Carcass, Sensory, Quality and Instrumental Color Characteristics of Serially Harvested Forage-Fed Beef

by

Clinton Wade Rowe

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Approved by

Christopher R. Kerth, Chair, Associate Professor of Animal Sciences
Russell B. Muntifering, Professor of Animal Sciences
Kyle R. Willian, Affiliate Professor of Animal Sciences
Christy L. Bratcher, Assistant Professor of Animal Sciences

Abstract

Striploins were used in two successive studies to evaluate the effects of serially harvested forage-finished steers and forage vs. grain-finished beef. The first study included six groups of steers that were serially harvested over 280 d while grazing forages. Average daily gain, fiber type determination, sensory evaluation, Warner-Bratzler shear force, thiobarbituric acid reactive substances (TBARS), fatty acid profile, instrumental color characteristics, and carcass characteristics were determined. Data were analyzed using mixed-model procedures with fixed effects of days on forage. Results indicate that animals that had increased days on forage had increased BW, HCW, LM areas and marbling scores. Fatty acid profiles were impacted by the different days on forage. Forage type appears to have no impact ($P > 0.05$) on sensory, quality or fatty acid profile characteristics in beef. In the second study, striploins from forage- or grain-fed cattle were procured for comparison of quality and sensory characteristics. Striploins were aged 21 d and cut into steaks for analyses of fresh and display quality and sensory evaluation to determine the effects of both grain-and forage-finishing on fresh and display steaks. Analyses included fresh Warner-Bratzler shear force, sensory evaluation, fatty acid profile, TBARS, and display Warner-Bratzler shear force, sensory evaluation and TBARS. Instrumental color characteristics were determined on display steaks while in simulated retail display. Data were analyzed using mixed-model procedures with finishing type and aging period as fixed effects. Results indicate that steaks from grain-finished cattle were more tender ($P < 0.05$) while steaks from forage-finished were juicier ($P < 0.05$). Steaks from grain-finished cattle had more ($P < 0.05$) mg fatty acid / mg meat than steaks from forage-fed cattle. Steaks from forage-fed cattle

had a lower ($P < 0.05$) n6:n3 ratio than steaks from grain-finished cattle. Results from both studies indicate that forage-finishing of beef can yield steaks with similar juiciness to grain-finished beef and greater amounts of n-3 fatty acids. However, tenderness and consistency issues need to be addressed in future research.

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Chapter I

Introduction

Cattle have been produced in the United States since the Spaniards brought them up from Mexico in the early 1500s (NCBA, 2010). Traditionally, the basic premise of the production of beef cattle through the years has been basically the same with the idea of putting as much weight on them as quickly as possible. For many years, beef cattle grazed native pasture lands all over the continental United States and when the cattle were ready, they were shipped to market. This was made famous by the old-time cattle drives that took place in the 1880s and 1890s. There have been many changes since the days of driving cattle.

After the Second World War, the cattle industry started to shift toward a more centralized feeding strategy (Schupp et al., 1980) that involved using feed grains to increase growth and finish on the cattle. Most of these feedlots are located in the High Plains and Midwest regions of the United States (Ward and Schroeder, 2002), because of the abundance of feed grains in the region. This localization of the feeding operations caused a shift in the locations of the packing plants that had been located in major cities to be moved closer to the where the cattle are fed (Ward and Schroeder, 2002).

The feeding section of the beef industry is very efficient in providing high quality beef products. However, in recent years, there has been a demand from consumers and producers to produce more forage-fed beef as an alternative to conventional grain-fed beef (ERS-USDA, 2010). The southeastern United States climate is not well suited for the production of cattle in a dry lot facility much like a feedlot because of the climate and remote distance to the packers and

feedstuffs. However, this region is very well suited for the production of forages (Allen et al., 1996). The southeastern states have a large number of brood cows that produce calf crops every year (USDA-NASS, 2007). For a number of years, these cattle have been sent to the feedlots in the High Plains and fed out on grain and harvested. This makes it virtually impossible for producers to keep their investment local, and pits them against producers in various sections of the United States. The idea of finishing beef cattle on forages is far from being a groundbreaking or new idea, but the idea of finishing cattle on forages at a commercial level in the 21st century is a novel concept. Over the past several years, the cost of feedstuffs for the beef cattle industry have risen dramatically (ERS, USDA, 2009). In previous years, forage-fed beef was marginally economically viable, but with rising cost associated with grain-feeding is now more viable.

The lack of widespread consumer acceptance of forage-fed beef over the years has occurred because of color of the muscle and fat, flavor and tenderness acceptability (Bowling et al., 1977). Reasons for the lack of consumer acceptance include forage type, growth rate and breed of cattle. The high-concentrate diet in the feedlot allows for many defects that are inherently found in an animal or breed to be diluted. However, that is generally not the case with forage-fed beef. The differences in forage type can have a major impact because of the plant constituents within each respective forage type. These plant constituents can elicit differing effects on the resulting meat of an animal, mainly in the fatty acid composition (Dewhurst et al., 2003).

Research Objectives

The use of forage-fed beef growth models has not been used on a large scale for many years. However, with the cost of inputs increasing, the increase in demand from consumers and a lack of new relevant research for producers and researchers, two projects were performed.

The objectives of the first project were to: 1) investigate differences in carcass and growth characteristics, fatty acid profiles and instrumental color characteristics of steers grazing cool and warm-season forages; 2) investigate effects of multiple aging periods on sensory and quality characteristics of steers grazing cool and warm-season forages.

The comparison of forage-finished beef with conventional beef was the second project undertaken. The objectives were to: 1) investigate differences in instrumental color characteristics during seven days of simulated retail display; 2) compare sensory and quality characteristics of conventional and forage-finished beef; 3) compare fatty acid profiles of conventional and forage-finished beef.

Chapter II

Review of Literature

Production Systems

Forage-Finishing Systems

There are many different systems that can be utilized depending on desired results and geographic location. Small grains are typically used in the cooler months of the year, and perennial grasses are used in the warmer months of the year. Differing geographic locations generally dictate the type of small grains used. The High Plains and Midwest regions will generally use a variety of wheat because it can be harvested to provide extra income after the cattle are removed (Malinowski et al., 2005). Other areas, where crop production is less significant, such as the Southeast, other forages such as rye, oats and annual ryegrass are used.

The warm-season forages used in beef production vary depending on geographic location. In the southern United States, bermudagrass (Taliaferro et al., 1974) and bahiagrass (Gates et al., 2001) can be used because of the moderate climate that allows these tropical grasses to flourish. In the eastern and northwest part of the United States, grasses such as fescue are used for the growing of cattle (Browning, Jr., 2003). There are advantages and disadvantages to each of the forages used. The tropical grasses such as bermudagrass, bahiagrass, and crabgrass are C4 metabolism type plants that require warm temperatures to grow. This is good in the summer months, but in spring and fall, production is limited because of temperature (Gates et al., 2001). Fescue is a C3 metabolism type plant that does better in moderate temperatures and has peak growth in the late spring and early fall months

(Henning et al., 1993). Fescue can become less palatable for animals in the hot dry months of the summer causing problems when forage production is needed (Henning et al., 1993). Endophyte-infection is another disadvantage of fescue. The endophyte-infected fescue causes decreased gains in cattle, elevated body temperature and other deleterious effects on cattle (Howard et al., 1992). There are endophyte-free varieties of tall fescue; however these varieties are less tolerant of drought conditions and grazing pressures resulting in near complete loss of stands within three to four years (Gunter and Beck, 2004).

Grain-Finishing Systems

Grain-finishing systems generally utilize cereal grains as well as some forage crops for the feed source. Grain-finishing allows for more energy to be in the diet compared with forage-finishing systems (Muir et al., 1998a), which increases cattle growth. Increased energy intake also increases the amount of fat deposition during the finishing phase because of excess energy after growing needs are met (Byers, 1982).

The feedlot industry has become more concentrated with fewer feedyards causing the same amount of cattle being marketed from fewer feedyards that are larger in size (Ward and Schroeder, 2002). This could possibly impact the types of feeds being fed and make the feedlot cattle more uniform.

Effects of forages and grain on growth rate

The differing forage types (C3, cool-season or C4, warm-season) have different effects on the plant growth characteristics with cool-season forages such as fescue having higher digestibility than warm-season forages such as bermudagrass (Fisher et al., 1991). This is because of the different components that are found between the forage types with warm-season forages having greater concentrations of NDF (Fisher et al., 1991). The different metabolism

types cause differences in the chemical composition of the plant which affects growth rates of the plants (Brown, 1984), which is of major concern because of the different geographic locations producing the forage-finished beef.

Cattle growth rates from consumption of forages are generally lower than from cattle consuming concentrates (Nuernberg et al., 2005; Steen et al., 2003; Mandell et al., 1998), which is causes by differences in available energy between the two feedstuffs (Mandell et al., 1998). The concentrate diet allows for more energy to be partitioned to growth versus maintenance. Mandell et al. (1998) found that when cattle were fed to a final backfat endpoint with two scenarios (Scenario 1: Cattle fed high-moisture corn to 4 mm backfat, cattle fed alfalfa silage and harvested when cattle fed high-moisture corn reached 4 mm backfat; Scenario 2: Cattle fed alfalfa silage to 4 mm backfat, cattle fed high-moisture corn and harvested when cattle fed alfalfa silage reached 4 mm backfat; all cattle were fed *ab libitum*) cattle fed a high-moisture corn diet had greater ADG than cattle fed alfalfa silages. However, carcass weights were different in Scenario 1, whereas Scenario 2, cattle carcass weights were not significantly different. Schaake et al. (1993), Schroeder et al. (1980), and Kerth et al. (2007) found that steers fed a concentrate diet had heavier carcass weights than steers fed forages. However, it must be noted that the steers fed a concentrate diet in the Schaake study and the Schroeder study were on feed longer and were older than their forage-fed counterparts. This increase in days of feeding is most likely the reason for the increase in carcass weights. However, most literature has a confounding factor involved. These factors generally involve the time of harvest of the cattle. Some studies harvested at a time endpoint (Brown et al., 2005; Mandell et al., 1998), whereas others harvested at a backfat or weight endpoint (Kerth et al., 2007; Mandell et al., 1998). As a result, the grain-fed animals were generally heavier when harvested at a time endpoint and younger when fed to a

backfat endpoint because of the higher plane of nutrition than their forage-fed counterparts (Muir, et al., 1998a). When cattle are fed only forages with a high, medium or low rate of growth, Hersom et al. (2004) reported that cattle reared on a high rate of growth had greater carcass weights than that of the other two groups. French et al. (2000) found that by using different levels of forages and grain among five different groups for a constant time basis; carcass weights were not significantly different among groups of cattle.

Types of forages, environment and climate

Carcass Characteristics

The amount of growth in cattle being fed at differing planes of nutrition can generally be determined through the measurement of ribeye area (REA; Guenther et al., 1965). The REA and backfat measurements allow researchers to determine the amount of saleable meat on a carcass (Abraham et al., 1980) and the amount of growth and carcass fat deposition (Guenther et al., 1965). Moreover, differing feeding regimes can alter REA and impact overall carcass value with the high-concentrate diet yielding a larger REA (Brown et al., 2005). In cattle fed for the same amount of time, Mandell et al. (1998) reported cattle fed a high-concentrate diet will have greater REA than that of forage-fed cattle.

Hedrick et al. (1983) found that in cattle of similar weight fed corn or forages, the cattle fed corn had greater REA than that of the forage-fed cattle. Likewise, Baublits et al. (2004) found that supplementing a forage diet with soyhull pellets when grazing fescue or orchardgrass pastures resulted in greater REA than cattle grazing fescue pastures with no supplementation. Neel et al. (2007) found that in cattle fed to a similar age, cattle fed in feedlot conditions had greater REA than cattle fed forages. However, Steen and Kilpatrick (2000) found that cattle fed differing amounts of rolled barley all had similar REA.

Muscle lean color and pH identify potential problems such as DFD (dark, firm and dry) appearance of the meat that consumers might find unappealing (USDA, 1997). In a study using 1,062 randomly selected cattle from three packing houses, Page et al. (2001) found a moderately negative correlation between pH and L* values. This indicates that as pH values increased, L* values decrease caused by decreased light scattering on the surface of the muscle, which makes it appear darker. Vestergaard et al. (2000a), Bruce et al. (2004), and Muir et al. (1998b) all found that forage-finished animals had higher pH values than their grain-fed counterparts. Nuernberg et al. (2005) found a breed type x production (forage- vs grain-finishing) interaction for pH. However, Varela et al. (2004), Xiong et al. (1996) and Bidner et al. (1981) found no differences in L* values between forage- and grain-finished animals.

Hedrick et al. (1983) found that when cattle of similar weight were fed either corn or forage, forage-fed animals had lower L* values or were darker than animals that were fed corn. Likewise, Vestergaard et al. (2000a), Varela et al. (2004), and Muir et al. (1998b) found that forage-finished cattle were darker than their grain-finished counterparts. Baublits et al. (2004) found that cattle supplemented with soyhulls while grazing either fescue or orchardgrass pastures had higher L* values when compared to cattle grazing fescue with no supplementation. Likewise, French et al. (2001) and Varela et al. (2004) found no differences between forage- and grain-finished cattle.

Forage-finished beef generally has more yellow colored fat than grain-finished cattle (Kerth et al., 2007; Schaake et al., 1993; Bidner et al., 1986), which is because of carotenoids that are found in the plants consumed by the cattle (Yang et al., 1992). High-concentrate diets have a bleaching effect on the fat because of low amounts of carotenoids, which then cause the fat to have a white color (Muir et al., 1998b; Craig et al., 1959). Bidner et al. (1985), Leheska et

al. (2008), and Crouse et al. (1984) found that steers fed a high-concentrate diet had less yellow-colored fat than steers allowed to graze ryegrass. Conversely, Baublits et al. (2004) found that supplementation of soyhulls to cattle grazing either orchardgrass or fescue had b* values or yellowness values similar to cattle grazing fescue only.

Forage-finished beef generally has a lower amount of marbling than grain-finished beef (Schaake et al., 1993; Bidner et al., 1985, 1986). This is directly related to the amount of energy that is in the diets, with the grain- or high-concentrate diet having more energy than forage diets (Mandell et al., 1998; Guenther et al., 1965). The high-concentrate diet provides for more energy to be partitioned to fat accretion (Byers, 1982). However, with the right combination of forage and biological type of cattle, a slight amount of marbling can be achieved (Brown et al., 2005).

Kerth et al. (2007) found that cattle grazing ryegrass had similar marbling scores when compared with cattle fed a high-concentrate diet. However, Reagan et al. (1977), Schroeder et al. (1980) and Mandell et al. (1998) found that steers fed a concentrate diet had greater amounts of marbling than their forage-fed counterparts. Likewise, Hersom et al. (2004), conducted a study using a forage-only model with steers assigned to either a high, medium or low rate of growth, reported steers with a high rate of growth had greater amounts of marbling than the other groups. However, the Schaake study and the Schroeder study fed concentrate-finished cattle longer and to an older age than the forage-fed cattle.

Backfat at the twelfth rib has quality implications because of the occurrences of cold shortening of sarcomeres (Jennings et al., 1978), and can be an indicator of the amount of intramuscular fat (marbling; Koch et al., 1982). Forage-finished beef generally has less backfat than cattle on high-concentrate diets (Steen et al., 2003; Schroeder et al., 1980; Bowling et al.,

1977), which is a function of the amount of energy in the diet (Byers, 1982). Genetic factors also contribute to the amount of fat accretion because of differences in mature size (Brown et al., 2005). Brown et al. (2005) suggested that the ideal animal for a forage-finished model would most likely be a small to medium biological type because of energy constraints associated with forages.

Mandell et al. (1993), Bidner et al. (1986), and Realini et al. (2004) found that cattle fed a high-concentrate diet had greater amounts of backfat when compared with their forage-fed counterparts. Similarly, Hersom et al. (2004) using a forage-only model with steers on a high, medium and low rate of growth, reported that steers fed on a high rate of growth had greater amounts of backfat than the other two groups.

Cool-season forages

Cool-season annuals such as annual ryegrass and other small grains like wheat and rye can elicit gains of 1.25 kg /d for animals consuming the forage (Beck et al., 2008). Hafley (1996) found using Marshall and Surrey ryegrass that gains of 1.1 to 1.50 kg/d were possible. Moreover, Roberts et al. (2009) found gains of 1.09 kg/d for Marshall ryegrass.

Fescue, a cool-season perennial is used as a forage, but can decrease ADG compared with ryegrass, wheat and rye (Beck et al., 2008). Lower gains in cattle grazing fescue are generally noticed because of the appearance of endophyte in the plant (Realini et al., 2005). This fungus produces a toxin that causes vasoconstriction and leads to increased rectal temperature and respiration rate, rough hair coat, and intolerance to heat (Howard et al., 1992). Realini et al. (2005) found that cattle grazing endophyte-infected fescue had decreased average daily gains, live weights and hot carcass weights at harvest compared with steers grazing novel endophyte-infected fescue.

Warm-season forages

Warm-season forages grow best at warm temperatures in the summer, late spring and early fall (Gates et al., 2001). The C4 plant metabolism pathways allows for greater plant growth (Brown, 1984), but as a consequence, changes in plant structure occurs and reduces digestibility thereby decreasing animal gains (Forster et al., 1993). Hill et al. (1993) using Tifton 78 and Tifton 85 varieties of bermudagrass found gains of 0.65 and 0.67 kg/d, respectively. Likewise, Galloway, Sr. et al. (1993) found gains of 1.77 kg/d using bermudagrass.

Cool vs. Warm-season forages

It is generally accepted that the cool-season forages are more digestible than that of warm-season forages because of structural differences in the plant (Akin, 1986). There are however drawbacks to both of the forage types. Most warm-season forages are found in established pastures and provide nutrients from late spring through early fall (Gates et al., 2001). Annual cool-season forages generally have to be planted in fall, allowed to germinate and established before they can grazed. Moreover, in late spring the plant will mature and the cattle will have to be moved or harvested; or if the forage is a dual crop for grain, cattle will have to be moved at the first hollow stem stage of maturity so that grain yield is not impacted (Reuter and Horn, 2002). However, some cool-season forages such as fescue are found in established pastures with fescue covering approximately 14 million hectares in the US (Thompson et al., 2001).

Forster et al. (1993) found that bermudagrass had greater amounts of NDF than the cool-season forages of ryegrass and wheat. The NDF content of forages has been negatively correlated with intake of forages and generally decreases gains. Brown et al. (1997) found that bermudagrass pastures produced heavier 205-d wts compared with calves grazing endophyte-

infected fescue. However, that study did not compare forage values, therefore the extent of the realized gains from bermudagrass cannot be distinguished on the basis of whether nutritive value was greater for bermudagrass or the endophyte in the fescue lowered gains.

Environment and climate

The environmental and climate conditions that cattle are subjected to can have large impacts on cattle growth (Winchester, 1964). Colder climates, especially cold winters can cause a shift in the fiber type distributions to a more oxidative state (Lefaucheur and Gerrard, 1998). Moreover, cold or hot climates can cause cattle to decrease the amount of intake, thereby limiting growth (Winchester, 1964).

Winchester (1964) suggested that ambient air temperature has an effect on the amount of intake and gains that an animal will realize. Winchester (1964) noted that the decreased intake of feedstuffs was most likely a function of the animal's body moderating physiological temperature by lowering the amount of heat production by means of reduced metabolism.

Muscle Biology Characteristics

Fiber types

Muscle fiber type composition in animals can vary depending on many different factors ranging from amount of mobility required to forage (Vestargaard et al., 2000a) to the ambient temperature (Lefaucheur and Gerrard, 1998). As activity increases, fiber types will transition from Type IIb → Type IIx → Type IIa → Type Ia and as activity decreases, the transition occurs in the opposite direction (Lefaucheur and Gerrard, 1998). Vestergaard et al. (2000a) found that forage-fed cattle had greater percentages of Type Ia fibers and lower percentages of Type IIb fibers compared with grain-fed cattle; however, fiber size was similar between the two groups.

Types of fibers

There are four major types of fibers found in bovine skeletal muscle. The forms are dictated by the myosin heavy chains which have four major isoforms. The Type I isoforms are considered to be slow-twitch and have predominately oxidative metabolism (Lefaucheur and Gerrard, 1998). These fibers are smaller in diameter, very fatigue resistant, and are generally found in support and locomotion muscles (Lefaucheur and Gerrard, 1998). Type Ia fibers are generally classified or termed as Type Ia, β-red or slow-twitch oxidative fiber types (Picard et al., 2002).

Type II fibers make up the other three major fiber types, are considered to be fast-twitch (Lefaucheur and Gerrard, 1998) and are larger in diameter than their Type I counterparts (Kirchofer et al., 2002; Johnston et al., 1975). The three major types are Type IIa, Type IIx and Type IIb. Type IIa fibers are considered the intermediate type and are classified as Type IIa, α-Red or fast-twitch oxidative-glycolytic (Picard et al., 2002). Type IIa fibers are an intermediate fiber type with a larger diameter than that of the Type Ia fiber (Kirchofer et al., 2002). Type IIa fibers have an oxidative metabolism and are moderately fatigue resistant (Picard et al., 2002).

Type IIb and Type IIX are the remaining fiber types to be discussed. The Type IIx and IIb fibers cannot be distinguished from each other when classified using conventional histochemical techniques (Picard et al., 2002). They are large in size with large amounts of glycogen storage to assist in their predominately glycolytic metabolism (Picard et al., 2002). Type IIb fibers are classified as Type IIb, α-White, or fast-twitch glycolytic (Picard et al., 2002)

Effects of fiber types

The effects of fiber types on meat quality have been studied for many years. The literature shows wide variation with regards to fiber type impact on tenderness in the LM with no relationship to any fiber types (Chang et al., 2003, Pork; Wegner et al., 2000, Beef; Whipple et

al., 1990, Beef), Type Ia impacting tenderness (Ryu and Kim, 2005, Pork; Therkildsen et al., 2002a, Beef) and Type IIb impacting tenderness (Therkildsen et al., 2002a, Beef; Karlsson et al., 1993, Pork).

