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PRODUCTION MANAGEMENT FACTORS AFFECTING INHERENT BEEF
FLAVOR: THE ROLE OF POST-WEANING FORAGE, ENERGY
SUPPLEMENTATION, FINISHING DIETS, AND AGING PERIODS

By

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Under the Supervision of Professor Chris R. Calkins

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PRODUCTION MANAGEMENT FACTORS AFFECTING INHERENT BEEF
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Kimberly A. Varnold, PhD

University of Nebraska, 2013

Advisor Chris R. Calkins

Projects were conducted to determine effects of diet and aging periods on inherent beef flavor characteristics by relating biochemical constituents of meat, consumer acceptability, and lexicon flavor notes of two different muscles. Prediction equations were also created. Warm-season grasses caused increased concentrations of moisture, heme iron, and zinc in *L. dorsi* steaks. Aging 28 d instead of 7 d caused increased pH, carbohydrate, and heme and non-heme iron concentrations in *B. femoris* steaks. Warm-season grasses caused decreased concentrations in a majority of fatty acids, specifically when supplementation was not provided. Few differences were observed with cool-season grasses. Provision of wet distillers grains with solubles (WDGS) as a supplemental energy source minimized a majority of effects. Aging longer than 7 d tended to dissipate desirability differences in both muscles. Finishing on WDGS, especially after supplementing with WDGS, caused declines in several consumer panel scores in *L. dorsi* steaks. Warm-season grasses were most detrimental towards consumer panel scores in *B. femoris* steaks. The least desirable flavor notes were associated with warm-season grasses most of which were improved with supplementation in both muscles. Clearly, grass type is important for both flavor development and consumer

preference. Even though several of the meat principle components were found to significantly influence consumer panel and lexicon flavor note scores, the regression coefficients were small. Several regression coefficients between lexicon notes and consumer panel scores were not only significant, but also large suggesting they may be good predictors of consumer acceptability. A majority of the lexicon flavor notes were shown to be altered by diet and aging. Grazing on cool-season grasses, or supplementing while being grazed on warm-season grasses, can alter flavor notes to create a product that is highly desirable to consumers. Providing supplementation, finishing on an all corn diet, and aging the meat also promoted desirable flavors.

Keywords: Aging, beef, diet, flavor, forages, supplementation

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INTRODUCTION

Consumers rely on flavor to evaluate a beef eating experience. Great flavor will cause a consumer to deem their eating experience as pleasurable and elicits many pleasurable emotions that will cause them to remember their meal.

There are many different biochemical components that help determine the flavor profile of a specific piece of meat. The maillard reaction is an important component of flavor development. It involves a reaction between the carbonyl groups of reducing sugars (carbohydrates) and amino acids in the presence of heat, as well as the degradation of fat. If the concentration of reducing sugars or specific types of amino acids were altered, then the flavor of a product would also be altered. In addition, many different fatty acids are associated with specific flavor notes, both desirable and undesirable.

Animal diet can have a significant impact on flavor development. Grass types, as well as the composition, vary among different geographical locations. Some of these variations can alter the biochemical components of the muscle. While grazing, it is common to supplement cattle to ensure their energy requirements are being met. Wet distillers grains with solubles (WDGS) are often one form of grain-based supplementation. By the addition of this grain source, the biochemical components of the meat may be changed which could later lead to flavor differences in the meat.

After the grazing period, most cattle are finished on a high concentrate diet. Although corn is the most common concentrate used, WDGS are often included as an economical alternative. Inclusion of a different grain source may cause many changes in the meat, both biochemical and flavor related.

As meat is aged, differences in flavor profile may also occur. Even within the same carcass, there can be profound differences between different muscles. Therefore, a study was conducted to investigate how flavor and the constituents that influence flavor are affected in two different muscles from cattle fed two different forages post-weaning, with or without supplemental energy, and finished on either a corn or WDGS diet. After varying the diets in this way, along with a simulated retail display, flavor differences were identified and the biochemical components responsible for these differences were quantified.

Objectives

1. Evaluate production factors (post-weaning forage grazing, energy supplementation during grazing, and finishing diets) associated with development of desirable endogenous beef flavor.
2. Assess the effects of aging on flavor of different muscles.
3. Relate biochemical constituents of muscle to specific flavor notes using the beef lexicon.
4. Assess relationships between production factors, biochemical constituents, the flavor lexicon and consumer flavor desirability.

