# INVESTIGATION OF BEEF QUALITY BY ANALYZING FATTY ACIDS AND EFFECTS OF PRE- AND POST-HARVEST MANAGEMENT

By

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A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE IN ANIMAL SCIENCES

WASHINGTON STATE UNIVERSITY DEPARTMENT OF ANIMAL SCIENCES

DECEMBER 2008

To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of TING JIANG find it satisfactory and recommend that it be accepted.

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#### Abstract

by Ting Jiang, M.S. Washington State University December 2008

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Fatty acids in meat have obtained a lot of attention from both scientists and consumers because fatty acids are demonstrated to be closely related with human health and meat is an important food source, especially, for developed countries. Beef has been the most consumed red meat in the US and the argument exists on whether beef is a healthy meat for humans. Therefore, the fatty acids of beef were analyzed in this thesis. Different from many studies, we analyzed fatty acid composition from different fat locations within a beef steak and compared fatty acid composition between raw and cooked steaks. The main results showed that conjugated linoleic acid level was almost doubled in outer subcutaneous fat compared to lean muscle tissue, but polyunsaturated fatty acids with chain lengths longer than 18 were detected almost exclusively in lean muscle. Cooking did not have huge impacts on the fatty acid composition of beef steaks. Although it is important to produce healthier meat for consumers, eating quality (palatability) should never be overlooked or compromised. Previous studies have proved that a lot of factors can influence beef palatability, including diet and postmortem management such as aging. Since few studies were conducted to study the interaction effects between diet and postmortem aging on beef palatability, we assigned different aging methods (dry- or wet-aging) within several diet regimes including two forage diets, one concentrate diet (heifers or steers), and one forage and

concentrate combined diet. In addition, sensory evaluations were conducted on both beef steaks and ground beef. There were no diet by aging interactions, and no diet or aging effect on palatability attributes of beef steaks. However, diet by aging interactions existed for ground beef aroma, tenderness, and juiciness. Diet had relatively small (P>0.05) effects on ground beef sensory attributes compared to aging (P<0.05). The specific effects of aging on ground beef palatability attributes differed depending on feeding systems. The results of this thesis will be very helpful in interpreting the results of previous studies, improving the design of future studies, and providing useful suggestions for practical beef production.

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#### Literature Review

#### Introduction

Meat quality describes the attractiveness of meat to consumers. The factors determining meat quality are very extensive, including eating quality, composition, food safety, and animal welfare (Wood, Enser, Fisher, Nute, Richardson, & Sheard, 1998). The National Beef Quality Audit (NBQA) is conducted every five years (1991-2005) to assess how the industry is doing in delivering quality beef to consumers. In the latest NBQA conducted between July 2005 and June 2006, lack of uniformity/consistency in quality was ranked the No. 1 defect in the U.S. beef industry by end-users. More specifically, inconsistency exists in beef palatability, marbling, and variation among and within quality grades. Palatability is mainly related with tenderness, flavor, and juiciness (Umberger et al., 2000). According to Miller, Carr, Ramsey, Crokett, and Hoover (2001), tenderness was the most important factor affecting beef palatability and consumers could distinguish the differences in beef tenderness, willing to pay more for more tender beef. In U.S., marbling level is currently used as a visual indicator of palatability in the beef quality grading system (USDA, 1997). The U.S. beef industry places a high value on marbling in the longissimus muscle. Furthermore, consumers are concerned about food safety and animal welfare and desire "Natural" or "Organic" products. To recapture the losing beef market share to poultry, it is critical to put consumers first and satisfy their expectations of beef.

However, it is important to note that improvement of beef quality is an integral task because many factors are associated with each other. When we are trying to improve one quality attribute, we also need to consider the effect of this improvement on other quality attributes. For instance, fatty acid content and composition of beef is associated with both human health and palatability attributes. Different diet regimes may have different impacts on beef quality by the influence on fatty acid content or composition of beef fat depots (subcutaneous, intermuscular, and intramuscular fat).

Grass-fed beef is currently obtaining extensive attention due to increased grain prices, consumers' desire for a healthier diet, and improved environment and animal welfare. Grass-fed beef is usually leaner and contains a higher proportion of n-3 polyunsaturated fatty acids (PUFA), which are considered beneficial for human health (Ritzenthaler et al., 2001). But eating quality may be compromised when pursuing this healthier beef product. In the US where marbling level is 20-80 mg/g muscle tissue, values above 30 mg/g were reported to be necessary for optimum tenderness (Smith et al., 1984; Dikeman, 1987). So, grass-fed beef may be tougher if the marbling level is too low. In addition, n-3 PUFA may lead to rancid flavor of beef because PUFA have the potential to make meat more susceptible to oxidation due to their unsaturated nature (Lee, Decker, Faustman, & Mancini, 2005). Also, some n-3 long chain PUFA (LCPUFA) may lead to a fishy flavor of grass-fed beef. As consumers are becoming more aware of the relationship between their diet and health, it is necessary to put more attention on the development of beef products which are more beneficial to human health. However, eating quality of beef should never be overlooked.

Improvement of beef quality is an integral task not only because of the association between quality attributes but also because of the possibility of multiple management technologies, preand post-harvest. One of the most popular options is postmortem aging (Dransfield, 1994). It has been well accepted that aging can increase meat tenderness. However, it is still unclear how other factors like diet regimes, animal breed, or carcass quality can influence the impact of aging on improving beef quality. The main purpose of this review was to discuss the function of fatty acids (FA) in human health and beef palatability and the effects of diet regimes and postmortem aging on beef quality.

#### Fatty acids in beef and human health

#### Fatty acid composition of beef and its relationship with human health

Enser, Hallett, Hewett, Fursey, and Wood (1996) investigated the fat content and composition of steaks or chops from equivalent parts of the loin in beef, lamb and pork (Table 1). Beef and lamb have a lower polyunsaturated: saturated fatty acids (PUFA: SFA) ratio but a more favorable n-6: n-3 fatty acids ratio than pork. Dietary fatty acid content and composition are closely related to human health.

Saturated fatty acids (C12:0, C14:0, C16:0) increased both low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol concentration as well as the LDL: HDL ratio (Wiseman, 1997). However, stearic acid (C18:0), a major SFA in beef, was considered to be neutral in its effect on plasma cholesterol in humans (Nuernberg et al., 2002). *Trans*-fatty acids (TVA) increased plasma LDL cholesterol and decreased HDL cholesterol concentration (Pond, Church, Pond, & Schoknecht, 2005; Wiseman, 1997). Unsaturated fatty acids (UFA) can be classified into two categories — monounsaturated fatty acids (MUFA) and PUFA. Polyunsaturated fatty acids perform many important functions in biological membranes and are precursors of various lipid regulators of cellular metabolism. The two families of PUFA, n-3 and n-6 FA, compete for the same enzymes to elongate and desaturate (Nuernberg et al., 2002). Monounsaturated fatty acids had a neutral effect on plasma cholesterol level while PUFA showed more complicated effects. N-6 fatty acids (mainly linoleic acid, C18:2) tended to decrease LDL blood cholesterol levels; n-3 fatty acids did not show consistent effects on plasma

cholesterol (Wiseman, 1997). However, certain n-3 LCPUFA consisting of linolenic acid (C18:3), eicosapentaenic acid (EPA; C20:5), and docosahexaenoic acid (DHA; C22:6) have the potential to prevent cardiovascular diseases (Bourre, 2005). Therefore, the balance between different categories of FA, particularly the SFA: PUFA and n-6: n-3 ratio, plays a vital role in human health. The recommended values for PUFA: SFA and n-6: n-3 are 0.45 and below 4.0, respectively (Wood & Enster, 1997).

Besides the n-3 LCPUFA, interest in CLA has increased remarkably over the last decade. Conjugated linoleic acids are a collection of positional and geometric isomers of linoleic acid, with conjugated double bonds. Twenty-four isomers have been identified in ruminant products (Cruz-Hernandez, Deng, Zhou, Hill, Yurawecz, & Dlmonte, 2004). In beef, 10 isomers of CLA were reported with CLA cis-9, trans-11 accounting for approximately 70% of total isomers (Dannenberger et al., 2004). Naturally occurring CLA originate principally from bacterial isomerisation or/and biohydrogenation of PUFA in the rumen and desaturation of TVA (t11-C18:1) in the adipose and mammary tissue (Fig. 1). Only a small proportion of CLA formed in the rumen is available for deposition in the muscle (Griinari & Bauman, 1999).

Ha, Grimm, and Pariza (1987) observed that CLA mixtures isolated from grilled beef or from isomerization of linoleic acid were responsible for the inhibition of mouse epidermal tumors. Since then CLA have received extensive attention. Conjugated linoleic acids may possess numerous positive properties for human health, such as anticarcinogenesis, antiadipogenesis, and antiatherogenesis. In addition, CLA may enhance the immune system and improve bone health (Belury, 2002; Schmid, Collomb, Sieber, & Bee, 2006). It should be noted that different isoforms of CLA could have different biological actions. For instance, CLA cis-9, trans-11 could be the active form to prevent tumors, alone or in combination with other isomers (Ha, Grimm, & Pariza,

1987; Ip, Singh, Thompson, & Scimeca, 1994; Pariza, Park, & Cook, 1999). CLA trans-10, cis-12 could be the active form affecting energy metabolism and reducing fat tissue deposition (Park, Storkson, Albright, Liu, & Pariza, 1999; Ryder, Portocarrero, & Song, 2001). In spite of so many encouraging potential attributes of CLA, some potential adverse effects of CLA have also been reported. Conjugated linoleic acid supplementation induced insulin resistance in several animal studies (DeLany, Blohm, Truett, Scimeca, & West, 1999; Tsuboyama-Kasaoka et al., 2000; Kelly, 2001; Roche et al., 2002). Fatty liver and spleen occurred concomitant with reduced body fat and weight gain in different animal models with CLA supplement (West et al., 1998, 2000; DeLany et al., 1999; Tsuboyama-Kasaoka, Takahashi, & Tanemura, 2000; Takahashi, Kushiro, Shinohara, & Ide, 2003). Although most studies regarding the physiological effects of CLA are based on animal models, the potential positive properties of CLA for humans are still intriguing.

Although beef contains a higher SFA level than pork, beef has a lower n-6: n-3 ratio and is an important dietary source of CLA for human. So, beef has the potential to become a functional food product in the future.

#### Functional food

A functional food is a food used to enhance certain physiological functions to prevent or even to cure diseases (Roberfroid, 2000). Functional foods usually involve certain methodologies including the addition or removal of a component, modification of food processing, genetic engineering and others, etc. To date, the predominant components added to foods include probiotics, prebiotics, synbiotics, and nutrients (López-Varela, González-Gross, & Marcos, 2002). 'Functional food' is a relatively modern term, which symbolizes that food can do more than just satisfy human energy and nutrient needs or gastronomic pleasure. The term 'functional food' was coined in Japan where most functional foods are on the market and the first legislation (Foods of Specified Health Use, FOSHU) was made to regulate what can be called functional food (Table 2; López-Varela et al., 2002). European and American countries, however, reached no consensus on the definition of a functional food. The USA prefers the term nutraceutical, while Europe has adopted the term 'functional food' in the FUFOSE (Functional Food Science in Europe) project.

Meat and meat products are an important part of dietary consumption especially in developed countries. N-3 fatty acids, predominantly EPA and DHA, are especially intriguing for human health. Previously, Simopoulos (1991) documented the function of n-3 FA on human health, growth, and development.

Human beings evolved while consuming a diet lower in SFA than today's diet (Eaton and Konner, 1985). Also, humans evolved on a diet with a n-6 : n-3 ratio being about 1:1; whereas this ratio was reported to be 10:1 to 25:1 twenty years ago in U.S. diet (Report of the National Cholesterol Education Program, 1988), which indicates that western diets are currently deficient in n-3 FA compared to the diet on which humans evolved (Simopoulos, 1991). Data from the TRANSFAIR study also indicated that the current intake of n-3 PUFA (especially  $\alpha$ -linolenic acid, ALA) might be inadequate for a substantial proportion of the population (Hulshof, van Erp-Baart, Anttolainen, Becker, Church, & Couet, 1999). Food with balanced fatty acid profiles may provide consumers with a healthier lifestyle as well as prevent or even help treat some diseases. For instance, the Nurses' Health Study indicated that replacement of 5% of the energy from SFA by PUFA could lower the risk of heart disease by 46% (Hu et al., 1997). Diets rich in ALA have been demonstrated to reduce the risk of Coronary Heart Disease (CHD) in several studies

(Dolecek, 1992; Ascherio, Rimm, Giovannucci, Spiegelman, Stampfer, & Willett, 1996; Hu et al., 1997; Pietinen et al., 1997).

In order to achieve healthier meat or meat derivatives labeled as functional food, undesired substances should be avoided or reduced to appropriate limits while the level of other substances with salubrious and functional properties should be increased. Three essential kinds of strategies are used to reach this aim: 1). management of animal production; 2). handling of meat raw materials; and 3). reformulation of meat derivatives (Jiménez-Colmenero, Carballo, & Cofrades, 2001). Feeding strategies have been successfully used to produce beef with DHA up to 20 times, vitamin E 7 times, and n-3 FA 6 times the normal level of traditional counterparts (Sloan, 2000). During the different stages of raw material preparation, external and internal fat can be removed by trimming and extracting to reduce the final fat content of retail cuts (Jiménez-Colmenero et al., 2001). The procedure of replacing part of the animal fat normally present in a food product with another more suited to human needs also can be used to alter fatty acid composition. Fish oils and vegetable oils have been used for this purpose in products such as patties and sausages (Marquez, Ahmed, West, & Johnson, 1989; Park, Rhee, Keeton, & Rhee, 1989; Liu, Huffman, & Egbert, 1991; Paneras, Bloukas, & Filis, 1998).

The content and composition of FA in meat are associated not only with human health but also with meat palatability. Feeding systems have been reported to influence the content and composition of FA in meat.

#### Fatty acids and beef palatability

#### Digestion and metabolism of dietary fat in cattle

The digestion and absorption of dietary lipids begins in the rumen (Van Soest, 1982). Lipids are transformed in the rumen before they arrive at the intestine. Lipolysis takes place through microbial lipases, galactosidases, and phospholipases and leads to free FA (FFA), on which later hydrogenation occurs (Doreau & Chilliard, 1997). However, the extent to which FA are saturated differs under different conditions. For example, the end-product of hydrogenation of C18 FA is stearic acid (C18:0), but when a large amount of linoleic acid (C18:2n-6) exists, hydrogenation may stop before complete saturation (Harfoot, Noble, & Moore, 1973). In addition to hydrolyze and biohydrogenate dietary FA, rumen microbes also synthesize various odd- and branched-chain FA, many with a *trans* configuration (Church, 1988). In general, microbial synthesis of FA is moderate.

After the modification of dietary lipids by ruminal microbes, most of the fat leaves the rumen and reaches the duodenum as highly saturated unesterified FA (Church, 1988); only a small amount of triglycerides escape the rumen fermentation (Van Soest, 1982). The amount of lipid arriving at the duodenum usually exceeds the amount of lipid fed due to the contribution of rumen microbial synthesis. Ruminants can absorb most FA with true digestibility near 100%, though the absorption rates of FA are different, depending on the extent of saturation and chain length (Van Soest, 1982).

The main site of lipogenesis in ruminants is the adipose tissue. About 90% of fat synthesis occurs there (Van Soest, 1982). Acetyl-CoA is the precursor for fatty acid synthesis, which is formed from acetate instead of glucose. Due to the saturating effect of the rumen on UFA, ruminant fat is characteristically hard and much more difficult to be changed by dietary lipid

than fat of non-ruminant animals. However, it is possible to alter the fatty acid composition of ruminant meat through different diet regimes because some dietary UFA can bypass the rumen for absorption and subsequent deposition in the adipose.

#### The influence of diet on beef fatty acid composition

Grass-fed beef had higher concentrations of n-3 PUFA than concentrate-fed beef (Scollan, Choi, Kurt, Fisher, Enser, & Wood, 2001). The concentration (g/100 g fatty acid methylesters) of CLA cis-9, trans-11 in intramuscular fat increased linearly with the increased grass intake (French et al., 2000). So, grass-fed beef has the potential to be a healthier alternative for consumers.

Data from ten experiments pertaining to the effect of feeding systems on fatty acid composition of beef are shown in Table 3. The proportion of C14:0 or C16:0 is either similar between feeding systems or higher in concentrate-based beef compared to pasture/grass-based beef. The proportion of linolenic acid (C18:3n-3) tends to be higher in grass/pasture-based beef compared to concentrate-based beef, significantly or not. The n-6: n-3 ratio is consistently lower in grass/pasture-based beef compared to concentrate-based beef.

Studies have analyzed the FA of beef from different feeding systems, but only a few of them related FA with palatability. Grass-fed beef has been criticized for lower palatability (Hedrick, 1983). Larick et al. (1987) suggested the greatest sensory difference between grain- and forage-fed beef was the flavor of fat.

