrick, 1979), whereas stearic acid is the primary determinant of fat hardness (i.e., lipid melting point) (Smith et al., 1998; Wood et al., 2004; Chung et al., 2006). Therefore, increasing the concentration of oleic

acid and/or decreasing stearic acid in beef would increase the fat softness and thereby increase juiciness of beef and beef products. However, in this study the concentrations of stearic and oleic acid in IM adipose tissue were unaffected by treatment. Correspondingly, treatments had no effect on lipid melting points. The greater rate of fatty acid synthesis observed in cattle infused with glucose (ruminally or abomasally) should have affected both IM and SC fatty acid composition (see Table 3). However, at the initiation of treatments, these steers were older than most steers studied in this laboratory (22 mo vs 12 mo). It is apparent that these steers had been fed pasture-based diets for an extended period before being fed the grain-based, finishing diet, based on the fatty acid composition of their adipose tissues. Subcutaneous adipose tissue of grain-fed cattle typically contains approximately 40% oleic (Chung et al., 2006; Brooks et al., 2011b), whereas the SC adipose tissue of the steers in the current study contained only 30% oleic acid). In steers this age, the adipose tissues would have been relatively mature and lipid-filled, so any contributions to total fatty acid composition during the 28-d duration of this experiment.

Brooks et al. (2011a) demonstrated that juiciness was the only sensory attribute that differed between rib steaks from calf-fed (corn-fed for 8 mo) and yearling-fed steers (corn-fed for 5.5 mo, beginning at 12 mo of age). Rib steaks from calf-fed steers contained more total lipid than yearling-fed steers (9.98 vs 7.75%; Brooks et al., 2011a) and the lipids in muscle from calf-fed steers contained more oleic acid than in yearling-fed steers (40.6 vs 38.2%; Brooks et al., 2011a). However, even though beef from the calf-fed steers contained more IM lipid than beef from the yearling-fed steers (as

predicted), the beef from the yearling-fed steers had a higher USDA marbling score than beef from the calf-fed steers (Small25 vs Small00; Brooks et al., 2011a).

Beef flavor is not a single attribute, but it is composed of multiple attributes that can be dynamic. Little research has been conducted to determine the effects of glucose and gluconeogenic precursors on flavor attributes. It is important that none of the treatments caused increases in linoleic acid. Research has demonstrated that increasing linoleic acid leads to negative beef flavor attributes (Melton et al., 1982; Baublits et al., 2009). We previously demonstrated that linoleic acid and arachidonic acid were negatively correlated with cooked beef fat flavor and demonstrated an increase in beef identity flavor attribute if these fatty acids were replaced with monounsaturated fatty acids (Blackmon et al., 2015). Baublits et al. (2009) also reported that positive beef flavor attributes were enhanced by increased percentages of SFA and MUFA, whereas PUFA had a greater effect on the perceived negative aspects of beef flavor. Polyunsaturated fatty acids oxidize readily and can increase alkanals four-fold in the aroma extracts from steaks (Elmore and Mottram, 2000). Cooked beef from animals fed lipid supplements high in n-6 PUFA contain higher levels of 2-alkyl-(2H)- thiapyrans and 2-alkylthiophenes in the volatiles (Elmore & Mottram, 2000). While thiapyrans have low odor potency, the reactions that form from them may remove potent aroma compounds, which modify meat aroma profile. The net effect is that positive attributes, such as beef flavor, decrease whereas negative attributes, such as rancid flavors, increase.

Conclusions

Although there were no treatment effects on adipose tissue fatty acid composition, propionate infusion generally decreased positive flavor attributes and increased negative flavor attributes. Thus, although propionate is a gluconeogenic precursor, oversupply of propionate in the rumen may negatively affect beef flavor.

CHAPTER V

GROUND BEEF PATTIES PREPARED FROM BRISKET, FLANK, AND PLATE HAVE UNIQUE FATTY ACID AND SENSORY CHARACTERISTICS[*]

Introduction

Approximately 40% of per capita beef consumption in the U.S. (30 kg/y) is consumed as ground beef, and low-income households consume more ground beef per capita than do high-income households (USDA, 2011). Furthermore, most of the ground beef available in retail outlets is relatively high in SFA and low in MUFA. Commercial ground beef in the U.S. contains fat with a MUFA:SFA ratio of 0.85–0.90, whereas ground beef from cattle fed high-grain diets has a MUFA:SFA greater than 1.10, and ground beef from grass-fed cattle has a MUFA:SFA ratio less than 0.75 (Gilmore et al., 2011; Turk and Smith, 2009).

We have demonstrated that fatty acid composition of ground beef can affect the concentration of high-density lipoprotein (HDL) cholesterol in men (Adams et al., 2010; Gilmore et al., 2011) and women (Gilmore et al., 2013). Ground beef from grass-fed steers that was high in SFA and trans-fatty acids decreased HDL cholesterol in mildly hypercholesterolemic men, whereas ground beef from grain-fed cattle naturally enriched with oleic acid returned HDL cholesterol concentrations to pre-intervention levels. Ground beef from grass-fed steers had no effect on HDL cholesterol concentrations in

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normocholesterolemic men, whereas high-oleic acid ground beef from grain-fed cattle increased HDL cholesterol (Gilmore et al., 2011), although ground beef from the grass-fed contained three times as much ALA as ground beef from grain-fed cattle (90 vs 30 mg per patty). Our most recent study (Gilmore et al., 2013) provided further support for increasing MUFA in ground beef; ground beef from Akaushi cattle (MUFA:SFA ratio = 1.43) increased HDL cholesterol concentrations in postmenopausal women, while there was no effect of chub pack ground beef (MUFA:SFA ratio = 0.86) on HDL cholesterol. These studies demonstrated that practical increases in MUFA in ground beef have positive effects on risk factors for cardiovascular disease, and are consistent with previous research that demonstrated the health benefits of replacing SFAs with MUFAs in the diet (e.g., Allman-Farinelli et al., 2005).