The distributions of each of the fiber types may have an important role in predicting and determining the resulting palatability of the meat. However, a clear relationship for intramuscular fat and tenderness relating to toughness has yet to be fully substantiated (Lee et al., 2010). May et al (1977) found no correlations between any fiber type and taste panel tenderness or juiciness. They did, however, find moderately positive correlations between fiber diameter and marbling, which indicates that increased growth resulted in increased fat deposition. Likewise, Ockerman et al. (1984) found a moderately positive correlation between tenderness and oxidative fiber types while the glycolytic fiber types had a moderately negative correlation with tenderness. However, juiciness attributes were not affected.

Fiber types can also be shifted depending on intrinsic and extrinsic factors (Lefaucheur and Gerrard, 1998). The most common way of altering fiber type ratios is through extrinsic effects such as environment and activity level (Lefaucheur and Gerrard et al., 1998). Extrinsic factors dictate the kind of metabolism needed to meet everyday physiological function, whether it is a high level of travel or generation of heat (Lefaucheur and Gerrard, 1998). The generation of heat is during times of cold weather generally done through the use of the tricarboxylic acid (TCA) cycle to generate ATP for use of heat production through metabolism (Lefaucheur and Gerrard, 1998). Animals that are required to travel long distances for food and water will also have a shift in the ratio of fiber types to the more oxidative types (Vestargaard et al, 2000a). This too is because of efficiency for the use of energy. Vestergaard et al. (2000a) showed in LM of cattle using two different production systems (intensive vs. extensive), an increase in

physical activity from the extensive system increased oxidative fiber types while decreasing glycolytic fiber types.

The growth pattern has been shown to alter both fiber type size and distribution. Wegner et al. (2000) reported that in *semitendinosus* muscles of cattle that, as age and time on feed increased, fiber diameter increased as well; however, they found no differences in fiber type distributions. Similarly, Johnston et al. (1975) found in LM of cattle that, as age and days on feed increased, fiber diameter increased for Type Ia fibers only, but fiber type distribution was not affected. Likewise, Vestergaard et al. (2000a) found that fiber type diameter increased as time fed increased for Type Ia and Type IIa fibers; however, Type IIb fibers were not affected. Conversely, May et al. (1977) found no differences between fiber type diameter and distribution in LM of three different breeds of cattle fed for three different time periods. Therkildsen et al. (2002b) found an increase in Type Ia fiber size in LM and *supraspinatus* muscles of cattle fed a higher energy diet compared with cattle fed a lower energy diet.

Color

Color pigments

The color of meat may be the most important attribute affecting consumer purchases (Kropf, 1980). This is because consumers can only see the product in the store, and the color of the meat can have a substantial influence on consumers' purchasing decisions (O'Sullivan et al., 2004). Consumers are not able to have a sample of the steak to check freshness and tenderness. Therefore, the only thing that they have to use in their decision is the color of the meat

The color of meat is dictated by myoglobin that is inherently found in meat (Faustman and Cassens, 1990). The differing colors of meat can range from a bright cherry-red to a brownish-red color (Faustman and Cassens, 1990). The bright cherry-red color is caused by

oxymyoglobin (Mancini and Hunt, 2005; Faustman and Cassens, 1990). Oxymyoglobin has a diatomic oxygen molecule attached at the sixth coordination site of the ferrous heme iron in the myoglobin molecule (Mancini and Hunt, 2005). Deoxymyoglobin causes a purplish color because of the lack of a ligand at the sixth coordination site in the ferrous heme iron (Mancini and Hunt, 2005). Metmyoglobin is caused by the heme iron oxidizing from the ferrous (Fe^{2+}) to the ferric (Fe^{3+}) state. The loss of an electron causes the heme iron to be unable to bind an oxygen ligand at the sixth coordination site and causes a brown color (Mancini and Hunt, 2005).

Lipid, myoglobin and protein oxidation

The oxidation of lipids and mainly myoglobin result in a product that is found undesirable by consumers, causing a loss in sales (Lanari et al., 1996). Oxidation of both lipids (Frankel, 1980) and myoglobin (Mancini and Hunt, 2005) is the result of the loss of an electron. This loss causes a conformational change in the myoglobin molecule and results in a brown color that consumers find undesirable (Lanari et al., 1996).

Lipid oxidation occurs when hydrogen is abstracted from a fatty acid (Frankel, 1980). There are two types of oxidation that occur in the meat system, photo-oxidation and auto-oxidation (Frankel, 1980). Photo-oxidation is the result of triplet oxygen being converted to an excited singlet state, which causes the oxygen molecule to need another electron to be stabilized (Frankel, 1980). The singlet oxygen will then steal an electron from a fatty acid, causing the abstracting of hydrogen resulting in a conformational change to the fatty acid (Frankel, 1980). Generally, the hydrogen is abstracted from an unsaturated fatty acid with increasing susceptibility as the amount of double bonds increase (Frankel, 1980). The result is that saturated fatty acids are the most stable and polyunsaturated fatty acids are the least stable.

Auto-oxidation is another form of oxidation that occurs in meat. It is the result of a free-radical caused by the loss of an electron from another molecule (Frankel, 1980). Inorganic ions and metals will steal an electron from a radical leaving the radical in an excited state (Frankel, 1980). The free-radical will then abstract hydrogen and an electron from a fatty acid causing a conformational change to the fatty acid (Frankel, 1980).

The main differences between the two pathways are that auto-oxidation can be slowed or stopped with the addition of chelators or antioxidants, which makes it much easier to control and stop. The pathway after the initial abstraction of hydrogen is similar between the two mechanisms. After the abstraction of the hydrogen and conformational change, the fatty acid undergoes transformations and cleavages (Frankel, 1980). This results in the production of aldehydes, ketones and epoxides that are thought to be the cause of off-flavors in meat as a result of oxidation (Faustman et al., 2010).

The feeding of forages to cattle can increase the amount of antioxidants in the muscle. The main antioxidant present from the forages is Vitamin E. Yang et al. (2002) found no differences between steaks from cattle on grain and forage diets supplemented with or without 2,500 IU vitamin E after seven days of display starting 24 h postmortem. However, Yang et al. (2002) found that, after 47 d aging in a vacuum bag, the steaks from the grain-fed animals showed lower TBARS values than the unsupplemented grain- and forage-fed treatments. In a study using two different breed types on either a forage- or concentrate-based diet, Nuernberg et al. (2005) found that cattle fed a forage-based diet had lower TBARS values on d 5 and 10 of display than their concentrate-fed counterparts.

Myoglobin oxidation occurs when the heme iron of the myoglobin protein loses an electron (Mancini and Hunt, 2005). This causes the ferrous (Fe^{2+}) iron to shift to the ferric (Fe^{3+})

iron state (Faustman and Cassens, 1990). The result is a conformational change to the myoglobin molecule and the heme iron loses the ability to bind a ligand at the sixth coordination site (Faustman and Cassens, 1990). The myoglobin pigment then has a brown color called metmyoglobin (Lanari et al., 1996). The transformation of iron from the ferric to the ferrous state and vice versa will occur until muscle antioxidants are expended, which would lead to the uninhibited formation of metmyoglobin (Lanari et al., 1996).

Faustman et al. (2010) stated that the mechanisms by which lipid oxidation occur could enhance the oxidation of myoglobin and have been described mainly through the reactivity of the primary and secondary products derived from unsaturated fatty acids. Moreover, Faustman et al. (2010) also stated that from a mechanistic view, the process of oxidation of oxymyoglobin to metmyoglobin generates reactive intermediates that can enhance further oxidation of both oxymyoglobin and unsaturated fatty acids. This would in part, explain both off-flavors and odors from meat that has undergone some oxidation.

Protein oxidation in the muscle has been shown to have deleterious effects on different aspects of meat quality, mainly tenderness (Lund et al., 2007). Two aspects of protein oxidation are the polymerization of proteins (Lund et al., 2007) and inactivation of the μ-calpains because of the oxidizing of cysteine residues in their active sites (Huff-Lonergan and Lonergan, 2005). Protein oxidation is linked to fatty acid oxidation (Viljanen et al., 2004; Srinivasan et al., 1996) with products from lipid oxidation such as malondialdehyde modifying proteins (Sayre et al., 2006). It would then be possible to postulate that myoglobin oxidation also impacts protein oxidation because of the linkage of myoglobin oxidation with lipid oxidation as proposed by Faustman et al. (2010).

Rowe et al. (2004) fed cattle supplemented with and without Vitamin E and with or without irradiation, reported that the increased oxidation caused by the irradiation decreased μ-calpains autolysis. This decrease in the amount of autolysis of the μ-calpains resulted in the increase of shear force values. Although some of the animals were fed vitamin E, it did not increase the amount of autolysis.

Shelflife

The color and shelf-life of meat is one of the most important quality traits of meat because of consumer purchasing criteria (Kropf, 1980). This is because consumers associate the appearance of the meat with freshness (Kropf, 1980). The type of diet that the animal consumes has a role in the shelf life of the meat products. This is because of the types and amounts of fatty acids deposited and the amount of antioxidants found in the muscle. Reagan et al. (1977) found when using a visual panel that steaks from forage –fed cattle had better color scores after six days of display than steaks from grain-fed cattle. Nuernberg et al. (2005) and Gatellier et al. (2005) found that forage-fed cattle had lower TBARS values than grain-fed cattle on d 10 and 6, respectively. However, Yang et al. (2002) found no differences between forage-fed and grain-fed cattle with either no supplementation or supplementation with vitamin E after 7 d of display.

Palatability and Quality traits

Warner-Bratzler Shear force

Warner-Bratzler shear force has been used for years to measure the tenderness of meat. While it is effective in offering a repeatable measure (Wheeler et al., 1996), it cannot measure juiciness and beef flavor. Shear force measures the amount of force that is required to slice through a piece of meat that it is approximately 1.27 cm thick. Wheeler et al. (1996) suggested that steaks are cooked to the same temperature, allowed to cool, and then five to six 1.27 cm-

diameter cores should be taken parallel with the fiber direction and sheared across of the muscle fiber direction. Baublits et al. (2006) found no differences in shear values between cattle supplemented with soyhulls on fescue and orchardgrass and cattle grazing fescue only. Kerth et al. (2007), Brown et al. (2007) and Leander et al. (1978) all found that animals fed a high-concentrate diet produced steaks with lower shear force values compared with animals on a forage-fed diet. However, Bruce et al. (2004), Muir et al. (1998b) and Bidner et al. (1981) found no differences between forage- and grain-finished cattle with regard to shear values.

Sensory evaluation

Sensory evaluation has been used for years to evaluate the quality attributes of meat. While shear force is a very good and repeatable measure as shown by Wheeler et al. (1996), it cannot take into effect intrinsic factors such as flavor and juiciness. Killinger et al. (2004) found that steaks with similar shear force values that had greater amounts of marbling were rated juicier, more flavorful and more acceptable than steaks with lower levels of marbling. The differing components of the meat sample as influenced by diet or feeding regime, whether it be differing types of forages or grains, can have large impacts.

Forage-finishing produced no differences in sensory tenderness compared with grain-finished animals (French et al., 2001; Davies, 1977; Camfield et al., 1997). Likewise, Roberts et al. (2009) found no differences in sensory tenderness of cattle fed either forage-only or supplemented with varying levels of corn while grazing forages. However, some research has shown that grain-fed animals yield more tender steaks than pasture-fed cattle (Kerth et al., 2007; Vestergaard et al., 2000b). Conversely, Bruce et al. (2004) found that forage-finished cattle had more tender steaks than grain-finished cattle.

Juiciness of forage-fed beef compared with grain-fed beef has been shown to be different in some research while other research shows no differences. May et al. (1992) and Nuernberg et al. (2005) both found no differences between forage- and grain-fed beef juiciness scores. Conversely, grain-fed beef has been shown to be juicier than that of forage-fed beef (Kerth et al., 2007; Sitz et al., 2005; Mandell et al., 1998).

Flavor of forage-fed beef compared with grain-fed beef is quite variable. May et al. (1992) and Nuernberg et al. (2005) both found no differences between forage- and grain-fed beef flavor. In contrast, others have reported grain-fed beef to be more flavorful than forage-fed beef (Mandell et al., 1998; Hedrick et al., 1983; Schroeder et al., 1980). Forages such as those found in Flint Hills pasture in Kansas, orchardgrass-clover, rye oats-ryegrass, forage sorghum, bluegrass-clover, fescue, fescue-orchardgrassclover, rye ryegrass-clover, arrowleaf clover, bermudaclover- sudan, millet and Coastal bermudagrass have been linked to lower or less desirable flavor ratings by sensory panels (Melton et al., 1990).

Fatty Acid Profiles

The fatty acid profiles of beef can be influenced by diet (Webb and O'Neill, 2008), but the fatty acid profile in beef may not easily be changed because of ruminal biohydrogenation (Wood and Enser, 1997). The fatty acid profiles of monogastric animals can be changed easily because the fatty acids are absorbed "as fed" with little change occurring to the fatty acids (Wood et al., 2008). In beef, the dietary lipids are hydrolyzed by microbial lipases; once liberated the unsaturated fatty acids are prone to biohydrogenation and isomerization by the rumen bacteria (Harfoot and Hazelwood, 1988). This results in a large production of stearic acid (C18:0), and results in a large proportional difference in the amounts of unsaturated fatty acids in the rumen lipids compared with post-ruminal lipids (Harfoot and Hazelwood,1988).

Fincham et al. (2009) found that, when comparing steaks from grain-finished cattle with steaks from pasture-finished cattle, amounts of C18:1 cis-9 increased as time on feed increased for steaks from grain-finished cattle, but no change was found for steaks from pasture-finished cattle. Furthermore, Fincham et al. (2009) reported that as time on feed increased, the amount of C:18:3 n-3 fatty acids increased in steaks from pasture-finished cattle, whereas steaks from grain-finished cattle,the amounts of the same fatty acid decreased.

Ashes et al. (1992), in a study using both *in vivo* and *in vitro* analyses, concluded that rumen bacteria are unable to efficiently hydrogenate EPA and DHA. Several studies have outlined the fact that PUFAs, particularly the n-3 fatty acids, can bypass the rumen and escape unchanged. Some ways that the PUFAs can escape biohydrogenation is by being encapsulated in a seed coat or intact organelle such as a chloroplast (Wood and Enser, 1997). Wachira et al. (2000) noticed using steers fed several different hay diets that some of the C18:3, n-3 fatty acids were able to escape the rumen and were incorporated into the fatty acid profile of the steers. They concluded that the C18:3n-3 fatty acids were predominately in the glycolipid form which is less susceptible to biohydrogenation because of its location in the cell structure. Moreover, Wachira et al. (2000) found that inclusion of linseed oil, which is high in C18:3n-3 nearly doubled the amount of C18:3n-3 found in the duodenum compared with the control animals. Wachira et al. (2000) speculated that this may be a feasible way of increasing the n-3 fatty acid concentrations in the muscle.

Ashes et al. (1992) concluded that the inability of the rumen microbes to hydrogenate EPA and DHA was most likely because of the absence of specific enzymes or steric factors. However, it must be noted that Ashes et al. (1992) found that DHA and EPA were not incorporated into the muscle triacylglycerides or adipose tissue, but rather in the muscle

phospholipids. They concluded that since muscle phospholipids make up such a small portion of the total fat, even the incorporation of DHA and EPA in the diet and phospholipid layers, it would not be enough to provide healthful benefits that are associated with the fatty acids.

Another way of obtaining DHA and EPA is through the elongation and desaturation of C18:3n-3. Sprecher (2000) reviewed the metabolism of n-3 and n-6 fatty acids and concluded that both DHA and EPA can be synthesized from linoleate and linolenate through a series of elongations, desaturations and β-oxidation steps.

Conjugated linoleic acids or CLAs have been touted to have positive healthful benefits such as antiadipogenic, anticarcinogenic, and anti-inflammatory properties to name a few (Bhattacharya et al., 2006). The potential benefits of these unique isomers have drawn much attention in recent years as the population becomes more aware of health aspects of life. Poulson et al. (2004), Maheca et al. (2009), and Fincham et al. (2009) all found that cattle fed a forage diet had higher amounts of CLA in their lipid tissue compared with grain-fed animals. However, Warren et al. (2008) found that the grain-fed animals had higher amounts of CLA compared with forage-fed animals.

Concentrations of the n-6 and n-3 fatty acids have gained in familiarity over the years for their prevention of cardiovascular disease (Carroll and Roth, 2002). The ratio of n-6:n-3 fatty acids has been a subject of meat as a potential source of the n-3 fatty acids (Wood et al., 2008). Leheska et al. (2008) found that forage-fed cattle had lower n-6:n-3 ratios than their grain-fed counterparts. Noci et al. (2005) found that heifers fed a concentrate diet and then allowed to graze forages for differing numbers of days prior to harvest had a negative linear relationship between days grazing and n-6:n-3 ratio. However, Laborde et al. (2001) found Simmental steers had higher n-6:n-3 ratios compared with Red Angus steers when fed a common diet. It must be

noted, that large breed differences may exist, but the data from this paper would only suggest it may occur, but could not substantiate it.

Aging and Protein Proteolysis

It has long been accepted that meat that is "aged" or allowed to set in a refrigerated setting for an extended period of time will become more tender (King et al., 2009; Jennings et al., 1978). This is because of the endogenous enzymes that are found in meat called μ-calpain (Hopkins and Thompson, 2002). The calpains degrade the proteins in the myofibrillar matrix of the muscle (Geesink et al., 2006). The μ-calpains are activated with a μM amount of calcium whereas the m-calpains are activated by a mM amount of calcium (for review see Huff-Lonergan et al., 2010).

There can be great variability among breeds for the amount of proteolysis that will occur; the Brahman breed, for example, has a greater amount of calpastatin in the muscle which inhibits the calpains, resulting in less tender products (Wheeler et al., 1990). Another factor that affects the amount of proteolysis is implants (Strydom et al., 2009). Strydom et al. (2009), using three different implants, reported that the amount of calpastatin was increased in the three implant treatments compared with the non-implanted control. Moreover, Strydom et al. (2009) also reported that the implanted treatments had higher shear force values compared with the non-implanted control.

Economics

Consumer Preference

Consumer preference for domestic corn-fed beef has been documented by several authors (Cox et al., 2006; Umberger et al., 2002). Umberger et al. (2002) reported that approximately 62 percent of participants in their study preferred grain-fed beef, however, they also found that

approximately 23 percent of participants preferred Argentine forage-fed beef. Furthermore, Cox et al. (2006) using American grain- and forage-fed beef concluded that approximately one-third to one-half of consumers preferred forage-fed beef to grain-fed beef in the southeastern United States. Cox et al. (2006) stated that there was a significant market for forage-fed beef. These available markets would allow producers another option when marketing cattle for optimum profit potential.

Chapter III

Sensory, Quality, Instrumental Color and Fiber Type Characteristics of Striploins from Serially Harvested Forage-Finished Steers

Abstract

Fall-born steers (n=60, 317 $\pm$ 29 kg) were serially harvested to determine the effects of days of grazing on sensory, quality, instrumental color and carcass characteristics of steers grazing cool- and warm-season forages. Steers grazed tall fescue until the beginning of the trial (Dec. 9, 2008). On the trial start date, steers were stratified by BW and Group 1 (about 12 mo of age) was humanely harvested and the remaining groups were placed on ryegrass and harvested every 56 d for a total of 6 groups. On June 23, 2009 steers were moved to a bermudagrass and fescue pasture for 28 d and then moved to a crabgrass pasture for the duration of the grazing period. Twenty-four h postmortem, HCW; backfat at the twelfth rib; marbling; LM area; kidney, pelvic and heart fat (KPH); bone maturity; pH; lean and fat L*, a*, b*; and yield grade were measured. Striploins from the left side of the carcass were removed from the carcasses 24 h postmortem, vacuum-packaged and aged for 13 d in dark storage at 2^{o} C. Strip loins were then removed from their vacuum packages and cut into 2.54-cm-thick steaks. The steaks were designated to one of three treatments: 14 d aging (14 d), 21 d aging (21 d) or 14 d aging followed by display (DIS) for seven d. The DIS steaks were overwrapped using PVC film on a foam tray. Color measurements (L*, a*, b*, hue angle, chroma, and 630/580 nm reflectance ratio) were taken on d 0 and 6 of retail display. Data were analyzed for a completely randomized design with days of grazing as a fixed effect and data for instrumental color were analyzed using mixed

model procedures with day as a repeated measure and days of grazing as a fixed effect. Group 6 had the greatest ($P < 0.05$) HCW, marbling score, backfat and yield grade compared with other groups. Group 1 had the smallest ($P < 0.05$) LM area, highest ($P < 0.05$) pH and the lowest ($P < 0.05$) HCW among groups. Day by Group interactions were significant ($P < 0.05$) because of changes in differences among Groups for all instrumental color characteristics. A treatment by Group interaction was significant ($P < 0.05$) for cooking loss. Group 2 had a higher ($P < 0.05$) shear value than Groups 1, 4, 5, and 6, but did not differ ($P > 0.05$) from Group 3. Results indicate that increased days on forage increased HCW, marbling and backfat for Group 6. Days of grazing did not ($P > 0.05$) increase fiber size after Group 3. Fatty acid profiles were not impacted ($P > 0.05$) with increased days of grazing.

Introduction

Forage-finished beef has gained popularity over the past ten years. The reasons for this are numerous, but some of the reasons used most are the increase in input costs of conventionally finished beef (Brown et al., 2007), human health (University of California, 2002), animal welfare (Vestergaard et al., 2000b) and environmental concerns (USDA-ERS, 2002). These reasons have caused many producers to retain cattle and finish them using forages instead of shipping them to the High Plains for feedlot finishing.