#### Fatty acids and beef palatability

The possible relationships between specific FA and beef aroma/flavor are listed in Table 4 (Melton, Amiri, Davis, & Backus, 1982; Mandell, Buchanan-Smith, & Campbell, 1998). Myristic (C14:0) and palmitic (C16:0) acid were positively related with fat and grassy aroma. Myristic and stearic (C18:0) acid were positively related with sour flavor; palmitic and stearic acid were positively related with sweet flavor. Stearic acid was also negatively related with fat aroma and liver flavor. Oleic acid (C18:1) was positively related with greasy, fat aroma and beef, sweet flavor; while negatively related with metallic aroma and sour flavor. Linolenic acid (C18:3) was positively related with metallic, grassy, milky-oily aroma and sour flavor; while negatively related with fat aroma and beef flavor. N-3 LCPUFA was associated with the fishy flavor of grass-fed beef (Vatansever et al., 2000). "Green" odor from meat of grass-fed animals was connected with hexanals derived from cis-9 C18:1 and linolenic acid (C18:3n-3); while "soapy" odors were connected with octanals derived from linoleic acid (C18:2n-6; Lorenz, Buettner, Ender, Nuernberg, Papstein, & Schieberle, 2002). As the length of grain feeding increased, the concentration of C18:3 in muscle phospholipids declined and the concentration of C18:2 increased. "Sweet" and "gamey" flavor declined, whereas "sour", "bloody", and "cooked-beeffat" flavor increased (Larick & Turner, 1990). The importance of n-6: n-3 value for flavor development in ruminant meat was also supported by studies in which protected lipid supplements were used. For instance, "meat" aroma and flavor decreased when the concentration of C18:2 in carcass fat increased from 2 to 20% by feeding protected sunflower seeds. High concentration of C18:2 resulted in a "sweet" and "oily" flavor (Park, Ford, & Ratcliffe, 1975). Muscle longissimus dorsi (LD) with high percentages of oleate (C18:1) generally scored higher in taste panel evaluations (Dryden & Marchello, 1970). Besides aroma and flavor, fatty acids can

also impact meat juiciness. The contents of myristate (C14:0) and palmitate (C16:0) were negatively correlated with juiciness rating for the same muscle; while UFA: SFA ratio had a positive correlation with juiciness (Waldman, Suess, & Brungardt, 1968). In addition to the composition of FA, it is also possible that grass-fed animals were unable to deposit sufficient lipid to ensure the favorable palatability attributes. Nuernberg et al. (2005) reported that the daily gain and intramuscular fat level of grass-based bulls were significantly lower than those of the concentrate-based bulls.

Lipid oxidation in meat is an important factor responsible for the formation of rancid odors and deterioration of flavor (Asghar, Gray, Buckley, Pearson, & Booren, 1988). Larick and Turner (1990) attributed flavor differences between forage- and concentrate-fed beef to both the content of PUFA and its lower oxidative stability. Beef from pasture-based diets tends to have higher proportions of n-3 PUFA than concentrate-fed beef. This increased the susceptibility to lipid oxidation in displayed products (Vatansever et al., 2000). Pasture-fed beef (both control and vitamin E supplemented) was more susceptible to lipid oxidation following aging than vitamin E supplemented grain-fed beef (Yang, Lanari, Brewster, & Tume, 2002). However, Realini, Duckett, Brito, Dalla Rizza, and De Mattos (2004) reported that steaks from pasture-fed steers had lower TBARS (thiobarbituric acid reactive substances) values than steaks from concentratefed steers, initially and during 21 days of display. This result might be explained by the greater concentrations of  $\alpha$ -tocopherol in the pasture-fed beef. Nuernberg et al. (2005) also found that grass-based beef was more oxidatively stable than concentrate-fed beef, especially at 10 days of retail display. So, the influence of diet (grass vs. concentrate) on lipid oxidation in beef is still undefined.

In general, n-3 PUFA tend to have a negative effect on beef palatability; oleic acid seems to

improve beef palatability. The saturation degree of FA may influence the juiciness of beef.

#### Marbling

#### Introduction of marbling

"Marbling" (intramuscular fat) is defined as the appearance of white flecks or streaks of adipose tissue between the bundles of muscle fibers in bovine skeletal muscle (Harper, Pethick, Oddy, Tume, Barendse, & Hygate, 2001). Under the microscope, marbling fat is comprised of adipocytes embedded in a connective tissue matrix close to blood capillary network (Harper & Pethick, 2004). Marbling is important in many markets like Japan, Korea, and the US. Marbling is one of the main factors used to determine beef quality grade in Japan (JMGA, 1988). The quality grading system in Korea is primarily determined by marbling score and additionally adjusted by other carcass traits (Moon et al., 2006). Park, Moon, Ko, Ha, Chang, and Joo (2002) suggested that the quality grade of Hanwoo carcass had the strongest relationship with the marbling score. In the United States, marbling level is also currently used as a visual indicator of palatability in the beef quality grading system (USDA, 1997).

The relationship between marbling score and beef palatability or consumer acceptance is undefined. How marbling might develop and what factors might influence marbling expression are also unclear. These questions will be discussed.

#### Marbling and beef palatability

The influence of marbling on the palatability of beef has been inconsistent. Some studies showed that higher marbling levels increased consumer acceptance of fresh beef steaks (Savell et al., 1987; Neely et al., 1998). But other reports indicated marbling level had no influence on the

palatability attributes of beef (Parrish, Jr., Olson, Miner, & Rust, 1973; Garcia de Siles, 1975). In a national consumer retail beef study (Savell et al., 1987), consumers in San Francisco and Kansas City gave consistently high palatability evaluation ratings, which were only slightly reduced as marbling level decreased from Slightly Abundant to Traces. In contrast, the ratings given by consumers in Philadelphia decreased sharply as marbling level decreased. Some studies reported that marbling had a positive relationship with beef tenderness (Berry, 1993; Wheeler, Cundiff, & Koch, 1996). In contrast, other researchers did not observe significant relationships between marbling level and beef tenderness (Reddy, Tuma, Grant, & Covington, 1970; Armbruster, Nour, Thonney, & Stouffer, 1983). The ability of marbling to maintain the tenderness of meat cooked to high end point temperatures is supported by some data (Luchak, Miller, Hale & Cross, 1990), but not by other data (Parrish et al., 1973). Wagyu is famous for its tenderness. A significant negative correlation (r=-0.83) was observed between shear force (SF) value and intramuscular fat% (IMF%; Ueda et al., 2007). Thompson (2004) demonstrated that there were positive curvilinear relationships between IMF% and consumer sensory scores for tenderness, juiciness, like flavor, and overall liking. These relationships plateaued at 15 to 17% IMF. Wheeler, Cundiff, and Koch (1994) reported a small, positive relationship of tenderness and juiciness with marbling score. Their data also supported the concept called "the window of acceptability" (Savell & Cross, 1989) which suggested 3 to 7% marbling fat would contribute to palatability but higher levels would contribute little more. However, there was a large amount of variation in SF values and sensory tenderness ratings within each marbling score (from Traces to Moderate; Wheeler et al., 1994). As a result, many tough (high SF; low tenderness rating) meats were in the "tender" group while many tender (low SF; high tenderness rating) meats were in the "tough" group. This may explain the inconsistent beef tenderness currently produced, even

though USDA quality grade is known. When tenderness differences were minimized, consumers found high-marbled steaks (high = upper 2/3 USDA Choice) juicier and more desirable in flavor and overall acceptability than low-marbled steaks (low = USDA Select) (Killinger, Calkins, Umberger, Feuz, & Eskridge, 2004a). Similarly, Thompson (2004) showed that when flavor and juiciness scores were adjusted for an independent measure of tenderness, gains in flavor and juiciness scores would plateau at the higher marbling fat percentage (15-20%) for beef served as grilled steaks to Australian consumers. However, consumers rated high- and low-marbled steaks similar (P>0.10) in flavor, juiciness, tenderness, and overall acceptability when evaluation was in their homes and the steaks were not necessarily matched for similar tenderness (Killinger, Calkins, Umberger, Feuz, & Eskridge, 2004b). Moon, Yang, Park, and Joo (2006) identified that physiological maturity and marbling level affected meat qualities of Hanwoo cows to the same extent. The high marbling group was rated the highest (P<0.05) in tenderness, flavor, and overall palatability, compared to low or intermediate marbling groups. But sensory panelists did not find the differences in juiciness among marbling groups.

The exact mechanism how marbling is associated with palatability is difficult to define (Millar, 1994). Millar proposed that increased IMF could influence the toughness of myofiber and connective tissue (CT) by decreasing bulk density. When meat was cooked, fat which is low density diluted the higher density, heat denatured proteins. So, a higher IMF% resulted in a lower density steak, requiring less resistance to bite through. The effect of marbling on CT toughness was discussed by Nishimura, Hattori, and Takahashi (1999). The development of adipose tissue in *longissimus* muscle appeared to disorganize the structure of the intramuscular connective tissue (IMCT) and contributed to the tenderization of highly marbled beef from Japan Black cattle during the late fattening period (Nishimura et al., 1999). The IMF was deposited mainly

between bundles of muscle fibers, within the perimysium. Typical structure of perimysium shown in *longissimus* muscle of 20 months old steers was not observed in 32 months old steers. The disorganization of perimysium caused by the development of fat cells could further lead to the partial breakdown of endomysium, which was bundled by perimysium. These structural changes are consistent with a decrease in mechanical strength of the IMCT. However, the mechanical strength of IMCT did not decrease markedly in *longissimus* muscle of steers before 24 months old or in *semitendinosus* muscle of steers during the whole 32 months, with less than 8% IMF. So, this tenderization effect might be applicable only to some breeds able to deposit large amounts of intramuscular fat (IMF). The IMF content might also affect beef flavor as the species-specific flavors are contained in fat (Hornstein & Wasserman, 1987). In contrast, Yeates, Edey, and Hill (1975) suggested the flavor elements in muscle were largely water soluble, against IMF as a contributor to flavor. In addition, a higher marbling level could stimulate salivation and give the perception of increased juiciness of meat during chewing. The lubrication effect of marbling would also help sustain the feel of juiciness in the mouth (Millar, 1994).

In summary, we cannot establish a confirmed positive relationship between marbling and beef palatability due to the controversial results from previous studies. However, the inconsistence may be due to the various marbling levels in different studies or due to the various circumstances under which the studies were conducted (e.g., tenderness controlled vs. tenderness uncontrolled).

#### Factors influencing the development and expression of marbling

Muscle satellite cells are one class of stem cells in skeletal muscle (Grounds, 1999). Muscle satellite cells could differentiate into adipocytes or osteocytes when isolated and grown in appropriate culture conditions (Asakura, Komaki, & Rudnicki, 2001; Wada, Inagawa-Ogashiwa,

Shimizu, Yasumoto, & Hashimoto, 2002). Preadipocyte is an intermediate form, through which a pluripotent stem cell develops into a more mature adipocyte when animals reach the appropriate age and are fed a suitable diet (Harper & Pethick, 2004). Many animal or environmental factors may influence the expression of marbling. Marbling is a late developing trait (Pethick, Harper, & Oddy, 2004), though the prerequisite adiposity of muscle occurs significantly earlier (Wegner, Albrecht, & Ender, 1998). The developmental order for fat depot is abdominal, intermuscular, subcutaneous, and finally intramuscular. However, this does not mean that the rate of fat accretion in intramuscular adipocytes is also late maturing. In fact, when fat deposition was described as proportions of total carcass fat developing within various depots, the proportional distribution of fat between carcass pools was found constant over a wide range of carcass fat contents (5-150 kg) (Johnson, Butterfield, & Pryor, 1972). Three studies (Duckett, Wagner, Yates, Dolezal, & May, 1993; Aoki, Nakanishi, Yamada, Harashima, & Yamazaki, 2001; Pugh, Pethick, Tudor, & McIntyre, 2002), representing different countries, production systems and genotypes, demonstrated that the rate of IMF% accretion is relatively similar (Pethick et al., 2004). This indicated that the initial or pre-feeding IMF level was important for the final IMF level. In agreement, Oddy, Smith, Dobos, Harper, & Allingham (2000) reported that IMF% at the end of a 147-day feeding period was related to the IMF% at feedlot entry or at 30 days after commencing feeding. So the important conclusion is that the backgrounding phase is critical for the deposition of IMF even though marbling is a late maturing trait (Pethick et al., 2004).

In the review of Muir, Deaker, and Brown (1998), when treatment groups were slaughtered at similar weights, grain-feeding (vs. forage-based feeding) was associated with increased marbling in 6 out of 14 experiments. In four of these six experiments, improved marbling was accompanied by greater subcutaneous fat depth and live weight gain prior to slaughter. Therefore,

higher energy concentration in grain diets might have resulted in the higher marbling levels. When cattle were fed a restricted grain diet to grow at the same rate as those fed grass, both treatment groups reached similar levels of IMF (Davis, Jr., Backus, & Melton, 1981). Nour, Thonney, Stouffer, and White Jr. (1983), who fed cattle either silage or grain, also found that carcass weight rather than diet or breed significantly affected marbling. Pethick et al. (2004) pointed out that feedlot feeding systems provided animals with more net energy available for fat synthesis compared to pasture-finishing system. Besides, less exercise in the feedlot could be important in reducing basal energy expenditure. Furthermore, higher CLA contents from grassfed cattle might be responsible for the inhibition of IMF accretion. Pethick et al. (2004) discussed the nutritional impact on marbling development. Adipocytes already present continued to grow in diameter by depositing fat (Hood & Allen, 1973) and were joined by new adipocytes (Leibel, Edens, & Fried, 1989). So, a nutritional strategy to increase both adipocyte number and size seems a right approach to increase marbling score. The substrates for lipogenesis de novo in ruminants are acetate and glucose/lactate (Pethick et al., 2004). However, marbling adipocytes showed a preference for glucose/lactate carbon while subcutaneous (s.c.) adipocytes tended to use acetate as a main source for lipogenesis (Smith & Crouse, 1984). So diets high in roughage, producing an excess of acetate compared to glucose, could lead to a reduced rate of lipogenesis for a given intake of metabolisable energy (Preston & Leng, 1987).

Fat tended to redistribute from adipose tissue into non-adipose tissues, specifically skeletal muscle, when mammals became old (Kirkland, 2002). However, this does not mean that all well marbled cattle are old. Wagyu and Angus cattle usually expressed marbling by less than 26 and 28 months old, respectively (Hocquette, Jurie, Ueda, Boulesteix, Bauchart, & Pethick, 2003). Harper and Pethick (2004) summarized gender effects on marbling levels (Table 5). Generally,

for a given slaughter weight and time on feed, heifers have higher marbling levels than steers; steers have higher marbling levels than bulls.

Breed may also be associated with marbling characteristics in cattle. McKeith, Savell, Smith, Dutson, and Carpenter (1985) found marbling score and quality grade of carcasses from largeframed steers (i.e., Charolais, Simmental, Brahman, and Limousin) were lower than those from medium- and small-framed steers (i.e., Angus, Hereford, Red Poll, and Jersey). Intermediate framed-early maturing and small framed-early maturing steers had higher (P<0.05) marbling score and quality grade than either large framed-late maturing or intermediate framedintermediate maturing steers (Camfield et al., 1999). Albrecht, Teuscher, Ender, and Wegner (2006) noted that marbling development differed among breeds (German Angus-beef cattle, Galloway- Smaller, environmentally resistant beef cattle, Holstein Friesian-dairy cattle, Belgian Blue-double muscled cattle). The intensive incorporation of IMF began at 6 mo of age in Holstein Friesian bulls, at 12 mo of age in German Angus and Galloway bulls, but the proportion and number of marbling flecks increased only at 24 mo of age in Belgian Blue bulls.

In conclusion, successful marbling manipulation is a substantial scientific challenge, which covers an array of disciplines from genetics, nutrition, biochemistry, to meat science. An integrated approach is needed (Bindon, 2004).

#### Influence of aging on beef quality

#### Introduction of Aging

Aging is holding meat for a period of time to enhance palatability (Anon., 1991). Wet and dry aging are the two fundamental techniques. Wet aging is the process of vacuum packaging meat into a highly moisture-impermeable bag and storing it under refrigeration for a specified length

of time. Traditional dry aging is the process of exposing unpackaged meat directly to cooler condition where temperature, humidity, and air-flow are strictly controlled (Ahnström, Seyfert, Hunt, & Johnson, 2006). Dry aging can be utilized for entire carcasses or individual subprimal cuts; while wet aging can be utilized for most primals before cutting into steaks or roasts, so wet aging can occur during shipping and storage (Sitz, Calkins, Feuz, Umberger, & Eskridge, 2006). Wet aging is more prevalently used because dry aging results in a high percentage of shrink, demands strict control of storage variables and requires more space. In contrast, wet aging space (Parrish, Jr., Boles, Rust, & Olson, 1991). However, dry aging remains popular for upscale restaurants due to the perceptions by consumers of premium quality, especially the dry-aged flavor (Ahnström et al., 2006).