Though health concerns are important to consumers, they also want a product that is highly palatable. The concentration of oleic acid in beef is positively correlated with overall palatability (Waldman et al., 1968; Westerling and Hedrick, 1979). Baublits et al. (2009) reported that the concentration of MUFA (and specifically oleic acid) in beef was positively correlated with beef/brothy ($r = 0.37$) and beef fat ($r = 0.44$) flavors, indicating that as the concentration of oleic acid increases, so do beefy flavors. Therefore, increasing the MUFA:SFA ratio would increase the palatability as well as the healthfulness of beef and beef products.

In cattle, it has not been practical to increase oleic acid in beef by feeding high-oleic oil or oil seeds (Chang et al., 1992; St. John et al., 1987), although fatty acid composition varies considerably across carcass fat depots and changes with animal age

(Smith et al., 2012; Turk and Smith, 2009). Adipose tissue overlying the brisket is unusually high in MUFA, especially oleic acid, and low in palmitic and stearic acid (Smith et al., 2012; Turk and Smith, 2009). The plate, which is typically used as raw materials for ground beef production, is intermediate in SFA content, whereas the flank has the highest proportions of SFA (Turk and Smith, 2009). Furthermore, the proportion of MUFA in SC adipose tissue overlying the brisket is high even in young cattle (Smith et al., 2012). For this reason, brisket fat trim can be taken at virtually any production age to produce ground beef consistently high in oleic acid. However, we do not know if the fatty acid compositions of the lean trims from the brisket, flank, and plate are similar to that of the overlying SC adipose tissues. The objective of this study was to determine the fatty acid composition, trained panel flavor descriptors, and lipid-derived and Maillard-derived volatiles of ground beef patties produced from lean and fat trims of brisket, flank, and plate primals. Additionally, we tested the effects of fat level (10, 20, and 30%) on fatty acid and flavor attributes of ground beef. We hypothesized that individual primal lean and fat trims can be used to formulate ground beef with unique fatty acid and sensory characteristics.

Materials and Methods

Sample collection

The study was carried out as a randomized complete block design in a 3 × 3 factorial arrangement. Four carcasses were selected and graded at random from carcasses being processed at Sam Kane Beef Processors Inc. at Corpus Christi, TX. The carcasses graded USDA Select and represented a variety of Bos taurus breed types and

backgrounds, produced at the Texas A&M University Research Center at McGregor. Primals were collected from both sides of the carcass and included the brisket, flank, and plate. After collection, carcasses were transported to Texas A&M University on ice, where they were frozen at -20°C.

Sample preparation

The day before the separation of lean and fat trim, primals were tempered at 4°C. Lean and fat trims were separated by dissection, and the lipid content of each fat and lean trim was measured by gravimetric chloroform:methanol lipid extraction (Folch, Lees, & Stanley, 1957). Lean and fat trims were combined to contain 10, 20, or 30% total fat, coarse ground (1.27 cm), vacuum packaged and stored at -20°C. A final grind (0.32 cm) was performed and patties were formed into 136-g patties, vacuum packaged and stored at -20°C until further testing, typically less than 14 d after sample collection. The ground beef preparation was divided into 5 production days.

Lipid extraction/fatty acid composition

Total lipids of raw patties were extracted by a modification of the method of Folch et al. (1957). One hundred milligrams of homogenized, fresh ground beef was extracted in chloroform:methanol (2:1, vol/vol) and FAME were prepared as described by Morrison and Smith (1964), modified to include an additional saponification step (Archibeque et al., 2005). The FAME were analyzed using a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 auto sampler, Varian Inc., Walnut Creek, CA). Separation of FAME was accomplished on a fused silica capillary column CP-Sil88 [100 m × 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands), with hydrogen as

the carrier gas (flow rate=35 mL/min) (split ratio 20:1). The initial oven temperature was 150°C; oven temperature was increased at 5°C/min to 220°C and held for 22 min. Total run time was 52 min. Injector and detector temperatures were at 270°C and 300°C, respectively. Individual fatty acids were identified using genuine external standard GLC-68D (Nu-Chek Prep, Inc., Elysian, MN). This method did not separate the 18:1*trans*-10 and 18:1*trans*-11 peaks in all samples, so these fatty acids were combined.

Slip points

Melting points of the subcutaneous adipose tissue lipids were approximated by determining slip points (Smith et al., 1998). After heating to approximately 45°C, extracted lipids were drawn 1 cm into glass capillary tubes and frozen at -20°C. After freezing, the capillary tubes were suspended vertically in a chilled water bath with the portion of the tube containing the lipid submerged. The water bath was heated gradually and the temperature of the water was monitored with a glass thermometer (model 9095-564, VWR International, Radnor, PA). Slip point is defined as the temperature at which the lipid began to move up into the capillary tube.