Tenderness of forage-finished and grain-finished beef has been investigated thoroughly showing either no differences (Bidner et al., 1986; Reagan et al., 1977) or grain-finished beef being more tender (Kerth et al., 2007; Vestergaard et al., 2000b). The literature has not yet addressed a serial harvest of cattle grazing forages for an extended period of time; however, there have been serial harvests of grain-finished beef. May et al. (1992) found that increased days on a

high-concentrate diet increased backfat thickness, BW, yield grades and dressing percentages. However, May et al. (1992) found no improvement in tenderness after 56 d feeding.

Other variables that have not been extensively examined are the effects of prolonged grazing on muscle fiber types, fatty acid profiles and carcass characteristics. Vestergaard et al. (2000a) found in a comparison of grain-finished cattle with forage-finished cattle that the amounts of oxidative fiber types increased in the LM of forage-finished cattle. The increase in activity because of travel required to forage is expected to increase oxidative fiber types (Lefaucheur and Gerrard, 1998). However, it is not yet known if fiber type distributions will shift with increased BW and foraging. The fatty acid profiles of forage-finished beef have been investigated with regards to the comparison of grain-finished and forage-finished beef (Fincham et al., 2009). However, a forage-only study has not yet addressed the effects of extended grazing on the impacts of the fatty acid profiles.

Therefore, the objective of this study was to investigate: 1) the effects of age at harvest on the sensory, quality and instrumental color characteristics and 2) the effects of differing aging periods and environments on sensory and quality characteristics.

Materials and Methods

Animals and Forages

Fall-born crossbred steers (n=60) were randomly chosen from the resident herd of the Alabama Agricultural Experiment Station E. V. Smith Research Center Beef Unit and assigned to one of six harvest dates. Steers were serially harvested every 56 d using six groups of ten head. Steers were weaned in early fall and allowed to graze fescue and bermudagrass pastures until the trial start date. On trial start date (Dec. 10, 2008), steers were weighed and harvest Groups 2 through 6 were placed on ryegrass. Harvest Group 1 was immediately harvested to

serve as a baseline for the remaining Groups. Remaining steers were weighed every 28 d and were allowed to graze as a sward to eliminate paddock differences. Steers were monitored visually daily to ensure animal health and conformed to the guidelines set forth by the Institutional Animal Case and Use Committee (IACUC) protocol number 2008-1490.

On June 23, 2009, steers were removed from ryegrass and placed on a fescue and bermudagrass pasture for 28 d. Remaining steers (Groups 5 and 6) were then moved to a crabgrass pasture for the duration of the project. All steers were humanely harvested at a USDA inspected small commercial processing facility in Bluffton, GA on their respective harvest dates. Harvest groups with days of grazing and forage types are presented in Figure 1.

Carcass Evaluation

Carcass data were recorded 24 h postmortem for each group. Carcass halves were split between the 12^{th} and 13^{th} rib and allowed to bloom for 30 min. The pH; LM area; lean color; fat color; kidney; pelvic and heart fat (KPH); backfat; marbling; and bone maturity were recorded by a trained evaluator. Boneless striploins were then removed from the left side of the carcasses, vacuum-packaged and stored at 2^{o} C for 13-d in the dark.

Sample Processing

Loins were aged for a total of 14-d. Steaks were then cut 2.54-cm-thick and assigned to treatments. Treatments included 14-d aging sensory (14 d Sen), shear (14 d Sh) and analytical (14 d TBARS); or 21-d sensory(21 d Sen), shear (21 d Sh), and analytical (21d TBARS); and display sensory (DIS Sen), shear (DIS Sh) and analytical (DIS TBARS). The 14 d steaks were cut from 14-d-aged loins, vacuum-packaged and frozen immediately. The 21 d and display (DIS) steaks were cut at the same time as the 14 d steaks from the same loins. The 21 d steaks were vacuum-packaged, placed in dark storage and frozen after an additional 7 d. The DIS

steaks were placed on a #2S Styrofoam tray with a polyabsorbent pad and overwrapped with polyvinyl chloride (PVC) film. The DIS steaks were then placed in a simulated retail display case for 7 d and then vaccum-packaged and frozen.

Instrumental Color and Retail Display

Steaks (DIS) were placed in a coffin-style retail display case at 4^{o} C for 7 d with the light intensity at 1100 1x using Sylvania© Designer Cool White Plus bulbs (F40/DCWP). Instrumental color characteristics were recorded on d 0 and 6 using a Hunter Miniscan XE Plus (Model 45/0-L, Hunter Associates Laboratory, Inc., Reston, VA, USA). The CIE L*, a*, and b* and reflectance values were determined from the average of two random readings from the surface of each steak using Illuminant D65 with a 10^{o} standard observer and a 3.5-cm aperture. Hue angle was calculated using $\tan^{-1}(b^*/a^*)$, and saturation index was calculated by square root $(a^{*2} + b^{*2})$. The 630/580 nm ratio was calculated by dividing the reflectance value at 630 nm by the reflectance value at 580 nm.

Shear force, cook loss and sensory evaluation

Steaks were allowed to thaw for 24 h prior to cooking. Steaks were then cooked by the method of Kerth et al. (2003). In brief, steaks were cooked on clam-shell-style grills for a constant time (6.75 min) to an internal temperature of 71^{o} C. Steaks were then cooled to 4^{o} C. Six 1.27-cm-thick round cores were then removed parallel to the muscle fiber from each steak. Cores were sheared once perpendicular to fiber direction using a TA.XT Plus Texture Analyzer (Texture Technologies Corp, Scarsdale, NY) with a Warner-Bratzler shear force attachment.

Cook loss values were determined by weighing steaks prior to cooking. After cooking, steaks were allowed to cool and were then reweighed. Cook loss was calculated using the following formula.

$$\frac{\text{Fresh weight} - \text{Cooked Weight}}{\text{Fresh Weight}} \times 100$$

Sensory evaluation steaks were allowed to thaw for 24 h prior to cooking. Steaks were cooked by the same method described for shear force. After cooking, steaks were cut into 1.3-cm x 1.3-cm x steak thickness cubes and placed in a warmer at 65^{o} C for no longer than 20 min.

Panelists were trained in accordance with AMSA (1995) guidelines. Samples were assigned random numbers prior to serving. Panelists were given unsalted crackers and water to cleanse their pallets between samples. Samples were evaluated for initial and sustained juiciness, initial and sustained tenderness, flavor intensity, and beef flavor on a scale of 1 to 8, with 1 = extremely dry, dry, tough, tough, bland, and extreme off-flavor, and 8 = extremely juicy, juicy, tender, tender, intense, and no off-flavor, respectively. Off flavors were described when identified and are presented as a percentage of the number of times it appeared within a sample. Two samples from each steak were evaluated by panelists in partitioned booths with red incandescent light.

Thiobarbituric Acid Reactive Substances

Thiobarbituric acid reactive substances (TBARS) were determined using a modified method of Wang et al. (2003). Five grams of meat free of excess fat and connective tissue were homogenized in 15 mL of 7.5% trichloroacetic acid, 0.1% propyl gallate, and 0.1% EDTA using a bullet style blender (Bella Cucini Rocket Blender, Bella Cucina Artful Food, Inc., Montreal Canada) for approximately 30 s. Samples were then centrifuged at 1,500 x *g* using a Beckman Coulter Allegra X-15 R (Beckman Coulter, Inc., Brea, CA, USA) swinging bucket rotor. After centrifugation, samples were filtered through no. 4 Whatman paper (Whatman, plc, Kent, UK). Samples were then loaded into a 96-well microplate (Greneir Bio-one, Frickenhausen, Germany)

in triplicate wells and incubated at 40° C for 130 min in a VWR Incubating Microplate Shaker (VWR International, LLC., West Chester, PA, USA). Microplates were then read using a Multiskan EX (Thermo Fisher Scientific, Waltham, MA, USA) absorbance reader at 540 nm.

Standards were made using a stock solution of tetraethoxypropane (TEP) at 1 mM/L. The stock solution was diluted to 0, 2, 4, 6, 8, 10, 20, and 30 μM/mL. A standard curve was then generated for each plate and used for the samples on each respective plate, and results are expressed as mg malondialdehyde / kg meat.

Histochemical fiber type analyses

Histochemical fiber type analyses were determined using the method of Solomon and Dunn (1988). At 24 h postmortem, 0.5 cm x 0.5 cm x 1.5 cm cubes were excised from the LM parallel to fiber direction and frozen in isopentane chilled in liquid nitrogen and then stored at -80° C. Samples were mounted on cork board using freezing gel with fiber orientation perpendicular to cork board. Sections were then cut 10 μm thick using a Microm HM 505 E microtome (Thermo Fisher Scientific, Waltham, MA, USA), transferred to slides and stained. Upon completion of staining, slides were covered with a cover slip using glycerol mounting gel. Fiber type determinations were then determined using a Motic BA 300 (Motic, Richmond, BC, Canada) microscope with a digital camera attachment. A total of eight pictures were taken from each slide so that approximately 100 fibers were represented for analyses.

Fibers types were determined by mitochondrial amount and shade of cell. Darkly shaded cells with large amounts of mitochondria were determined to be β-red fibers. Fibers that displayed moderately dark shading with moderate amounts of mitochondria were determined to be α-red fibers, and lightly shaded fibers with low amounts of mitochondria were determined to be α-white fibers.

Fiber sizes were determined by calculating total area within the cell. To obtain an accurate measurement, the microscope was calibrated using the certified calibration slide from Motic. Measurements for area are presented as μm^2, and numbers of fibers are presented as percentages of total.

Fatty Acid Profiles

Fatty acid profiles were determined using the method of O'Fallon et al. (2007). One gram of muscle tissue was placed in 30 mL glass tube. Then, 5.3 mL of MeOH, 0.7 mL of 10 N KOH, and 2 mg trinondecanoin (C:19:0; internal standard) was added to the sample. Samples were then incubated for 90 min at 55^o C in a water bath with a shaker attachment. Samples were then removed and allowed to cool in tap water. After cooling, 0.58 mL of 24 N H_2SO_4 was added to the sample. Samples were then incubated in a shaker/water bath for 90 min at 55^o C, then were removed from water bath, placed in tap water and allowed to cool. After cooling, 3 mL of hexane was added to samples. Samples were then vortex-mixed for 5 min followed by centrifugation for 5 min at 1,500 x *g*. The hexane layer was then removed and placed in fatty acid vials and stored at -20^o C until analyses.

Fatty acid profiles were determined using an Agilent Technologies 6890N Network GC System using a 60m long, 0.25 mm inner diameter, 0.1 µm film thickness DB-23 High Resolution Gas Chromatography column (Agilent Technologies, Santa Clara, CA, USA). The GC was programmed for an oven temperature of 130^o C for 1 min, 130-170^o C at 6.5^o C/ min, 170-215^o C at 2.75^o C/ min, 215-230^o C at 40^o C/ min and had a constant temperature of 230^o C for 3 min with a split ratio of 50:1. The detector and injector temperature was set at 250^o C. The injector was set at 1µL of sample with helium as the carrier gas and a flow rate of 1 mL/min. The fatty acid methyl ester (FAME) standards (Nu-Check Prep, Inc.) were ran daily to identify

retention times of FAMEs and the peaks were then integrated so that individual FAMEs could be identified.

Statistical Analyses

Data were analyzed using SAS 9.1 (SAS Institute, Inc., Cary, NC, USA). Instrumental color data were analyzed using mixed-model procedures with the fixed effects of days of grazing. Day was treated as a repeated measure. All other data were analyzed using generalized linear models with the fixed effect of days of grazing and aging period. Least squares means were generated using the LSMEANS option of SAS and, when the model P-Value was significant ($P < 0.05$), means were separated using the pair-wise t-test with the PDIFF option in SAS.

Results

Carcass Characteristics

Group 6 had the greatest ($P < 0.05$) BW, and HCW among the groups (Table 1). Groups 4 and 5 had similar ($P > 0.05$) BW and HCW, and had lower ($P < 0.05$) BW and HCW than group 6, respectively. Groups 2 and 3 had similar ($P > 0.05$) BW, but group 3 had a greater ($P < 0.05$) HCW than group 2. Group 1 had the lowest ($P < 0.05$) BW and HCW among the Groups.

Group 6 had the greatest amount ($P < 0.05$) of backfat at the 12^{th} rib and marbling score among the groups. Groups 4 and 5 had similar ($P > 0.05$) amounts of backfat and marbling scores, but were lower ($P < 0.05$) than group 6. Groups 1, 2 and 3 had similar ($P > 0.05$) amounts of backfat. However, Group 1 had lower ($P < 0.05$) marbling scores than Groups 2 and 3, which were similar ($P > 0.05$).

Group 5 had a greater ($P < 0.05$) LM area than groups 1, 2, and 3, but was similar ($P > 0.05$) to groups 4 and 6. Groups 3 and 4 had similar ($P > 0.05$) LM area and Group 1 had the smallest ($P < 0.05$) LM area.

Group 6 had a greater ($P < 0.05$) amount of KPH than Groups 1, 2, 3, and 5, but was similar ($P > 0.05$) to group 4. Group 1 had lower ($P < 0.05$) amounts of KPH than Groups 2, 4, 5, and 6, but was similar ($P > 0.05$) to Group 3. Group 1 also had the highest ($P < 0.05$) 24 h pH among the Groups, whereas Groups 2, 3, 4, 5, and 6 had similar ($P > 0.05$) pH values.

Group 6 had the greatest ($P < 0.05$) yield grade among the Groups. Groups 1, 2, 4, and 5 were similar ($P > 0.05$) and Groups 3 and 5 had similar ($P > 0.05$) yield grades. Group 2 had greater ($P < 0.05$) ADG than Groups 3, 5, and 6, but was similar ($P > 0.05$) to Group 4. Group 3 had similar ($P > 0.05$) ADG to Group 4, and Groups 5 and 6 were similar ($P > 0.05$), but had lower ($P < 0.05$) ADG than the other Groups.

All Groups had similar ($P > 0.05$) muscle L* values (Table 2). Group 6 had greater ($P < 0.05$) muscle a* value than Groups 1, 3, 4, and 5, but was similar ($P > 0.05$) to Group 2. Group 4 had lower ($P < 0.05$) muscle a* value than Groups 2 and 5, but was similar ($P > 0.05$) to Groups 1, 3 and 4. Group 2 had a greater ($P < 0.05$) muscle b* value than Groups 1, 3, 4, and 5, but was similar ($P > 0.05$) Group 6. Group 4 had a lower ($P < 0.05$) muscle b* value than Groups 2, 3, 5, and 6, but was similar ($P > 0.05$) Group 1.

Group 6 had the highest ($P < 0.05$) fat L* value, while Groups 2, 3, 4, and 5 were similar ($P > 0.05$). There were no differences ($P > 0.05$) for fat a* values among the Groups. Group 6 had a higher ($P < 0.05$) fat b* value than Groups 2 and 4, and was similar ($P > 0.05$) to Groups 3 and 5. Group 4 had a lower ($P < 0.05$) fat b* value than Groups 3, 5, and 6, and was similar ($P > 0.05$) to Group 2.

Instrumental Color Characteristics

Group x day interactions were significant ($P < 0.05$) for L*, a*, and b* values (Table 3). Group 1 had the lowest ($P < 0.05$) L* value on d 0, while Group 2 had a higher ($P < 0.05$) L* value than Groups 1, 4, and 6 on d 0, but was similar ($P > 0.05$) to Groups 3 and 5. Group 1 had a higher ($P < 0.05$) L* value than Groups 3, 4, 5, and 6 on d 6, but was similar ($P > 0.05$) to Group 2. Groups 1 and 6 had similar ($P > 0.05$) a* values on d 0, whereas Groups 2, 3, 4, and 5 had similar ($P > 0.05$) a* values on d 0, but were lower ($P < 0.05$) than Groups 1 and 6. Groups 5 and 6 had similar ($P > 0.05$) a* values on d 6. Group 1 had the highest ($P < 0.05$) b* value among the Groups on d 0. Groups 2, 3, 4, and 5 had similar ($P > 0.05$) b* values on d 0. Group 2 had the highest ($P < 0.05$) b* value on d 6. Groups 1, 3, and 4 had similar ($P > 0.05$) b* values on d 6, Group 5 had a lower ($P < 0.05$) b* value than Groups 1, 2, 3, and 4, but was similar ($P > 0.05$) to Group 6 on d 6.

Group x day interactions were significant ($P < 0.05$) for hue angle, Chroma, and 630/580 nm ratio (Table 4). Group 1 had the highest ($P < 0.05$) hue angle on d 0 whereas Groups 2, 3, 4, 5, and 6 had similar ($P > 0.05$) hue angle values on d 0. Group 2 had the highest ($P < 0.05$) hue angle value on d 6. Groups 1, 3 and 6 had similar ($P > 0.05$) hue angle values and Groups 3 and 5 had similar ($P > 0.05$) hue angles values on d 6. Group 4 had the lowest ($P < 0.05$) hue angle value on d 6. Group 1 had the highest ($P < 0.05$) Chroma value on d 0, whereas Groups 2, 3, 4, and 5, had similar ($P > 0.05$) Chroma values on d 0. Group 2 had the highest ($P < 0.05$) Chroma value on d 6. Groups 1, 3, and 4 had similar ($P > 0.05$) Chroma values and Groups 5 and 6 were similar ($P > 0.05$) for Chroma values on d 6. Group 1 had the greatest ($P < 0.05$) 630/580 nm ratio on d 0 and d 6 among the Groups. Group 4 had a lower ($P < 0.05$) 630/580 nm ratio than Groups 1, 2, 3 and 5 on d 0 and 6, but was similar ($P > 0.05$) to Group 6 on both days.

Cook loss, Warner-Bratzler Shear Force and TBARS

Group 1 had greater ($P < 0.05$) amounts of cook loss for all aging periods, but was similar ($P > 0.05$) to Group 2 for the 14-d and 21-d aging periods (Table 5). Group 3 had a lower ($P < 0.05$) amount of cook loss than Groups 1 and 2 for the 14-d aging, but was similar to Groups 4, 5, and 6. Group 6 had a lower ($P < 0.05$) cook loss for the 21-d aging period than Groups 1, 2, and 3, but was similar ($P > 0.05$) to Groups 4 and 5. Group 5 had a lower ($P < 0.05$) cook loss than Group 1 for the DIS aging period, but was similar ($P > 0.05$) to Groups 2, 3, 4, and 6.

Groups 1 and 5 had similar ($P > 0.05$) shear values for all aging periods. Groups 2, 3, 4 and 6 had decreasing shear values over time and greater ($P < 0.05$) 14-d shear values than DIS. Groups 1, 2, 3, 5 and 6 were similar ($P > 0.05$) for the DIS and 21-d aging periods. However, Group 4 had lower ($P < 0.05$) shear values for DIS steaks than its 21-d counterpart.

Groups 1, 2, 3, and 4 had similar ($P > 0.05$) TBARS values for the 14-d aging period and Groups 5 and 6 had similar ($P > 0.05$) values. All Groups were similar ($P > 0.05$) for the 21-d aging period. Groups 1 and 2 had similar ($P > 0.05$) values for the DIS aging period and were higher ($P < 0.05$) than the other Groups. Groups 3, 4, 5 and 6 had similar ($P < 0.05$) values for the DIS analysis.

Sensory Evaluation

Group x aging period interactions were significant ($P < 0.05$) for initial and sustained juiciness (Table 6). The Group x aging period interaction occurred because Groups 2, 3 and 4 had similar ($P > 0.05$) values for all aging periods, whereas Groups 1, 5, and 6 had at least one aging period different ($P < 0.05$). The interaction also occurred for sustained juiciness because Groups 2, 3, and 6 had similar ($P > 0.05$) values for all aging periods, whereas Groups 1, 4 and 5 had at least one aging period different ($P < 0.05$) from the other analysis.

Group x aging period interactions for initial and sustained tenderness were not significant (Table 7; $P > 0.05$). However, because of missing data from Group 3, 21-d analysis treatment, means were separated as an interaction because the main effects values are not estimable. Group 1 had a higher ($P < 0.05$) rating for initial and sustained tenderness than Groups 2, 3, 4, and 6 for 14-d and Groups 2 and 3 DIS steaks, but was similar ($P > 0.05$) to Group 5 for the 14-d steaks and Groups 4, 5, and 6 for the DIS steaks for both initial and sustained tenderness. Group 3 had a lower ($P < 0.05$) initial tenderness rating for the 14-d aging period than Groups 1, 5 and 6, but was similar ($P > 0.05$) to Groups 2 and 4. Groups 2 had the lowest ($P < 0.05$) initial tenderness rating for the DIS aging period. Group 2 also had a lower ($P < 0.05$) sustained tenderness value for the DIS aging period than Groups 1, 4, 5, and 6, but was similar ($P > 0.05$) to Group 3.

There was a Group x aging period trend ($P = 0.06$) for beef flavor intensity and a Group x aging period interaction ($P < 0.05$) for off flavor intensity (Table 8). Groups 1, 2, 3, 4, and 5 had similar ($P > 0.05$) values for beef flavor intensity among their respective aging periods whereas within Group 6, the 14-d aging period had a lower ($P < 0.05$) value than the 21-d and DIS aging periods. The Group x aging period interaction for off flavor intensity occurred because Group 6 had similar ($P > 0.05$) values among aging periods and the other Groups had differing ranks among their respective aging periods. Group 1 had the highest ($P < 0.05$) value for the 14-d off flavor, whereas Groups 2, 3, 4, 5 and 6 had similar ($P > 0.05$) off flavor ratings. Group 5 had a higher ($P < 0.05$) value for the 21-d off flavor than Groups 1, 2, and 6, and was similar ($P > 0.05$) to Group 4. Group 3 had a higher ($P < 0.05$) value for DIS off flavor than Groups 1, 2, 5, and 6, and was similar ($P > 0.05$) to Group 4.

Groups 1, 4 and 5 had no other off flavors reported (Table 9). However, Group 2 had other off flavors for the 21-d and DIS aging period Groups, Group 3 had other off flavors for the

14-d and DIS aging period Groups, and Group 6 had other off flavors for the DIS aging period Group. All of the reported other off flavors were similar ($P < 0.05$). The other off flavors are the flavors that appear but are not either salty, grassy, bitter, rancid, metallic, livery or bloody. The other off flavors reported by the panelist were fishy and sour. There were no differences for bloody off flavors among the Groups.