Fresh meat is aged to enhance the palatability of the product. It has been well accepted that postmortem aging can increase meat tenderness, however, controversy exists about palatability aspects other than tenderness. For instance, Hodges, Cahill, and Ockerman (1974) reported beef flavor intensity increased in USDA Choice short loins but decreased in USDA Standard short loins after 15d of dry aging. Aging increased sensory tenderness and decreased rancid off-flavor of *Gluteus Medius, Infraspinatus, Psoas Major, Recuts Femoris, and Teres Major* during the 14 days of aging (Stetzer, Tucker, McKeith, & Brewer, 2007). Wet-aged strip loins were more flavorful and juicier than unaged loins (Binder, Montgomery, Bagley, & McMillin, 1985). In contrast, other studies showed that aging increased bitter and sour off-flavors and decreased desirable beefy, brothy, browned caramel, and sweet flavors (Spanier, Flores, McMillin, & Bidner, 1997; Gorraiz, Beriain, Chasco, & Insausti, 2002). In fact, many factors can influence the effects of postmortem aging on beef quality, such as aging method (wet-, dry-, or modified

atmosphere- aging), aging period, and meat quality grade. The complex influence of aging on beef quality needs to be discussed.

#### Changes of muscle during postmortem aging

Tenderness of meat is determined by the properties of myofibrils and the intramuscular connective tissue (IMCT) and is improved with postmortem aging time (Nishimura, Liu, Hattori, & Takahashi, 1998). The tenderization of meat during aging occurs in two steps: a rapid first phase and a slow phase thereafter (Takahashi, 1996). The rapid phase of tenderization is mainly due to the structural weakening of myofibrils, and the later slow phase is chiefly attributed to the structural weakening of IMCT (Liu, Nishimura, & Takahashi, 1995). The shear force of raw semitendinosus muscle decreased (P<0.05) rapidly up to 10d, then decreased gradually and reached 56% of initial value after 35d of postmortem aging. About 29 and 41% of total tenderization occurred after 10 and 14d aging, respectively. While the mechanical strength of the IMCT remained unchanged up to 10d of postmortem aging and then decreased linearly till 35d (Nishimura et al., 1998). However, the structural weakening of myofibrils occurred significantly during the first 10d of aging (Takahashi, 1996). The K20 values (20% total compression) reflects the mechanical resistance of the myofibrillar structure and the K80 values (80% total compression) is an index of CT strength. The K20 values decreased between 54 and 66% after 35d aging and the highest rate of tenderization occurred in the first week of aging (60.7-77.6% of total tenderization); whereas the K80 values did not change during aging (Monsón et al., 2004).

Aging results from proteolytic degradation of muscle proteins both by endogenous enzymes and by structural damage to Z-lines and/or M-lines (Novakofski & Brewer, 2006). It is likely that multiple proteases and proteins are responsible for aging. Myosin degradation was not considered an important factor during low temperature aging, while calpain was critical in degrading muscle structure (Bechtel & Parrish, 1983). Besides, degradation of Z-line proteins (Goll, Thompson, Taylor, & Ouali, 1998) and/or costameres, titan, and nebulin (Taylor, Geesink, Thompson, Koohmaraie, & Goll, 1995) might be important during aging. Considering the enzymatic systems, calpains would be responsible for the tenderization occurring immediately after slaughter (Koohmaraie, 1994), and cathepsins would be responsible for myofibril fragmentation at longer aging times (Kim, Homma, Ikeuchi, & Suzuki, 1995). However, the mechanism for weakening IMCT is still unknown (Nishimura et al., 1998).

#### Dry aging vs. Wet aging

Although it has been suggested that no major difference in cut yield exists between wet- and dry-aging (Troy, 1999), it is also reported that beef shrank 8 to 10% during dry aging (Bishoff, 1984) and that weight loss for dry aged strip loins was about 12% (Parrish et al., 1991), making dry aging a costly alternative. Wet aging significantly reduces shrinkage by up to 50% compared to dry aging (Taendler & Heinz, 1970). However, dry aged beef required shorter cooking time and resulted in lower cooking loss than wet aged beef (Warren & Kastner, 1992). This was also supported by Jeremiah and Gibson (2003), who found that conventionally dry aged cuts required shorter cooking time and got less cooking loss than boneless, wet aged cuts. However, Jeremiah and Gibson (2003) indicated that controlled atmosphere, boneless, display-ready cuts were less tender than boneless, wet aged cuts and that conventionally aged controls or controlled atmosphere, boneless, display-ready cuts were less juicier or had less desirable flavor than wet aged cuts. However, Parrish et al. (1991) found slight differences in juiciness, flavor intensity, and flavor desirability between dry- and wet-aged loin and rib steaks. But scores for tenderness

and overall palatability were significantly higher for wet-aged steaks. Nevertheless, Warren and Kastner (1992) indicated no difference in tenderness between dry- and wet-aged products.

#### Aging Period

The aging period of beef is normally thought of as the time, in days, from slaughter till the carcass is broken down into retail cuts. The optimum aging time for beef is not consistent, for it usually depends on other factors, such as individual muscle, quality grade, and breed.

Eilers, Tatum, Morgan, and Smith (1996) found tenderness of beef was improved as postmortem aging period was increased. But the greatest improvement happened at different aging time for different cuts (strip loin, LM; top sirloin, GM; and top round, SM). LM and SM steaks achieved the greatest improvement in tenderness by 12d; while the tenderness of GM steaks exhibited linear response to increased aging time and continued to increase up to 24d. Regarding the within-class variation in SF value, aging for 12d or more decreased it for LM; aging from 6d to 24d decreased it for SM; whereas aging had no effect on it for GM. For GM, CT and overall tenderness ratings were higher (P < 0.05) for steaks aged 21d than those aged for 7d, without differences in flavor or juiciness (George-Evins, Unruh, Waylan, & Marsden, 2004). Other studies also reported similar tenderness change for GM during aging (18d vs. 4d, 35d vs. 21d) (Savell, McKeith, Murphey, Smith, & Carpenter, 1982; Harris, Miller, Savell, Cross, & Ringer, 1992). However, it should be noted that both Savell et al. (1982) and Harris et al. (1992) failed to detect an improvement in SF by 18d or 21d. This might be attributed to the higher content of CT. Smith, Culp, and Carpenter (1978) found no improvement in SF after 5d of aging for vastus medialis (VM), after 8d of aging for complexus (CP) and serratus ventralis (SV), and after 11d for biceps femoris (BF), infraspinatus (IF), LM, rectus femoris (RF), semimembranosus

(SM), *semitendinosus* (ST), and *spinalis dorsi* (SP) from USDA Choice. So, 11 or more days of aging would maximize tenderness of the muscles from USDA Choice grade.

For GM, IF, and *psoas major* (PM), aging longer than 21d generally decreased beef flavor identity. Aging these steaks to 21 or 35d increased metallic and rancid flavors (Yancey, Dikeman, Hachmeister, Chambers IV, & Milliken, 2005). But for LM, steaks dry-aged for 14 and 21d had slightly greater beef and brown-roasted flavors and were juicier than those aged for 7d. Increasing the aging time from 14 to 21d did not have further effect on flavor or tenderness (Campbell, Hunt, Levis, & Chambers IV, 2001). In contrast, Sapp, Williams, and McCann (1999) indicated LM steaks aged for 21d were more tender and palatable (P < 0.05) than those stored for 7 or 14d. Juiciness, beef flavor intensity, and off flavor incidence did not change (P > 0.05) across aging time. However, there was a decrease in juiciness for *longissimus thoracis* (LT) from Rubia Gallega yearling calves as the aging time increased (Oliete et al., 2005). Therefore, aging period seems to have different impacts on different muscles, regarding beef palatability attributes. Even for the same muscle, we have to weigh the impacts on different palatability attributes when trying to decide the best aging period.

Meat quality grade had a significant effect on beef juiciness, flavor desirability and overall palatability (Parrish et al., 1991). Prime steaks scored higher in flavor intensity than Choice or Select steaks. Prime and Choice steaks scored higher in tenderness than Select steaks. However, SF values for upper 2/3 Choice GM muscles aged for 14d were similar to those of Select GM muscles aged for 28d (4.53±0.15 vs. 4.58±0.14; Gruber, Tatum, Scanga, Chapman, smith, & Belk, 2006). In general, SF values of upper 2/3 Choice muscles decreased more rapidly from 2 to 10d postmortem aging than corresponding Select muscles. Gruber et al. (2006) listed the length

of aging for a muscle of each quality grade to reach a majority of its respective aging response (Table 6). Generally, Select muscles required 20d or longer than Premium Choice muscles. Bratcher, Johnson, Littell, and Gwartney (2005) suggested that CP, IF, SV, *triceps brachii* (TB), and *vastus lateralis* (VL) muscles be aged for 7d if from upper 2/3 Choice while for at least 14d if from Select. Novakofski and Brewer (2006) proposed that there was a paradox during postmortem aging. SF value of the toughest steaks decreased the most (-36%), while the value of the most tender steaks increased (16%) during the 14d aging. Yet all steaks achieved the same degree of tenderness after aging, regardless of initial tenderness. Steaks from utility grade cattle improved the most (42%) while steaks from other grades improved less (Standard 30%, Choice & Prime 20%, Commercial 18%, Select 13%). Therefore, although quality grade is considered a predictor of beef palatability, it is still possible that postmortem aging could influence the quality differences, especially tenderness, between final products from different quality grade meat. It is necessary to take quality grade into consideration when trying to determine the better aging method or optimum aging times.

Campo, Sañudo, Panea, Albertí, and Santolaria (1999) indicated that breed (Continental breeds) significantly affected the aging process in cattle slaughtered at 450 kg live weight. In crossbred steers and heifers representing diverse breed types (0-62.5% Bos indicus), the influence of aging depended on the potential tenderness of the breed (Shackelford, Wheeler, & Koohmaraie, 1997). The optimal instrumental hardness was achieved after 5d of aging for LM from Limousin breed (6-7 months old) and after 7d of aging for LM from Charolais breed (6-7 months old). There was no significant difference in sensory hardness between the two breeds at the beginning of aging, but by 7d of aging Charolais meat was more tender than Limousins meat (Revilla & Vivar-Quintana, 2006). Monsón, Sañudo, and Sierra (2004; 2005) chose four breeds representing

different biotypes (dairy: Spanish Holstein, dual purpose: Brown Swiss, meat type: Limousin, high muscularity: Blonde d'Aquitaine) to study the influence of breed and aging time on beef quality. There were significant differences (P<0.001) among breeds in the quality of total and insoluble collagen in the LT and *lumborum* muscle, with similar collagen solubility (41-44%) except for the muscle from Brown Swiss (33%). In general, meat from breeds specialized for beef production had lower compression and WBSF values when aged for less than a week. The differences in tenderness between breeds could be related to the quantity, solubility and space organization of the collagen, fatness and calpain and calpastatin activity. Nevertheless, aging had a larger influence on myofibrillar tenderness than breed and tended to eliminate the breed effect on textural variables as well as individual differences within the breed. The major increase in tenderness was achieved during the first two weeks of aging. At 14d of aging, Blonde d'Aquitaine obtained 83.0% of total tenderization seen between 1 and 35d, Brown Swiss obtained 89.5%, whereas, Limousin and Spanish Holstein achieved total tenderization at 7d and 14d of aging, respectively. Compo et al. (2000) observed that the tenderization rate was 99.7%, 89.9%, and 88.7% of the total for fast growth, dual purpose and unimproved breed, respectively, during the first week of 21d aging. The highest values of acceptability were observed between 3 and 7d of aging for Limousin and Brown Swiss, between 3 and 14d for Blonde d'Aquitaine, and between 14 and 21d for Spanish Holstein.

In conclusion, aging is an effective way to improve beef post-harvest quality (especially tenderness). But more research is needed to find out the optimal aging period for meat from different individual muscles, quality grades, or breeds.

#### Conclusion

Beef fat is characteristically more saturated than fat of non-ruminant animals, which may negatively affect human health. However, beef has a more favorable n-6: n-3 FA ratio compared to pork; also, beef is one of the important dietary sources of CLA. Although it is much more difficult to change the fatty acid composition of beef by dietary manipulation compared to nonruminant animals, grass-based feeding system or fat supplementation has the potential to increase the content of beneficial n-3 PUFA and CLA and therefore decrease SFA:PUFA and n-6:n-3 ratio. Fat content and fatty acid composition is also associated with beef palatability. In general, n-3 PUFA are considered to have a negative impact on beef flavor while oleic acid (C18:1) is believed to be positively related with beef eating quality. But more research is needed to further explore the complex relationship between FA and beef palatability and how diet regimes can influence beef quality. Postmortem aging is prevalently used to improve beef palatability (especially tenderness), however, the effect of aging on palatability attributes rather than tenderness is still controversial. More research is needed to investigate the factors that may influence aging period. In this thesis, two studies were conducted i) to analyze the effect of fat location on fatty acid composition of beef steaks from cattle on various diets and explore the effect of cooking on fatty acid profile; and ii) to investigate the influence of diet (grass vs. grain) and aging (dry vs. wet) on beef steaks or ground beef palatability attributes.

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Table 1

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няп	acia	content	OT 10	nn i	muscie i	m\sigma	σ	musciel	1 n	STEAKS	or	cnoi	ng.	trom	SIII	nermar	Kets.
I un	uoru	content	01 10	JIII	musere (	1115/	5	masere	111	Steaks	O1	Uno	00	nom	Su	perman	ROUD

Fatty acid	Beef	Lamb	Pork
12:0 (lauric)	2.9	13.8	2.6
14:0 (myristic)	103	155	30
16:0 (palmitic)	962	1101	526
18:0 (stearic)	507	898	278
18:1 trans	104	231	-
18:1 (oleic)	1395	1625	759
18:2n-6 (linoleic)	89	125	302
18:3n-3 (a-linolenic)	26	66	21
20:3n-6	7	2	7
20:4n-6 (arachidonic)	22	29	46
20:5n-3 (eicosapentaenoic)	10	21	6
22:5n-3 (docosapentaenoic)	16	24	13
22:6n-3 (docosahexaenoic)	2	7	8
Total	3835	4934	2255
PUFA:SFA <sup>a</sup>	0.1 1	0.15	0.58
n-6:n-3	2.11	1.32	7.22

<sup>a</sup>PUFA: SFA = (18:2n-6+18:3n-3) / (12:0+14:0+16:0)

(Enser et al. 1996)

## Table 2

Japanese 'FOSHU' criteria for functional food

- 1. They are food (not capsules, pills or power) on the basis of naturally occurring food components
- 2. They can and should be consumed as part of a normal daily diet
- 3. They have a defined function on the human organism:
- to improve immune function
- to prevent specific diseases
- to support recovery from specific diseases
- to control physical and psychic complaints
- to slow down the ageing process

(López-Varela, González-Gross, & Marcos, 2002)

Table 3		
Effect of feeding systems on fatty	y acid com	position of beef

Reference	Noci, Monahan, French & Moloney	Purchas, Knight, &	French et al.	Dannenberger et al. (2004)
	(2005)	(2005)	(2000)	(2001)
Breed Type	crossbred Charolais heifers	Angus-cross heifers	continental crossbred steers	German Holstein bulls
Feeding System Difference	duration of grazing before slaughter	concentrate vs. pasture	grass, grass silage, concentrate	concentrate vs. pasture+concentrate finishing
Muscle	longissimus	Longissimus lumborum(LL); triceps brachii(TB)	longissimus	longissimus
Fatty acid content	not significant	LL-concentrate higher	not significant	not significant
SFA	quadtratic effect	LL higher; small differces between the groups	linear decrease with decreased concentrate	
MUFA	not significant	TB higher; small differces between the groups	not significant	
PUFA	linear increase with duration	TB higher; small differces between the groups	grass higher	
P:S	cubic effect	not significant	linear increase with decreased concentrate	
n-6FA	not significant		not significant	not significant
n-3FA	cubic effect		linear increase with decreased concentrate	pasture+concentrate finishing significantly higher
n-6/n-3	linear decrease with duration	concentrate significantly higher	linear decrease with decreased concentrate	pasture+concentrate finishing significantly lower
C18:1			not significant	
C14:0	quadtratic effect	concentrate significantly higher	not significant	concentrate significantly higher
C16:0	cubic effect	not significant	linear decrease with decreased concentrate	concentrate significantly higher
C18:2n-6		concentrate significantly higher		concentrate higher (not significant)
C18:3n-3	cubic effect	pasture significantly higher		pasture+concentrate finishing significantly higher
C18:2/C18:3		concentrate significantly higher		