Trained sensory analysis

Descriptive flavor analysis was conducted by a trained, 4-member descriptive panel for 4 consecutive days after 5 d of training. Panelist evaluated samples using a descriptive flavor analysis, using the Spectrum Universal intensity scale where 0=none and 15=extremely intense for each attribute (Adhikari et al., 2011; AMSA, 1995). Each panelist evaluated 36 different samples, each coming from a different source of primal and fat percentage. Samples were assigned randomly to a different order for each day of

sensory evaluation. Each sample was presented with a three-digit random code and placed in a small clear plastic serving cup. Panelists were seated in individual booths separated from the sample preparation area. Panelists were also given salt-free saltine crackers and double-distilled, deionized water as palate cleansers to be used prior to evaluation of each sample. The patties were thawed for 4 h in a cooler at 4°C on each day of the evaluation. Patties were cooked in electric skillets (Rival, Inc., Boca Raton, FL., Model CKRVSK11 Skillet) set at 204°C to an average internal temperature of 71°C. Internal temperatures were monitored by iron-constantan thermocouples (Omega Engineering, Stanford, CT) inserted into the geometric center of each patty and monitored using a digital thermometer (Omega Engineering, model HH501BT type T, Stamford, CT) with a type T thermocouple (Omega Engineering, model TMQSS). Once patties reached 71°C they were cut into eight individual pieces, providing two samples for each panelist. Samples were served a minimum of 4 min apart.

Gas chromatography with mass spectrometry

Samples were thawed and cooked the same as described for consumer sensory analysis at the same time samples were prepared for sensory analysis. Once samples were cooked, they were placed in a glass jar (473 mL) with a Teflon piece under the metal lid and then placed in a water bath at 60°C, where the headspace was collected with a solid-phase micro-extraction (SPME) Portable Field Sampler (Supelco 504831, 75 μm Carboxen/polydimethylsiloxane, Sigma- Aldrich, St. Louis, Mo). Upon first receiving the SPME fibers, each fiber was conditioned for 1 h at 280°C in the GC injection port. The headspace above each meat sample in the glass jar was collected for 2

h on the SPME. Upon completion of collection, the SPME was injected in the injection port, where the sample was desorbed at 280°C. The sample was loaded onto the multi-dimensional gas chromatograph into the first column (30m× 0.53mmID / BPX5 [5% phenyl-polysilphenylene-siloxane] × 0.5 μm, SGE Analytical Sciences, Austin, TX), which is nonpolar and separates compounds based on boiling point. Through the first column, the temperature started at 40°C and increased at a rate of 7°C/min until reaching 260 °C. Upon passing through the first column, a program was designed to leave the heart-cut and cryo-trap open to forward the compounds to the second column (30 m × 0.53 mm ID [BP20-polyethylene glycol] × 0.50 μm, SGE Analytical Sciences), which separates compounds due to polarity. The gas chromatography column was then split at a three-way valve with one column going to the mass spectrometer (Agilent Technologies 5975 series MSD, Santa Clara, CA) and one column going to each of the two sniff ports, which were heated to a temperature of 115°C, and fitted with glass nose pieces. The sniff ports and software for determining flavor and aroma are a part of the AromaTrax program (MicroAnalytics-Aromatrax, Round Rock, TX) (Harbison, 2012).

Statistical analysis

For fatty acid data, data were analyzed by single-factor analysis of variance by the Super Anova program (Abacus Concepts, Inc., Berkeley, CA), with primal and fat percentages as the main effects; the model also tested the primal × fat percentage interaction. Means were separated by the Fisher's Protected LSD method contained in the same software program. Differences between means were considered significant at $P < 0.05$. For sensory data, data were analyzed using the GLM procedure of SAS (v9.3, SAS Institute Inc., Cary, NC) with an alpha < 0.05. An initial analysis was conducted to examine the effects of panelist and panelist × primal and fat level interaction to validate the sensory panel data. Panelists × primal and fat level interactions were not significant ($P > 0.05$). Therefore, sensory data were averaged across panelists within a sample in the final analysis, the main effects were primal, fat level; the model also tested the fat level × primal interaction; these were analyzed as fixed effects. Sensory day and order served were defined as random effects. Least squares means were calculated and when significance was defined in the Analysis of Variance table, least squares means were separated using the pdiff function of SAS. The effects of primals and the fat level for lipid derived volatiles and Maillard derived volatiles were analyzed using JMP® Software (JMP®, Version 9.0.0, SAS Institute Inc., Cary, NC, 1989–2010).

Results

Fatty acid composition of ground beef patties

There were several differences in fatty acid composition in the fat and lean trims from the brisket, flank and plate (Table 8). The proportion of oleic acid was greatest in brisket and least in flank trims ($P = 0.02$); the plate was intermediate (data pooled across fat and lean trims). The brisket contained lesser proportions of palmitic acid and stearic acid than the flank or plate ($P \leq 0.003$). The brisket and flank contained greater proportions of ALA than the plate, and the brisket and plate contained greater proportions of DHA than the flank. Primal × fat trim interactions were observed for stearic acid and ALA ($P \leq 0.04$). Stearic acid was highest in the flank fat trim and lowest in the brisket fat and lean trims, whereas ALA was highest in brisket fat trim and lowest in plate fat trim. Lean trim contained a greater proportion of arachidonic acid and EPA than fat trim ($P \leq 0.04$) (Table 8).

Table 8. Fatty acid composition of lean and fat trim from the brisket, flank, and plate