The DIS aging period of Group 2 had a higher ($P < 0.05$) value for rancid off flavors than Group 6; however, it was similar ($P > 0.05$) to the DIS aging periods of Groups 1, 3, 4, and 5 (Table 10). Group 4 had the highest ($P < 0.05$) rancid value for the 21-d aging period among the Groups. There was no ($P > 0.05$) trend observed for the grassy off flavors. While there are differences ($P < 0.05$) among the Groups and aging periods, each Group had at least two aging periods with similar values. Group 5 DIS aging period had a higher ($P < 0.05$) value for grassy flavor than Groups 1, 2, 3, and 6, and was similar ($P > 0.05$) to the DIS aging period Group of Group 4.

Fiber types

Group x fiber type interactions were significant ($P < 0.05$) for both area and percentages (Table 11). The interaction area occurred because Groups 1 and 2 had similar ($P > 0.05$) values for Type Ia fibers and Type IIa fibers, but were different ($P < 0.05$) for Type IIb fibers. Likewise, Groups 3, 4, 5, and 6 had similar ($P > 0.05$) values for Type Ia and Type IIa fibers, respectively. Group 3 had greater ($P < 0.05$) areas for Type Ia, Type IIa and Type IIb fibers than Groups 1 and 2, but was similar ($P > 0.05$) to Groups 4, 5 and 6 for Type Ia, Groups 4 and 5 for Type IIa and Groups 4 and 5 for Type IIb fibers. The interaction of Group x fiber type percentages occurred because of percentages of the Type Ia and Type IIb fibers were different among Groups. Groups 1, 4, and 6 had similar ($P > 0.05$) percentages of Type Ia and Type IIb

fibers whereas Groups 2 and 3 had higher ($P < 0.05$) percentages of Type Ia fibers than Type IIb. Group 5 had a lower ($P < 0.05$) percentage of Type Ia fibers than Type IIb fibers. Type IIa fibers were similar ($P > 0.05$) among the Groups. Group 2 had a higher ($P < 0.05$) percentage of Type IIa fibers than Groups 4 and 5 and was similar ($P > 0.05$) to Groups 1, 3, and 6. Group 5 had a higher ($P < 0.05$) percentage of Type IIb fibers than Groups 2, 3, and 6, but was similar ($P > 0.05$) to Groups 1, and 4.

Fatty Acid Profiles

Results for fatty acid profiles for mg / g meat and percent of profile are presented in Tables 12 and 13, respectively. Group 3 had a greater ($P < 0.05$) amount of C:16:1 t than Groups 1 and 6, while having a similar ($P > 0.05$) value to Groups 2, 4, and 5. Group 5 had a greater ($P < 0.05$) amount of C:18:1 n7 than Group 1, while having similar ($P > 0.05$) values to Groups 2, 4, 5 and 6. Group 1 a higher ($P < 0.05$) value for C:18:2 n6 than Groups 2, 3, 4 and 6, while being similar ($P > 0.05$) to Group 5. Group 5 had a greater ($P < 0.05$) value than Group 6 for C:18:3 n3 while being similar ($P > 0.05$) to Groups 1, 2, 3, and 4. Group 1 had a higher ($P < 0.05$) value for C:20:3 n6 than Groups 3, 4, and 6, but was similar ($P > 0.05$) to Groups 2, and 5. Likewise, Group 1 had a greater ($P < 0.05$) value for C:20:4 than Groups 2, 4, and 6, while being similar ($P > 0.05$) to Groups 3, and 5. Group 5 had a higher ($P < 0.05$) value than Group 6 for C:22:0 but was similar ($P > 0.05$) to Groups 1, 2, 3, and 4. Group 4 had a greater ($P < 0.05$) amount of C:22:5 than Groups 2 and 6, while being similar ($P > 0.05$) in value to Groups 1, 3, and 5. All Groups were similar ($P > 0.05$) for total amounts of saturated fatty acids and monounsaturated fatty acids. However, Group 5 had a greater ($P < 0.05$) amount of polyunsaturated fatty acids than Groups 2, 3, 4, and 6, but was similar ($P > 0.05$) to Group 1.

Group 6 had a higher ($P < 0.05$) n6:n3 ratio than Groups 2, 3,4, and 5, but was similar ($P > 0.05$) to Group 1.

Group 1 had a higher ($P < 0.05$) percentage of 14:0 than Groups 3, 4, and 5 and was similar ($P > 0.05$) to Groups 2, and 6. Group 6 had a greater ($P < 0.05$) percentage of 16:0 and was similar to Group 4. Group 3 had the highest ($P < 0.05$) percentage of 16:1 t among the Groups. Group 6 had a greater ($P < 0.05$) percentage of 18:1 n9 than Group 3, but had similar ($P > 0.05$) values to Groups 1, 2, 4, and 5. Group 3 had a higher ($P < 0.05$) percentage of 18:2 n6 than Groups 4 and 6, and was similar ($P > 0.05$) to Groups 1, 2 and 5. Likewise, Group 3 had a greater ($P < 0.05$) percentage of 18:3 n3 than Groups 1, 4, 5 and 6, and was similar ($P > 0.05$) to Group 2. Group 3 had a greater ($P < 0.05$) percentage of 20:2 than Groups 2, 5, and 6, but was similar ($P > 0.05$) to Groups 1, and 4. Group 3 also had higher ($P < 0.05$) percentages of 20:4 and 20:3 n6 than Groups 4, 5 and 6, but was similar ($P > 0.05$) to Groups 1 and 2, respectively. Group 3 had the greatest ($P < 0.05$) amount of 22:0 and 22:5 and was different from all other Groups. Group 6 had a greater ($P < 0.05$) percentage of saturated fatty acids than Group 3, but had similar ($P > 0.05$) values to Groups 1, 2, 4 ,and 5. Group 6 also had a higher ($P < 0.05$) percentage of monounsaturated fatty acids than Groups 1, 2, and 3, and was similar ($P > 0.05$) to Groups 4 and 5. Group 3 had a greater ($P < 0.05$) amount of polyunsaturated fatty acids than Groups 2, 4, 5 and 6, but was similar ($P > 0.05$) to Group 1.

Discussion

Carcass Characteristics

Greater BW as well as HCW from Groups 4, 5, and 6 were expected because the Groups were older at time of harvest and had a greater number of days grazing forages. The literature supports this conclusion, as a number of authors have found this same result with both forages

and grain (Therkildsen et al., 1998; Westerling and Hedrick, 1979; Harrison et al., 1978). Moreover, the differences in the Groups in backfat measurement suggests that the rate of muscle growth had slowed and the rate of fat accretion had increased. This is further supported by the fact that the amounts of backfat continued to increase with Group 6 having more than the other Groups, whereas Groups 4, 5, and 6 were all similar for LM areas. Moreover, it is supported by the marbling scores of the cattle. With the exception of Group 1 for marbling scores, backfat thickness and marbling scores were similar. This would also indicate that, as cattle age and days on forage increased, the amount of fat deposition increased as well. Moreover, the amount of growth from protein accretion slowed, allowing more energy from the diet to be partitioned to fat accretion. Therkildsen et al. (1998), Duckett et al. (1993), and Harrison et al. (1978) all found that as days on feed increased, the amounts of backfat, LM area and marbling scores also increased.

Group 1 had greater 24-h pH values than the other Groups. This is most likely because of the low plane of nutrition that the first Group experienced prior to the trial start date. As detailed in the Materials and Methods section, the steers were held on a predominately fescue and bermudagrass pasture and the trial start date was Dec. 10. These forages would have very low nutritive levels at this time during the year (Gates et al., 2001; Hennig et al., 1993). This low plane of nutrition would most likely cause low levels of glycogen stores in the muscles. This would then cause a lack of available glycogen stores immediately postmortem and would result in a failure of pH decline (Priolo et al., 2001). The other Groups were similar for pH values and were on a higher plane of nutrition.

Lean L* values for the Groups were not different although the pH values of Group 1 were greater. However, the differences for a* and b* showed no clear trend. More than likely, the

differences in the a* and b* values were the result of differences in oxygenation rate related to inherent differences in the physiology of the steers in the project. Mancini and Hunt (2005) noted in their review of color that factors such as the meat's temperature, oxygen partial pressure, meat pH, and competition by other respiratory processes affect oxygen penetration into the muscle and oxymyoglobin thickness. This could explain the differences between the Groups a* values at 24 h with some Groups possessing respiratory processes with lower oxygen needs than the other Groups because of lower postmortem metabolic rates. The L* values for fat color were similar for Groups 2, 3, 4, and 5 with Group 6 having the highest L* value. This could possibly be the result of increased fat accretion. However, if this was the case, it would be expected that the other color values would change similarly as well. This was not the case as fat a* values showed no differences among Groups. Moreover, the b* values showed no trend among Groups or forage types, and within forage types showed there were no differences. Baublits et al. (2004) found that feeding soyhulls to cattle grazing on either fescue or orchardgrass did not change fat yellowness compared with cattle grazing fescue only. The reason for the differences in the fat b* values is not known. Yang et al. (1992) showed that the carotenoids are responsible for the yellow fat color of forage-finished beef. It could possibly be that the function of the maturity of the plants being grazed impacted the amount of carotenoids available to the animal. This would then allow for increased carotenoids to be deposited in the adipose tissue thereby increasing fat b* values.

Instrumental Color Characteristics

The L* values for steaks during simulated retail were lower for Group 1than the other Groups. This was most likely caused by the higher 24 h pH value decreasing the amount of light scattering on the surface of the muscle (Norman et al., 2004). This interaction of day x Group

was caused in part by Group 1 having a similar value on d 0 as on d 6 whereas the other Groups had a general decline in L* values from d 0 to d 6. Rowe et al. (2009) noticed a decrease in L* values over 7 d of display while Braden et al. (2007) found no differences over 5 d of display. The a* values showed a decline from d 0 to d 6. O'Sullivan et al. (2004) found that over 17 d of display, a* values decreased. During that same time, O'Sullivan et al. (2004) found that metmyoglobin percent increased. This is expected because of increased myoglobin oxidation caused by the inability of the muscle to carry out metmyoglobin reduction mechanisms (Lanari et al., 1996). Moreover, competition of the mitochondria for oxygen and ultimately the use of NADH causes a depletion of NADH which is needed for the reduction of metmyoglobin (Mancini and Hunt, 2005). This loss in the reduction properties then causes an increase in the amounts of metmyoglobin, resulting in a brown color on the surface of the muscle and decreasing a* values (Mancini and Hunt, 2005). Likewise, the decrease in b* values is most likely caused by the same mechanisms.

The Group x day interaction for hue angle was caused in part because of Group 1 had similar values on d 0 and d 6, whereas the other Groups had a general increase in hue angle values. Hue angle is a measure of the trueness of red color with lower values indicating a more true red. The increase in values is caused by the decrease of oxymyoglobin and an increase in metmyoglobin. Moreover, since hue angle is calculated using a* and b* values, a change in those values would elicit changes in hue angle (Yancey and Kropf, 2008). Stivarius et al. (2002) found that ground beef over 7 d in display, had increased hue angle values. The Group x day interaction for Chroma was caused in part again by Group 1 having a higher value on d 0 but was similar in value to other Groups on d 6. Chroma is a measure of vividness with larger values indicating a more vivid color. The Chroma values had a generalized decrease in value over the 7

d of display. Like hue angle, Chroma is measured by calculating the a* and b* values (Yancey and Kropf, 2008). Changes in either one or both of those values would change the value of Chroma (Yancey and Kropf, 2008). As can be seen by the a* and b* values, the increase in hue angle signifying a loss in true redness and a decrease in Chroma signifying a loss in vividness is mimicked by the a* and b* values. With the exception of Group 1, the values for the 630/580 nm ratio remained similar which indicates that the oxidation of oxymyoglobin to metmyoglobin was low. However, it was not low enough to keep the other color measures from deteriorating. Stivarius et al. (2002) found that ground beef over 7 d in display had decreased 630/580 nm ratio. However, in the current study, the values remained similar.

Cook loss, Warner-Bratzler Shear Force and TBARS

The cook loss results do not show any apparent trends. French et al. (2001) found results similar to those of the present study, with Groups 2 and 6 had different amounts of marbling, but cook loss values were similar among the Groups. Kerth et al. (2007) found that cattle with similar amounts of marbling had similar cook loss values. Moreover, Group 1 had the highest pH among the Groups but had the greatest numerical cook loss for each aging period. Sawyer et al. (2008) found that dark-cutting beef (high pH) had lower cook loss than that of the normal pH meat. The current study contradicts this. Although Group 1 would not have been considered dark-cutting, it would still be considered to be above normal. Shanks et al. (2002) postulated that during long aging periods (14 d or more), muscle loses its inherent ability to hold moisture. However, in the current study, the lack of a trend among the treatment Groups suggests that inherent differences between animals and muscles are likely the cause of differences between Groups. Seideman et al. (1982) found, using loins from grain-fed and forage-fed cattle with two different storage times (7 d and 21d), no differences between production system (forage vs.

grain) and storage time on cook loss. The storage time results are in agreement with the current study, with all of the Groups having at least two similar values within aging periods.

The results for Warner-Bratzler shear force show a trend of lower shear values for increased time. Although the 21-d and DIS steaks were the same age, the DIS steaks were displayed in a retail display case for 7 d. The temperatures in the display case were higher (4^{o} C vs. 2^{o} C) than the temperatures in the cooler where the 21-d steaks were kept. It is possible that this increase in average temperatures increased the amount of proteolysis occurring in the muscle, which caused increased tenderization compared with the other Groups. Koohmaraie (1992) found that increased temperatures increased the amount of proteolysis of proteins within purified myofibrils. Although Koohmaraie compared 25^{o} C and 5^{o} C for the temperature effects on proteolysis, warmer temperatures would still increase proteolysis, just to a lesser extent than what Koohmarie's results indicated. With the exception of Group 1, all of the DIS steaks had significantly lower shear values than their 14-d counterparts. Moreover, with the exception of Group 5, mean numerical values decreased from 14-d steaks to DIS steaks. Seideman et al. (1982) found that increased storage time (7 d vs. 21 d) decreased shear values. May et al. (1992) found a moderately negative relationship with days fed and shear values, indicating that, as days on feed increased, shear values decreased. However, in the current study, Group 1 had similar values to Group 5 and 6 for all analyses, which would indicate that in this study, days fed had little impact on shear values.

The results from the TBARS analyses are much like the shear force and cook loss analyses had no trend present among the Groups, although Groups 3, 4, 5, and 6 had lower TBARS values for DIS steaks than Groups 1 and 2. The reason for the 21-d values being greater than the DIS values in some cases is not known. The relative low values indicate that even

though there were moderate to high levels of polyunsaturated fatty acids, which are highly prone to oxidation, the antioxidant status of the muscle was able to keep fatty acid oxidation low. Mercier et al. (2004) concluded that pasture-finishing has an important effect on the antioxidant status, such as vitamin E and antioxidant enzymes compared with animals on a mixed-grain diet. Although the current study did not compare muscles from grain-fed and forage-fed cattle, the antioxidant status of the muscle would still be increased. Likewise, Arnold et al. (1993) found that feeding vitamin E to grain-fed cattle decreased TBARS values compared with unsupplemented controls. Furthermore, Arnold et al. (1993) found that, as time in display increased, TBARS values for the unsupplemented controls increased, whereas the vitamin E-supplemented controls remained similar across days of display. This could be the reason for the differences in the results of the current study. It could be possible that the different steaks expended the amount of antioxidants at differing rates causing differences in the amount of oxidation.

Sensory Evaluation

The results for initial and sustained juiciness showed a Group x aging period interaction. It should be noted that, while the interaction occurred, there were not large differences within each Group. Each Group had at least two aging periods that were similar in value, which indicates that the amount of juiciness was not impacted greatly by the different aging periods. Xiong et al. (1996) and Sapp et al. (1999) both found no differences among treatments for aging periods and juiciness. However, French et al. (2001) found aging time to have an impact on juiciness scores of beef. However, even though they found differences, the differing aging periods impacted juiciness differently, which suggest that another variable might have a larger impact than aging period. The 14-d aging period for initial and sustained juiciness indicates a

trend that as days on forage increased, the amount of juiciness increased. Conversely, May et al. (1992) and Schaake et al. (1993) both found, using a concentrate diet with differing feeding times, that as days on feed increased, there were no differences between the first and last groups. Furthermore, their data did not show any trends among the groups for increased or decreased juiciness as days on feed increased.

The initial and sustained tenderness results are much like the results for initial and sustained juiciness. Each of the Groups had at least two values for the aging period being similar. Furthermore, increased days of grazing did not have an apparent affect on perceived tenderness. Likewise, Schaake et al. (1993) found no differences in tenderness between the first and last Group of cattle fed for different periods of times. However, May et al. (1992) found that after 56 d of feeding a high-concentrate diet, sensory tenderness values were greater than those cattle fed for fewer days. Moreover, after 84 d, May et al. (1992) found there to be no improvement in tenderness ratings. Comparisons of forage-fed and grain-fed cattle often result in the grain-fed beef having better tenderness ratings. However, the literature does not offer any results for the comparison of days of grazing in a serial harvest scenario. This makes extrapolation of the results difficult. However, according to the present data, Group 1 had similar values for initial and sustained tenderness as Group 6, which indicates that improvements in tenderness would be difficult to achieve for forage-fed beef models in the current study.

The results from the beef flavor intensity ratings indicate that improvements in beef flavor are low. Although Groups 5 and 6 had higher ratings for the 14-d aging period and Group 6 had higher ratings than Group 1 for the 21-d and DIS aging period, improvements were still low. It would be expected that with the great difference in intramuscular fat content that Group 6 would have much higher scores than Group 1. The data suggest that there may be more variables

than just fat content impacting beef flavor, which is in agreement with Calkins and Hodgen (2007). They noted that many compounds contributing to the flavor of beef are water-soluble, which would explain at least in part, why the fatty acid content had little effect on beef flavor.

The results for off-flavor intensity show that the amount of off-flavor was low. However, like the other sensory results, with the exception of Groups 3 and 4, all the other Groups have at least 2 aging periods with similar values. The reason for the 21-d aging period having a higher value than the DIS aging period is unknown. DIS steaks were expected to have a greater value than the others because of the 7 d in display. However, this was not the case.

There were no differences in bloody, livery, salty, bitter and metallic off-flavors. This may be because the steaks were not enhanced. Furthermore, strip loins have not been implicated for having off flavors such as livery and metallic. The appearance of rancid and other off-flavors are likely that of oxidation. Greene and Cumuze (1982) found a correlation between TBARS values and rancid taste in beef, which would indicate that as oxidation increased, the amount and appearance of rancid off-flavors increased. Furthermore, Rhee and Myers (2003) found a high positive correlation with TBARS values and cardboard aromatics in goat meat. The appearance of grassy off-flavors was expected because the cattle were fed forages, which would cause an increase in the polyunsaturated fatty acids leading to the increased incidence of grassy off flavors (Priolo et al., 2001).

Fiber Types

Within each respective Group, all fibers had different mean areas, with Type Ia having the smallest area and Type IIb having the largest. The Type Ia fibers showed a lack of significant growth after Group 3 indicating that the growth potential for these fiber types were realized with the plane of nutrition given. Johnston et al. (1975) found cattle fed for 153 d had

larger Type IIa and IIb area than cattle fed for 233 d, although they were not significantly larger. Lefaucheur and Gerrard (1998) stated that genetic factors between breeds can influence fiber type composition. The breed crosses used in this study could be the reason for the disparity in fiber type size and distribution. Greenwood et al. (2009) found that nutritionally restricted cattle had a loss in muscle mass compared with the unrestricted Group. However, this cannot explain the discrepancy within the Groups of the current study because of increased LM areas and BW. Vestergaard et al. (2000a) found that fiber areas were similar for the Type IIb fibers for the 460-kg extensive production Group compared with the 360-kg extensive production group. The lack of difference show that when using different groups of animals for different harvest times, inherent differences between animals can increase variability.

The percent of the fiber types were not dramatically different among the Groups, most likely because of similar planes of nutrition among groups during the project. Although the cattle grazed forages and the quality of the forages can be variable during different times of the year, it would still be expected that the plane of nutrition would be similar. Greenwood et al. (2009) found that nutritionally restricted cattle had an increase in the amounts of Type Ia oxidative fiber types, indicating that the plane of nutrition can shift fiber types. Moreover, they found that when the nutritionally restricted cattle were given adequate feed, the fiber types shifted to be more like that of the conventionally fed cattle, which would indicate that the animals metabolic needs dictate the type of metabolism needed to sustain life. Lefaucheur and Gerrard (1998) noted that the general idea of the shift of fiber types was from Type IIb →IIx→IIa→ when activity level increased. The animals from the present study had a high activity level, which would increase the Type Ia and IIa fibers. Moreover, this would keep the oxidative fiber types more prevalent compared with cattle being finished using a concentrate

diet. Vestergaard et al. (2000a) found that the extensive-production (pasture) cattle had greater percentages of Type Ia fibers than intensive-production (concentrate diet) cattle. Moreover, Vestergaard et al. (2000a) found that the extensive cattle had greater vascularization compared to the intensive Group indicating greater blood flow for the oxidative fiber types.