Table 3	(continue)	)
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Reference	Gatellier, Mercier, Juin, & Renere. (2005)	Realini, Duckett, Brito, Dalla Rizza, & De Mattos (2004)	Varela et al. (2004)
Breed Type	Charolais steers, heifers, and cows	Hereford steers	Rubia Gallega steers
Feeding System Difference	pasture vs. mixed diet finishing	pasture vs. concentrate	pasture vs. concentrate finishing
Muscle	M. longissimus dorsi	longissimus	longissimus thoracis
Fatty acid content	mixed diet significantly higer	concentrate significantly higer	
SFA	steers-mixed diet significantly higher	not significant	not significant
MUFA	not significant	concentrate significantly higer	not significant
PUFA	cows-pasture significantly higher	pasture significantly higer	not significant
P:S		pasture significantly higer	not significant
n-6FA	cows-pasture significantly higher		concentrate higher
n-3FA	pasture significantly higher		pasture higher
n-6/n-3	heifers-pasture significantly lower	pasture significantly lower	concentrate significantly higher
C18:1			not significant
C14:0		concentrate significantly higer	not significant
C16:0	steers-mixed diet significantly high	concentrate significantly higer	concentrate finishing significantly higher
C18:2n-6	cows-pasture significantly higher	pasture significantly higer	concentrate finishing significantly higher
C18:3n-3	pasture significantly higher	pasture significantly higer	pasture higher (not significant)
C18:2/C18:3			concentrate significantly higher

Table 3 (continue)

NeterinicDisploy, Nail, & Rogers. (1991)Database tail. (2006)Notice tail. (2005)Breed TypeBritish and Britishxcontinental steers and heifers; USDA choice and select steaksGermanFeeding Systemgrain vs. forageforage-fed (fescue, fescue+soyhull, orchard+soyhull) vs. retail productionConcentrate vs. grass-basedMusclelongissimus dorsi (toin), gluteus medius (rump)IongissimusIongissimusconcentrate vs. grass-basedFatty acid contentnot significantChoice steaks>fescue+soyhull, fescue+soyhull>select steaks, fescueconcentrate significantly higherMUFAloin-grain significantly highernot significantrescue+soyhull, choice> fescue+soyhull, choice> fescue+soyhull, choice> fescue+soyhull, choice> fescue+soyhull, choice> fescue+soyhull, choice> fescue+soyhull, choice> fescue+soyhull, choice> fescue+soyhull, fescue> fescue+soyhullont significantn-6FAnot significantfescue, soyhull, fescue> fescue+soyhullnot significantn-6fn-3choice>select> orchard+soyhull, fescue> fescue+soyhullgrass-based significantn-6/n-3choice>select> rochard+soyhull, fescue+soyhull> rescuegrass-based significantly lowerfescue fescue rescuenot significantconcentrate significantn-6/n-3not significantfescue+soyhull>choice> rescuegrass-based significantn-6/n-3not significantfescue+soyhull>choice> rescuenot significantn-6/n-3not significantfescue+soyhull>choice> rescuenot significantn-6/n-3 </th <th>Poforonco</th> <th>Gregory Alan &amp;</th> <th>Baublits et al</th> <th>Nuemberg et al</th>	Poforonco	Gregory Alan &	Baublits et al	Nuemberg et al
Rogers. (1991)         (2005)         (2005)           Breed Type         British and Britishxcontinental steers and heifers; USDA choice and select steaks         German Simmental(GS), German Holstein(GH) bulls           Feeding System         grain vs. forage         forage-fed (fescue, fescue+soyhull, orchard+soyhull) vs. retail production         concentrate vs. grass- based           Muscle         longissimus dorsi (foin), gluteus medius (rump)         longissimus         longissimus           Fatty acid content         not significant         Choice steaks>fescue+soyhull, fescue+soyhull>select steaks, fescue         concentrate significantly higher           FTA         rump-grain significantly lower         not significant         not significant           PUFA         not significant         fescue>select> orchard+soyhull, choice> fescue+soyhull         grass-based significant           PUFA         not significant         fescue>select> orchard+soyhull, choice> fescue+soyhull         not significant           n=SFA         fescue>soyhull         grass-based significant         significant           n=GFA         fescue>soyhull         grass-based significant         significant           n=GFA         fescue>soyhull         grass-based significant         significant           n=GFA         fescue>soyhull, fescue>soyhull>         grass-based significant         significant	Kelelelice	Degere		(2005)
Breed Type         British and Britishxcontinental steers and heifers; USDA choice and select steaks         German Simmental(GS), German Holstein(GH) bulls           Feeding System         grain vs. forage         forage-fed (fescue, fescue+soyhull, orchard+soyhull) vs. retail production         concentrate vs. grass- based           Muscle         longissimus dorsi (loin), gluteus medius (rump)         longissimus         longissimus fescue+soyhull>select steaks, fescue         concentrate significantly higher           Fatty acid content         not significant         Choice steaks>fescue+soyhull, fescue+soyhull>select steaks, fescue         concentrate significantly higher           FUFA         not significant         fescue>select> orchard+soyhull, choice> fescue+soyhull         ond significant           PUFA         not significant         fescue>select> orchard+soyhull, choice> fescue+soyhull         orchard+soyhull, choice> fescue+soyhull           n-6FA         select, choice> refscue+soyhull         not significant           n-6FA         select, choice> fescue+soyhull         grass-based significantly higher           n-6FA         fescue>soyhull         grass-based significantly higher           n-6FA         choice>select> orchard+soyhull, fescue> fescue+soyhull>choice         grass-based significantly higher           n-6FA         loin-grain significantly higher         fescue+soyhull>choice> fescue         grass-based significantly higher <t< th=""><th></th><th>Rogers.</th><th>(2006)</th><th>(2005)</th></t<>		Rogers.	(2006)	(2005)
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Feeding System         grain vs. forage         forage-fed (fescue, fescue+soyhull, orchard+soyhull) vs. retail production         concentrate vs. grass- based           Muscle         longissimus dorsi (loin), gluteus medius (rump)         longissimus         longissimus           Fatty acid content         not significant         Choice steaks>fescue+soyhull, fescue+soyhull>select steaks, fescue         concentrate vs. grass- based           FAT         rump-grain significantly lower         not significant         concentrate significant           MUFA         toin-grain significant         fescue+soyhull-select steaks, fescue         grass-based           PUFA         not significant         fescue+soyhull, choice> rechard+soyhull, choice> rescue+soyhull, choice> rescue+soyhull, choice> rescue+soyhull, fescue> fescue+soyhull, fescue> fescue+soyhull, fescue> rescue+soyhull, fescue> rescue+soyhull, fescue> rescue+soyhull, fescue> rescue+soyhull>choice         grass-based significantly higher           n-6FA         loin-grain significant         fescue+soyhull, fescue> rescue+soyhull>choice         grass-based significantly nowr fescue           n-6fn-3         loin-grain significant         choice>select> rescue+soyhull, fescue+soyhull> rescue         not significant rescue+soyhull, orchard+soyhull>           fescue         not significant         fescue+soyhull, orchard+soyhull> rescue         not significant rescue+soyhull, orchard+soyhull>           fescue         not significant         fescue+so				bulls
System DifferenceGenerate solution orchard+soyhully vs. retail productionbasedMusclelongissimus dorsi (loin), gluteus medius (rump)longissimuslongissimusFatty acid contentnot significantChoice steaks>fescue+soyhull, fescue+soyhull>select steaks, fescueconcentrate significantly higherSFArump-grain significantly lowernot significantconcentrate significantly fescue+soyhull>select steaks, fescuePUFAnot significantfescue>select> orchard+soyhull, choice> fescue+soyhullgrass-based significantly higherPUFAnot significantfescue>select> orchard+soyhull, choice> fescue+soyhullnot significantly significantly higherP:Snot significantfescue>select> orchard+soyhull, choice> fescue+soyhullnot significantn-6FAselect, choice> fescue+soyhullnot significantn-3FAfescue>soyhull>fescue> fescue+soyhull>choicegrass-based significantly highern-6/n-3loin-grain significantly fescuefescue+soyhull> choice>select> fescue+soyhull, orchard+soyhull> choice>select> fescue+soyhull, orchard+soyhull> choice>select>fescueont significantly lowerC18:0not significantfescue+soyhull, orchard+soyhull> fescueoncentrate significantly higherC18:10not significantfescue+soyhull, orchard+soyhull> fescueoncentrate significantly higherC18:2n-6rump-grain significantlyfescue+soyhull, orchard+soyhull, fescueconcentrate significantly higherC18:2n-6rump-grain sig	Feeding	grain vs. forage	forage-fed (fescue fescue+sovhull	concentrate vs. grass-
System         Oscilation System Production         Description           Muscle         longissimus dorsi (loin), gluteus medius (rump)         longissimus         longissimus           Fatty acid content         not significant         Choice steaks>fescue+soyhull> fescue+soyhull>select steaks, fescue         concentrate significantly higher           SFA         rump-grain significantly lower         not significant         not significant           PUFA         not significant         fescue>select> orchard+soyhull, choice> fescue+soyhull, choice> fescue+soyhull, choice> fescue+soyhull, choice> fescue+soyhull, choice> fescue+soyhull, choice> fescue+soyhull, fescue> fescue+soyhull, fescue> fescue+soyhull, fescue> fescue+soyhull         not significant           n-6FA         select, choice> refescue+soyhull, fescue> fescue+soyhull         not significant           n-3FA         fescue>select> orchard+soyhull, fescue> fescue+soyhull> choice>select> orchard+soyhull, fescue> fescue+soyhull> choice>select> orchard+soyhull>choice         grass-based significantly ligher           n-6/n-3         choice>select> orchard+soyhull, fescue+soyhul> fescue         orchard+soyhull> choice>select> orchard+soyhull> fescue           C18:10         loin-grain significantly higher         fescue+soyhull, orchard+soyhull> fescue         ont significantly higher           C18:2n-6         not significant         fescue+soyhull, orchard+soyhull> fescue         orchard+soyhull, fescue+soyhull, orchard+soyhull> fescue         grass-based sig	Svetom	grain vo. lorage	orchard+sovbull) vs. retail production	based
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Table 4 Possible effects of	of fatty acids on beef aroma or fl	avor
Aroma/Flavor	FA positively related	FA negatively related
Greasy aroma	C16:1, C18:1	
	010.0	040.4

Greasy aroma	C16:1, C18:1	
Metalic aroma	C18:3	C18:1
Fat aroma	C14:0, C16:0, C16:1, C18:1	C15:0, C18:0, C18:3, C19:1, C20:1, C20:4
Grassy aroma	C14:0, C16:0, C18:3	
Milky-oily aroma	C18:0, C18:3	
Sour flavor	C14:0, C18:0, C18:3	C16:1, C18:1
Beef flavor	C18:1	C18:3
Sweet flavor	C16:0, C16:1, C18:0, C18:1, C18:2	
Salty flavor	C18:2	
liver flavor	C16:1	C18:0

(Melton, Amiri, Davis, & Backus, 1982; Mandell, Buchanan-Smith, & Campbell, 1998)

# Table 5Relative influence of gender classes on marbling levels

	Gender	Class <sup>a</sup>		Study
Cows Heifers		Steers	Bulls	
		1	2	Shackleford et al. (1992)
		1	2	Huerta-Leidenz et al. (1991)
1	2			Vincent et al. (1991)
	1	2	3	Jones et al. (1990)
		1	2	Johnson et al. (1988)
		1	2	Jones et al. (1986)
	1	2		Slanger et al. (1985)
		1	2	Ockerman et al. (1984)

<sup>a</sup>Gender class with the highest marbling level in each study is indicated by '1'; gender classes with the next highest marbling levels in each study are indicated by '2' and then '3' (Harper & Pethick, 2004)

## Table 6

Change in shear force from 2 through 28d postmortem (aging response, kg) and the length of aging (d) needed for the majority of this change to occur for USDA Select and upper two-thirds Choice beef muscles

	USDA S	Select	Upper two-thirds USDA Choice			
Muscle	Aging response	Aging time <sup>a</sup>	Aging response	Aging time <sup>a</sup>		
Biceps femoris	1.1	26	0.5	6		
Complexus	1.7	27	1.5	23		
Gluteus medius	1.6	27	1.1	21		
Infraspinatus	1.4	25	1.4	18		
Longissimus dorsi	2.5	26	2.0	15		
Psoas major	1.3	27	1.1	25		
Rectus femoris	1.3	25	1.0	15		
Semimembranosus	2.3	23	1.4	16		
Semitendinosus	1.6	23	1.4	18		
Serratus ventralis	1.0	24	0.9	25		
Spinalis dorsi	1.3	23	1.0	13		
Supraspinatus	1.6	23	1.4	14		
Tensor fasciae latae	1.1	22	1.0	12		
Teres major <sup>ь</sup>	_	_	0.7	21		
Triceps brachii	1.6	21	1.4	16		
Vastus lateralis	1.6	20	1.5	21		
Vastus medialis	1.9	25	1.8	21		

<sup>a</sup>Aging times for muscles with aging responses  $\geq 2.2$  kg (high), 2.1 to 1.8 kg (moderately high), 1.7 to 1.1 kg (moderate), 1.0 to 0.7 kg (moderately low), and  $\leq 0.6$  kg (low) correspond to the day that at least 96, 95, 94, 90, and 85% of the aging response was completed, respectively.

<sup>b</sup>Warner-Bratzler shear force of Select teres major did not decrease with increasing time of postmortem storage. (Gruber et al., 2006)



Fig.1. Biosynthesis of CLA cis-9, trans-11 (Schmid et al., 2006)

Effect of sampling fat location and cooking on fatty acid composition of beef steaks

#### Abstract

Research was conducted to investigate the impact of sampling fat location and cooking on fatty acid content and composition of beef steaks. Twenty-one crossbred steers were pastured and then fed a moderate grain diet until they reached a similar slaughter weight and marbling grade. Two longissimus muscle steaks from each steer were collected, one of them was cooked. Raw samples were dissected to obtain outer subcutaneous fat (OSC), inner subcutaneous fat (ISC), seam fat, marbling, and lean muscle. Cooked samples were dissected to obtain OSC, ISC, seam fat, surface muscle, and inner muscle. Total fat content was higher in subcutaneous and seam fat compared to marbling which in turn had far more fat than muscle (P < 0.05). The levels of transvaccenic acid and c9, t11-CLA were lower (P<0.05) in lean muscle than in subcutaneous fat or marbling. Seam fat and marbling contained higher (P<0.05) saturated fatty acids than subcutaneous fat and lean muscle. The saturated: monounsaturated fatty acids ratio was also higher (P<0.05) in seam fat and marbling. The levels of linoleic and linolenic acid, and total polyunsaturated fatty acids were highest (P<0.05) in lean muscle. Most n-6 and n-3 fatty acids except linoleic and linolenic acid were not detected in subcutaneous fat, seam fat, or marbling but were present in lean muscle. However, there was no difference (P<0.05) in n-6: n-3 ratio among fat locations. The fatty acid composition characteristics of cooked beef steaks were similar to that of raw steaks except for that n-6: n-3 ratio was higher (P<0.05) in muscle than that in subcutaneous or seam fat. In conclusion, fatty acid composition of steaks differs depending on the location of fat. Cooking did not significantly influence total fat content or the levels of most fatty acids of subcutaneous and seam fat. But n-6: n-3 ratio in subcutaneous and seam fat decreased (P<0.05) after cooking.

*Keywords:* Beef steak; Fat location; Fatty acid composition; Cooking

## 1. Introduction

It has been well known that the fatty acid composition of ruminant meat like beef differs from non-ruminant meat like pork. Polyunsaturated: saturated fatty acids (PUFA: SFA) ratio is lower in beef than in pork due to ruminal bio-hydrogenation. However, beef does have a more beneficial n-6: n-3 ratio than pork (Enser et al., 1996). Furthermore, beef is an important dietary source of conjugated linoleic acids (CLA) for humans, which were reported to possess a range of health promoting biological properties including antitumor and anticarcinogenesis (De la Torre et al., 2006). Therefore, studies have been conducted to explore health aspects of beef by analyzing fatty acid profile. Another relationship of interest is between fatty acids (FA) and beef palatability, especially flavor. Grass-fed beef was criticized for a fishy flavor, which was believed to be a result from higher n-3 PUFA levels in grass-fed beef compared to traditional concentrate-fed beef (Vatansever et al., 2000). In contrast oleic acid (C18:1n9) was associated with favorable beef palatability attributes (Dryden & Marchello, 1970). Therefore, fatty acid analysis also plays an important role in beef eating quality.

However, it should be noted that lipid in meat includes neutral (storage) and polar (membrane) fractions, with PUFA located primarily in the polar fraction. Adipose tissue is mostly composed by neutral lipids, predominantly triglycerides; while lean muscle mainly contains polar lipids from cell membrane, predominantly phospholipids. Therefore, fatty acid composition from different fat locations within beef muscle would be different.

Therefore, the main objective of the study was to explore the effect of sampling fat location within beef steaks on fatty acid composition. The effect of cooking on fatty acid profiles of subcutaneous and seam fat was also evaluated. The results will be helpful with establishing sampling protocols and interpreting fatty acid profiles of muscle or adipose tissues.