	Brisket		Flank		Plate			*P*-values		
	Fat	Lean	Fat	Lean	Fat	Lean	SEM[1]	Primal	Trim	Primal x trim
Fatty acids (g/100 g of total lipid)										
14:0	4.23	3.78	4.11	4.36	4.36	3.89	0.10	0.76	0.10	0.76
14:1n-5	1.10	1.02	0.95	2.98	1.03	0.81	0.36	0.46	0.45	0.39
16:0	25.1	25.3	27.5	27.7	27.6	26.1	0.50	0.001	0.42	0.26
16:1n-7	3.74	3.44	3.53	3.00	3.27	2.66	0.20	0.50	0.27	0.95
18:0	12.5[c]	12.4[c]	16.4[a]	14.2[ab]	13.1[b]	14.7[ab]	0.40	0.003	0.76	0.05
18:1t10 + t11	3.49	3.18	4.37	3.68	3.12	3.51	0.20	0.11	0.50	0.36
18:1n-9	39.1	40.0	34.3	36.7	38.7	36.0	1.70	0.02	0.82	0.17
18:1n-7	0.71	0.40	0.23	0.36	0.29	0.08	0.20	0.12	0.36	0.41
18:2n-6	2.64	3.45	2.96	3.43	2.78	4.25	0.20	0.51	0.01	0.47
18:3n-3	0.22[a]	0.16[ab]	0.19[ab]	0.12[bc]	0.05[c]	0.13[bc]	0.02	0.02	0.45	0.04
20:4n-6	0.26	0.39	0.08	0.28	0.32	0.53	0.10	0.08	0.04	0.89
20:5n-3	0.03	0.22	0.01	0.06	0.05	0.11	0.01	0.12	0.01	0.18
22:6n-3	0.42	0.27	0.01	0.03	0.09	0.04	0.08	0.04	0.51	0.17
MUFA:SFA[d]	1.07	1.08	0.81	0.94	0.97	0.89	0.03	0.004	0.65	0.19

[1]SEM, standard error of the mean. Data are means, $n = 4$ for each primal and trim.[abc]Means in the same group (Primal or Trim) with common superscripts are not different ($P > 0.05$).

[d]MUFA= total monounsaturated fatty acids (14:1n-5 + 16:1n-7 + 18:1n-9 + 18:1n-7 + 20:1n-9). SFA= total saturated fatty acids (14:0 + 16:0 + 18:0 + 20:0)

Fat percentage had no effect on the proportion of fatty acids in ground beef patties formulated from the brisket, flank and plate ($P \geq 0.13$; Table 9). Brisket ground beef contained higher proportions of palmitoleic acid and oleic acid ($P < 0.001$) and lesser proportions of palmitic acid and stearic acid ($P \leq 0.04$) than ground beef formulated from the flank or the plate. The MUFA:SFA ratio was highest for the brisket and lowest for the plate, but there was no difference in the n-6:n-3 ratio across primals or fat levels (Table 9).

Slip points

The mean slip point values for lipids from the brisket, flank, and plate were 33.7°C, 38.0°C and 34.9°C, respectively (Table 9). The slip point was highest for the flank and lowest for the brisket; the plate was intermediate and not different from either the brisket or the flank ($P = 0.001$). Fat percentage had no effect on slip point ($P = 0.41$).

Trained panel sensory panel attributes

Table 7 contains the definitions and reference standards for meat descriptive flavor aromatics and basic taste sensory attributes and their intensities. The brisket and plate had higher fat-like values than the flank ($P = 0.006$), and the brisket had higher bloody/serumy values than the flank ($P = 0.02$) (Table 10). Although the descriptor scores were very low, the brisket patties also had higher green hay-like flavors than the flank ($P \leq 0.02$), but the brisket and plate were not different for these flavor descriptors. Fat level increased fat flavor values ($P = 0.008$) and green hay-like flavor values ($P = 0.01$), which increased as fat level increased.

Table 9. Fatty acid composition of raw ground beef patties formulated at three fat levels from the brisket, flank, and plate

	Primal			Fat percent				*P*-values[1]	
	Brisket	Flank	Plate	10	20	30	SEM[2]	Primal	Fat %
Fatty acids (g/100 g of total lipid)									
14:0	3.43	3.63	3.61	3.48	3.55	3.64	0.08	0.54	0.70
14:1n-5	0.77	0.56	0.68	0.67	0.74	0.60	0.04	0.14	0.38
16:0	23.6^{b}	25.1^{a}	24.2^{ab}	24.2	24.3	24.5	0.23	0.04	0.80
16:1n-7	3.33^{a}	2.46^{c}	2.97^{b}	2.88	2.94	2.98	0.09	0.001	0.93
18:0	14.2^{c}	19.1^{a}	16.4^{b}	16.3	16.5	16.6	0.44	0.001	0.65
18:1t10 + t11	4.65	4.63	4.42	4.47	4.57	4.66	0.21	0.89	0.93
18:1n-9	39.4^{a}	34.6^{c}	37.1^{b}	37.2	37.2	36.8	0.49	0.001	0.66
18:1n-7	0.22	0.17	0.25	0.19	0.29	0.17	0.05	0.87	0.62
18:2n-6	3.47	3.45	2.99	3.58	3.21	3.09	0.16	0.44	0.48
18:3n-3	0.42	0.36	0.40	0.39	0.42	0.38	0.02	0.42	0.69
20:4n-6	0.16	0.16	0.15	0.16	0.15	0.16	0.01	0.75	0.88
20:5n-3	0.12	0.12	0.11	0.11	0.11	0.12	0.01	0.80	0.85
22:6n-3	0.13	0.06	0.10	0.11	0.07	0.11	0.02	0.26	0.50
MUFA:SFA[3]	1.03^{a}	0.77^{c}	0.99^{b}	0.91	0.91	0.89	0.02	0.001	0.64
n-6:n-3[4]	5.49	6.68	5.20	6.21	5.73	5.32	0.27	0.09	0.45
Cooking loss	30.3	34.4	33.1	30.0	32.9	34.9	1.01	0.24	0.14
Slip point	33.7^{b}	38.1^{a}	34.9^{ab}	34.9	36.1	35.4	0.47	0.001	0.41

[1]Primal x Fat % interactions were not significant ($P > 0.05$). [abc]Means in the same row with common superscripts are not different ($P > 0.05$).

[2]SEM, standard error of the mean. Data are means, $n = 12$.

[3]MUFA= total monounsaturated fatty acids (14:1n-5 + 16:1n-7 + 18:1n-9 + 18:1n-7 + 20:1n-9). SFA= total saturated fatty acids (14:0 + 16:0 + 18:0 + 20:0). [4]n-6:n-3 = (18:2n-6 + 20:4n-6)/(18:3n-3 + 20:5n-3 + 22:6n-3).