LITERATURE REVIEW

Consumers and beef flavor

Humans recognize flavor using five different receptors: sweet, salty, sour, bitter, and umami. Umami is the taste receptor responsible for recognizing meaty, savory, and delicious notes in food, such as those found in beef steaks (Cattleman's Beef Board and National Cattleman's Beef Association, 2010). Out of all the different palatability characteristics of meat, flavor is often the one attribute that most people rely on for a pleasurable eating experience. Reicks (2006) had consumers rate the importance of tenderness, juiciness, flavor, product consistency, ease of preparation, nutritional value, natural, organic, and price per pound. Consumers consistently rated the attribute of flavor to be the most important factor to them when they were purchasing a beef steak or roast.

Flavor is so important that studies have shown consumers are willing to pay more for a more flavorful steak. In a study conducted by Umberger et al. (2002), consumers sampled and evaluated cuts of beef from different USDA quality grades, production practices, and countries of origin. Consumers were willing to pay considerably more for cuts they had identified as having a pleasurable flavor. Consumers that had a stronger flavor preference also had a larger bid differential. Feuz and Umberger (2001) also learned that consumers were willing to pay significantly more for beef that had a pleasurable flavor. In their study, consumers were willing to pay \$1.30 or more per pound for a steak they thought had a good flavor when compared to a less desirable steak. In some cases, consumers were willing to pay up to \$1.63 more per pound.

Similarly, Platter et al. (2003) showed that even the smallest change in consumer sensory ratings, which included flavor, can have drastic effects on overall acceptance of a steak. Using a 9-point hedonic scale (1 = like extremely and 9 = dislike extremely), they found that when consumer flavor ratings decreased from a 3 to a 5 the probability of that steak being rated as acceptable also decreased rapidly, from >85% to $\leq 10\%$. When it comes to beef flavor, consumers know what they want and are willing to pay for it.

Beef Flavor

Out of all the different palatability characteristics of meat, flavor tends to be one of the most important. As discussed earlier, Umberger et al. (2002) determined that consumers were willing to pay considerably more for cuts they had identified as having a pleasurable flavor, meaning flavor is often an attribute that most people use to rate an eating experience. There are many components of meat that contribute to the flavor development.

The formation of 'meat' flavors is due to the Maillard reaction, a reaction that occurs when amino acid compounds react with the carbonyl groups of reducing sugars in the presence of heat, and the degradation of fats while cooking (Mottram, 1998, Calkins and Hodgen, 2007). Mottram (1998) stated that the reducing sugars, in particular ribose, and amino acids that react in the Maillard reaction are water-soluble precursors for flavor. The main water-soluble precursors are free amino acids, peptides, nucleotides, other nitrogenous compounds, free sugars, sugar phosphates, and nucleotide-bound sugars. The products that are formed from the Maillard reaction can be changed into furfural, furanone derivatives, hydroxyketones, and dicarbonyl compounds, all of which all

contribute to meat flavor. These intermediates can then react with other volatiles in different reactions which will create more and different flavor compounds.

Amino Acids

The water-soluble components of meat, such as amino acids and carbohydrates, contribute to the development of a 'meaty' flavor in animal tissue (Koutsidis et al., 2008). Elmore and Mottram (2006) and Macy et al. (1964a, b) also discovered that amino acids may be of importance to meat flavor. Macy et al. (1964a, b) revealed that all twenty amino acids are prevalent in beef. When beef is cooked, concentrations of taurine and alanine decreased while histidine and methionine increased. The increases in concentration were due to the hydrolysis of carnosine and/or anserine while the decreases were due to the compounds themselves being broken down. Other amino acids degraded during cooking included glutamic acid, glycine, lysine, serine, cysteine, methionine, leucine, and isoleucine. Elmore and Mottram (2006) and Macy et al. (1964a, b) speculated that the products of degradation were partly responsible for the browning of meat, as well as the resulting flavors and odors, which holds true to what is known about the Maillard reaction.

Carbohydrates

Just like for free amino acids, Macy et al. (1964a, b) also stated that carbohydrates, another water-soluble component, may be very important potential flavor precursors due to their function in the Maillard reaction. In their research, Macy et al. (1964b) found that glucose is the most prevalent carbohydrate in meat with fructose and ribose present in smaller amounts. Further studies showed that fructose was the most

stable to heating while ribose was the most labile. They deduced that the products resulting from reducing sugars and amino acids reacting with each other in the Maillard reaction may be important in browning and flavor development.