#### 2. Materials and Methods

#### 2.1. Animals and diets

Animals were harvested at the University of Nevada, Reno (UNR) Meat Laboratory following USDA humane slaughter procedures. Fifteen Wagyu sired half Angus steers and six Angus steers grazed a mixed grass pasture and were then fed grain diets in the feedlot for different periods of time until they reached a similar slaughter weight of about 525 kg and minimum small<sup>0</sup> marbling degree measured by ultrasound. The grain diet was composed of commercial feed and early bloom alfalfa hay (Tables 1 and 2). Hot carcass weight, rib eye area, back fat, yield grade, and quality grade were measured on carcass.

## 2.2. Sample collection and preparation

Two longissmus muscle steaks were collected from each steer carcass at 48h postmortem; one of them was thawed at 3-4 °C for 48h and then cooked on a preheated Farberware Open Hearth Grills (model R4550; Farberware, Bronx, NY). Internal central temperature was monitored by a 12-Channel Scanning Thermocouple Thermometer (Model 692-8010, Barnart, Barrington, IL). Steaks were turned over when the temperature reached 40 °C and were removed at 71 °C. Raw steaks were dissected to obtain outer subcutaneous fat (OSC), inner subcutaneous fat (ISC), seam (intermuscular) fat, marbling (intramuscular fat), and lean muscle. Cooked steaks were dissected to get OSC, ISC, seam fat, surface muscle, and inner muscle. Samples were cut into small pieces with razor blade, about 0.05 g for adipose tissue and 0.8-1.0 g for muscle. Samples were kept in 16 x 125 mm screw-cap culture tubes and stored in a freezer (-20 °C) for subsequent fatty acid analysis.

## 2.3. Fatty acid methyl esters (FAME) synthesis and fatty acid analysis

Fatty acid content of the samples was determined according to O'Fallon, Busboom, Nelson, Gaskins (2007). One ml of C13:0 internal standard (0.5 mg C13:0 / ml MeOH), 0.7 ml 10 N KOH in water, and 5.3 ml MeOH were added to the samples, then tubes were incubated in a 55 <sup>o</sup>C water bath for 1.5 h with vigorous hand-shaking for 5 s every 20 min. After the tubes were cooled below room temperature in a cold tap water bath, 0.58 ml 24 N H<sub>2</sub>SO<sub>4</sub> in water was added. Tubes were mixed by inversion and incubated at 55 °C again with precipitated K<sub>2</sub>SO<sub>4</sub> present. After FAME synthesis, tubes were cooled in a cold tap water bath. Then 3.0 ml of hexane was added and the tubes were vortex-mixed and then centrifuged for 5 min. The hexane layer, containing the FAME, was transferred into 2 ml gas chromatography (GC) vials. The vials were capped and stored at -20 °C until GC analysis. The fatty acid composition of FAME was determined by capillary GC on a SPTM-2560, 100 m × 0.25 mm × 0.20 µm capillary column (Supelco, Bellefonte, PA) installed on a Hewlett Packard 5890 GC (Hewlett Packard, Farmington Hills, MI). Initial oven temperature was 140 °C held for 5 min, then the temperature was increased to 240 °C at a rate of 4 °C min<sup>-1</sup> and held for 20 min. Helium was used as the carrier gas at a flow rate of 0.5 ml min<sup>-1</sup> and column head pressure was 280 kPa. Both the injector and detector were set at 260 °C. The split ratio was 30:1. Fatty acids were identified by comparing their retention times to those of methylated FA standards (Nu-Chek Prep Inc., Elysian, MN).

## 2.4. Calculations

Saturated fatty acids (SFA) were the sum of C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, and C20:0. Monounsaturated fatty acids (MUFA) were the sum of C14:1n5, C16:1n7, C17:1n7,

C18:1n7, and C18:1n9. Polyunsaturated fatty acids (PUFA) were the sum of n-6 and n-3 fatty acids. N-6 fatty acids were the sum of C18:2n6, C18:3n6, C20:2n6, C20:3n6, C20:4n6, and C22:4n6; n-3 fatty acids were the sum of C18:3n3, C20:5n3, C22:5n3, and C22:6n3. The indices of  $\Delta$ 9-desaturase and elongase were calculated by 100\*[C18:1n9 / (C18:0 + C18:1n9)] and 100\* [(C18:0 + C18:1n9) / (C16:0 + C18:0 + C16:1n7 + C18:1n9)], respectively (Nuernberga et al., 2002). Fatty acids were not listed in the result tables (Table 3-6) if the levels were too low to detect.

#### 2.5. Statistical analysis

Samples were collected in a completely randomized design for raw and cooked steaks with fat location as treatment. Duplicate GC results were averaged and analyzed by ANOVA using PROC GLM of SAS (SAS Inst. Inc., Gary, NC). Tukey's test was used to make all pairwise comparisons among raw or cooked data. Data from common treatments for raw and cooked steaks were analyzed in a two-factorial treatment design, with two levels of cooking (raw and cooked) and three levels of fat location (outer s.c., inner s.c., and seam fat). Student's t-test was used to make pairwise comparisons between raw and cooked samples.

#### 3. Results

#### 3.1. Carcass measurements

The steers were slaughtered at  $661 \pm 14$  d old when they reached a similar slaughter weight (526.1 ± 8.40 kg) and marbling grade (680 ± 24, Modest) after 158 ± 28 d of consuming a moderate grain diet. Carcass measurements were:  $306 \pm 6.04$  kg hot carcass weight, 13 (average Choice) ± 0.22 quality grade,  $76.7 \pm 1.38$  cm<sup>2</sup> rib eye area,  $0.8 \pm 0.05$  cm back fat, and  $2.7 \pm 0.09$  yield grade. Breed (Wagyu or Angus sired steers) had no effect on the carcass measurements

(data were not shown). The steers were kept in the feedlot for short  $(42 \pm 19 \text{ d})$ , medium  $(126 \pm 19 \text{ d})$ , or long  $(306 \pm 19 \text{ d})$  periods of time, however, this did not significantly influence the carcass measurements either, except that hot carcass weight from steers kept in the feedlot for the medium time was heavier (P<0.05) than those kept for the short time (327 vs. 292 \pm 8 kg).

#### 3.2. Fatty acid composition of raw beef steaks

There was no interaction between days in the feedlot and sampling fat locations for fatty acid composition. However, days in the feedlot affected the level of CLA and SFA: MUFA ratio of beef steaks. The level of CLA linearly (P<0.05) increased while SFA: MUFA ratio showed a cubic trend (P<0.05) as days in the feedlot increased. There was no effect of breed on fatty acid composition of beef steaks. However, interaction (P<0.05) existed between sampling fat location and breed for TVA. For steaks from Angus sired steers, TVA level increased from OSC to ISC, then decreased in the order of Seam fat, marbling, and lean muscle. For Wagyu sired steers, TVA level increased in lean muscle.

Fatty acid content and profile of OSC, ISC, seam fat, marbling and lean muscle tissue from raw beef steaks were shown in Table 3. Total fatty acid content was higher in subcutaneous and seam fat compared to marbling which in turn had far more fat than muscle (P<0.05). The OSC, ISC, seam fat, marbling, and lean muscle contained 841.1, 836.2, 844.3, 582.6,  $32.3 \pm 12.7$  mg fatty acid / g tissue, respectively.

The levels of trans-vaccenic acid (TVA) and c9, t11-CLA were higher (P<0.05) in subcutaneous fat and marbling compared to lean muscle. The level of TVA was highest in marbling and seam tissue, intermediate in ISC and OSC, lowest in lean muscle (2.3, 2.2, 2.1, 1.7, and 1.1 % of total FA, respectively). The level of c9, t11-CLA was highest in subcutaneous fat

and marbling (0.5 % and 0.5 %, respectively), intermediate in seam fat (0.4 %), and lowest in lean muscle (0.3 %).

Seam fat and marbling contained higher (P<0.05) SFA than subcutaneous fat or lean muscle. The SFA: MUFA ratio was also higher (P<0.05) in seam fat and marbling (1.3 and 1.2, respectively) than in subcutaneous fat or lean muscle (1.0 and 1.0, respectively).

The levels of linoleic, linolenic acid, and total PUFA were highest (P<0.05) in lean muscle, about 2 to 4 times the levels in adipose tissues. There were no differences (P>0.05) in the levels of linoleic, linolenic acid, and total PUFA among subcutaneous, seam fat, and marbling.

Most n-6 and n-3 FA except linoleic and linolenic acid were not detected in adipose tissues but were present in lean muscle. However, there was no difference (P<0.05) in n-6: n-3 ratio among fat locations.

Delta-9 desaturase enzyme activity index in C18 fatty acids was highest (P<0.05) in OSC (80.9), followed by ISC (76.8) and lean muscle (75.2). The index was lowest (P<0.05) in marbling and seam (69.7 and 70.1, respectively). Elongase enzyme activity index on C16-C18 fatty acids was higher (P<0.05) in lean muscle and marbling than in subcutaneous or seam fat.

#### 3.3. Fatty acid composition of cooked beef steaks

Fatty acid content and profile of OSC, ISC, seam fat, surface muscle, and inner muscle tissue from cooked beef steaks were shown in Table 4. As expected, total fatty acid content was much higher (P<0.05) in subcutaneous and seam fat (832.7 and 789.7 mg fatty acid / g tissue, respectively) compared to the muscle tissues (88.8 mg fatty acid / g tissue).

The levels of TVA and c9, t11-CLA were higher (P<0.05) in subcutaneous and seam fat than muscle tissue. The levels of TVA in OSC, ISC, seam fat, and muscle were 1.7, 2.0, 2.0, and

1.0% of total FA, respectively. The level of c9, t11-CLA was highest (P<0.05) in OSC (0.5%), followed by ISC (0.4%) and seam fat (0.4%). The levels of c9, t11-CLA in adipose tissues (subcutaneous or seam fat) were about 2 to 3 times higher than the level in muscle.

Seam fat contained higher (P<0.05) SFA than subcutaneous fat or muscle tissue. The SFA: MUFA ratio was also higher (P<0.05) in seam fat (1.3) compared to subcutaneous fat (1.0) or muscle (1.0). There were no differences (P>0.05) between subcutaneous fat and muscle in SFA level or SFA: MUFA ratio.

The levels of linoleic, linolenic acid, and total PUFA were higher (P<0.05) in muscle compared to adipose tissues. The proportion of PUFA in muscle exceeded twice the proportion in adipose tissues.

Most n-6 and n-3 FA, except linoleic and linolenic acid, were not detected in adipose tissues but were present in muscle. However, after cooking the levels of docosadienoic (C22:2n6) and docosahexaenoic acid (C22:6n3) in muscle were too low to detect. The n-6: n-3 ratio was higher (P<0.05) in muscle than in adipose tissues (2.5 vs. 2.0%).

Delta-9 desaturase enzyme activity index was highest (P<0.05) in OSC (81.9), followed by ISC (77.2) and muscle (76.3). The index was lowest (P<0.05) in seam fat (69.7). Elongase enzyme activity index was higher (P<0.05) in muscle compared to adipose tissues. The index of OSC was higher (P<0.05) than that of ISC or seam fat.

#### 3.4. The influence of cooking on FA composition of beef steaks

There were no interactions between location (OSC, ISC, or Seam) and cooking (raw or cooked) for fatty acid composition of beef steaks. Therefore, the main effects of fat location and cooking on fatty acid content and composition were shown in Tables 5 and 6, respectively.

There was no difference (P>0.05) in total fatty acid content among fat locations. The level of TVA was lower (P<0.05) in OSC compared to ISC or seam fat; while the level of c9, t11-CLA was higher (P<0.05) in subcutaneous fat than in seam fat. The level of SFA was lower (P<0.05) while the level of MUFA was higher (P<0.05) in subcutaneous fat than in seam fat. As a result, SFA: MUFA ratio was lower (P<0.05) in subcutaneous fat. The proportions of linoleic acid, and total PUFA was lower (P<0.05) in OSC compared to ISC or seam fat. Most n-6 and n-3 FA except linoleic and linolenic acid were undetectable; there was no difference (P>0.05) in n-6: n-3 ratio among fat locations. Delta-9 desaturase enzyme activity index decreased (P<0.05) from OSC to ISC and seam fat.

There was no influence (P>0.05) of cooking on total fatty acid content of subcutaneous and seam fat. For most fatty acids, their level didn't change (P>0.05) after cooking. However, the level of linolenic acid increased (P<0.05); n-6: n-3 ratio decreased (P<0.05). The proportions of myristoleic (C14:1n5), pentadecanoic (C15:0), heptadecenoic (C17:1n7), and arachidic (C20:0) acid also changed (P<0.05) after cooking.

#### 4. Discussion

#### *4.1. Fatty acid composition of beef*

The CLA level in our study is similar to the level Chin, Liu, Storkson, Ha, & Pariza (1992) has reported for ground beef (4.3 mg/g lipid). There are also other studies which observed higher concentrations of CLA in ground beef, ranged from 6.6 (Shantha, Crum, & Decker, 1994) to 12.6 mg/g lipid (Realini, Duckett, & Windham, 2004). Different nutritional background of animals is likely to explain the differences at least to some extent. Two pathways to produce

CLA in ruminant animals have been suggested: (1) incomplete ruminal biohydrogenation of dietary fatty acids, especially C18:2n-6 (Kepler & Tove, 1967); and (2) tissue desaturation of ruminally derived C18:1*trans*-11 (Santora, Palmquist, & Roerhig, 2000). Linolenic acid (C18:3n-3) is a predominant fatty acid in pasture grasses and can be converted to C18:1*trans*-11 by ruminal biohydrogenation, which will be ultimately converted to CLA in adipose tissue by delta-9 desaturase enzyme (Noci, Monahan, French, & Moloney, 2005). Noci et al. (2005) also found that the relative proportion of CLA in the neutral lipid fraction was greater than that in the polar lipid fraction, which agrees with our observation (adipose vs. muscle).

Waldman, Suess, and Brungardt (1968) determined the fatty acid composition of outer and inner subcutaneous fat, *longissimus dorsi* (LD) marbling and *chuck* seam fat. In line with our results, they found the concentration of SFA increased from external to internal sample locations. In the current study, more C18:0 and less C16:1 was in internal than external fat depots. However, Westerling and Hedrick (1979) found no difference in total SFA between subcutaneous fat and marbling.

Marino, Albenzio, Girolami, Muscio, Sevi, & Braghieri (2006) evaluated fatty acid composition of LD muscle from Podolian young bulls on diets with different forage: concentrate ratios during finishing period. The level of MUFA (33.81%) was much lower while the level of PUFA (18.63%) and n-6: n-3 (6.72) ratio were much higher compared to the results we got from raw LD marbling (43.28%, 2.10%, 2.40) and lean muscle (45.10%, 7.59%, 2.48). The favorable concentration of PUFA from Podolian young bulls may be a result from the lean nature of this breed (Carnovale & Nicoli, 2000) because the contribution of phospholipids to total FA is proportionately greater when fatty acid content is lower. Enser, Hallett, Hewitt, Fursey, and Wood (1996) evaluated the fatty acid composition of adipose tissue and muscle from beef sirloin

steaks. Their results supported ours, especially, C20 and C22 PUFA were present in the muscle but their concentrations in adipose tissue were too low to measure. Cattle in US are usually fatter than those in Europe. But in their study (Enser et al., 1996) small amount of adipose tissue was left adhering to the muscle to make it similar to that consumed by consumers. As a result, the fat content of muscle was at the upper end of the reported range for European cattle but similar to ours.

Delta-9 desaturase is the enzyme responsible for the conversion of all SFA to their corresponding MUFA in ruminant animals. It also converts TVA (C18:1trans-11) to its corresponding CLA isomer (C18:2 *cis*-9, *trans*-11). Delta-9 desaturase is encoded by the stearoyl coenzyme A desaturase (SCD) gene, which is essential for bovine preadipocyte differentiation (Smith, Lunt, Chung, Choi, Tume, & Zembayashi, 2006). Bovine adipose tissue had considerably higher delta-9 desaturase activity and gene expression than muscle. This was demonstrated by Archibeque, Lunt, Gilbert, Tume, & Smith (2005), who also found that s.c. adipose tissue had approximately twice delta-9 desaturase activity of marbling. The result was consistent with a higher level of MUFA in subcutaneous fat than in marbling. Their observations supported our results. The study of delta-9 desaturase in beef is especially important for the markets in certain countries like Korea and Japan where fat softness is also an important component of beef carcass quality in addition to marbling (JMGA, 1988). Stearic acid (C18:0) is one of the main FA indicating fat hardness. Increased conversion of stearic acid to oleic acid will increase fat softness because beef lipids enhanced with oleic acid have lower melting point (Chung et al., 2006). The concentration of oleic acid has been reported to be positively correlated with beef overall palatability (Westerling & Hedrick, 1979), which may be related to fat softness.