Table 10. Flavor descriptors and off-flavors in cooked ground beef patties formulated from the brisket, flank, and plate

	Primal			Fat Percent				*P*-values[1]	
	Brisket	Flank	Plate	10	20	30	RSME[2]	Primal	Fat %
Beef identity	7.14	7.44	7.44	3.71	3.59	3.55	0.57	0.47	0.43
Brown/roasted	0.72	0.82	0.78	0.82	0.56	0.62	0.83	0.37	0.70
Bloody/serumy	1.84^{a}	1.33^{b}	1.55^{ab}	1.65	1.58	1.49	0.35	0.02	0.72
Fat-like	3.53^{a}	2.97^{b}	3.64^{a}	3.01^{b}	3.37^{b}	3.77^{a}	0.38	0.006	0.008
Metallic	2.06	1.85	2.02	1.99	1.95	1.99	0.33	0.40	0.94
Green hay-like	0.07^{a}	0.01^{b}	-0.01^{bc}	-0.02^{c}	0.04^{b}	0.05^{ab}	0.04	0.004	0.01
Umami	0.02	0.11	0.08	0.08	0.08	0.04	0.19	0.60	0.90
Overall sweet	0.38	0.64	0.51	0.46	0.54	0.53	0.26	0.13	0.80
Sweet	0.40	0.67	0.39	0.40	0.47	0.60	0.25	0.07	0.35
Bitter	1.59	1.36	1.47	1.44	1.28	1.71	0.39	0.47	0.11
Sour	2.06	1.49	2.06	1.96	1.69	1.96	0.52	0.06	0.45
Salty	1.29	1.38	1.42	1.33	1.43	1.34	0.24	0.56	0.60
Sour Aromatic	0.09	0.04	0.22	0.12	0.15	0.08	0.17	0.16	0.75
Heated Oil	0.12	0.20	0.11	0.07	0.23	0.14	0.23	0.70	0.36
Warmed-over Flavor	0.11	0.04	-0.01	0.09	0.03	0.03	0.13	0.20	0.55
Refrigerator_Stale	0.15	0.25	0.03	0.19	0.09	0.16	0.21	0.20	0.60
Medicinal	0.10	0.21	0.02	0.04	0.19	0.11	0.15	0.09	0.12
Burnt	0.18	-0.01	0.37	0.06	0.07	0.41	0.66	0.57	0.53

[1]Primal x fat % interactions were not significant ($P > 0.05$). Data are means, $n = 12$. [abc]Least square means in the same row with common superscripts are not different ($P > 0.05$).
[2]RMSE = Root Mean Square Error.

Correlations among fatty acids, trained panel descriptive sensory panel flavor attributes, and slip points

Beef identity increased as linoleic acid and arachidonic acid decreased ($P < 0.05$) (Table 11), and patties with higher proportions of arachidonic acid had higher values for bloody, metallic and sour flavors ($P < 0.01$). As myristoleic acid increased, salty flavor decreased, and myristic acid and myristoleic acid were moderately and positively correlated to metallic flavors ($P < 0.05$). The proportion of palmitic acid was not correlated to any sensory panel flavor attributes, but palmitoleic acid was negatively related to umami, sweet, and salty flavors and positively related to sour flavors. As stearic acid increased, umami, overall sweet, sweet, and heated oil flavors increased and sour flavor decreased. Higher proportions of 18:1*trans*-10 plus 18:1*trans*-11 resulted in lower burnt flavors, and higher proportions of oleic acid resulted in lower heated oil and medicinal flavor attributes. Slip points were positively correlated with the proportions of palmitic acid and stearic acid and negatively correlated with oleic acid (Table 11).

Lipid-derived volatiles

Patties from the brisket had less 2-heptenal, decane, nonane, 2-octanone, dodecane, nonenal, heptanal, pentanal, octane, and octanal, but more butanoic (butyric) acid and 2-nonenal than those from the plate; flank patties were intermediate ($P < 0.05$) (Table 12). Patties with 20 or 30% fat generally had greater amounts of lipid-derived volatiles except for dodecane and nonenal (tallow, fatty). The 20% fat patties had higher amounts of nonane, octane, and octanal than the 10 or 30% fat patties ($P \leq 0.01$).

Table 11. Simple correlations coefficients between fatty acid composition, trained descriptive sensory panel flavor attributes, and slip points