Fatty Acid Profiles

The lack of differences in the fatty acid profiles indicate that forage type and days on forage had little or no impact on the types of fatty acids deposited. Fincham et al. (2009) found using four biopsy periods over 140 d increased amounts of elaidic acid (18:1 t-9) after the first (d 28) sampling period, decreased saturated fatty acids and increased monounsaturated fatty acids after 112 d from cattle fed a mixture of timothy hay, soybean meal and soybean hulls (Neel et al., 2007). This would be in partial agreement with the results of the current study. Moreover, in the Fincham study, the cattle grazed multiple paddocks utilizing combinations of triticale/ annual ryegrass, alfalfa/ orchardgrass, and a cool-season / legume mixture. The lack of differences across time would indicate that forage type did not have a large impact on the fatty acid profile similar to the results of the current study. Noci et al. (2005) found that as days on forage increased, total PUFA increased as well as certain fatty acids such as eladic acid, docosopentonoic acid, and linolenic acid, to name a few. However, it must be noted that in the Noci et al. (2005) study, the animals were all harvested at the same age endpoint. Furthermore, when the cattle were not grazing, they were fed a silage and concentrate diet. This would impact the fatty acid profile and still make extrapolation to the current study difficult.

Conclusions

Improvements in tenderness from increased days of grazing may be difficult to achieve using forage-fed beef growth models. The lack of improvements in tenderness indicate that harvesting at a younger age or fewer days of grazing may be a possibility for increasing animal turnover which has been an issue for forage-fed beef. Muscle fiber growth characteristics and their impact on quality characteristics have still not been elucidated. The forages used in this study indicate that it is possible to change forages without having great impact on palatability or fatty acid profiles. Further research into forage types and quality characteristics are needed.

Chapter IV

Sensory, Quality, and Instrumental Color Characteristics of Striploins from Forage-Finished and Grain-Finished Beef

Abstract

Striploins from forage-finished (n=17) and grain-finished (n=18) cattle were procured to compare quality, sensory and instrumental color characteristics. Loins from forage-finished cattle were procured from steers that grazed ryegrass through the spring and bermudagrass and fescue through the summer. Steers were harvested in late August. Striploins were removed from the carcasses 24 h postmortem, vacuum-packaged and stored in the dark at 2^{o} C for a total of 21 days. Loins from grain-finished cattle were procured from a commercial Midwest processor and were of USDA Select quality. Loins were transported to the Lambert-Powell Meats Lab and stored in dark at 2^{o} C until loins were 21 d of age from time of harvest. Histochemical samples were obtained from loins of forage-finished cattle 24 h postmortem, and samples from loins of grain-finished cattle were obtained 96 h postmortem. Samples were frozen in isopentane chilled in liquid nitrogen and stored at -80^{o} C until time of analyses. After completion of aging, loins were cut into 2.54 cm-thick steaks and analyzed for either fresh shear force, sensory, thiobarbituric acid reactive substances (TBARS), fatty acid analyses or display shear force, sensory and TBARS. Fresh steaks were individually vacuum-packaged and frozen until time of analyses. Display steaks were placed on a Styrofoam tray with a polyabsorbent pad, overwrapped with polyvinyl chloride film and placed in a display case for instrumental color

characteristics during simulated retail display. Upon completion of display, steaks were vacuum packaged and frozen until time of analyses. Shear force, cook loss, sensory evaluation and TBARS data were analyzed using generalized linear models with the fixed effects of feed type and aging period (fresh or display). Instrumental color data were analyzed using mixed model procedures with feed type, and day as fixed effects. Results indicate that loins from grain-finished cattle had greater ($P < 0.05$) L*, b*, hue angle, and Chroma values during display. Grain-finished cattle had muscles with greater ($P < 0.05$) fiber sizes while forage-finished cattle had muscles with greater ($P < 0.05$) percentages of Type Ia fiber types. Meat from forage-finished cattle had higher ($P < 0.05$) juiciness ratings whereas meat from grain-finished cattle had greater ($P < 0.05$) tenderness ratings for sensory evaluation. Warner-Bratzler shear force values were less ($P < 0.05$) for steaks from grain-finished cattle than forage-finished cattle. Grain-finished cattle had LM with greater ($P < 0.05$) percentages of saturated fatty acids whereas LM from forage-finished cattle had lower ($P < 0.05$) n6:n3 ratios. Steaks from grain-finished cattle had significantly ($P < 0.05$) lower shear values, greater fiber size and higher sensory tenderness ratings. Steaks from forage-finished cattle had increased ($P < 0.05$) sensory juiciness and lower ($P < 0.05$) n6:n3 ratios.

Introduction

Grass- or forage-based systems have gained in popularity over the past ten years because of an increase in input costs (Brown et al., 2007), human health (University of California, 2002), animal care (Vestergaard et al., 2000a) and environmental (USDA-ERS, 2002) concerns. However, forage-based beef production systems generally cause decreased rates of growth (French et al., 2001; Bidner et al., 1986; Bidner et al., 1981), more yellow fat color (Kerth et al., 2007; Realini et al., 2004;), and a grassy flavor (Baublits et al., 2006). Tenderness remain an

issue and Vestergaard et al. (2000b), Mitchell et al. (1991) and Bowling et al. (1977) have shown that grain-fed cattle produced more tender steaks. However, Kerth et al. (2007), Mandell et al. (1998), and Bidner et al. (1986) found no differences in tenderness of steaks between grain- and forage-finished cattle.

The fatty acid profile of beef has been a topic of discussion for several years, focusing on the n-6:n-3 fatty acid ratio. Carroll and Roth (2002) reviewed the role of omega-3 fatty acids and concluded that there is considerable evidence for the use of omega-3 fatty acids for the prevention of heart disease. Several authors have shown that forage-finished beef has a lower n-6:n-3 fatty acid ratio (Duckett et al., 2009; Nuernberg et al., 2005; Enser et al., 1998), which may help in the prevention and management of diseases such as cardiovascular disease, auto immune diseases and diabetes, and have suggested a ratio of 4:1 or less (Simopoulos, 2002).

Grain-based finishing systems have been used extensively over the past 40 years to produce animals with high rate of growth, and highly marbled cuts of meat. Therefore, the objectives of this study was to: 1) compare forage-finished cattle with conventional grain-finished cattle for quality, sensory and instrumental color characteristics and 2) compare non-displayed steaks to displayed steaks on sensory and quality characteristics.

Materials and Methods

Muscles and Sample Processing

A total of 35 striploins from forage-finished and grain-finished beef carcasses were obtained from the Lambert-Powell Meats Laboratory and a major commercial meat processing facility, respectively. Striploins from forage-finished cattle (n=17) were obtained from the Lambert-Powell Meats Laboratory from cattle that had grazed forages. Forage-finished cattle were placed on ryegrass, rye and oats in late fall at the Wiregrass Research and Extension Center

in Headland, AL and allowed to graze until forages could no longer support the animals growth. At that time, animals were transported to the Stan Wilson Beef Teaching Unit in Auburn, AL and placed on a predominantly fescue and bermudagrass pasture. Cattle grazed from May to late August with *ab libitum* access to water, vitamin and mineral. Cattle were then transported to the USDA inspected Lambert-Powell Meats Laboratory in Auburn, AL for humane harvest. Twenty-four hours postmortem, striploins from the left side of the carcasses were removed, vacuum-packaged and stored in the dark for a total of 21 d from time of harvest.

Striploins from grain-finished cattle (n=18) were obtained from a major meat processing facility. Striploins were obtained from the same day of production at random and were USDA Select quality. Loins were received at the Lambert-Powell Meats Laboratory and stored in the dark for a total of 21 d from time of harvest.

Histochemical fiber type samples were obtained from each striploin prior to aging. The LM samples from the striploins of forage-finished cattle were obtained 24 h postmortem. However, samples from the striploins of the grain-finished cattle were obtained 96 h postmortem.

Upon completion of the aging period, loins were removed from their vacuum-packages and cut into 2.54-cm-thick steaks. Steaks were analyzed for shear force, sensory, TBARS, fatty acid analyses, and display shear force, sensory and TBARS. All steaks were individually vacuum-packaged and frozen until time of analyses.

Instrumental Color and Retail Display

Fresh (never frozen) steaks aged for 21 d from time of harvest were cut to 2.54-cm.thick and placed on Styrofoam trays with an absorbent pad, overwrapped with polyvinyl chloride (PVC) film then placed in a coffin-style retail display case at 4^{o} C for 7 d with the light intensity

at 1,100 1x using Sylvania© Designer Cool White Plus bulbs (F40/DCWP). Instrumental color characteristics were recorded on d 0 and 6 of retail display using a Hunter Miniscan XE Plus (Model 45/0-L, Hunter Associates Laboratory, Inc., Reston, VA, USA). The CIE L*, a*, b* and reflectance values were determined from the average of two random readings from the surface of each steak using Illuminant D65 with a 10^{o} standard observer and a 3.5 cm aperture. Hue angle was calculated using $\tan^{-1}(b^*/a^*)$, and saturation index was calculated by square root $(a^{*2} + b^{*2})$. The 630/580 nm ratio was calculated by dividing the reflectance value at 630 nm by the reflectance value at 580 nm.

Shear force, cook loss and sensory evaluation

Steaks were allowed to thaw at 2^{o} C for 24 h prior to cooking. Upon completion of thawing, steaks were cooked using the method of Kerth et al. (2003). Steaks were cooked on clam-shell style grills for a constant time of 6.75 min to an average internal temperature of 71^{o} C. Upon completion of cooking, steaks were cooled to 4^{o} C. Six 1.27-cm-thick round cores were then taken parallel to the fiber direction of each steak. Cores were sheared once perpendicular to fiber direction using a Warner-Bratzler shear machine (Model 1955; G. R. Electric Manufacturing, Manhattan, KS).

Cook loss values were determined by weighing steaks prior to cooking. After cooking, steaks were allowed to cool and then were reweighed. Cook loss was calculated using the following formula.

$$\frac{\text{Fresh weight} - \text{Cooked Weight}}{\text{Fresh Weight}} \times 100$$

Sensory evaluation steaks were allowed to thaw at 2^{o} C for 24 h prior to cooking. Steaks were cooked by the same method described for shear force. After cooking, steaks were cut into

1.3-cm X 1.3-cm X steak thickness cubes and placed in a warmer at 65° C for no longer than 20 min.

Panelists were trained in accordance with AMSA (1995) guidelines. Samples were assigned random numbers prior to serving. Panelists were given unsalted crackers and water to cleanse their pallets between samples. Two samples from each steak were evaluated by panelists in partitioned booths with red incandescent light. Samples were evaluated for initial and sustained juiciness, initial and sustained tenderness, flavor intensity, and beef flavor on a scale of 1 to 8, with 1 = extremely dry, dry, tough, tough, bland, and extreme off-flavor, and 8 = extremely juicy, juicy, tender, tender, intense, and no off-flavor, respectively. When off-flavors were present, panelist identified the off-flavor as other, bloody, livery, metallic, rancid, grassy, bitter or salty. Off-flavors are reported as the percentage of times that it was present within a sample.

Fatty Acid Profiles

Fatty acid profiles were determined using the method of O'Fallon et al. (2007). Briefly, 1 g of muscle tissue was placed in 30 mL glass tube. Then, 5.3 mL of MeOH, 0.7 mL of 10 N KOH in water, and 2 mg trinondecanoin (C:19:0; internal quantification standard) was added to the sample and then incubated for 90 min at 55° C in a water bath with a shaker attachment. Samples were then removed and allowed to cool in tap water to room temperature. After cooling, 0.58 mL of 24 N H_2SO_4 was added to the sample and samples were then incubated in a shaker/water bath for 90 min at 55° C. Samples were removed from water bath, placed in tap water and allowed to cool to room temperature. After cooling, 3 mL of hexane was added to samples and then vortex-mixed for 5 min followed by centrifugation for 5 min at 1,500 x *g*. The hexane layer was then removed, placed in vials and stored at -20° C until analyses.

Fatty acid profiles were determined using an Agilent Technologies 6890N Network GC System using a 60m long, 0.25 mm inner diameter, 0.1 µm film thickness DB-23 High Resolution Gas Chromatography column (Agilent Technologies, Santa Clara, CA, USA). The GC was programmed for an oven temperature of 130^{o} C for 1 min, 130-170^{o} C at 6.5^{o} C/ min, 170-215^{o} C at 2.75^{o} C/ min, 215-230^{o} C at 40^{o} C/ min and had a constant temperature of 230^{o} C for 3 min with a split ratio of 50:1. The detector and injector temperature was set at 250^{o} C. The injector was set at 1µL of sample with helium as the carrier gas with a flow rate of 1 mL/min. The fatty acid methyl ester (FAME) standards (Nu-Check Prep, Inc.) were run daily to identify retention times of FAME. The FAME peaks were then integrated so that individual FAME could be identified.

Histochemical fiber type analyses

Histochemical fiber type analyses were determined using the method of Solomon and Dunn (1988). In brief, 24 h (forage-finished cattle) and 96 h (grain-finished cattle) postmortem, 0.5 cm x 0.5 cm x 1.5 cm cubes were excised from the LM parallel to fiber direction and frozen in isopentane chilled in liquid nitrogen and then stored at -80^{o} C. Samples were mounted on cork board using freezing gel with fiber orientation perpendicular to the cork board. Sections were then cut 10 µm thick using a Microm HM 505 E microtome (Thermo Fisher Scientific, Waltham, MA, USA) and transferred to slides. Slides were then stained and covered with a cover slip using glycerol mounting gel. Fiber type determinations were determined using a Motic BA 300 (Motic, Richmond, BC, Canada) microscope with a digital camera attachment. A total of eight pictures were taken from each slide so that approximately 100 fibers were represented for analyses.

Fibers were determined by mitochondrial amount and shade of cell. Darkly shaded cells with large amounts of mitochondria were determined to be β-red fibers. Fibers that displayed moderately dark shading with moderate amounts of mitochondria were determined to be α-red fibers and lightly shaded fibers with low amounts of mitochondria were determined to be α-white fibers.

Fiber sizes were determined by calculating total area within the cell. To obtain an accurate measurement, the microscope was calibrated using the certified calibration slide from Motic. Measurements for area are presented as μm^2, and total number of fibers are presented as percentages of total fibers.

Thiobarbituric Acid Reactive Substances

Thiobarbituric reactive substances (TBARS) were determined using the method of Wang et al. (2002). Five grams of meat was minced and placed in a 50 mL centrifuge tube. Then, 15 mL of 7.5% TCA solution was added and samples were homogenized using a bullet blender (Bella Cucini Rocket Blender, Bella Cucina Artful Food, Inc., Montreal Canada) for 30 sec. Samples were centrifuged at 1,500 *g* for 10 min at 2^o C using a Beckman Coulter Allegra X-15 R (Beckman Coulter, Inc., Brea, CA, USA) swinging bucket rotor followed by filtering through No. 4 Whatman paper (Whatman, plc, Kent, UK). Three millileters of sample was placed in a 16-mL glass tube in duplicate. Then, 3 mL of 40 mM TBA was added to the sample and incubated at 40^o C for 90 min. Samples were then placed in cold water for 20 min and standards were prepared and incubated with the samples. Samples were read using a spectrophotometer (Beckman Coulter DU 730, Beckman Coulter, Inc., Brea, CA, USA) at 532 nm. Results are presented as equivalents of mg malondialdehyde / kg meat.

Standards were made using a stock solution of tetraethoxypropane (TEP) at 1 mM/L. The stock solution was diluted to 0, 2, 4, 6, 8, 10, 20, and 30 μM/mL. A standard curve was then generated for each run and used for the samples on each respective run.

Statistical Analyses

Data were analyzed using SAS 9.1 (SAS Institute, Inc., Cary, NC, USA). Instrumental color data were analyzed using mixed model procedures with the fixed effects of feed type, and Day was analyzed as a repeated measure. All other data were analyzed by ANOVA with the fixed effects of feed type and aging period. Least squares means were generated and separated using pair-wise t-test when the model P-Value was significant ($P < 0.05$).

Results

Instrumental Color Characteristics

Results for instrumental color characteristics are presented in Table 14. Steaks from grain-finished cattle had greater ($P < 0.05$) L* values on d 0 and d 6 of display than the steaks from forage-finished cattle. There was also a day effect with L* values being greater ($P < 0.05$) on d 0 than on d 6 of display. There was a day X feed type interaction for a* values because steaks from grain-finished cattle had greater ($P < 0.05$) a* values on d 0, but were similar ($P > 0.05$) to steaks from forage-finished cattle on d 6 of display. Steaks from grain-finished cattle had greater ($P < 0.05$) b* values on d 0 and d 6 of display. There was also a day effect with b* values being greater ($P < 0.05$) on d 0 than d 6 of display.

There was a day x feed type interaction ($P < 0.05$) for hue angle because steaks from grain-finished cattle had a greater increase in value from d 0 to d 6 of display than steaks from forage-finished cattle. Steaks from grain-finished cattle had greater ($P < 0.05$) Chroma values on both d 0 and d 6 of display. Chroma values were greater ($P < 0.05$) d 0 than on d 6 of display.

There was a day x feed type interaction for 630/580 nm ratio because of steaks from forage-finished cattle had a lower ($P < 0.05$) value than steaks from grain-finished cattle on d 0, but had a greater ($P < 0.05$) value than steaks from grain-finished cattle on d 6 of display.

Fiber type area and percentages

Muscles from grain-finished cattle had larger ($P < 0.05$) average fiber areas than forage-finished cattle (Table 15). Likewise, Type IIb fibers were larger ($P < 0.05$) than Type IIa and Type Ia fibers, Type Ia fibers were smaller ($P < 0.05$) than Type IIa and Type IIb fibers, and Type IIa fibers were smaller ($P < 0.05$) than Type IIb fibers and larger ($P < 0.05$) than Type Ia fibers. Muscle from grain-finished cattle had more ($P < 0.05$) Type IIa and IIb than Type Ia fibers. Likewise, muscle from forage-finished cattle had more ($P < 0.05$) Type IIb and Ia fibers than Type IIa fibers.

Sensory Evaluation, cook loss, shear force and TBARS

There were no feed type x aging period interactions ($P > 0.05$; Table 16) for sensory evaluation. Steaks from forage-finished cattle had greater ($P < 0.05$) initial juiciness values than steaks from grain-finished cattle. Moreover, the fresh steaks had greater ($P < 0.05$) initial juiciness values than the display steaks. Steaks from forage-finished cattle had greater ($P < 0.05$) sustained juiciness values than steaks from grain-finished cattle and fresh steaks had greater ($P < 0.05$) sustained juiciness ratings than the display steaks.

Steaks from grain-finished cattle had greater ($P < 0.05$) ratings for both initial and sustained tenderness. However, there were no differences ($P > 0.05$) for aging periods. Beef flavor intensity values were similar ($P > 0.05$) for steaks from grain-finished and forage-finished cattle. However, fresh steaks had greater ($P < 0.05$) beef flavor intensity ratings than display steaks. Steaks from grain-finished cattle had lower ($P < 0.05$) off-flavor ratings than steaks from

forage-finished cattle. Moreover, fresh steaks had lower ($P < 0.05$) off-flavor ratings than the display steaks.

Cook loss values were similar ($P > 0.05$) between steaks from grain-finished and forage-finished cattle as well as for fresh and display steaks. Grain-finished cattle had lower ($P < 0.05$) shear force values than forage-finished cattle for both age periods.

Steaks from forage-finished cattle had greater ($P < 0.05$; Table 17) mean ratings for other off-flavors. Moreover, fresh steaks had lower ($P < 0.05$) other off-flavor ratings than display steaks. Other off-flavors are those that are not identified in the descriptors and were identified by panelist as fishy or sour. Steaks from forage-finished cattle had a greater ($P < 0.05$) rancid rating for fresh steaks than fresh grain-finished steaks. However, display steaks from forage-finished and grain-finished cattle had similar ($P > 0.05$) rancid ratings. Steaks from forage-finished and grain-finished cattle were not different ($P > 0.05$) with either aging period for bloody, bitter, grassy, livery, salty, and metallic off-flavors .

Steaks from grain-finished and forage-finished had similar ($P > 0.05$) TBARS values for fresh steaks; however, steaks from grain-finished cattle had greater ($P < 0.05$) amounts of malondialdehyde for the display aging period than display steaks from forage-finished cattle.

Fatty Acid Profiles

Results for fatty acid profiles for mg / g meat and percentage of total fatty acids are presented in Tables 18 and 19, respectively. Steaks from grain-finished cattle had greater ($P < 0.05$) concentrations of the fatty acids 12:0, 15:0, 16:0, 16:1, 17:0, 17:1, 18:0, 18:1 n9 t, 18:2 n6, 20:3 n6, 20:4, and 22:4 than steaks from forage-finished cattle. Likewise, steaks from forage-finished cattle had greater ($P < 0.05$) concentrations of 18:3 n3, 20:3 n3, 22:0, and 22:5 than steaks from grain-finished cattle. Steaks from grain-finished cattle had greater ($P < 0.05$)

concentrations of saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. However, this was expected because of the fact that the grain-finished cattle most likely had greater amounts of fat in the muscle.

Meat from grain-finished cattle had higher ($P < 0.05$) percentages of the fatty acids 14:0, 15:0, 16:0, 17:0, 18:1 n9 t, and 22:4 than meat from forage-finished cattle. However, steaks from forage-finished cattle had greater ($P < 0.05$) percentages of the fatty acids 13:1, 15:1, 16:1 t, 16:1, 18:2 n6t, 18:3 n3, 20:3 n3, 22:0, and 22:5 than steaks from grain finished cattle. Meat from grain-finished cattle had greater ($P < 0.05$) percentages of saturated fatty acids and also had a greater ($P < 0.05$) n6:n3 ratio than meat from forage-finished cattle.

Discussion

Instrumental Color Characteristics

The L* values for the steaks from grain-finished cattle were greater than the steaks from forage-finished cattle for both d 0 and d 6 of display. Nuernberg et al. (2005) and Realini et al. (2004) found that steaks from grain-finished cattle had higher L* values than steaks from their forage-finished counterparts. Baublits et al. (2004) found that supplementation of soyhulls to forage-finished cattle increased L* values in steaks compared with the unsupplemented control. Conversely, French et al. (2001) found no differences between steaks from forage-finished and grain-finished cattle for L* values. The day x feed interaction for a* values resulted from steaks from grain-finished cattle had greater values on d 0 but similar values on d 6 of display compared with steaks from forage-finished cattle. Steaks from forage-finished and grain-finished cattle had similar a* values on d 6 of display, most likely because of the oxidation of the myoglobin molecule. Braden et al. (2007) found that a* values decreased over time while beef steaks were in display.