Cysteine and Ribose

The Maillard reaction occurs when amino acids and carbohydrates react with each. One of the most important interactions in the Maillard reaction is the one between the amino acid cysteine and the carbohydrate ribose. When cysteine and ribose react with each, under heating, lots of aromatic volatiles are formed (Farmer et al., 1989). The major component of these is sulphur-containing heterocyclic compounds, such as thiols and thiopenes. There are several thiols that possess meaty odor characteristics including 3-mercapto-2-methyl-2,3-dihydrothiophene, 2-ethylbenzenethiol, and pyrazine methanethiol, 2-methyl-3-furanthiol, Bis(1-mercaptoethyl)sulfide to name a few (Maga, 1976).

Two degradation products that were found in high volumes were 2-methyl-3-furanthiol and 2-furanmethanethiol. Both of these compounds are known to be important parts of aroma volatiles for beef and meat (Gasser and Grosch, 1988). Gasser and Grosch also reported that Bis(2-methyl-3-furyl)disulfide, the disulfide of 2-methyl-3-furanthiol, possesses a meat-like odor. Other compounds that were revealed by Farmer et al. (1989) when cysteine reacted with ribose included several 2-acylthiophenes, dihydro-3(2H)-thiophenone and its 2-methyl derivative, thiazole, several alkylthiazoles, and 3,5-dimethyl-1,2,4-trithiolane. All of the aforementioned compounds are also known to exist

in the volatiles of cooked beef and meat and contribute to the meaty aroma (van Straten and Maarse, 1983).

When Farmer et al. (1989) added phospholipid to the reaction of cysteine and ribose, there was an increase in meaty aroma, showing that phospholipid is also important for aroma production in the Maillard reaction. Not only did phospholipid increase the intensity of meaty aroma, but it also increased the amount of compounds that create a meaty aroma, like the ones listed previously. There are many other volatiles present in this reaction that contribute to the overall meat flavor, but only the ones that are specific to a meaty, beefy, or roasted flavor have been discussed.

Fatty Acids

Although the water-soluble compounds are responsible for the 'meaty' flavor, lipids produce the species-specific flavor (Koutsidis et al., 2008). When lipids are broken down during cooking, the fatty acids (FA) within the lipids create unique flavor profiles. Larick and Turner (1990) and Melton et al. (1982) were able to identify specific FA that promoted a desirable cooked beef flavor when their presence was increased as well as FA that promoted off-flavors in beef.

For example, a study conducted by Larick and Turner (1990) found myristic acid (C14:0), myristoleic acid (C14:1), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), and oleic acid (C18:1) to be significantly ($P \leq 0.05$), positively correlated to cooked beef fat flavor. Melton et al. (1982) also showed that palmitoleic acid (C16:1) and oleic acid (C18:1) were positively correlated ($P < 0.05$) with cooked beef fat flavor, as well as heptadecenoic acid (C17:1). Conversely, pentadecanoic acid (C15:0), linolenic

acid (C18:3), and arachidonic acid (C20:4) were negatively correlated ($P < 0.01$) with cooked beef fat flavor. As levels of FA increased in beef, the cooked beef fat flavor decreased. Also, stearic acid (C18:0) was negatively correlated ($P < 0.001$) with cooked beef fat flavor (Melton et al., 1982), which is opposite of what Larick & Turner (1990) reported.

Not all of the flavors that FA create are pleasant. Heptanoic acid, octanoic acid (C8:0), nonanoic acid, and decanoic acid are significantly ($P \leq 0.05$) correlated with grassy flavor in ground beef (Larick et al., 1987). Melton et al. (1982) discovered that C15:0, stearic acid (C18:0), linolenic acid (C18:3), and arachidonic acid (C20:4) were all associated with milky-oily, sour, and fishy flavors. They also found that C20:1 was positively correlated to sour flavor and C19:1 was positively correlated to fishy flavor. Conversely, myristoleic acid (C14:1), palmitoleic acid (C16:1), heptadecenoic acid (C17:1), and oleic acid (C18:1) were all negatively correlated to milky-oily, sour, and fishy flavors ($P < 0.05$). Higher levels of these FA would be only desirable when they decrease the incidence of other, less desirable off-flavors.

Levels of myristoleic acid (C14:1) and palmitoleic acid (C16:1) increased the incidence of liver flavor ($P < 0.05$), but increasing levels of stearic acid (C18:0) decreased ($P < 0.001$) the incidence (Melton et al., 1982). Jenschke et al. (2008) also showed other fatty acids in the subcutaneous fat of cattle that were related to liver flavor. They reported that C18:2(n-6*t*) was inversely related ($P = 0.04$) while C20:1(n-9) and CLA 9c, 11t were directly related ($P \leq 0.05$) to liver flavor. An additional study by Jenschke et al. (2007a) found that as the content of both C20:2(n-6) and C20:5 increased,

the frequency of liver-like off flavor also increased. In contrast, as both C20:3(n-6) and C22:5 increased, the frequency of liver-like off flavor decreased.