	Fatty acids									
Flavor descriptors	14:0	14:1n-5	16:0	16:1n-7	18:0	18:1*trans*	18:1n-9	18:1n-7	18:2n-6	20:4n-6
Beef	0.04	-0.24	-0.03	-0.07	0.20	0.08	-0.01	0.09	-0.32*	-0.38*
Brown	0.17	-0.04	0.20	0.10	-0.01	-0.10	0.01	-0.09	-0.29	-0.05
Bloody	-0.07	0.17	-0.02	-0.08	-0.07	-0.10	-0.07	-0.03	0.31	0.38**
Fat	-0.08	0.05	-0.03	0.07	-0.15	-0.13	0.05	-0.03	0.01	0.15
Metallic	0.35*	0.36*	0.24	0.13	-0.15	-0.14	-0.08	-0.23	0.08	0.33**
Green Hay	-0.17	-0.01	-0.22	0.01	-0.09	0.25	0.04	0.27	0.01	0.10
Umami	-0.11	-0.25	-0.08	-0.33*	0.34*	-0.04	-0.27	-0.08	0.03	-0.27
Osweet	-0.04	-0.10	0.02	-0.20	0.31*	0.06	-0.14	-0.25	-0.11	-0.24
Sweet	-0.23	-0.23	-0.06	-0.41**	0.40**	-0.02	-0.26	-0.13	0.10	-0.05
Sour	0.27	0.28	0.02	0.32*	-0.35*	0.08	0.13	0.01	0.01	0.39**
Salty	-0.23	-0.35*	0.05	-0.33*	0.28	0.09	-0.31	-0.09	0.27	0.01
Bitter	-0.01	0.02	-0.01	0.06	-0.15	-0.10	0.09	-0.01	0.01	0.25
Sour A	0.13	-0.01	-0.05	0.15	-0.14	-0.03	0.08	-0.22	-0.09	-0.16
Heated Oil	0.05	-0.05	0.16	-0.30	0.31*	0.30	-0.36*	-0.06	-0.05	0.07
WOF	0.01	0.18	-0.16	0.24	-0.27	0.05	0.23	0.14	0.15	0.13
Medicinal	0.20	-0.09	0.31	-0.29	0.27	0.10	-0.45**	-0.03	0.06	0.05
Burnt	-0.09	0.02	0.13	0.03	-0.06	-0.41**	-0.07	0.02	0.03	0.19
Refrigerator Stale	0.09	0.12	0.09	0.02	0.14	0.20	-0.05	0.03	-0.05	0.04
Slip points	0.19	-0.08	0.36*	-0.31	0.53***	-0.27	-0.39*	0.10	-0.30	-0.18

*$P < 0.05$
**$P < 0.01$
***$P < 0.001$

Table 12. Lipid-derived volatiles of cooked ground beef patties formulated from the brisket, flank, and plate[3]

	Primal			Fat Percent				*P*-values[1]	
	Brisket	Flank	Plate	10	20	30	RSME[2]	Primal	Fat %
2-Heptenal (soapy, fatty, almond, fishy)	0[b]	7,543[ab]	20,910[a]	3,437	16,223	8,792	16,205	0.03	0.25
Decane	0[b]	537[b]	6,269[a]	1,293	2,208	3,304	5,106	0.02	0.68
Nonane (sour, burnt, cracker)	0[c]	2973[b]	6,999[a]	339[b]	8,407[a]	1,225[b]	2,342	0.001	0.001
2-Octanone (fruity, musty)	0[b]	897[a,b]	4,354[a]	3,811	897	541	3,763	0.04	0.14
Dodecane	0	0	3,076	2,316	0	759	3,146	0.06	0.28
Nonenal (tallow, fatty)	6,588[b]	24,027[ab]	49,214[a]	27,852	26,536	25,440	33,891	0.04	0.98
Butanoic acid (putrid, vomit)	144	954	124	366	378	477	279	0.08	0.95
2-Nonenal (cardboard, paper)	40,088[a]	1,459[b]	0[b]	516	4,379	36,652	35,668	0.04	0.07
Heptanal (nutty, fatty, green)	48,857[b]	348,699[a]	608,451[a]	24,777	521,378	236,853	290,064	0.001	0.08
2-Octene	2,912	30,043	14,937	7,575	13,038	27,279	24,579	0.08	0.21
Pentanal (almond, malt, pungent, acrid)	52,873[b]	151,822[a]	75,703[b]	58,414	135,802	86,182	68,880	0.01	0.07
Octane (meaty)	69,044[b]	153,473[a,b]	237,684[a]	102,345[b]	235,892[a]	102,345[b]	96,101	0.004	0.01
Octanal (orange,soapy, lemon,green)	244,414[b]	455,150[a]	531,138[a]	315,838[b]	561,526[a]	353,339[b]	157,458	0.002	0.006

[1]Primal x fat% interactions were not significant ($P > 0.05$). Data are means, $n = 12$. [abc]Least square means in the same row with common superscripts are not different ($P > 0.05$).
[2]RMSE = Root Mean Square Error [3]Units are equivalent to total ion counts.

Table 13. Maillard-derived volatiles of cooked ground beef patties formulated from the brisket, flank, and plate[3]

	Primal			Fat Percent				*P*-values[1]	
	Brisket	Flank	Plate	10	20	30	RSME[2]	Primal	Fat %
Trimethyl pyrazine (nutty, roasted, smokey, burnt)	0[b]	9,609[ab]	66,841[a]	0[b]	7,6450[a]	0[b]	62,821	0.05	0.02
Butyl-cyclopentane	0[b]	724[ab]	4,250[a]	0[b]	4,363[a]	612[b]	3,849	0.05	0.05
1-Butylpentyltrifluoromethane sulfonate	0[b]	717[a]	0[b]	0[b]	717[a]	0[b]	650	0.04	0.04
Hexamethyl-cyclotrisiloxane	987[b]	5,151[ab]	10,519[a]	4,204[ab]	11,725[a]	728[b]	7,843	0.04	0.02
Thiobis-methane	1,609[a]	0[b]	414[ab]	1,100	923	0	1,429	0.05	0.21
2-Furancarboxaldehyde (meaty, caramel)	8,838[a]	1,349[b]	9,558[a]	6,798	5,518	7,428	7,313	0.05	0.84
Benzene, methyl	11,080[b]	7,957[b]	34,489[a]	32,749[a]	17,290[ab]	3,488[b]	21,620	0.03	0.02
1-(1H-pyrrol-2-yl)-Ethanone	39,046	13,646	21,370	38,391	22,275	13,396	26,545	0.13	0.13
Benzeneacetaldehyde	65,336[b]	45,403[b]	147,840[a]	121,161[a]	114,853[a]	22,564[b]	87,543	0.04	0.03
Benzene ethanol	906.4	590	152	633	0	1,016	1,173	0.38	0.19
3-Hydroxy-2 butanone (buttery)	248,338	170,138	41,757	87,559	100,224	272,449	189,973	0.07	0.08
Trimethyl pyrazine (nutty, roasted, smokey, burnt)	0[b]	9,609[ab]	66,841[a]	0	76,450[a]	0[b]	62,821	0.05	0.02
Butyl-cyclopentane	0[b]	724[ab]	4,250[a]	0	4,363[a]	612[b]	3,849	0.05	0.05

[1]Primal x fat % interactions were not significant ($P > 0.05$). Data are means, $n = 12$. [abc]Least square means in the same row with common superscripts are not different ($P > 0.05$).
[2]RMSE = Root Mean Square Error. [3]Units are equivalent to total ion counts.