## 4.2. Effect of cooking on fatty acid composition of beef

Scheeder, Casutt, Roulin, Escher, Dufey, & Kreuzer (2001) concluded that cooking only slightly, but significantly, decreased the proportion of SFA while increased the proportion of PUFA in ground beef patties from Brown Swiss bulls fed different diets. More specifically, the levels of C14:0, C16:0, C18:0, and C18:1 trans decreased and concomitantly the level of total PUFA was increased. Their results were supported by previous studies (Ono, Berry, & Paroczay, 1985; Ramamurti, 1986; Rodriguez-Estrada, Penazzi, Caboni, Bertacco, & Lercker, 1997). The proportional change in SFA and PUFA may result from drip loss which contains mainly triglycerides from adipose tissue. However, in our study the levels of SFA and PUFA both increased a little bit after cooking, not significantly. The levels of C14:1n5 and C18:3n3 increased (P<0.05) while the levels of C15:0, C17:1n7, and C20:0 decreased (P<0.05) by cooking. The n-6/n-3 ratio also decreased, which may be explained by the increased level of C18:3n3. In another study, Johnson, Williams, Neel, and Reagan (1994) found no difference in fatty acid composition between raw and cooked ground beef. It should be noticed that in our study beef steaks were used to measure the influence of cooking on fatty acid composition while the previous studies we have discussed so far used ground beef. However, it is still unclear whether this can explain the different results we got compared to the above previous studies. Nevertheless, the results and explanations from previous studies about beef steaks differed from those about ground beef.

Duckett and Wagner (1998) investigated the effect of cooking on fatty acid composition of marbling from beef steaks. They concluded that cooking increased the percentages of stearic acid (C18:0) and SFA while reduced the percentage of PUFA, which was in contrast to the studies with ground beef. Harris, Harberson, Savell, Cross, and Smith (1992) found that cooking had no

influence on the fatty acid composition of total lipid extracts from beef muscle. However, it is possible that the changes in fatty acid composition during cooking would be overlooked with total lipid extracts analyzed (Duckett & Wagner, 1998) because there is a great difference in fatty acid composition between neutral (storage component of lipid) and polar (membrane component of cells) lipid fraction. In neutral lipid fraction, cooking reduced percentages of oleic acid and MUFA with a resultant increase in percentages of stearic acid and SFA; in polar lipid fraction, cooking reduced percentages of linoleic, linolenic acid, and PUFA with a resultant increase in percentages of stearic acid and SFA (Duckett & Wagner, 1998).

## 5. Conclusions

The fatty acid composition of steaks differs depending on the location of fat. CLA level was almost doubled in OSC compared to lean muscle in this study, but PUFA with chain lengths longer than 18 were detected almost exclusively in lean muscle. The fatty acid composition characteristics of cooked beef steaks were similar to that of raw steaks except that n-6: n-3 ratio was higher (P<0.05) in muscle compared to subcutaneous or seam fat. Cooking did not significantly influence total fatty acid content of beef steaks or the levels of most fatty acids, except for myristoleic, pentadecanoic, heptadecenoic, linolenic and arachidic acid. In addition, n-6: n-3 ratio decreased (P<0.05) after cooking.

It is easier to sample subcutaneous and seam fat for fatty acid analysis, but beef steaks are usually trimmed before eating. Therefore sampling muscle tissue should be more accurate when assessing the contribution of FA to human health or beef palatability. In addition, contamination of s.c. or seam fat should be avoided because only a small amount of adipose tissue could have a big influence on the results of fatty acid profile for muscle tissue.

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Table 1

	0
Ingredients	%,DM
Wheat millrun	3.69
Rice bran-high fat	0.93
barley screens	15.44
corn-steam flake	11.02
barley-steam flake	10.99
salt	0.53
Calcium Carbonate	0.26
magnesium oxide, 54% Mg	0.06
Sod Sesouicarb	2.15
Selenium, 0.06%	0.02
Herd bldr flavr	0.03
dairy ade vit pmx	0.01
vitE 25%	0.03
Rout mold inhib	0.05
Rumensin 80	0.02
Trace miner pmx	0.05
Alfalfa hay, early bloom	54.72
Total	100

Ingredient composition of the grain diet

Table 2

Nutrent composition of the gran			
Nutrients	Unit	Level	
Protein	%	18.23	
Fat	%	3.10	
NDF	%	27.84	
ADF	%	19.11	
Dry Matter	%	89.49	
Ca	%	0.94	
Р	%	0.30	
Mg	%	0.29	
K	%	1.65	
Salt	%	0.54	
VitA	IU/g	6.58	
VitD	IU/g	1.13	
VitE	IU/Kg	96.97	
Monensin	g/ton	29.84	

Nutrient composition of the grain diet
				Sampling Site <sup>a</sup>	, b									
Fatty acid	Structure	OSC	ISC	Seam	Marbling	Muscle	SE							
				- mg/g tissue			_							
Total		841.1c	836.2c	844.3c	582.6b	32.3a	12.67							
				- % of total FA			_							
Lauric	C12:0	0.1b	0.1b	0.1b	0.0a	0.0ab	0.01							
Myristic	C14:0	3.7bd	3.9cd	4.2e	3.5b	2.5a	0.07							
Myristoleic	C14:1n5	0.5c	0.6d	0.6cd	0.3b	0.2a	0.03							
Pentadecanoic	C15:0	1.7cd	1.4bc	1.1ab	0.8a	1.8d	0.09							
Palmitic	C16:0	30.9bd	29.9b	31.6cd	30.3bc	27.9a	0.37							
Palmitoleic	C16:1n7	6.6d	5.7c	4.6b	3.8a	4.0a	0.14							
Heptadecanoic	C17:0	1.3b	1.5bc	1.5c	1.8d	1.1a	0.03							
Heptadecenoic	C17:1n7	1.1c	1.0b	0.9bc	0.8bc	0.3a	0.07							
Stearic	C18:0	8.8a	11.1b	14.2c	15.1c	12.4b	0.39							
Vaccenic	C18:1n7	3.7	3.6	3.1	3.4	3.1	0.16							
Trans-vaccenic	C18:1n7t	1.8b	2.1bc	2.2c	2.3c	1.1a	0.10							
Oleic	C18:1n9	37.1c	36.5c	33.3a	34.9b	37.5c	0.40							
Linoleic	C18:2n6	1.1a	1.2a	1.2a	1.4a	3.5b	0.14							
Linolelaidic	C18:2n6t	0.5c	0.6c	0.4bc	0.3ab	0.2a	0.05							
c-9,t-11 CLA	C18:2c9t11	0.5b	0.5b	0.4ab	0.5b	0.3a	0.04							
Gamma- Linolenic	C18:3n6	0.0a	0.0a	0.0a	0.0a	0.1b	0.02							
Linolenic	C18:3n3	0.6a	0.6a	0.6a	0.7a	1.2b	0.04							
Eicosatrienoic	C20:3n6	0.0a	0.0a	0.0a	0.0a	0.3b	0.03							
Arachidonic	C20:4n6	0.0a	0.0a	0.0a	0.0a	1.3b	0.07							
Eicosapentaenoic	C20:5n3	0.0a	0.0a	0.0a	0.0a	0.3b	0.02							
Docosadienoic	C22:2n6	0.0a	0.0a	0.0a	0.0a	0.1b	0.01							
Docosatetraenoic	C22:4n6	0.0a	0.0a	0.0a	0.0a	0.1b	0.01							
Docosapentaenoic	C22:5n3	0.0a	0.0a	0.0a	0.0a	0.5b	0.03							
Docosahexaenoic	C22:6n3	0.0a	0.0a	0.0a	0.0a	0.07b	0.02							
SFA <sup>c</sup>		46.5ab	47.8b	52.6c	51.5c	45.8a	0.49							
MUFA <sup>d</sup>		49.0c	47.2c	42.5a	43.3ab	45.1b	0.48							
SFA:MUFA		1.0a	1.0a	1.3b	1.2b	1.0a	0.02							
PUFA <sup>e</sup>		1.7a	1.8a	1.8a	2.1a	7.6b	0.32							
n-6: n-3 <sup>f</sup>		2.1	2.3	2.2	2.4	2.5	0.18							
Delta-9 desaturase <sup>g</sup>		80.9c	76.8b	70.1a	69.7a	75.2b	0.74							
Elongase <sup>h</sup>		55.0a	57.2h	56 7h	59 5c	61 1c	0 40							

Table 3Fatty acid composition of different fat locations within raw beef steaks

<sup>a</sup> OSC = outer subcutaneous fat; ISC = inner subcutaneous fat.

<sup>b</sup> Means within the same row with different letters are statistically different (P<0.05).

<sup>c</sup> SFA = C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + 20:0.

<sup>d</sup> MUFA = C14:1n5 + C16:1n7 + C17:1n7 + C18:1n9 + C18:1n7.

<sup>e</sup> PUFA = C18:2n6 + C18:3n6 + C18:3n3 + C20:3n6 + C20:4n6 + C20:5n3 + C22:2n6 + C22:4n6 + C22:5n3 + C22:6n3.

		Sampling Site <sup>a, b</sup>								
Fatty acid	Structure	OSC	ISC	Seam	S. mus	l. mus	SE			
				mg/g tissue	<u> </u>					
Total		837.5b	827.9b	789.7b	92.5a	85.1a	18.15			
			9	% of total fa	t					
Lauric	C12:0	0.1	0.1	0.1	0.1	0.1	0.01			
Myristic	C14:0	3.7b	3.9bc	4.1c	3.0a	2.9a	0.08			
Myristoleic	C14:1n5	0.7b	0.7b	0.7b	0.4a	0.4a	0.03			
Pentadecanoic	C15:0	1.4b	1.3b	1.0a	0.9a	0.9a	0.07			
Palmitic	C16:0	31.0ab	30.2a	31.1b	30.2a	29.9a	0.31			
Palmitoleic	C16:1n7	6.8c	5.9b	4.5a	4.3a	4.1a	0.15			
Heptadecanoic	C17:0	1.4b	1.5b	1.6c	1.2a	1.2a	0.03			
Heptadecenoic	C17:1n7	0.5b	0.6b	0.4ab	0.2a	0.2a	0.08			
Stearic	C18:0	8.7a	10.8b	14.5d	12.4c	12.4c	0.39			
Vaccenic	C18:1n7	3.8d	3.6cd	3.1bc	2.4a	2.5ab	0.14			
Trans-vaccenic	C18:1n7t	1.7b	2.0b	2.0b	1.0a	0.9a	0.09			
Oleic	C18:1n9	37.4b	36.7b	33.5a	39.5c	39.7c	0.43			
Linoleic	C18:2n6	1.1a	1.2a	1.2a	2.2b	2.3b	0.07			
Linolelaidic	C18:2n6t	0.6c	0.6c	0.4b	0.3a	0.3ab	0.04			
c-9,t-11 CLA	C18:2c9t11	0.5c	0.4bc	0.4b	0.2a	0.2a	0.03			
Linolenic	C18:3n3	0.6a	0.6a	0.6a	0.8b	0.9b	0.02			
Arachidic	C20:0	0.0a	0.0a	0.0a	0.1b	0.1b	0.01			
Arachidonic	C20:4n6	0.0a	0.0a	0.0a	0.5b	0.7b	0.04			
Eicosapentaenoic	C20:5n3	0.0a	0.0a	0.0a	0.1b	0.1b	0.01			
Docosapentaenoic	C22:5n3	0.0a	0.0a	0.0a	0.2b	0.3c	0.02			
SFA <sup>c</sup>		46.1a	47.7a	53.2b	47.9a	47.5a	0.49			
MUFA <sup>d</sup>		49.4c	47.4b	42.2a	46.8b	46.9b	0.49			
SFA:MUFA		1.0a	1.0a	1.3b	1.0a	1.0a	0.02			
PUFA <sup>e</sup>		1.7a	1.9a	1.8a	3.9b	4.3b	0.13			
n-6: n-3 <sup>f</sup>		2.0a	2.0a	2.0a	2.5b	2.4b	0.08			
Delta-9 Desaturase <sup>g</sup>		81.9c	77.2b	69.7a	76.2b	76.3b	0.77			
Elongase <sup>h</sup>		54.7a	56.8b	56.8b	60.0c	60.4c	0.42			

Table 4 Fatty acid composition of different fat locations within cooked beef steaks

<sup>a</sup> OSC = outer subcutaneous fat; ISC = inner subcutaneous fat; S.mus = surface muscle; I.mus = inner muscle.

<sup>b</sup> Means within the same row with different letters are statistically different (P<0.05). <sup>c</sup> SFA = C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + 20:0.

<sup>d</sup> MUFA = C14:1n5 + C16:1n7 + C17:1n7 + C18:1n9 + C18:1n7.

<sup>e</sup> PUFA = C18:2n6 + C18:3n6 + C18:3n3 + C20:3n6 + C20:4n6 + C20:5n3 + C22:2n6 + C22:4n6 + C22:5n3 + C22:2n6 + C22C22:6n3.

f n-6 = C18:2n6 + C18:3n6 + C20:2n6 + C20:3n6 + C20:4n6 + C22:4n6;

n-3 = C18:3n3 + C20:5n3 + C22:5n3 + C22:6n3.

<sup>g</sup> Delta-9 desaturase = 100\*[C18:1n9 / (C18:0 + C18:1n9)].

<sup>h</sup> Elongase = 100\*[(C18:0+C18:1n9)/(C16:0+C18:0+C16:1n7+C18:1n9)].

		•	Location <sup>a, b</sup>					
Fatty acid	Structure	OSC ISC		Seam	SE			
			—mg/g tissı	ie				
Total		839.3	832.0	817.0	12.49			
			—% of total fa	nt				
Lauric	C12:0	0.1	0.1	0.1	0.01			
Myristic	C14:0	3.7a	3.9a	4.2b	0.07			
Myristoleic	C14:1n5	0.6	0.7	0.7	0.03			
Pentadecanoic	C15:0	1.5c	1.4b	1.0a	0.05			
Palmitic	C16:0	31.0ab	30.1a	31.8b	0.28			
Palmitoleic	C16:1n7	6.7c	5.8b	4.6a	0.19			
Heptadecanoic	C17:0	1.3a	1.5b	1.6c	0.02			
Heptadecenoic	C17:1n7	0.8	0.7	0.6	0.06			
Stearic	C18:0	8.8a	10.9b	14.4c	0.38			
Vaccenic	C18:1n7	3.7b	3.6b	3.1a	0.08			
Trans-vaccenic	C18:1n7t	1.8a	2.0b	2.1b	0.07			
Oleic	C18:1n9	37.3b	36.6b	33.4a	0.44			
Linoleic	C18:2n6	1.1a	1.2b	1.2b	0.02			
Linolelaidic	C18:2n6t	0.6b	0.6b	0.4a	0.04			
c-9,t-11 CLA	C18:2c9t11	0.5b	0.5b	0.4a	0.03			
Linolenic	C18:3n3	0.6	0.6	0.6	0.01			
SFA <sup>c</sup>		46.3a	47.7a	52.9b	0.54			
MUFA <sup>d</sup>		49.2b	47.3b	42.3a	0.56			
SFA:MUFA		1.0a	1.0a	1.3b	0.02			
PUFA <sup>e</sup>		1.7a	1.9b	1.8b	0.03			
n-6: n-3 <sup>f</sup>		2.0	2.1	2.1	0.06			
Delta-9 Desaturase <sup>g</sup>		81.4c	77.0b	69.9a	0.79			
Elongase <sup>h</sup>		54.9a	57.0b	57.0b	0.37			

Table 5The effect of fat locations on fatty acid composition of beef steaks

<sup>a</sup> OSC = outer subcutaneous fat; ISC = inner subcutaneous fat.

<sup>b</sup> Means within the same row with different letters are statistically different (P < 0.05).

<sup>c</sup> SFA = C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + 20:0.

<sup>d</sup> MUFA = C14:1n5 + C16:1n7 + C17:1n7 + C18:1n9 + C18:1n7.

<sup>e</sup> PUFA = C18:2n6 + C18:3n6 + C18:3n3 + C20:3n6 + C20:4n6 + C20:5n3 + C22:2n6 + C22:4n6 + C22:5n3 + C22:6n3.

f n-6 = C18:2n6 + C18:3n6 + C20:2n6 + C20:3n6 + C20:4n6 + C22:4n6;

n-3 = C18:3n3 + C20:5n3 + C22:5n3 + C22:6n3.

<sup>g</sup> Delta-9 desaturase = 100\*[C18:1n9 / (C18:0 + C18:1n9)].

<sup>h</sup> Elongase = 100\* [(C18:0 + C18:1n9) / (C16:0 + C18:0 + C16:1n7 + C18:1n9)].