The day x feed interaction for hue angle was due to a larger increase in value from d 0 to d 6 of display for the steaks from grain-finished cattle compared to the steaks from forage-finished cattle. The hue angle is a measure of trueness of red color, with lower values indicating a more true value. The increase in hue angle was most likely caused by an increase in oxidation of the myoglobin, resulting in a brown color on the surface of the muscle. Moreover, since hue angle is calculated using a* and b* values, a change in those values would elicit changes in hue angle (Yancey and Kropf, 2008). Braden et al. (2007) found an increase in hue angles over 5 d of display using inside beef rounds. Koger et al. (2010) found over 7 d of display that TBARS values increased while a* values decreased, which would indicate increased myoglobin oxidation causing a loss of redness and ultimately a higher hue angle value. Chroma values were higher for steaks from grain-finished cattle on both d 0 and d 6 of display. Chroma is a measure of vividness with larger numbers indicating a more vivid color. The 630/580 nm ratio showed an interaction of day X feed. Although both values decreased significantly over the 7 d of display, steaks from forage-finished cattle had greater values on d 6 of display. Steaks from grain-finished cattle had a higher 630/580 nm ratio on d 0, likely as the result of increased oxygen penetration followed by an increase in oxymyoglobin to metmyoglobin. The data shows that there is a roughly 0.6% difference in the d 0 values and 0.5% in the d 6 values. It appears that the steaks from grain-finished cattle had a slightly greater affinity for oxymyoglobin on d 0, and that the steaks from forage-finished cattle preserved myoglobin better through display from increased antioxidant status. Mercier et al. (2004) concluded that pasture-finishing has an important effect on the antioxidant status, such as vitamin E and antioxidant enzymes compared with animals on a mixed-grain diet, which could explain why the steaks from forage-finished cattle had greater 630/580 nm ratios on d 6.

Fiber Types

Type IIb fibers had the largest area while the Type Ia fibers had the smallest, which was expected because literature reports that the Type IIb fibers are the largest followed by Type IIa and Type Ia (Klont et al., 1998). Moreover, muscle from grain-finished cattle had greater fiber areas than muscle from forage-finished cattle, which would be expected because of the higher plane of nutrition that the grain-finished cattle experienced compared with the forage-finished cattle. Greenwood et al. (2009) found that an increased plane of nutrition resulted in larger muscle fiber areas. Conversely, Vestergaard et al. (2000a) found no differences between production systems (intensive vs. extensive) for LM fiber area. However, Vestergaard used bulls instead of steers which could have potentially increased growth of the fibers compared with steers.

Fiber percentage showed a fiber type x feed interaction due to percentages of Type IIa and Type IIb fibers were similar for muscles from grain-finished cattle, whereas Type IIb and Type IIa fibers were different for muscles from forage-finished cattle. The decrease in the amounts of Type Ia fibers for muscles from grain-finished cattle compared with the Type IIa and Type IIb fibers is expected because of decreased physical activity and a higher plane of nutrition. Likewise, the increase in Type Ia fibers compared with the Type IIa fibers is expected because the forage-finished cattle had increased physical activity from grazing large expanses. Greenwood et al. (2009) found that nutrition-restricted cattle had an increase in the percentages of oxidative Type Ia fibers. Likewise, Vestergaard et al. (2000a) found an increase in the amount of Type Ia fibers for cattle raised on pasture compared with cattle fed a concentrate ration in a small pen. Moreover, as activity increases, the amount of oxidative fiber types will increase; likewise, as activity decreases the amount of glycolytic fiber types will increase

(Lefaucheur and Gerrard, 1998). This explains why the grain-finished cattle had a lower percentage of Type Ia fibers compared to forage-finished cattle.

Sensory Evaluation, Cook Loss, Shear Force and TBARS

Initial and sustained juiciness ratings were greater for steaks from forage-finished cattle. Juiciness decreased for display steaks compared with fresh steaks. French et al. (2001) found that juiciness scores increased with aging time. Conversely, Jennings et al. (1978) found no differences in juiciness using two aging periods (10 or 20 d). The literature is inconclusive for sensory panel juiciness with regards to diet with Bruce et al. (2004) found that steaks from forage-finished cattle were juicier, Sitz et al. (2005) found that steaks from grain-finished cattle were juicer, and some authors found no differences between steaks from forage- and grain-fed cattle (Mitchell et al., 1991; Purchas and Davies, 1974; Camfield et al., 1997).

Initial and sustained tenderness ratings were greater for steaks from grain-finished cattle for both aging periods than steaks from forage-finished cattle. The literature is inconclusive for tenderness. Some authors finding no differences between steaks from forage- and grain-finished cattle (Bidner et al., 1986; Schaake et al., 1993; Reagan et al., 1977), some authors reported steaks from grain-finished cattle more tender (Mitchell et al., 1991; Harrison et al., 1978; Bowling et al., 1977) and some found steaks from forage-finished cattle more tender (Bruce et al., 2004). It could be postulated that grain-finished cattle would have greater amounts of fat, which would cause increased salivation and lubrication between the teeth. Jennings et al. (1978) found that steaks with higher marbling were rated more tender than steaks with lower amounts of marbling. Killinger et al. (2004) also found using paired loins with similar shear force values but differing degrees of marbling, that the higher marbled steaks were rated as more tender than the lower marbled steaks by panelist.

Beef flavor intensity was similar for steaks from both grain-finished and forage-finished cattle. However, beef flavor intensity ratings were lower for display steaks compared with the fresh steaks. The literature is inconclusive for flavor intensity of the beef. Some authors reported no differences between steaks from forage- and grain-fed cattle (Schaake et al., 1993; Bruce et al., 2004; Bowling et al., 1977), some reported steaks from grain-finished cattle higher rated (Mitchell et al., 1991; Purchas and Davies; 1974; Camfield et al., 1997), and other found steaks from forage-finished cattle with higher ratings (Reagan et al., 1977). Off-flavor intensity ratings were higher for steaks from forage-finished cattle for both fresh and display steaks. Rhee and Myers (2003) found a positive correlation with TBARS and cardboard-like flavor, which could explain why beef flavor scores decreased from the fresh steaks to the display steaks. The increase in off-flavors would likely dilute the beef flavor of the steaks causing lower scores while increasing the off-flavor scores.

The appearance of the other off-flavors was the result of the 7 d of display. This was likely caused by the oxidation of both proteins and fatty acids. The oxidation could have created intermediate compounds that, when heated, reacted to give off flavors such as fishy and sour. Moreover, higher values of rancid flavors occurring in the display steaks than fresh steaks indicates increased oxidation, which has been documented (Greene and Cumuze, 1982). More than likely the other off-flavors were the result of this increased oxidation.

The increased TBARS value for the display steaks from grain-fed cattle compared with the display steaks from forage-finished cattle was expected. Yang et al. (2002) have reported that feeding forages increased the amount of the precursor to vitamin E (α-tocopherol) in the muscle which serves as an antioxidant compared with grain-fed cattle. This antioxidant capacity would potentially decrease fatty acid oxidation, although myoglobin oxidation might still

proceed. Gatellier et al. (2005) found that steaks from pasture-fed cattle had lower TBARS values compared with steaks from concentrate-finished cattle. Mercier et al. (2004) concluded that pasture-finishing has a greater effect on the antioxidant status, such as vitamin E and antioxidant enzymes than animals on a mixed-grain diet. Vitamin E can be an effective antioxidant for enhancing both color and lipid stability (Faustman et al., 1998).

The similarity of cook loss values between steaks from grain- and forage-finished cattle was unexpected. The literature is inconclusive. Some authors found no differences between steaks from grain- and forage-finished cattle (Brown et al., 2007; Camfield et al., 1997; Bowling et al., 1977), some reported steaks from grain-finished cattle with higher cook loss (Bruce et al., 2004) and others found steaks from forage-finished cattle with higher cook loss (Brown et al., 2007).

The lower shear values for grain-finished cattle were expected because of increased fat accretion and growth rate. The literature is inconclusive as some authors reported no differences (Schaake et al., 1993; Bruce et al., 2004; Sitz et al., 2005) and some reported grain-finished cattle with lower shear values (Leander et al., 1978; Bowling et al., 1977; Brown et al., 2007). However, no studies were found in which steaks from forage-finished cattle had lower shear values than steaks from grain-finished cattle.

Fatty Acid Profiles

Steaks from grain-finished cattle had greater amounts of total fatty acids within the samples, resulting in greater amounts of most of the major fatty acids. This is expected because of increased fat content in the muscle. The steaks from forage-finished cattle did however have greater amounts of the omega-3 fatty acids, which was expected because forages have been well

documented to increase the amounts of these fatty acids in the muscle (French et al., 2000; Realini et al., 2004; Steen et al., 2003).

Similar to the amounts of fatty acids, steaks from grain-finished cattle had greater percentages of the saturated fatty acids whereas the steaks from forage-finished cattle had greater percentages of the omega-3 fatty acids. However, Yang et al. (2002), Nuernberg et al. (2005) and Realini et al. (2004) found no differences between steaks from forage-finished and grain-finished cattle for saturated fatty acid percentages whereas French et al. (2000) found that steaks from grain-finished cattle had greater amounts of saturated fatty acids. Moreover, steaks from grain-finished beef had a greater n6:n3 fatty acid ratio than steaks from forage-finished cattle. It has been well documented that steaks from forage-finished cattle have lower n6:n3 ratios (Nuernberg et al., 2005; Steen et al., 2003; French et al., 2000).

Conclusions

Steaks from grain-finished cattle had greater quality characteristics than steaks from forage-finished beef. However, improvements in the fatty acid profiles of the forage-finished beef indicate that it may be a healthy option for consumers desiring foods with high amounts of n-3 fatty acids. Off-flavor remains an issue with forage-finished beef. Further research is needed in the area of flavor chemistry of forage-finished beef to elucidate the differing components responsible for the characteristic forage-finished beef flavor.

Chapter V

Implications and Conclusions

Results from the first study indicate that increasing the number of days on forage is necessary to improve carcass characteristics with regards to BW and finish. However, sensory and quality data show inconclusive results for the amount of time needed on forage. Moreover, the lack of differences in fatty acid profiles suggests that ryegrass and crabgrass could possibly be used to maintain similar quality and sensory characteristics as well as fatty acid characteristics.

Results from the second study indicate that muscles from grain-finished cattle have larger fiber sizes, most likely from increased planes of nutrition. Moreover, steaks from grain-finished cattle were more tender than the steaks from forage-finished cattle. However, the steaks from forage-finished cattle were rated juicier than the steaks from grain-finished cattle. The fatty acid profiles indicate that the steaks from grain-finished cattle had greater amounts of saturated fatty acids and larger n6:n3 ratio whereas the steaks from forage-finished cattle had greater amounts of the omega 3 fatty acids present.

Results from both studies indicate that the large scale production of forage-finished beef can produce beef of similar quality as conventional grain-fed beef. However, research is needed to investigate key areas of tenderness and growth characteristics of forage-finished cattle. Moreover, research into breed related-differences of forage-finished cattle is needed to identify possible genetic and physiological differences.

Chapter VI

Tables and Figures

Table 1. Least squares means for carcass characteristics of serially harvested forage-fed beef steers.

Group[1]	BW, kg	HCW, kg	Backfat, cm	LM Area, cm^2	KPH, %	24 h pH	Bone Maturity	Marbling Score[2]	Yield Grade	ADG, kg
1	328.22^{d}	160.57^{e}	0.215^{c}	45.10^{d}	1.00^{d}	6.04^{a}	146.00^{b}	182.00^{d}	2.1^{b}	-
2	398.94^{c}	210.24^{d}	0.432^{c}	57.29^{c}	1.625^{b}	5.80^{b}	134.00^{cd}	269.00^{c}	2.0^{b}	1.15^{a}
3	430.50^{c}	239.22^{c}	0.315^{c}	71.61^{b}	1.175^{cd}	5.78^{b}	132.00^{d}	289.00^{c}	1.5^{c}	0.93^{b}
4	504.53^{b}	275.29^{b}	0.853^{b}	75.42^{ab}	1.725^{ab}	5.79^{b}	140.00^{bc}	349.00^{b}	2.3^{b}	1.05^{ab}
5	496.78^{b}	268.71^{b}	0.725^{b}	81.35^{a}	1.300^{c}	5.79^{b}	134.00^{cd}	351.00^{b}	1.6^{bc}	0.74^{c}
6	545.22^{a}	301.46^{a}	1.104^{a}	75.23^{a}	1.875^{a}	5.74^{b}	158.00^{a}	401.00^{a}	2.8^{a}	0.77^{c}
SEM	14.01	7.97	0.05	2.90	0.08	0.07	2.13	14.08	0.17	0.05
P-Value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.038	< 0.001	< 0.001	< 0.001	< 0.001

^{a,b,c,d,e}Means within a column lacking a common superscript differ ($P < 0.05$).

[1]Groups- Harvested every 56-d; Group 1: Control, no grazing; Groups 2, 3, and 4, 56, 112, 168 d grazing ryegrass, respectively; Group 5, 168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 28 d grazing crabgrass; Group 6,168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 84 d grazing crabgrass.

[2]Marbling Score: 350 – The first number (3) is the level and the last two numbers (50) are the degree; 1=Practically devoid, 2=Traces, 3= Slight, 4=Small.

Table 2. Least squares means for instrumental color characteristics of muscle and fat at the 12th rib 24 h postmortem of serially harvested forage-fed beef steers

Group[1]	Muscle			Fat		
	L*[2]	a*[3]	b*[4]	L*[2]	a*[3]	b*[4]
1	29.59	19.60[c]	15.52[de]	-	-	-
2	29.50	22.01[ab]	19.39[a]	72.18[b]	9.05	24.40[bc]
3	28.03	20.83[bc]	17.26[bc]	70.73[b]	10.40	26.52[ab]
4	28.24	18.69[c]	13.97[e]	71.26[b]	9.51	22.84[c]
5	29.85	20.42[bc]	15.82[cd]	72.26[b]	9.74	26.78[ab]
6	30.34	22.95[a]	17.92[ab]	76.56[a]	10.41	28.61[a]
SEM	1.27	0.58	0.61	0.95	0.90	1.19
P-Value	0.752	< 0.001	< 0.001	< 0.001	0.790	0.013

[a,b,c,d,e]Means within a column lacking a common superscript differ (P < 0.05).

[1]Groups- Harvested every 56-d; Group 1: Control, no grazing; Groups 2, 3, and 4, 56, 112, 168 d grazing ryegrass, respectively; Group 5, 168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 28 d grazing crabgrass; Group 6,168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 84 d grazing crabgrass.

[2]L* - Lightness: 0=Black; 100= White.

[3]a* - Redness: -100=Green; 100=Red.

[4]b*- Yellowness: -100=Blue; 100=Yellow.

Table 3. Least squares means for group x day interaction for L*, a* and b* values of simulated retail display loin steaks of serially harvested forage-fed beef steers

Group[1]	L*[2]		a*[3]		b*[4]	
	Day 0	Day 6	Day 0	Day 6	Day 0	Day 6
1	33.35^{c}	33.85^{c}	21.67^{a}	9.25^{c}	21.43^{a}	9.62^{e}
2	38.98^{a}	32.74^{cd}	18.84^{b}	9.19^{c}	14.71^{bc}	11.64^{d}
3	37.64^{ab}	30.47^{ef}	19.50^{b}	9.02^{c}	14.83^{bc}	9.18^{ef}
4	37.05^{b}	29.84^{f}	19.19^{b}	9.80^{c}	14.71^{bc}	8.76^{ef}
5	37.88^{ab}	31.75^{de}	19.31^{b}	7.78^{d}	14.06^{c}	7.54^{g}
6	37.35^{b}	31.49^{de}	20.83^{a}	7.77^{d}	15.19^{b}	8.44^{fg}
SEM	0.5318		0.4316		0.3895	
P-Value						
Group	< 0.001		< 0.001		< 0.001	
Day	< 0.004		< 0.001		< 0.001	
Interaction	< 0.001		< 0.001		< 0.001	

^{a,b,c,d,e,f,g}Means within an effect lacking a common superscript differ (P < 0.05).

[1]Groups- Harvested every 56-d; Group 1: Control, no grazing; Groups 2, 3, and 4, 56, 112, 168 d grazing ryegrass, respectively; Group 5, 168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 28 d grazing crabgrass; Group 6,168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 84 d grazing crabgrass.

[2]L* - Lightness: 0=Black; 100= White.

[3]a* - Redness: -100=Green; 100=Red.

[4]b*- Yellowness: -100=Blue; 100=Yellow.

Table 4. Least squares means for group x day interaction for Hue Angle, Chroma and 630/580 nm ratio values of simulated retail display loin steaks of serially harvested forage-fed beef steers

Group[1]	Hue Angle[2]		Chroma[3]		630/580 nm Ratio[4]	
	Day 0	Day 6	Day 0	Day 6	Day 0	Day 6
1	44.91^{cd}	46.29^{bc}	30.49^{a}	13.39^{e}	6.86^{a}	6.10^{b}
2	37.93^{f}	51.15^{a}	23.92^{c}	14.93^{d}	4.91^{c}	5.08^{c}
3	37.19^{f}	45.48^{bcd}	24.50^{bc}	12.92^{ef}	5.34^{c}	5.33^{c}
4	37.32^{f}	41.72^{e}	24.19^{c}	13.18^{e}	2.34^{f}	2.47^{ef}
5	35.98^{f}	44.25^{d}	23.89^{c}	10.86^{g}	3.12^{d}	3.02^{de}
6	36.06^{f}	47.12^{b}	25.78^{b}	11.52^{g}	2.40^{ef}	2.67^{def}
SEM	0.7077		0.5544		0.2311	
P-value						
Group	< 0.001		< 0.001		0.630	
Day	< 0.001		< 0.001		< 0.001	
Group x day	< 0.001		< 0.001		0.056	

^{a,b,c,d,e,f,g}Means within an effect lacking a common superscript differ (P < 0.05).

[1]Groups- Harvested every 56-d; Group 1: Control, no grazing; Groups 2, 3, and 4, 56, 112, 168 d grazing ryegrass, respectively; Group 5, 168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 28 d grazing crabgrass; Group 6,168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 84 d grazing crabgrass.

[2]Hue Angle: Lower values indicate truer red color.

[3]Chroma: Higher values indicate more vivid red color.

[4]630/580 nm ratio: Higher numbers indicate larger oxymyoglobin to metmyoglobin proportion.

Table 5. Least squares means for cook loss, Warner-Bratzler shear force and TBARS of serially harvested forage-fed beef steers

	Cook loss, %			Warner-Bratzler Shear Force, kg			TBARS[2], mg / kg of meat		
Group[1]	14d	21d	DIS	14d	21 d	DIS	14d	21 d	DIS
1	28.88^{ab}	31.74^{a}	27.03^{bc}	6.18^{ef}	5.33^{ef}	4.66^{f}	1.07^{abc}	0.88^{cde}	1.36^{ab}
2	26.79^{bc}	28.68^{ab}	23.15^{de}	8.55^{a}	8.27^{ab}	7.02^{bcd}	0.92^{bcd}	0.88^{cde}	1.50^{a}
3	22.97^{def}	-	22.44^{def}	8.11^{abc}	-	5.43^{ef}	0.72^{cde}	-	0.40^{ef}
4	23.89^{cde}	21.65^{def}	21.87^{def}	7.16^{abcd}	6.27^{de}	4.43^{f}	0.89^{bcde}	0.49^{def}	0.46^{def}
5	24.00^{cde}	23.27^{de}	19.85^{f}	5.96^{ef}	6.17^{ef}	4.58^{f}	0.00^{f}	0.84^{cde}	0.42^{def}
6	24.67^{cd}	20.90^{ef}	21.66^{def}	6.80^{cd}	5.14^{ef}	4.77^{f}	0.00^{f}	0.72^{cde}	0.59^{cde}
SEM	1.1213			0.5223			0.1806		
P-value									
Group	< 0.001			< 0.001			< 0.001		
Aging period	< 0.001			< 0.001			0.184		
Interaction	0.024			0.311			< 0.001		

^{a,b,c,d,e,f}Means within an effect lacking a common superscript differ ($P < 0.05$)

[1]Groups- Harvested every 56-d; Group 1: Control, no grazing; Groups 2, 3, and 4, 56, 112, 168 d grazing ryegrass, respectively; Group 5, 168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 28 d grazing crabgrass; Group 6,168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 84 d grazing crabgrass.

[2]TBARS: Thiobarbituric Reactive Substances- Expressed as mg malondialdehyde / kg of meat

Table 6. Least squares means for initial and sustained juiciness for sensory evaluation of serially harvested forage-fed beef steers

Group[1]	Initial Juiciness[2]			Sustained Juiciness[2]		
	14 d	21 d	DIS	14 d	21 d	DIS
1	5.31^{cdef}	4.94^{f}	5.35^{cde}	5.03^{cdefg}	4.64^{h}	5.26^{abc}
2	5.37^{cde}	5.07^{def}	5.02^{ef}	4.97^{cdefgh}	4.71^{gh}	4.80^{defgh}
3	5.54^{abc}	-	5.31^{cdef}	5.19^{abcd}	-	5.07^{cdefg}
4	5.47^{bc}	5.40^{cd}	5.18^{cdef}	5.10^{cdef}	5.20^{abc}	4.76^{fgh}
5	5.83^{ab}	5.46^{c}	5.24^{cdef}	5.52^{a}	5.13^{bcdef}	4.79^{efgh}
6	5.84^{a}	5.42^{cd}	5.51^{abc}	5.50^{ab}	5.15^{abcde}	5.15^{abcde}
SEM		0.13			0.14	
P-value						
Group		< 0.001			0.004	
Aging period		0.010			0.071	
Interaction		0.021			< 0.001	

^{a,b,c,d,e,f,g,h}Means within an effect lacking a common superscript differ ($P < 0.05$).