Maillard-derived volatiles

Trimethyl pyrazine (an important nutty, roasted, smokey, burnt aroma), butyl-cyclopentane, and 1-butylpentyltrifluoromethanesulfonate were absent in samples from the brisket, and thiobis-methane was not present in flank patties, while each was present in the other beef patty types ($P \leq 0.05$) (Table 13). Brisket patties had higher amounts of 2-furancarboxaldehyde (a meaty, caramel aroma) than flank patties ($P < 0.05$), whereas the plate patties generally contained more Maillard-derived volatiles than the other patty types.

Discussion

One finding of this study is that ground beef derived from each of the three primals had unique profiles of lipid-derived and Maillard-derived volatiles. Though water-soluble compounds are responsible for the "meaty" flavor, lipids produce the species-specific flavors (Koutsidis et al., 2008). When lipids undergo oxidation or Maillard reaction during cooking, the volatile compounds they produce contribute to create unique flavor profiles (Wood et al., 2004). In this study, MUFA were not associated with beef flavor attributes. This is in contrast to the findings of Melton et al. (1982), who demonstrated that palmitoleic acid and oleic acid were positively correlated and stearic acid negatively correlated with beef flavor attributes. Instead, in this study stearic acid was positively associated with umami, sweet, and heated oil attributes. Similar to Melton et al. (1982), linoleic acid and arachidonic acid were negatively correlated with cooked beef fat flavor. Overall, stearic acid was highly correlated with

beef flavors, contrary to our hypothesis that oleic acid would be positively correlated to cooked ground beef flavor attributes.

Aroma descriptors associated with each aromatic compound are reported based on previous research by others (see Calkins and Hodgen, 2007; Moon et al., 2006 for review). Aromas and flavors associated with beef are derived from a complex interaction of volatiles. Many of the individual compounds (e.g., butyric acid) by themselves may have an undesirable aroma but contribute significantly to the positive beef aroma. Many of the most important volatiles contain sulfur (rotten egg aroma) at high concentrations, but have meaty/brothy aromas at very low concentrations. Raw meat has very little flavor or aromas (Mottram, 1998). Aroma/flavor compounds are generated during the cooking process through the heating of the meat product and are generated through two general paths: thermal degradation of lipid and non-polar compounds; and Maillard reaction products. The latter are derived from the thermal reaction of amino acids with carbohydrates (mostly sugars, especially ribose, derived from the degradation of DNA/RNA) at higher temperatures and in the absence of water. In general, ground beef patties from the plate generated higher amounts of lipid-derived volatiles than those from the brisket. Brisket patties had greater 2-furancarboxaldehyde (a meaty, caramel aroma) and 2-nonenal (cardboard, paper), whereas patties from the plate generally had greater amounts of both lipid-derived and Maillard-derived volatiles.

Because of the direct relationship between lipids and the lipid-derived classification of volatiles, it was expected that the fat percentage in the ground beef would affect the amounts of lipid-derived volatiles. Mottram (1998) reported that the

amount and types of fat interfere with many of the chemical reactions that generate volatiles; while this is mostly recognized in Maillard products, it is plausible that higher concentrations of fat (> 20%) block the formation of some of these lipid-derived volatiles. This phenomenon also can be seen in the impact of fat percentage on the Maillard products reported here; patties that contained the highest fat level also had the least volatiles in all but one of the detected Maillard compounds. This agrees with Mottram (1998) in that the formation of many of the Maillard products is retarded as the percentage fat in the meat increases, as it directly interferes with chemical reactions within the Maillard reaction.

We previously demonstrated that fat trim from the brisket contained a higher percentage of MUFA than fat trim from the flank and plate (Smith et al., 2012; Turk and Smith, 2009), and these results were confirmed in this study. Oleic acid is the most abundant fatty acid in ground beef produced from fat and lean trim of commercial beef cattle (reviewed in Adams et al., 2010; Turk and Smith, 2009). This is due to the high SCD activity present in adipose tissue and muscle of beef cattle (Archibeque et al., 2005; St. John et al., 1991). Stearoyl-CoA desaturase is responsible for the synthesis of MUFA from SFA in animal tissues and its activity is highly sensitive to dietary manipulation and animal age. In beef cattle fed grain-based diets, the concentration of MUFA (and especially oleic acid) increases with time on feed (Chung et al., 2006; Gilmore et al., 2011; Huerta-Leidenz et al., 1996; Smith et al., 2012), as SCD activity and gene expression increase with time fed a high-grain diet (Brooks et al., 2011; Chung et al., 2007). Slip point values differ between adipose tissue depot depending on the size and

extent of differentiation of adipocytes (i.e., larger adipocytes have higher concentrations of MUFA) (Smith et al., 2006). As the percentage of stearic acid decreases in adipose tissue, slip point also decreases due to the high melting point of stearic acid (approximately 70 °C) (Wood et al., 2004). The current study is consistent with earlier studies (Chung et al., 2006; Smith et al., 1998; Wood et al., 2004) that reported that the proportion of stearic acid in lipids has the greatest effect on lipid melting points.