			Cooking <sup>a</sup>	
Fatty acid	Structure	Raw	Cooked	SE
			-mg/g tissue—	
Total		840.5	818.4	10.20
			% of total fat—	
Lauric	C12:0	0.1	0.1	0.01
Myristic	C14:0	3.9	3.9	0.06
Myristoleic	C14:1n5	0.6a	0.7b	0.03
Pentadecanoic	C15:0	1.4b	1.2a	0.04
Palmitic	C16:0	30.8	31.1	0.23
Palmitoleic	C16:1n7	5.6	5.8	0.16
Heptadecanoic	C17:0	1.4	1.5	0.02
Heptadecenoic	C17:1n7	0.9b	0.5a	0.05
Stearic	C18:0	11.3	11.3	0.31
Vaccenic	C18:1n7	3.5	3.5	0.07
Trans-vaccenic	C18:1n7t	2.0	1.9	0.06
Oleic	C18:1n9	35.7	35.9	0.36
Linoleic	C18:2n6	1.2	1.2	0.02
Linolelaidic	C18:2n6t	0.5	0.5	0.03
c-9,t-11 CLA	C18:2c9t11	0.5	0.4	0.02
Linolenic	C18:3n3	0.6a	0.6b	0.01
SFA <sup>b</sup>		49.0	49.0	0.44
MUFA <sup>c</sup>		46.2	46.3	0.46
SFA:MUFA		1.1	1.1	0.02
PUFA <sup>d</sup>		1.8	1.8	0.03
n-6: n-3 <sup>e</sup>		2.2b	2.0a	0.05
Delta-9 Desaturase <sup>f</sup>		75.9	76.3	0.64
Elongase <sup>g</sup>		56.3	56.1	0.30

Table 6 The effect of cooking on fatty acid composition of subcutaneous and seam fat from beef steaks

<sup>a</sup> OSC = outer subcutaneous fat; ISC = inner subcutaneous fat.

<sup>b</sup> Means within the same row with different letters are statistically different (P<0.05). <sup>c</sup> SFA = C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + 20:0.

<sup>d</sup> MUFA = C14:1n5 + C16:1n7 + C17:1n7 + C18:1n9 + C18:1n7.

 $^{e}$  PUFA = C18:2n6 + C18:3n6 + C18:3n3 + C20:3n6 + C20:4n6 + C20:5n3 + C22:2n6 + C22:4n6 + C22:5n3 + C22:6n3.

f n-6 = C18:2n6 + C18:3n6 + C20:2n6 + C20:3n6 + C20:4n6 + C22:4n6;

n-3 = C18:3n3 + C20:5n3 + C22:5n3 + C22:6n3.

<sup>g</sup> Delta-9 desaturase = 100\*[C18:1n9 / (C18:0 + C18:1n9)].

<sup>h</sup> Elongase = 100\*[(C18:0+C18:1n9)/(C16:0+C18:0+C16:1n7+C18:1n9)].

The influence of diet and aging on beef palatability

#### Abstract

Research was conducted to investigate the influence of diet and aging on beef steak and ground beef palatability and lipid oxidative stability. Crossbred calves were assigned to Feedlot (finished on alfalfa and grain), Forage1 (triticale and annual rye grass), Forage2 (triticale and kale), or Combination (grazing rye, fescue and orchard, finished on alfalfa and grain) dietary treatment. Heifers were finished on alfalfa and grain (EBA). Steaks and ground beef samples were collected from these animals. Steaks were either dry- or wet-aged for 14d. Ground beef samples were dry-aged, wet-aged for 14d, or not aged. Trained sensory panels were conducted to evaluate palatability attributes of beef steaks and ground beef. There was no (P>0.05) effect of diet or aging on cooking weight loss. Diet and aging treatment did not (P>0.05) influence the palatability of beef steaks. Diet did not (P>0.05) influence the palatability of ground beef, either. However, aging impacted (P < 0.05) ground beef sensory attributes and the influences depended on dietary treatment or possibly animal sex. In general, aging negatively affected ground beef palatability; furthermore, dry-aging seemed to have more negative effects than wet-aging. In addition, dietary and aging treatments had no (P>0.05) impact on lipid oxidative stability of raw ground beef.

Keywords: Diet; Aging; Beef steaks; Ground beef; Palatability; Lipid oxidation

#### 1. Introduction

Palatability is one of the important factors in beef quality. Palatability is related to tenderness, flavor, and juiciness (Umberger, Feuz, Calkins, & Killinger, 2000). Some pre- and post-harvest management has been demonstrated to impact beef palatability attributes. For example, grass-fed beef has been criticized for lower palatability (Hedrick, 1983). Larick et al. (1987) suggested the

greatest sensory difference between grain- and forage-fed beef was the flavor of fat. In addition, Mitchell, Reed, & Rogers (1991) reported that forage finishing had a negative effect on beef tenderness. However, Marino, Albenzio, Girolami, Muscio, Sevi, & Braghieri (2006) found no influence of diets, with different forage to concentrate ratios, on beef flavor or tenderness determined by instrumental or sensory approach. Nevertheless, post-harvest techniques could be used to improve eating quality of beef or minimize the impact of diet systems. One of the most popular options is postmortem aging. It has been well accepted that postmortem aging can increase meat tenderness. However, controversy still exists about the influence of aging on palatability aspects other than tenderness, like aroma, flavor, and juiciness (Gorraiz, Beriain, Chasco, & Insausti, 2002; Stetzer, Tucker, McKeith, & Brewer, 2007).

Most studies investigating the influence of diet or aging on beef palatability used beef steaks as experimental subjects, and few studies have been conducted to explore the interaction between diet systems and aging methods. Therefore, the objectives of this paper were to study the influence of diet and aging on the palatability attributes for both beef steaks and ground beef and to demonstrate the interaction between diet and aging.

#### 2. Materials and methods

#### 2.1. Animals and pre-, post-harvest management

Crossbred calves were assigned to one of the following dietary treatments: Feedlot (S; traditional finished in feedlot on alfalfa and grain), Forage1 (TR; grazing triticale and annual rye grass, finished on forage harvested from the other half of the field), Forage2 (TK; grazing triticale and kale, finished on forage harvested from the other half of the field), and Combination

(grazing a pasture mix of rye, fescue and orchard, finished on alfalfa and grain). Heifers were finished on alfalfa and grain (Feedlot, H).

Animals were harvested at the University of Nevada, Reno (UNR) Meat Laboratory following USDA humane slaughter procedures. Five and four animals were sub-sampled within each diet treatment, respectively, to get steaks and ground beef samples. Two longissimus muscle steaks and three trimmed triceps muscle samples were collected from each animal. Steaks were either wet-aged in vacuum bags or dry-aged on the carcass for 14d. Trimmed triceps muscle samples were un-aged (removed from carcass at 2d postmortem, vacuum packaged, and frozen immediately), dry-aged on the carcass, or wet-aged in vacuum bag for 14d.

#### 2.2. Sample preparation

Trimmed triceps muscle was cut into pieces and ground by table top meat grinder (model MG-203100; BUNZL Processor Division, Kennewick, WA) under cooler condition. Samples were ground twice with coarse cutting plate (1cm diameter) and fine cutting plate (0.5cm diameter). 10 cm-diameter and 1.5 cm-thick patties were made by a patty maker (Progressive International, Kent, WA). Steaks and patties were stored at -20 °C. A total of fifty beef steaks and sixty ground beef samples were measured cooking loss ([(weight before cooking – weight after cooking) / weight before cooking]\*100) and evaluated palatability attributes by a trained laboratory panel, separately.

Steaks were thawed at 3-4 °C for 48h, weighed and then cooked on a preheated ( $229 \pm 5$  °C) Farberware Open Hearth Grills (model R4550; Farberware, Bronx, NY). Geometric center temperature was monitored by a 12-Channel Scanning Thermocouple Thermometer (Model 692-8010, Barnart, Barrington, IL). Steaks were turned over when central temperature reached 40 °C and removed at 71 °C (3.5-4 degree of doneness; Romans, Costello, Carlson, Greaser, & Jones, 2001). Steaks were weighed; then a 2.5 cm-thick, 4 cm-long slice was remove from the lateral end of each steak, parallel to the muscle fibers for slice shear force (SSF) measurement (Wheeler, Shackelford, & Koohmaraie, 2007). These standardized slices were cooled to room temperature (21 °C) and measured on a TA-XT2 Texture Analyzer (Texture Technologies, Scarsdale, NY) fitted with a flat blade designed for SSF. The slices were positioned so that they would be sheared in the center, perpendicular to the muscle fibers along the 4 cm dimension of the slices. The left of the steak was cut into 1×1×2.5 cm cubes without visible connective or fat tissue, served warm for sensory evaluation. Steam stable was used when necessary to keep samples warm before serving.

Frozen patties were weighed and cooked on a preheated ( $182 \pm 2 \, {}^{\circ}C$ ) George Foreman grill (model GR12; Salton, Miramar, FL) for about 8min to reach a central temperature of 68  ${}^{\circ}C$ , monitored by a 12-Channel Scanning Thermocouple Thermometer (Model 692-8010, Barnart, Barrington, IL). Then the patties were removed, weighed again and wrapped in aluminum foil (where temperature of patties increased about 3  ${}^{\circ}C$ ) until being cut into twelve pie-shaped pieces for sensory evaluation.

#### 2.3. Sensory evaluation

Two separate nine-member trained sensory panels were conducted to evaluate palatability attributes of beef steaks and ground beef (AMSA, 1995). Samples were randomly assigned with diet and aging treatment as balanced as possible across sessions. Six warm samples were served to panelists in individual booths per session under fluorescent light ( $512 \pm 13$  Lux, measured by Traceable Dual-range Light Meter, Control, Friendswood, TX). Steaks were evaluated for flavor,

off-flavor, initial tenderness, sustained tenderness, and juiciness; ground beef was evaluated for beef aroma, off-aroma, beef flavor, off-flavor, tenderness, and juiciness, both on a 10-cm unstructured line scale labeled at each end (Stone & Sidel, 1985). Each panelist was supplied unsalted crackers to cleanse the palate, distilled water to rinse, and a cup for expectoration. A ruler was used to determine the panelists' scores and the results were expressed in centimeters.

#### 2.4. Lipid oxidation measurement

All raw ground beef samples and 55% cooked ground beef samples were collected to assess the amount of lipid oxidation. Approximately 100-200mg tissue pieces were pre-weighed and homogenized with an Omni Tissue Homogenizer (Omni Int., Marietta, GA) in 1ml of RIPA Buffer (50mM Tris-HCl, pH 8.0, with 150mM sodium chloride, 1.0% Igepal (NP-40), 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate) (Sigma R0278) with a protease inhibitor cocktail (Sigma P2714). Tissue homogenates were centrifuged at 8,000rpm (3000 x g) at 4°C for 10 minutes in a Sorvall table top centrifuge (Thermo Fisher Scientific, Waltham, MA). 100ul of the supernatant from each sample was used for the 2-thiobarbituric acid reactive substances (TBARS) assay. 200ul of 10% (w/v) Trichloroacetic Acid (TCA) was added to the 100ul of supernatant from each sample to precipitate protein from the sample. They were incubated on ice for 15 minutes. The samples were then centrifuged at 6.000 rpm (2200 x g) at 4°C for 15 minutes to pellet the precipitated protein. Protein is removed from the assay to reduce interference. 200ul of the supernatant of each sample was combined with 200ul of 0.67% (w/v) 2-thiobarbituric acid (TBA). The samples were boiled at 100°C for 10 minutes. The absorbance of each sample was read at 532nm. Known Malondialdehyde (MDA) standards were used to determine the amount of MDA (nmoles / g wet weight of tissue) present in each sample.

#### 2.5. Statistical analysis

Normality of data was confirmed by Proc univariate of SAS (SAS Inst. Inc., Gary, NC). The consistency of panelist performance across test sessions was tested by a completely randomized design (CRD) with a model including panelist, sample, session, and panelist by session interaction. Residual error was used as the error term. Taste panel data were averaged across panelist, then analyzed as split-plot design with diet in whole-plot, aging and diet by aging interaction in sub-plot. Animal (diet) was the error term for diet and aging by animal (diet) interaction was the error term for aging and diet by aging interaction. Slice analysis of diet by aging interaction was conducted when the interaction was significant (P<0.05; Winer, 1971) to test simple effect of diet or aging. Data were further analyzed in a CRD with diet or aging as treatment when simple effect was indicated significant by slice analysis. Tukey's test was used to make all pairwise comparisons. Correlations among variables were measured by Pearson's Correlation Coefficient (simple). All analyses were conducted using SAS (SAS Inst. Inc., Gary, NC).

#### 3. Results

#### 3.1. Cooking weight loss and shear force

There was no interaction (P>0.05) between diet and aging for SSF or cooking weight loss. There were no (P>0.05) effects of diet or aging on SSF ( $17.4 \pm 3.8$  kg) or cooking weight loss for steak ( $22.4 \pm 6.4\%$ ) or ground beef ( $33.4 \pm 4.7\%$ ).

#### 3.2. Sensory evaluation

Data from one panelist was dropped to eliminate the interaction (P>0.05) between panelist and session, so the performance of panelists was consistent across test sessions. There was no interaction (P>0.05) between diet and aging, no effect (P>0.05) of diet or aging on sensory attributes evaluated for beef steaks (Table 1). The average score of Flavor, Off-flavor, Initial Tenderness, Sustained Tenderness, or Juiciness was 5.8, 1.4, 6.5, 6.1, and 6.4, respectively.

There was no interaction between diet and aging for ground beef flavor, off-aroma, or off-flavor. Therefore, main effect was used to assess the influence of diet or aging on these sensory attributes. Diet had no impact (P>0.05) on these attributes; but aging had significant effect on beef flavor. Evaluation scores for off-aroma and off-flavor were  $0.8 \pm 0.06$  and  $0.5 \pm 0.12$ , respectively. Un-aged ground beef had more (P<0.05) beef flavor than aged groups ( $5.8 \pm 0.11$  of un-aged vs.  $5.3 \pm 0.11$  of dry-aged or  $5.4 \pm 0.11$  of wet-aged). In addition, the p-value for the effect of diet on ground beef flavor was relatively small (0.053), Tukey's pairwise comparison showed that the major difference came from diet Feedlot (H) ( $5.8 \pm 0.18$ ) and Forage (TK) ( $5.0 \pm 0.18$ ; p = 0.056).

There were interactions (P<0.05) between diet and aging for ground beef aroma, tenderness, and juiciness (Fig. 1-3). Diet had no impact on these sensory attributes; but aging significantly affected these attributes. Slice analysis showed that aging impacted (P<0.05) beef aroma of ground beef from Feedlot (S) or Forage + Feedlot dietary treatment, tenderness of beef from Feedlot (H), Feedlot (S), or Forage (TK), and juiciness of beef from Feedlot (H), Feedlot (S), or Forage (TR). Simple effect of aging within these dietary treatments was analyzed and the results were shown in Fig. 4-6. Dry-aging decreased beef aroma by 17.7 and 12.1% in Feedlot (S) and by 23.3 and 25.8% in Forage + Feedlot, respectively, compared to un-aged or wet-aging. The

trained panelists could tell the difference of 0.7 based on the 10cm unstructured line scale, which indicated the high acuity of the panelists. Different from the results of slice analysis, the effect of aging on beef tenderness was not significant for Feedlot (H) group using Tukey's comparison, but the p-value was small (P = 0.11). Tenderness score of dry-aged ground beef (5.6) was lower than the un-aged (6.6) or wet-aged (6.3) from Feedlot (H). Wet-aging decreased (P<0.05) tenderness of ground beef by 18.6% compared to the un-aged from Feedlot (S). It is interesting that the effect of aging in Feedlot (S) was different from that in Feedlot (H), which might indicate that sex could also influence the effect of aging. Similar to Feedlot (H), in Forage (TK) group dry-aging decreased (by 12.1%; P<0.05) tenderness compared to un-aged or wet-aging. The panelists could tell the difference of 0.8 based on 10cm unstructured line scale, indicating their high acuity. Similar to the effect of aging on beef tenderness for Feedlot (H), the effect of aging on beef juiciness was not significant in Tukey's comparison (P = 0.17), though slice analysis demonstrated significance (P<0.05). Dry-aging (3.7) seemed to decrease juiciness compared to un-aged (4.8) or wet-aging (5.0) in Feedlot (H) group. Aging decreased (P < 0.05) juiciness compared to un-aged in Feedlot (S) group; dry- and wet-aging decreased juiciness score by 25 and 33.9%, respectively. In Forage (TR) group, dry-aging decreased (P<0.05) juiciness by 26% compared to wet-aging. The panelists could tell the difference of 1.3 based on the 10cm unstructured line scale. Complete sensory evaluation scores for ground beef were shown in Table 2.

#### *3.3. Lipid oxidation*

There was no interaction (p = 0.07) between diet and aging treatments on TBARS of raw ground beef. Dietary or aging treatments had no impact on TBARS of raw samples, either (p=

0.11; p=0.97; Table 2). TBARS of raw and cooked ground beef patties were  $22.8 \pm 3.87$  and  $12.08 \pm 1.44$  nmoles/g wet weight of tissue, respectively. TBARS of raw samples was not correlated with that of cooked samples (r = 0.27; p = 0.12), which meant lipid oxidation status of ground beef had changed during cooking. TBARS of cooked samples were not correlated with off-aroma (r = 0.17; p = 0.34) or off-flavor (r = 0.15; p = 0.42).