[1]Groups- Harvested every 56-d; Group 1: Control, no grazing; Groups 2, 3, and 4, 56, 112, 168 d grazing ryegrass, respectively; Group 5, 168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 28 d grazing crabgrass; Group 6,168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 84 d grazing crabgrass.

[2]5= Slightly juicy; 4= Slightly dry.

Table 7. Least squares means for initial and sustained tenderness for sensory evaluation of serially harvested forage-fed beef steers

Group[1]	Initial Tenderness[2]			Sustained Tenderness[2]		
	14 d	21 d	DIS	14 d	21 d	DIS
1	5.99^{a}	5.09^{bcdef}	5.55^{abc}	5.65^{a}	4.58^{bcdef}	5.19^{abc}
2	4.49^{fgh}	4.67^{efgh}	4.05^{h}	3.79^{ghi}	4.33^{defgh}	3.39^{i}
3	4.13^{gh}	-	4.82^{defg}	3.65^{hi}	-	4.20^{efghi}
4	4.65^{efgh}	5.52^{abc}	5.23^{bcde}	4.10^{fghi}	5.12^{abcd}	4.72^{bcdef}
5	5.67^{ab}	5.47^{abcd}	4.88^{cdef}	5.33^{ab}	5.28^{abc}	4.51^{cdefg}
6	5.23^{bcde}	5.33^{abcde}	4.94^{cdef}	4.75^{bcdef}	5.08^{abcde}	4.60^{bcdef}
SEM		0.24			0.28	
P-value						
Group		< 0.001			< 0.001	
Aging period		0.002			< 0.001	
Interaction		0.133			0.279	

^{a,b,c,d,e,f,g,h,i}Means within an effect lacking a common superscript differ ($P < 0.05$).

[1]Groups- Harvested every 56-d; Group 1: Control, no grazing; Groups 2, 3, and 4, 56, 112, 168 d grazing ryegrass, respectively; Group 5, 168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 28 d grazing crabgrass; Group 6,168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 84 d grazing crabgrass.

[2]5= Slightly tender; 3=Moderately tough.

Table 8. Least squares means for beef flavor and off flavor intensity for sensory evaluation of serially harvested forage-fed beef steers

Group[1]	Beef Flavor Intensity[2]			Off Flavor Intensity[2]		
	14 d	21 d	DIS	14 d	21 d	DIS
1	4.66^{cd}	4.62^{d}	4.80^{bcd}	2.36^{bc}	1.43^{d}	1.31^{d}
2	4.78^{bcd}	4.78^{bcd}	4.56^{d}	1.58^{d}	2.51^{b}	1.70^{d}
3	4.99^{abc}	-	4.84^{bcd}	1.57^{d}	-	2.57^{b}
4	4.92^{abcd}	4.62^{d}	4.86^{bcd}	1.54^{d}	3.23^{a}	2.40^{b}
5	5.07^{ab}	4.78^{bcd}	5.09^{ab}	1.42^{d}	3.49^{a}	1.33^{d}
6	4.79^{bcd}	5.24^{a}	5.28^{a}	1.37^{d}	1.80^{cd}	1.50^{d}
SEM		0.13			0.20	
P-value						
Group		< 0.001			< 0.001	
Aging period		0.391			< 0.001	
Interaction		0.067			0.002	

^{a,b,c,d}Means within an effect lacking a common superscript differ (P < 0.05).
[1]Groups- Harvested every 56-d; Group 1: Control, no grazing; Groups 2, 3, and 4, 56, 112, 168 d grazing ryegrass, respectively; Group 5, 168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 28 d grazing crabgrass; Group 6,168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 84 d grazing crabgrass.
[2]5Slightly intense beef flavor, moderate off flavor; 1= Extremely bland, no off flavor.

Table 9. Least squares means for other and bloody off flavors sensory evaluation of serially harvested forage-fed beef steers

Group[1]	Other[2], %			Bloody[2], %		
	14 d	21 d	DIS	14 d	21 d	DIS
1	0.00[b]	0.00[b]	0.00[b]	1.43	3.81	0.00
2	0.00[b]	5.03[a]	2.86[ab]	0.00	0.00	1.43
3	1.67[ab]	-	5.00[a]	1.67	-	0.00
4	0.00[b]	0.00[b]	0.00[b]	0.00	0.00	0.00
5	0.00[b]	0.00[b]	0.00[b]	1.67	0.00	0.00
6	0.00[b]	0.00[b]	4.00[a]	0.00	0.00	0.00
SEM		1.42			0.94	
P-value						
Group		0.026			0.274	
Aging period		0.104			0.517	
Interaction		0.379			0.236	

[a,b]Means within an effect lacking a common superscript differ (P < 0.05).
[1]Groups- Harvested every 56-d; Group 1: Control, no grazing; Groups 2, 3, and 4, 56, 112, 168 d grazing ryegrass, respectively; Group 5, 168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 28 d grazing crabgrass; Group 6,168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 84 d grazing crabgrass.
[2]Means are expressed as a percentage of the number of times that the off-flavor was identified within a sample.

Table 10. Least squares means for rancid and grassy off flavors sensory evaluation of serially harvested forage-fed beef steers

Group[1]	Rancid[2], %			Grassy[2], %		
	14 d	21 d	DIS	14 d	21 d	DIS
1	5.10^{e}	6.30^{de}	42.05^{a}	9.82^{cd}	5.03^{d}	14.62^{cd}
2	19.35^{cd}	7.14^{de}	49.00^{a}	15.59^{cd}	14.13^{cd}	15.81^{cd}
3	9.53^{de}	-	42.38^{a}	14.29^{cd}	-	9.29^{cd}
4	20.24^{cd}	39.67^{ab}	38.33^{ab}	5.95^{d}	14.00^{cd}	28.33^{ab}
5	13.34^{cde}	6.00^{de}	40.95^{ab}	5.00^{d}	11.33^{cd}	34.53^{a}
6	6.67^{de}	4.00^{e}	27.43^{bc}	10.00^{cd}	18.00^{bc}	7.43^{cd}
SEM		5.14			4.01	
P-value						
Group		< 0.001			0.222	
Aging period		< 0.001			0.001	
Interaction		0.004			< 0.001	

[a,b,c,d,e]Means within an effect lacking a common superscript differ ($P < 0.05$).
[1]Groups- Harvested every 56-d; Group 1: Control, no grazing; Groups 2, 3, and 4, 56, 112, 168 d grazing ryegrass, respectively; Group 5, 168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 28 d grazing crabgrass; Group 6,168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 84 d grazing crabgrass.
[2]Means are expressed as a percentage of the number of times that the off-flavor was identified within a sample.

Table 11. Least squares means for fiber type area and percentage of serially harvested forage-fed beef steers

	Area, μm^2			Percentage		
Group[1]	Type Ia	Type IIa	Type IIb	Type Ia	Type IIa	Type IIb
1	1821.07^{ij}	2308.76^{gh}	3182.94^{de}	37.61^{abc}	28.36^{f}	34.03^{cde}
2	1760.35^{j}	2557.80^{fg}	3619.96^{c}	40.88^{a}	28.72^{f}	30.40^{ef}
3	2280.13^{gh}	3436.25^{cd}	4997.83^{a}	38.97^{ab}	30.27^{ef}	30.83^{ef}
4	2052.04^{hij}	3062.65^{de}	4799.95^{a}	34.56^{bcde}	30.87^{ef}	34.41^{bcde}
5	2165.12^{ghij}	3051.58^{def}	4644.60^{ab}	32.28^{def}	30.53^{ef}	37.19^{abc}
6	2203.85^{ghi}	2976.92^{ef}	4247.85^{b}	36.26^{abcd}	31.86^{def}	31.88^{def}
SEM		152.27			1.68	
P-value						
Group		< 0.001			1.00	
Fiber type		< 0.001			< 0.001	
Interaction		0.001			0.001	

^{a,b,c,d,e,f,g,h,i,j}Means within an effect lacking a common superscript differ (P < 0.05).

[1]Groups- Harvested every 56-d; Group 1: Control, no grazing; Groups 2, 3, and 4, 56, 112, 168 d grazing ryegrass, respectively; Group 5, 168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 28 d grazing crabgrass; Group 6,168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 84 d grazing crabgrass.

Table 12. Least squares means for fatty acid profiles (mg / g meat) of serially harvested forage-fed beef steers

Fatty Acid, mg/g	Group[1]						SEM	P-value
	1	2	3	4	5	6		
12:0	0.017	0.031	0.000	0.005	0.011	0.008	0.008	0.096
12:1	0.029	0.000	0.000	0.000	0.000	0.000	0.012	0.386
13:0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-
13:1	0.203	0.185	0.182	0.134	0.179	0.130	0.034	0.576
14:0	0.495	0.446	0.150	0.390	0.516	0.428	0.104	0.166
14:1	0.113	0.092	0.036	0.121	0.115	0.082	0.028	0.267
15:0	0.089	0.079	0.35	0.60	0.109	0.082	0.023	0.282
15:1	0.095	0.111	0.158	0.085	0.125	0.089	0.024	0.259
16:0	3.695	3.811	2.292	4.255	5.197	4.458	0.712	0.102
16:1 t	0.074^{b}	0.109^{ab}	0.138^{a}	0.132^{a}	0.135^{a}	0.090^{b}	0.014	0.009
16:1	0.462	0.467	0.320	0.605	0.613	0.536	0.090	0.194
17:0	0.174	0.154	0.072	0.152	0.230	0.172	0.038	0.126
17:1	0.151	0.143	0.091	0.159	0.157	0.138	0.022	0.266
18:0	2.459	2.551	1.624	2.560	3.876	2.885	0.524	0.085
18:1 n9 t	0.061	0.044	0.026	0.064	0.059	0.060	0.014	0.372
18:1	0.138	0.217	0.395	0.115	0.122	0.090	0.105	0.328
18:1 n9	5.253	5.239	3.131	6.149	7.530	6.269	1.012	0.067
18:1 n7	0.449^{a}	0.379^{ab}	0.254^{b}	0.511^{a}	0.535^{a}	0.445^{a}	0.061	0.023
18:2 n6 t	0.087	0.066	0.025	0.056	0.121	0.065	0.025	0.146
18:2 n6	1.316^{a}	0.937^{b}	0.943^{b}	0.943^{b}	1.273^{a}	0.867^{b}	0.073	<0.001
18:3 n6	0.000	0.000	0.031	0.000	0.044	0.045	0.023	-
19:1	0.000	0.000	0.000	0.000	0.000	0.035	0.015	0.462
18:3 n3	0.425^{a}	0.416^{a}	0.420^{a}	0.451^{a}	0.507^{a}	0.233^{b}	0.053	0.012
20:0	0.000	0.031	0.000	0.000	0.008	0.018	0.014	0.540
20:1 n15	0.041	0.016	0.015	0.038	0.042	0.030	0.012	0.414
20:1 n12	0.000	0.009	0.000	0.004	0.007	0.013	0.006	0.572
20:1 n9	0.000	0.000	0.000	0.000	0.007	0.015	0.005	0.154
20:2	0.081	0.052	0.062	0.074	0.054	0.049	0.009	0.143
20:3 n6	0.156^{a}	0.128^{a}	0.116^{b}	0.117^{b}	0.140^{ab}	0.093^{b}	0.010	0.002
20:4	0.653^{a}	0.537^{b}	0.564^{ab}	0.476^{b}	0.553^{ab}	0.339^{c}	0.039	<0.001
20:3 n3	0.089	0.084	0.076	0.104	0.097	0.055	0.013	0.129
22:0	0.371^{a}	0.326^{a}	0.347^{a}	0.341^{a}	0.387^{a}	0.221^{b}	0.029	0.001
20:5	0.000	0.032	0.000	0.000	0.000	0.015	0.014	0.501
22:1	0.000	0.000	0.000	0.000	0.000	0.004	0.001	0.466
22:2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-
22:4	0.000	0.000	0.000	0.000	0.004	0.000	0.001	0.466
22:3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-
24:0	0.000	0.000	0.000	0.000	0.000	0.006	0.002	0.466
22:5	0.345^{abc}	0.321^{bc}	0.409^{ab}	0.423^{a}	0.414^{a}	0.289^{c}	0.032	0.014
22:6	0.000	0.049	0.026	0.021	0.018	0.000	0.016	0.322
24:1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-
SFA	7.299	7.429	4.522	7.762	10.335	8.277	1.393	0.111
MUFA	7.069	7.010	4.745	8.118	9.625	8.026	1.202	0.110
PUFA	3.152^{ab}	2.620^{b}	2.671^{b}	2.665^{b}	3.225^{a}	2.051^{c}	0.195	<0.001
n6:n3	1.837^{ab}	1.303^{bc}	1.299^{bc}	1.127^{c}	1.777^{b}	2.191^{a}	0.230	0.009

[a,b,c]Means within a row lacking a common superscript differ ($P < 0.05$).

[1]Groups- Harvested every 56-d; Group 1: Control, no grazing; Groups 2, 3, and 4, 56, 112, 168 d grazing ryegrass, respectively; Group 5, 168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 28 d grazing crabgrass; Group 6,168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 84 d grazing crabgrass.

Table 13. Least squares means for percentage of fatty acid profile of serially harvested forage-fed beef steers

Fatty Acid, %	Group						SEM	P-value
	1	2	3	4	5	6		
12:0	0.087	0.237	0.000	0.018	0.031	0.030	0.073	0.225
12:1	0.181	0.000	0.000	0.000	0.000	0.000	0.071	0.386
13:0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-
13:1	1.156	1.186	1.551	0.877	1.074	0.883	0.260	0.460
14:0	2.689^{a}	2.188^{ab}	1.247^{c}	1.961^{b}	1.981^{b}	2.145^{ab}	0.240	0.005
14:1	0.596	0.402	0.305	0.575	0.432	0.393	0.104	0.318
15:0	0.501	0.373	0.299	0.296	0.379	0.402	0.064	0.237
15:1	0.549	0.739	1.392	0.569	0.727	0.591	0.216	0.068
16:0	20.847^{bc}	21.301^{bc}	19.162^{c}	22.459^{ab}	21.600^{c}	23.878^{a}	0.695	< 0.001
16:1 t	0.419^{c}	0.771^{b}	1.159^{a}	0.799^{b}	0.659^{bc}	0.528^{bc}	0.105	< 0.001
16:1	2.563	2.671	2.670	3.116	2.591	2.897	0.166	0.133
17:0	0.993	0.852	0.574	0.797	0.842	0.844	0.096	0.094
17:1	0.860	0.872	0.744	0.866	0.815	0.771	0.072	0.432
18:0	14.172	14.601	13.552	13.804	15.810	15.219	0.561	0.058
18:1 n9 t	0.341	0.174	0.212	0.373	0.260	0.286	0.066	0.308
18:1	0.826	1.279	3.672	0.687	0.734	0.681	0.996	0.232
18:1 n9	29.570^{ab}	29.932^{ab}	25.588^{b}	32.390^{a}	31.432^{a}	33.542^{a}	1.588	0.013
18:1 n7	2.607	2.381	2.092	2.726	2.396	2.451	0.199	0.309
18:2 n6 t	0.480	0.256	0.192	0.285	0.428	0.288	0.079	0.105
18:2 n6	7.768^{a}	6.447^{ab}	8.007^{a}	5.490^{b}	6.437^{ab}	5.334^{b}	0.660	0.019
18:3 n6	0.000	0.000	0.245	0.000	0.409	0.504	0.242	0.124
19:1	0.000	0.000	0.000	0.000	0.000	0.154	0.068	0.462
18:3 n3	2.485^{b}	2.706^{ab}	3.617^{a}	2.627^{b}	2.329^{b}	1.190^{c}	0.353	< 0.001
20:0	0.000	0.214	0.000	0.000	0.015	0.071	0.088	0.472
20:1 n15	0.206	0.056	0.116	0.179	0.168	0.124	0.051	0.371
20:1 n12	0.000	0.024	0.000	0.013	0.014	0.052	0.018	0.324
20:1 n9	0.000	0.000	0.000	0.000	0.013	0.061	0.017	0.082
20:2	0.488^{ab}	0.326^{bcd}	0.509^{a}	0.435^{abc}	0.228^{d}	0.306^{cd}	0.062	0.009
20:3 n6	0.924^{ab}	0.871^{ab}	0.987^{ab}	0.683^{bc}	0.726^{bc}	0.582^{c}	0.092	0.019
20:4	3.922^{ab}	3.819^{ab}	4.787^{a}	2.787^{bc}	2.901^{bc}	2.211^{c}	0.415	< 0.001
20:3 n3	0.521	0.507	0.654	0.597	0.446	0.309	0.086	0.082
22:0	2.187^{b}	2.145^{b}	2.967^{a}	2.016^{bc}	2.057^{bc}	1.396^{c}	0.261	0.004
20:5	0.000	0.220	0.000	0.000	0.000	0.064	0.091	0.461
22:1	0.000	0.000	0.000	0.000	0.000	0.017	0.007	0.466
22:2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-
22:4	0.000	0.000	0.000	0.000	0.008	0.000	0.003	0.466
22:3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-
24:0	0.000	0.000	0.000	0.000	0.000	0.026	0.011	0.466
22:5	2.062^{bc}	2.148^{bc}	3.456^{a}	2.475^{b}	2.097^{b}	1.774^{c}	0.254	< 0.001
22:6	0.000	0.303	0.245	0.101	0.060	0.000	0.105	0.201
24:1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-
SFA	41.476^{a}	41.911^{a}	37.800^{b}	41.350^{a}	42.714^{a}	44.010^{a}	1.178	0.013
MUFA	39.874^{b}	40.488^{b}	39.501^{b}	43.170^{ab}	41.217^{ab}	43.429^{a}	0.976	0.015
PUFA	18.651^{ab}	17.602^{b}	22.700^{a}	15.481^{bc}	16.069^{bc}	12.560^{c}	1.590	0.001
n6:n3	1.837^{ab}	1.303^{bc}	1.299^{bc}	1.127^{c}	1.777^{b}	2.191^{a}	0.230	0.009

^{a,b,c}Means within a row lacking a common superscript differ (P < 0.05)

[1]Groups- Harvested every 56-d; Group 1: Control, no grazing; Groups 2, 3, and 4, 56, 112, 168 d grazing ryegrass, respectively; Group 5, 168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 28 d grazing crabgrass; Group 6,168 d grazing ryegrass, 28 d grazing bermudagrass and tall fescue, 84 d grazing crabgrass.

Table 14. Least squares means for instrumental color characteristics for simulated retail display of LM steaks from forage- and grain-finished cattle

Characteristic	Day 0		Day 6		SEM	P-Value		
	Grain	Forage	Grain	Forage		Day	Finishing type	Day x finishing type
L*[1]	43.38[a,y]	39.84[b,y]	39.69[a,z]	35.64[b,z]	0.35	< 0.001	< 0.001	0.240
a*[2]	23.04[c]	20.72[d]	6.53[e]	6.89[e]	0.20	< 0.001	< 0.001	< 0.001
b*[3]	18.94[a,y]	14.85[b,y]	13.91[a,z]	9.56[b,z]	0.22	< 0.001	< 0.001	0.481
Hue Angle[4]	39.42[e]	35.50[f]	64.87[c]	54.06[d]	0.55	< 0.001	< 0.001	< 0.001
Chroma[5]	29.85[a,y]	25.50[b,y]	15.38[a,z]	11.88[b,z]	0.26	< 0.001	< 0.001	0.067
630/580 nm[6]	5.67[c]	5.07[d]	1.28[f]	1.76[e]	0.09	< 0.001	0.548	< 0.001

[a,b]Means within a row lacking a common superscript differ (P < 0.05) for finishing type

[y,z]Means within a row lacking a common superscript differ (P < 0.05) for day

[c,d,e,f]Means within a row lacking a common superscript differ (P < 0.05) for day x feed interactions

[1]L* - Lightness: 0=Black; 100= White

[2]a* - Redness: -100=Green; 100=Red

[3]b*- Yellowness: -100=Blue; 100=Yellow

[4]Hue Angle: Lower values indicate truer red color

[5]Chroma: Higher values indicate more vivid red color

[6]630/580 nm ratio: Higher numbers indicate larger oxymyoglobin to metmyoglobin proportion

Table 15. Least squares means for fiber type area and percent from striploins of forage- and grain-finished cattle

Item	Area, μm^2		%	
	Grain	Forage	Grain	Forage
Type Ia	2388.78^{a,z}	1858.44^{b,z}	26.67^{d}	36.60^{c}
Type IIa	2986.25^{a,y}	2456.85^{b,y}	37.77^{c}	28.60^{d}
Type IIb	4357.59^{a,x}	3506.93^{b,x}	38.55^{c}	35.12^{c}
SEM	130.76	145.07	1.83	1.95
P-value				
Finishing type	< 0.001		1.00	
Fiber type	< 0.001		0.007	
Interaction	0.406		<0.001	

^{a,b}Within a variable, means lacking a common superscript differ (P < 0.05) for finishing type

^{y,z}Within a variable, means lacking a common superscript differ (P < 0.05) for fiber type

^{c,d}Within a variable, means lacking a common superscript differ (P < 0.05) for the interaction

Table 16. Least squares means for sensory evaluation, cook loss and Warner-Bratzler shear force of LM steaks from forage- and grain-finished cattle