The MUFA:SFA ratio of brisket fat trim was unusually low in the current study (1.07). In previous studies, we demonstrated that the MUFA:SFA ratio in brisket was greater than 1.4 at 9 mo of age, and increased to over 1.6 by 14 mo of age (Smith et al., 2012; Turk & Smith, 2009). Similarly, the MUFA:SFA ratios of the flank and plate were less than that reported previously (Smith et al., 2012; Turk and Smith, 2009). The primals used for the current study were obtained at random from a commercial abattoir, and graded USDA Select. We have demonstrated that the MUFA:SFA ratio increases with USDA Quality Grade (Brooks et al., 2011; Chung et al., 2006), and the low MUFA:SFA ratios observed in the current study in part can be explained by the lesser quality grades of these beef carcasses. However, the unusually low MUFA:SFA ratios also suggest that these cattle spent a limited amount of time on a grain-based diet; pasture feeding of cattle strongly depresses SCD activity and the concentration of MUFA in beef cattle (Brooks et al., 2011; Chung et al., 2006, 2007; Duckett et al., 2009; Gilmore et al., 2011; Leheska et al., 2008).

The MUFA:SFA ratios we observed across types of ground beef (0.77–1.03) were of sufficient magnitude to elicit different responses in risk factors for

cardiovascular disease. In normocholesterolemic men, consumption of low-MUFA ground beef produced from pasture fed cattle (MUFA:SFA = 0.71) had no effect on HDL-cholesterol concentrations, but consumption of high-MUFA ground beef from grain-fed cattle (MUFA:SFA = 1.10) significantly increased HDL-cholesterol concentrations (Gilmore et al., 2011). In that study, the ground beef patties contained 24% total fat, so ground beef from grass-fed and grain-fed cattle contained 10.1 and 13.3 g oleic per 114 g patty. In the current study, 10, 20, and 30% fat ground beef from the brisket would have contained an estimated 4.1, 9.0, and 13.4 g oleic acid per 114 g patty, so the amount of oleic acid in 30% fat brisket ground beef was similar to that in ground beef from grain-fed cattle used in our previous study.

The ground beef formulated in this study contained small but perhaps significant amounts of ALA. We estimate that 30% ground beef (regardless of primal source) would have contained 130mg ALA per 114 g patty. This is higher than we reported previously for 24% fat ground beef from grass-fed cattle (90 mg per 114-g patty). We do not know the background of these cattle, but the relatively high proportion of ALA suggests that they spent a significant amount of time consuming grasses before entering the feedlot. The Daily Reference Intake (RDI) for ALA (average for men and women, all ages) is 1.4 g/d (reviewed by Gebauer et al., 2006), so a ground beef patty from this study containing 30% total fat would provide approximately 9% of the RDI for ALA. Thus, ground beef formulated from the brisket not only contributes to dietary oleic acid intake, but also provides at least a portion of the RDI for ALA.

Conclusions

The results from this study have demonstrated that ground beef produced from brisket, flank and plate had unique fatty acid and functional characteristics. Brisket had a higher fat-like flavor attribute than the flank, and also was lower in unpleasant headspace volatiles and highest in pleasant headspace volatiles. We conclude that these primals can be used to produce ground beef products with distinctive compositional and flavor attributes. Furthermore, the differences in fatty acid composition are sufficient to elicit different responses in risk factors for cardiovascular disease. The current study and our previous research with brisket adipose tissue (Smith et al., 2012; Turk and Smith, 2009) will provide the impetus for the industry to produce beef products that include brisket lean and/or brisket fat trim.

CHAPTER VI

SUMMARY

We predicted that "glucogenic" diets, which provide more glucose for absorption from the small intestine would promote marbling development, hence juiciness, to a greater extent than propiogenic diets. High concentrate diets doubled glucose incorporation into fatty acids in subcutaneous adipose tissue as measured *in vitro*, but had no effect on acetate incorporation into fatty acids in intramuscular adipose tissue. Intramuscular adipose tissue uses glucose for a greater proportion of the carbon source for *de novo* fatty acid biosynthesis than SC adipose tissue.

Contrary to our hypothesis, marbling scores were not different among treatment groups, although the acetate-infused steers had the highest fat thickness and yield grades. The greater adiposity of the acetate-infused steers indicates that the increased availability of acetate for fatty acid synthesis was sufficient to promote greater carcass fatness than was seen with the other treatments. It also is possible that the excessive fatness of the acetate-infused steers caused the depression in *de novo* fatty acid synthesis.

The rate of acetate incorporation into fatty acids in subcutaneous adipose tissue of the acetate-infused steers was less than 25% of the rate in the other treatment groups. Also, propionate infusion, and not glucose infusion, caused the greatest rates of glyceride-fatty acid synthesis in intramuscular adipose tissue.

Infusion of acetate elicited the highest taste panel juiciness and steaks from the acetate, abomasal glucose-infused, and ruminal glucose-infused steers were higher in the flavor attributes brown/roasted, fat-like, and overall sweet, and lower in bitter off-flavor, than steaks from the propionate-infused steers. This study demonstrated that propionate infusion decreased positive flavor attributes, even though propionate is a gluconeogenic precursor.

Though consumers want to enjoy a palatable meal, they are very health conscious. Ground beef from cattle fed high-grain diets has higher MUFA:SFA ratios than conventional ground beef. Practical increases in MUFA, especially oleic acid, have been shown to increase HDL cholesterol and other positive risk factors for cardiovascular disease. Though not practical to increase oleic acid in beef by feeding high-oleic oil, fatty acid composition varies considerably across carcass fat depots. The results from these studies have demonstrated that ground beef produced from brisket, flank and plate had unique fatty acid and functional characteristics and positive flavor characteristics.

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