#### 4. Discussion

#### 4.1. Effect of diet on beef palatability

Previous studies reported a negative effect of forage finishing on tenderness (Mitchell, Reed, & Rogers, 1991) and flavor (Melton, 1983) of meat. Two possible reasons for less desirable flavor of grass-fed beef have been proposed: a lower fat content (Harrison, Smith, Allen, Hunt, Kastner, & Kropf, 1978) and the fatty acid composition (Westerling & Hedrick., 1979). Brown, Melton, Riemann, & Backus (1979) found that grass-fed ground beef still had a less desirable flavor than grain-fed ground beef when they contained the same amount of fat. Similarly, in the study by Melton, Amiri, Davis, and Backus (1982) ground beef from grass-fed steers had a less desirable flavor than ground beef from limited grain-fed steers. Since the ground beef prepared in their study showed no differences in fat content, the differences in flavor of ground beef due to diet were not related to fat content. Based on the assessment from untrained panelists, there was no trend toward higher intensity of green flavor in the ground beef with less desirable flavor from grass-fed steers. Instead, the reasons for the less desirable ground beef flavor were the lack of beef fat flavor and some other undesirable notes. In our study, diets only affected the offflavor of un-aged ground beef. Off-flavor score of beef from Forage(TR) diet was higher than that from Feedlot(H), Feedlot(S), or Combination diet. Melton et al. (1982) also discussed the

influence of diets on fatty acid composition of beef and the correlation of certain fatty acids with beef flavor score. Grass-based diet caused higher concentrations of C18:3 in ground beef. C18:3 had the largest negative correlation of all fatty acids with desirable beef flavor score. However, in the study by Marino, Albenzio, Girolami, Muscio, Sevi, & Braghieri (2006) diets with different forage to concentrate ratios had no influence on beef flavor or tenderness which was determined by both instrumental and sensory approach. Similarly, we also found no effect of diets on flavor, off-flavor, tenderness, or juiciness of beef steaks.

The influence of diet on beef palatability is likely due to the alteration of fatty acid composition of beef muscle (Ford, Park, & Ratcliff, 1976) because degradation of lipid is one of the main sources of volatiles in cooked meat (Mottram, 1994). Heat-induced oxidation of fatty acids, particularly unsaturated fatty acids, produces compounds that may have intrinsic flavors and these products may further react with Maillard products, producing other compounds that may contribute to flavor (Elmore, Mottram, Enser, & Wood, 1997). C20 and C22 PUFA are present only in the polar lipids (Ashes, Siebert, Gulati, Cuthbertson, & Scott, 1992) and the phospholipids are considered to be the main targets for lipid oxidation reactions because of their higher degree of unsaturation. In addition, these PUFA are exposed to proteins and other catalysts of lipid oxidation, which also are components of cell membranes (Boylston, Morgan, Johnson, Wright, Busboom, & Reeves, 1996). The oxidative degradation of fatty acids involves a free radical mechanism (Frankel, 1980). The initial step, which involves the formation of an alkyl radical from an unsaturated fatty acid (UFA), is critical for the rate of the oxidative degradation reaction. These radicals are formed much more readily from PUFA like C20:4, C20:5, and C22:6 than from C18:1 or C18:2. Once the free radical reaction started, the subsequent chain reaction is less dependent on the degree of saturation of fatty acids. Therefore,

PUFA appear to induce the thermal degradation of oleic and linoleic acids, which are most abundant UFA and di-UFA in meat, respectively. This theory was demonstrated in a study by Elmore, Mottram, Enser, and Wood (1999).

#### 4.2. Effect of aging on beef palatability

A previous study (Stetzer, Tucker, McKeith, & Brewer, 2007) demonstrated that aging increased sensory tenderness and decreased rancid off-flavor of beef steaks from round, chuck, and loin muscle. These results disagree with what we found in the present study and there were several possible reasons to explain the difference. First of all, they did not have an un-aged treatment like we did. Second, they used steaks instead of ground beef, which was mechanically ground and therefore tenderness modified. Finally, beef in their study was enhanced with phosphate/salt-containing solutions, which may have mask off-flavors originally present or developed after aging. In another study (Campbell, Hunt, Levis, & Chambers IV, 2001) dryaging for 14 or 21d increased (P<0.05) tenderness, juiciness and dry-aged flavor of loin steaks were small, with tenderness score 10.6 vs. 10.0, juiciness 8.4 vs. 8.3, aged flavor 10.6 vs. 9.7 (based on a 15-point scale). It should be noted that statistical significance does not mean biological difference sometime.

Parrish, Jr., Boles, Rust, and Olson (1991) found only slight differences in tenderness (6.20 vs. 6.36) and overall palatability scores (6.18 vs. 6.28) of rib and loin steaks dry- or wet-aged for 21d (P<0.01), based on an 8-point descriptive scale. There was no difference in juiciness, flavor intensity or desirability between dry- and wet-aged groups. Similar, we also found no influence of aging method on beef steak palatability attributes. Nevertheless, Sitz, Calkins, Feuz,

Umberger, and Eskridge (2006) suggested that the impact of aging technology depended on USDA grade of carcass. Consumers detected no difference for sensory traits between wet-aged and dry-aged Choice strip loin steaks; but they found wet-aged Prime strip loin steaks more desirable than dry-aged samples. But again the sensory evaluation scores were very similar for wet- and dry-aged steaks, though statistically significant.

Bruce, Beilken, and Leppard (2005) studied the influence of different production and aging regimes on sensory attributes of *m. longissimus thoracis et* lumborum steaks. Diet (grass- and grain-finished) and aging (unaged and aged) counted for 44.1 and 22.9% of the total variance, respectively. Aging beef (from steers with small production variation) up to 14d increased (P<0.05) tenderness, however, beef aroma and flavor were only improved slightly; while juiciness was unchanged. They also indicated that aging could not efficiently decrease the variation in tenderness or juiciness of steaks from different production regimes.

### 4.3. Lipid oxidation and beef quality

Oxidative damage is the major non-microbial factor that causes the quality deterioration of muscle foods (Descalzo et al., 2005). Oxidation is indicated by a conversion of the red muscle pigment myoglobin to brown metmyoglobin and the development of rancid odors and flavors due to the degradation of PUFA in the tissue membranes (Wood & Enser, 1997). Susceptibility of muscle foods to oxidative reactions is mainly attributed to their high concentrations of prooxidants (Rhee & Ziprin, 1987) and unsaturated lipids (Asgar, Gray, Buckley, Pearson, & Booren, 1988). Lipid oxidation is a major deterioration reaction and is positively correlated with pigment oxidation (Liu, Lanari, & Schaefer, 1995). Chan, Faustman, and Decker (1997) reported that secondary products of lipid oxidation (e.g. aldehydes) could accelerate oxymyoglobin

oxidation. In contrast to our observations, dietary treatments have been reported to affect lipid oxidative stability of beef. Nuernberg et al., (2005) reported that the oxidative stability of muscle from grass-based bulls was significantly higher compared to concentrate-based bulls due to a higher concentration of vitamin E in the muscle of grass-based bulls. But with similar contents of  $\alpha$ -tocopherol, pasture-fed beef was more susceptible to lipid oxidation following aging than vitamin E supplemented grain-fed beef, due to a higher proportion of peroxidisable lipids in pasture-fed beef (Yang, Lanari, Brewster, & Tume, 2002). However, it should be noticed that in current study TBARS were measured on ground beef instead of steaks, which may be one reason for different results from previous studies.

### 5. Conclusion

Diet or aging had no effect on palatability attributes of beef steaks. In another word, trained panelists did not detect differences between forage-based and concentrate-based steaks or between dry- and wet-aged steaks. The impact of aging on ground beef palatability differed among diet systems. In general, 14d-aging compromised ground beef palatability instead of improving it. Furthermore, dry-aging seemed to have more negative effects than wet-aging. In addition, diet or aging treatments had no impact on lipid oxidative stability of raw ground beef.

The acuity of the trained panelists seemed relatively high, so it is possible that consumers may not tell the differences caused by aging for the ground beef. Actually aging had no positive impact on ground beef palatability in this study. Therefore, this study indicated that it was not necessary to age ground beef. In addition, the trained panel did not tell the difference between dry- and wet-aged steaks, so wet-aging was suggested for cheaper and more efficient production.

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		Diet <sup>a</sup>											
	Feedlot	(heifer)	Feedlo	t(steer)	Fora	ge(TR)	Forag	ge(TK)	Forage-	+Feedlot			
	Aging												
Attribute <sup>b</sup>	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	SE		
Flavor	6.3	6.1	5.1	5.7	5.9	6.6	5.1	5.9	5.7	6.1	0.47		
Off-flavor	1.6	1.5	1.1	1.2	1.6	2.2	1.9	1.0	1.1	1.3	0.32		
Initial Tenderness	6.4	6.7	6.5	6.8	6.3	6.6	6.4	6.2	6.6	6.5	0.37		
Sustained Tenderness	6.8	6.7	5.4	5.9	6.0	5.7	6.2	6.3	5.9	6.1	0.50		
Juiciness	7.1	7.1	5.7	6.4	6.4	6.3	6.3	6.5	6.3	6.3	0.55		

# Table 1 Sensory evaluation scores of beef steaks (10 cm unstructured line scale)

<sup>a</sup>Feedlot(heifer) = Heifers finished on alfalfa and grain, EBA;

Feedlot(steer) = Steers finished on alfalfa and grain, Feedlot;

Forage(TR) = Steers grazing triticale and annual rye grass, Forage1;

Forage(TK) = Steers grazing triticale and kale, Forage2;

Forage+Feedlot = Steers grazing rye, fescue, and orchard, then finished on alfalfa and grain, Combination.

<sup>b</sup> There was no diet\*aging interaction, no effect of diet or aging on sensory attributes evaluated for beef steaks (P>0.05).

								Diet <sup>a</sup>								
	Fe	edlot(heife	er)	Fe	edlot(stee	er)		Forage(TR) Aging	)		Forage(TK	)	Foi	Forage+Feedlot		
Attribute <sup>b</sup>	Un-aged	Dry	Wet	Un-aged	Dry	Wet	Un-aged	Dry	Wet	Un-aged	Dry	Wet	Un-aged	Dry	Wet	SE
Beef Aroma	6.3	5.5	5.7	6.2	5.1	5.8	5.1	5.6	5.6	5.4	5.3	5.1	6.0	4.6	6.2	0.31
Off-aroma	0.4	0.9	0.7	0.6	1.1	0.5	1.4	0.7	0.8	0.8	1.0	0.9	0.6	1.1	0.8	0.18
Beef Flavor	6.1	5.9	5.4	6.3	5.6	5.3	5.2	5.2	5.7	5.4	4.9	4.8	6.1	5.0	5.7	0.31
Off-falvor	0.6	1.5	1.4	0.8	2.4	1.5	2.1	2.3	1.2	1.5	1.2	2.4	1.2	1.7	1.5	0.47
Tenderness	6.6	5.6	6.3	7.0	6.2	5.7	6.3	5.9	6.6	6.6	5.8	6.6	6.1	6.3	5.7	0.29
Juiciness	4.8	3.7	5.0	5.6	4.2	3.7	4.7	3.7	5.0	4.3	3.6	4.1	4.4	4.2	4.3	0.42
						-	TBARS <sup>c</sup> , nmoles	/ mg wet	weight of tis	sue						
	13.5	13.1	16.5	7.9	9.1	7.9	7.8	20.8	22.1	15.6	12.6	6.9	16.3	7.7	7.0	3.92

#### Table 2 Sensory evaluation scores of ground beef (10 cm unstructured line scale)

<sup>a</sup>Feedlot(heifer) = Heifers finished on alfalfa and grain, EBA;

Feedlot(steer) = Steers finished on alfalfa and grain, Feedlot;

Forage(TR) = Steers grazing triticale and annual rye grass, Forage1;

Forage(TK) = Steers grazing triticale and kale, Forage2;

Forage+Feedlot = Steers grazing rye, fescue, and orchard, then finished on alfalfa and grain, Combination.

<sup>b</sup> There were diet\*aging interactions for beef aroma, tenderness, and juiciness of ground beef (P<0.05).

<sup>C</sup> TBARS = 2-thiobarbituric acid reactive substances ;

There was no diet\*aging interaction, no diet or aging effect on TBARS of raw gound beef (P>0.05).



Fig.1. Interaction between diet and aging for ground beef aroma (P<0.05)

<sup>a</sup> Feedlot(H) = Heifers finished on alfalfa and grain, EBA;

Feedlot(S) = Steers finished on alfalfa and grain, Feedlot;

Forage(TR) = Steers grazing triticale and annual rye grass, Forage1;

Forage(TK) = Steers grazing triticale and kale, Forage2;

Forage+Feedlot = Steers grazing rye, fescue, and orchard, then finished on alfalfa and grain, Combination.





<sup>a</sup> Feedlot(H) = Heifers finished on alfalfa and grain, EBA;

Feedlot(S) = Steers finished on alfalfa and grain, Feedlot;

Forage(TR) = Steers grazing triticale and annual rye grass, Forage1;

Forage(TK) = Steers grazing triticale and kale, Forage2;

Forage+Feedlot = Steers grazing rye, fescue, and orchard, then finished on alfalfa and grain, Combination.



Fig.3. Interaction between diet and aging for juiciness of ground beef (P<0.05)

<sup>a</sup> Feedlot(H) = Heifers finished on alfalfa and grain, EBA;

Feedlot(S) = Steers finished on alfalfa and grain, Feedlot;

Forage(TR) = Steers grazing triticale and annual rye grass, Forage1;

Forage(TK) = Steers grazing triticale and kale, Forage2;

Forage+Feedlot = Steers grazing rye, fescue, and orchard, then finished on alfalfa and grain, Combination.





<sup>†</sup> Feedlot(S) = Steers finished on alfalfa and grain, Feedlot;

Forage+Feedlot = Steers grazing rye, fescue, and orchard, then finished on alfalfa and grain, Combination.

\* Within Feedlot(S) or Foraged+Feedlot, data with different letters were statistically different (P<0.05). Standard errors for Feedlot(S) and Forage+Feedlot were 0.17 and 0.23, respectively.



Fig.5. Effect of aging on tenderness of ground beef

\* Feedlot(H) = Heifers finished on alfalfa and grain, EBA; Feedlot(S) = Steers finished on alfalfa and grain, Feedlot; Forage(TK) = Steers grazing triticale and kale, Forage2;

\* Within Feedlot(H), Feedlot(S) or Forage(TK), data with different letters were statistically different (P<0.05). Standard errors for Feedlot(H), Feedlot(S) and Forage(TK) were 0.30, 0.27, and 0.18, respectively.





† Feedlot(H) = Heifers finished on alfalfa and grain, EBA; Feedlot(S) = Steers finished on alfalfa and grain, Feedlot; Forage(TR) = Steers grazing triticale and annual rye grass, Forage1;

\* Within Feedlot(H), Feedlot(S) or Forage(TR), data with different letters were statistically different (P<0.05). Standard errors for Feedlot(H), Feedlot(S) and Forage(TR) were 0.47, 0.28, and 0.30, respectively.

## Conclusion

Fatty acid composition of beef steaks differed depending on the location of fat. Especially, it is necessary to notice the difference between fat depots and muscle tissue because it is easier to sample subcutaneous and seam fat for fatty acid analysis but beef steaks are usually trimmed before eating. Therefore, sampling muscle tissue should be more accurate to evaluate the contribution of fatty acids to human health or beef palatability. In addition, contamination of subcutaneous or seam fat should be avoided because only a small amount of adipose tissue could have a big influence on the results of fatty acid profile for muscle tissue due to the relatively high fat content of fat depots compared to muscle tissue. Cooking did not have dramatic influence on fatty acid composition of subcutaneous or seam fat. However, further analysis is needed to compare the fatty acid composition of muscle tissue before and after cooking.

Although previous studies reported that grass-based beef was less palatable than concentratebased beef and that dry-aged beef steaks possessed a unique desirable dry-aged flavor, diet or aging treatments did not (P>0.05) influence the palatability of beef steaks in current study. Therefore, whether it is worthy to dry age beef with a higher cost is still a question. For ground beef, diet by aging interaction existed for beef aroma, tenderness, and juiciness. Diet did not significantly influence ground beef palatability. However, aging impacted (P<0.05) ground beef sensory attributes and the influences depended on dietary treatment or possibly animal sex. In general, aging negatively affected ground beef palatability. Therefore, this study indicated that it would not be necessary to age ground beef. In the future, it will be interesting to analyze fatty acid composition of the ground beef to demonstrate the influence of diet on fatty acids and the potential relationship between fatty acids and beef quality.