Characteristic	Fresh		Display		SEM	P-Value		
	Grain	Forage	Grain	Forage		Finishing type	Age	Finishing type x Age
Initial Juiciness[1]	5.58^{b,y}	6.03^{a,y}	5.41^{b,z}	5.58^{a,z}	0.08	< 0.001	< 0.001	0.125
Sustained Juiciness[1]	5.17^{b,y}	5.83^{a,y}	5.00^{b,z}	5.30^{a,z}	0.09	< 0.001	< 0.001	0.068
Initial Tenderness[2]	6.43^{a}	5.93^{b}	6.34^{a}	5.81^{b}	0.09	< 0.001	0.314	0.862
Sustained Tenderness[2]	6.16^{a}	5.51^{b}	6.04^{a}	5.51^{b}	0.11	< 0.001	0.384	0.826
Beef Flavor Intensity[3]	5.29^{y}	5.43^{y}	4.75^{z}	4.87^{z}	0.10	0.239	< 0.001	0.876
Off Flavor Intensity[3]	1.36^{b,z}	1.61^{a,z}	3.81^{b,y}	4.45^{a,y}	0.18	0.012	< 0.001	0.314
Cook loss, %	24.06	22.60	24.06	22.67	1.14	0.2209	0.976	0.974
WB Shear Force, kg	2.99^{b}	4.13^{a}	2.94^{b}	3.61^{a}	0.15	< 0.001	0.071	0.130

^{a,b}Means within a row lacking a common superscript differ (P < 0.05) for finishing type

^{y,z}Means within a row lacking a common superscript differ (P < 0.05) for age

[1]8= Extremely juicy; 1= Extremely dry

[2]8= Extremely tender; 1=tough

[3]8= Extremely intense beef flavor, off flavor; 1= Extremely bland, no off flavor

Table 17. Least squares means for sensory evaluation off flavors, and TBARS of LM steaks from forage- and grain-finished cattle

Characteristic	Fresh		Display		SEM	P-Value		
	Grain	Forage	Grain	Forage		Finishing type	Age	Finishing type x Age
Other	$0.00^{b,z}$	$0.00^{a,z}$	$1.19^{b,y}$	$5.95^{a,y}$	1.29	0.005	0.033	0.061
Rancid	4.23^{e}	18.45^{d}	75.99^{c}	75.29^{c}	3.68	0.063	< 0.001	0.041
Bloody	0.00	0.88	0.00	0.00	0.50	0.373	0.373	0.373
Bitter	0.00	0.00	1.19	2.14	1.07	0.648	0.113	0.648
Grassy	8.45	15.53	7.45	10.62	3.18	0.103	0.344	0.531
Livery	0.00	0.00	0.00	0.00	0.00	-	-	-
Salty	0.00	0.00	0.00	0.00	0.00	-	-	-
Metallic	2.98	0.75	1.19	1.43	1.18	0.388	0.629	0.285
TBARS[1]	0.42^{d}	0.55^{d}	1.64^{c}	0.74^{d}	1.14	< 0.001	0.014	0.001

[a,b]Means within a row lacking a common superscript differ ($P < 0.05$) for finishing type

[y,z]Means within a row lacking a common superscript differ ($P < 0.05$) for age

[c,d,e,]Means within a row lacking a common superscript differ ($P < 0.05$) for interaction

[1]TBARS: Thiobarbituric Reactive Substances- Expressed as mg malondialdehyde / kg of meat

Table 18. Least squares means for fatty acid profiles (mg / g meat) of from LM from forage- and grain-finished cattle

Fatty Acid, mg / g meat	Grain	Forage	SEM	P-value
12:0	0.027[a]	0.008[b]	0.006	0.037
12:1	0.000	0.000	-	-
13:0	0.005	0.000	0.002	0.107
13:1	0.033	0.042	0.008	0.433
14:0	1.356[a]	0.533[b]	0.250	0.026
14:1	0.291	0.181	0.054	0.160
15:0	0.261[a]	0.073[b]	0.052	0.015
15:1	0.425	0.461	0.058	0.663
16:0	11.317[a]	5.514[b]	1.679	0.020
16:1 t	0.129	0.089	0.016	0.095
16:1	1.244	0.810	0.183	0.103
17:0	0.725[a]	0.207[b]	0.136	0.011
17:1	0.456[a]	0.208[b]	0.080	0.036
18:0	6.049[a]	3.016[b]	0.889	0.021
18:1 n9 t	0.450[a]	0.010[b]	0.113	0.009
18:1	0.740	0.158	0.222	0.072
18:1 n9	15.646	8.66	2.554	0.062
18:1 n7	0.709	0.261	0.193	0.110
18:2 n6 t	0.112	0.095	0.031	0.706
18:2 n6	3.023[a]	1.402[b]	0.222	< 0.001
18:3 n6	0.024[a]	0.014[b]	0.009	0.425
19:1	0.000	0.000	-	-
18:3 n3	0.084[b]	0.354[a]	0.019	< 0.001
20:0	0.016	0.008	0.006	0.380
20:1 n15	0.073	0.067	0.018	0.804
20:1 n12	0.019	0.009	0.006	0.301
20:1 n9	0.057	0.019	0.015	0.076
20:2	0.039	0.031	0.009	0.527
20:3 n6	0.215[a]	0.107[b]	0.017	< 0.001
20:4	0.793[a]	0.478[b]	0.038	< 0.001
20:3 n3	0.000[b]	0.039[a]	0.007	< 0.001
22:0	0.063[b]	0.241[a]	0.016	< 0.001
20:5	0.000	0.000	-	-
22:1	0.000	0.000	-	-
22:2	0.000	0.000	-	-
22:4	0.089[a]	0.007[b]	0.010	< 0.001
22:3	0.000	0.000	-	-
24:0	0.000	0.000	-	-
22:5	0.170[b]	0.301[a]	0.014	< 0.001
22:6	0.000	0.000	-	-
24:1	0.006	0.000	0.004	0.324
SFA	19.817[a]	9.599[b]	2.983	0.021
MUFA	20.277[a]	10.974[b]	3.023	0.037
PUFA	4.549[a]	2.828[b]	0.277	< 0.001
n6:n3	13.450[a]	2.523[b]	1.175	< 0.001

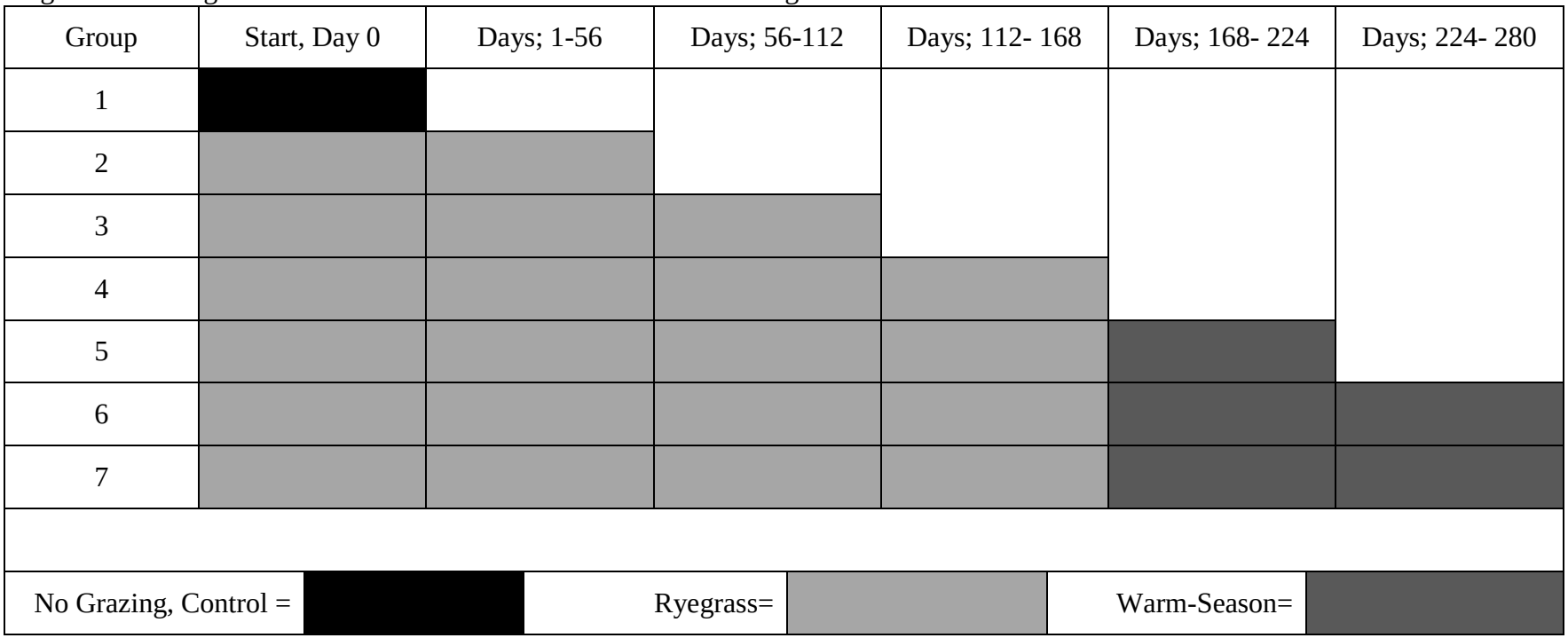
[a,b]Within a row, means lacking a common superscript differ ($P < 0.05$)

Table 19. Least squares means for fatty acid profiles (%) of LM from forage- and grain-finished cattle

Fatty Acid, %	Grain	Forage	SEM	P-value
12:0	0.042	0.019	0.009	0.073
12:1	0.000	0.000	-	-
13:0	0.005	0.000	0.002	0.078
13:1	0.097[b]	0.223[a]	0.044	0.049
14:0	2.649[a]	1.931[b]	0.199	0.015
14:1	0.603	0.897	0.223	0.359
15:0	0.485[a]	0.226[b]	0.047	< 0.001
15:1	1.618[b]	2.802[a]	0.407	0.047
16:0	24.741[a]	22.084[b]	0.656	0.007
16:1 t	0.304[b]	0.439[a]	0.033	0.007
16:1	2.787[b]	3.464[a]	0.107	< 0.001
17:0	1.408[a]	0.693[b]	0.100	< 0.001
17:1	0.932	0.879	0.043	0.386
18:0	13.173	12.387	0.371	0.143
18:1 n9 t	1.741[a]	0.024[b]	0.366	0.002
18:1	1.601	0.532	0.456	0.107
18:1 n9	32.803	35.741	1.052	0.057
18:1 n7	1.197	0.587	0.347	0.222
18:2 n6 t	0.164[b]	0.371[a]	0.061	0.022
18:2 n6	8.371	7.475	0.937	0.503
18:3 n6	0.028[b]	0.031[a]	0.014	0.008
19:1	0.000	0.000	-	0.875
18:3 n3	0.230[b]	2.043[a]	0.195	< 0.001
20:0	0.018	0.016	0.010	0.883
20:1 n15	0.157	0.299	0.083	0.233
20:1 n12	0.021	0.020	0.010	0.919
20:1 n9	0.076	0.042	0.021	0.255
20:2	0.081	0.110	0.030	0.499
20:3 n6	0.685	0.518	0.105	0.271
20:4	2.839	2.685	0.420	0.797
20:3 n3	0.000[b]	0.176[a]	0.037	0.002
22:0	0.242[b]	1.487[a]	0.172	< 0.001
20:5	0.000	0.000	-	-
22:1	0.000	0.000	-	-
22:2	0.000	0.000	-	-
22:4	0.275[a]	0.016[b]	0.038	< 0.001
22:3	0.000	0.000	-	-
24:0	0.000	0.000	-	-
22:5	0.612[b]	1.784[a]	0.187	< 0.001
22:6	0.000	0.000	-	-
24:1	0.004	0.000	0.003	0.324
SFA	42.769[a]	38.844[b]	0.0962	0.007
MUFA	43.946	45.949	0.785	0.080
PUFA	13.285	15.208	1.523	0.378
n6:n3	13.450[b]	2.523[b]	1.175	< 0.001

[a,b]Means within a row lacking a common superscript differ (P < 0.05)

Figure 1. Grazing and harvest schedule for the serial harvest of forage-finished steers



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Appendices

Appendix A

Histochemical Fiber type analyses

Reference

Solomon, M. B., and Dunn, M. C. 1988. Simultaneous histochemical determination of 3 fiber types in single sections of ovine, bovine, and porcine skeletal muscle. J. Anim. Sci. 66:255-264.

A. Solutions

a. Acid Pre-incubation solution

100 mL $CaCl_2$ (0.18 M)
3 mL Glacial Acetic Acid
890 mL ddH_2O
Adjust pH to 4.15

Note: Even though the pH is adjusted at this step it will need to be adjusted again depending on the species being typed

b. Rinse

12.1 g Tris Base
100 mL $CaCl_2$ (0.18 M)
900 mL ddH_2O
Adjust pH to 7.8

c. ATPase Incubation Medium: (Make fresh immediately before use)

40 mL Batch	200 mL Batch
2.68 mL 2-Amino 2-Methyl 1-Propanol (1.5 M)	13.40 mL
4.0 mL $CaCl_2$	20.00 mL
0.148 g KCL	0.740 g
0.0608 g ATP	0.304 g
32.0 mL ddH_2O	160.00 mL
(QS to 40 mL)	(QS to 200 mL)

Adjust pH to 9.4; incubate tissues to 37^{o} C

d. β-NADH Incubation Solution **(Make fresh daily)**

10 mL 0.2 M Tris Buffer, pH 7.4
10 mg Tetranitro Blue Tetrazolium (TNBT)
8 mg Nicotineamide Adenine Dinucleotide, reduced

Note: No matter how long you mix this it will not all go into solution, therefore, it MUST BE FILTERED

e. Tris Buffer, pH 7.4

75 mL 0.2 M Tris Base (12.11 g / 500 mL)
126 mL 0.1 M HCL (50 mL of 1 N HCL, QS to 500 mL)
(1 N HCL is 41.7 mL HCL / 458 mL ddH_2O)

174 mL ddH_2O
As a preservative, add a few drops of Chloroform

f. Ehrlich's Hematoxylin Stain
6 g hematoxylin
300 mL ethyl alcohol, absolute
9 g Aluminum Ammonium Sulfate
300 mL ddH_2O
300 mL Glycerin (Glycerol)
0.72 g Sodium Iodate
30 mL Glacial Acetic Acid
Mix and Filter

g. 0.18 M $CaCl_2$ = 26.46 g / L of ddH_2O

h. 1% (w/v) $CaCl_2$ = 10 g / L ddH_2O

i. 2% $CoCl_2$ (w/v) = 10 g / 500 mL ddH_2O

j. 2% (v/v) Ammonium Sulfide (Mix under hood) 10 mL / 500 mL ddH_2O

k. 50% (v/v) Ethyl Alcohol = 250 mL EA / 250 mL ddH_2O

l. Glycerol Gelatin/Flouromount

B. Sample preparation

a. Cut section of muscle transverse to fiber orientation to approximately 1 inch thick. Cut sample parallel to fiber orientation to approximately 0.65 cm x 0.65 cm x 2 cm. Then freeze in isopentane chilled in liquid nitrogen. Place sample in whirl pac bags.

b. After placing in whirl pac bags, place whirl pac bag in vacuum bag and vacuum package. Keep frozen and store at -80^{o} C.

c. Upon time of analysis, remove sample from ultracold and place in cryostat. Set cryostat to -20^{o} C and cut thickness to 10µm. Fix samples to cork board with freezing media and make sure that fibers are perpendicular to cork board.

d. Freeze cork board to the chuck in the cryostat using freezing media.

e. After freezing is finished, place chuck in cryostat arm and slice the sample until the whole cross section of the sample is exposed. Slice a section and affix to slide. Repeat 4 to 6 as needed.

C. Staining Sequence for Bovine

a. Start solutions that need to made or pH adjusted
b. Turn on oven to 37^{o}C
c. Cut slices for slides
d. β-NADH solution, drop on slides, incubate for 45 min @ 37^{o}C
e. Drop distilled water on slide, let stand for 30 sec.
f. Drop acid preincubation solution on slide, let stand at room temp for 10 min.
g. Drop rinse solution on slide for 1 min. pH 7.8
h. Repeat step 7
i. Drop ATPase incubation solution on slide, pH 9.4, incubate @ 37^{o}C for 30 min.
j. Drop 1% $CaCl_2$ on slide, let stand for 30 sec.
k. Repeat step 10.
l. Repeat step 10
m. Drop 2% $CaCl_2$ on slide, let stand for 3 min.
n. Drop distilled water on slide, let stand for 30 sec.
o. Repeat step 14
p. Repeat step 14
q. Repeat step 14
r. Drop 2% Ammonium sulfide on slide, **DO THIS UNDER HOOD**, let stand for 3 min.
s. Place slides into container, run distilled water continuously for 3 min.
t. Place slides in hematoxylin for 5 min
u. Repeat step 19
v. Place slides in 50% EtOH for 2 min.
w. Drain and mount slides with gel and place cover slip on them.
x. Let stand for a while before handling and viewing.

Results:

β-Red fibers:	Fibers that stain dark purple
α-Red fibers:	Fibers that stain intermediate
α-White Fibers:	Fibers that stain light purple

Note:

- For "cleaner" slides, filter the NADH solution before using it
- If three fiber types are not identifiable, increase the pH of the acid preincubation step until desired intensity is obtained

Appendix B

Sensory Evaluation form

Trained Sensory Evaluation Form

Name_____ Date_____ Time_____ Project_____

Sample No	Initial Juiciness	Sustained Juiciness	Initial Tenderness	Sustained Tenderness	Flavor Intensity	Off Flavor	Off Descriptor
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							

Juiciness	Tenderness	Flavor Intensity	Off Flavor	Off Flavor Descriptors
8=Extremely Juicy	8=Extremely Tender	8=Extremely Intense Beef	8=Extreme Off Flavor	8=Metallic
7=Very Juicy	7=Very Tender	7=Very Intense Beef	7=Intense Off Flavor	7=Salty
6=Moderately Juicy	6=Moderately Tender	6=Moderately Intense Beef	6=Very Off Flavor	6=Livery
5=Slightly Juicy	5=Slightly Tender	5=Slightly Intense Beef	5=Moderate Off Flavor	5=Grassy
4=Slightly Dry	4=Slightly Tough	4=Slightly Bland	4=Modest Off Flavor	4=Bitter
3=Moderately Dry	3=Moderately Tough	3=Moderately Bland	3=Small Off Flavor	3=Bloody
2=Very Dry	2=Very Tough	2=Very Bland	2=Slight Off Flavor	2=Rancid
1=Extremely Dry	1=Extremely Tough	1=Extreme Bland	1=No Off Flavor	1=Other-Explain

Appendix C

Thiobarbituric Reactive Substances Assay

Reference

Wang, B., Pace, R. D., Dessai, A. P., Bovell-Benjamin, A., Phillips, B. 2002. Modified extraction method for determining 2-thiobarbituric acid values in meat with increase specificity and simplicity. J. Food Sci. 67:2833-2836.

A. Solutions

a. TCA Extraction solution

7.5% (w/v) trichloroacetic acid
0.1% (w/v) EDTA
0.1% (w/v) Propyl Gallate

b. 80 mM TBA solution

1.15 g Thiobarbituric acid into 100 mL ddH_2O

c. Standard Solution

Make a 1 mM solution by adding 240 µL of tetraethoxypropane to 1L

B. Standards

a. Dilute 1mM stock solution to 80 nM /L

b. Then make standards following the table below in individual tubes

mg / kg TEP	TEP (µL)	TCA (µL)	Pipette Setting
0	0	2000	1000 x 2
2	50	1950	975 x 2
4	100	1900	950 x 2
6	150	1850	925 x 2
8	200	1800	900 x 2
10	250	1750	875 x 2
20	500	1500	750 x 2
30	750	1250	625 x 2

C. Sample preparation and extraction procedure

a. Mince meat sample and weigh out 5 g

b. Place meat into a 50 mL centrifuge tube and add 15 mL TCA Extraction solution

c. Homogenize meat for 20-30 sec using a blender

d. Place lid back on centrifuge tube

e. Centrifuge at 1,500 x *g* for 15 min

f. Remove from centrifuge and filter through No. 4 Whatman paper

D. Incubation and Reading

a. Load 96-well microplate

b. Each sample should be loaded in triplicate with 125 µL / well (See diagram below for details

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	A	A	A	I	I	I	Q	Q	Q
B	2	2	2	B	B	B	J	J	J	R	R	R
C	4	4	4	C	C	C	K	K	K	S	S	S
D	6	6	6	D	D	D	L	L	L	T	T	T
E	8	8	8	E	E	E	M	M	M	U	U	U
F	10	10	10	F	F	F	N	N	N	V	V	V
G	20	20	20	G	G	G	O	O	O	W	W	W
H	30	30	30	H	H	H	P	P	P	X	X	X

c. After sample are loaded, pipette 125 µL of TBA Solution into each well

d. Incubate for 130 min at 40^{o} C

e. Remove plates from incubator and read at 540 nm on plate reader

Appendix D

Fatty Acid Methyl Esters

Reference

O'Fallon, J. V., Busboom, J. R., Nelson, M. L., Gaskins, C. T. 2007. A direct method for fatty acid methyl ester synthesis: Application to wet meat tissues, oils, and feedstuffs. J. Anim. Sci. 85:1511-1521.

A. Solutions and Chemicals

a. Hexane

b. Methanol (MeOH)

c. 10 N KOH

d. 24 N H_2SO_4

B. Direct Fatty Acid Methylation

a. Mince meat sample and weigh out 1 g

b. Place 1 g meat into a 16 mL screwtop tube

c. Add 5.3 mL of MeOH, 0.7 mL of KOH and standard to meat sample in tube. Place cap on tube and place in water bath at 55^o C. If waterbath has a shaker attachment, turn shaker attachment on to desired setting. If there is no shaker attachment, vortex samples for 5 s every 20 min. Incubate for 90 min.

d. After incubation, place samples in cold tap water and allow to cool to below room temperature.

e. After cooling, add 0.58 mL of 24 N H_2SO_4. Mix tube by inversion and make sure K_2SO_4 precipitate is present. Place tube back in water bath and incubate for 90 min at 55^o C. If there is no shaker attachment, vortex samples for 5 s every 20 min.

f. Repeat step d.

g. After cooling, add 3 mL of hexane and vortex for 5 min.

h. Centrifuge tubes for 5 min at 1,500 x *g*.

i. Remove hexane layer and place in fatty acid vial.

j. Place fatty acid vials in freezer until time of analysis