It appears that at first, stearic acid is not associated with liver off flavor, but as it becomes more unsaturated it begins to change. The type of change all depends on the location of the double bond. Stearic acid (C18:0) is converted to oleic acid (C18:1) which becomes linoleic acid (C18:2) by an enzyme in ruminant animals. If the double bonds in linoleic acid are in the conjugated 9*cis*, 11*trans* position they will continue to be unrelated to liver off flavor. However, if one of the double bonds is in the n-6*trans* location then the compound tends to be linked with liver off flavor (Jenschke et al., 2008). According to Smith et al. (2006), the enzyme Δ^9 desaturase is the enzyme responsible for converting saturated fatty acids (SFA) into monounsaturated fatty acids (MUFA). Δ^9 desaturase is encoded by the stearoyl coenzyme A desaturase gene, which can also convert MUFA into polyunsaturated fatty acids (PUFA). It is due to the Δ^9 desaturase enzyme and the stearoyl coenzyme A desaturase gene working together that converts trans-vaccenic acid (C18:1*trans*-11) into its C18:2*cis*-9,*trans*-11 isomer. Therefore, if one could control the expression of both the Δ^9 desaturase is encoded by the stearoyl coenzyme A desaturase gene, they could also control the prevalence of liver off flavor.

Hundreds of volatiles that are responsible for meat flavor have been identified from the degradation of lipid. Most of them are aliphatic hydrocarbons, aldehydes, ketones, alcohols, carboxylic acid, esters, and some aromatic compounds (Mottram, 1998). Different volatiles create different flavor notes that are present in meat. Farmer

and Patterson (1991) identified several volatiles that contributed to the meaty and roasted flavor of meat. Yancey et al. (2006) identified several volatiles that caused a more intense livery off-flavor as their content in a sample increased. All of these compounds are derived from the fatty acid components of the lipid. By manipulating the FA content, a more desirably flavored beef product may be created. A study focusing on how various diets can affect FA profiles and flavor is needed.

Minerals

Few have studied the effects of minerals on beef flavor. One study saw that as sodium concentrations increased, so did the intensity of liver-like off flavor, but only minimally (Jenschke et al., 2007a). The same study also showed that zinc was associated with stronger off-flavor intensity while phosphorous and calcium were associated with lower off-flavor intensity. The magnitude of the effects were minimal.

Of all the minerals, iron has been the one researched most. Yancey et al. (2006) determined that an increase in total iron content in a meat sample paralleled the amount of livery off-flavor ($P < 0.05$). This relationship was only observed in certain muscles. In contrast, Jenschke et al. (2007a) found an increase in occurred iron content with lower intensities of off-flavors ($P < 0.001$), although the contribution was minimal. Yancey et al. (2006) also saw significant positive correlations between myoglobin content and livery off-flavor ($P < 0.05$). This correlation was present in all muscles sampled.

When beef was cooked, non-heme iron content increased (Schricker and Miller, 1983). The increase was believed to be due to the breakdown of heme iron in

hemoglobin and myoglobin. Perhaps the increased amount of non-heme iron contributes to the livery off-flavor by catalyzing oxidation.

pH

The ultimate pH of beef may also have an effect on flavor notes. Meynier and Mottram (1995) reported different flavor notes were observed when pH changed. For example, when the pH was at 4.5 their glycine and lysine model systems had a caramel-like aroma. When the pH was increased it changed to a roasted aroma. Similarly, at a pH of 4.5 the cysteine model had what was described as a strong sulphurous and unpleasant aroma. The aroma became more roasted-meat-like at higher pH values. Moreover, Meynier and Mottram (1995) found that all of the Maillard reaction products were strongly affected by pH. Increasing the pH change an unpleasant aroma and make it more desirable.

Animal Diets

Forage feeding

Many components of meat affect its flavor, but the diet of the animal can manipulate these components and therefore change the flavor of the beef. This manipulation begins with the type of forage cattle graze post-weaning before they enter a feedlot. Grazing on sorghum sudangrass caused the flavor of ground beef to have more of a sweet and gamey aftertaste, but less of a sour and cooked beef fat flavor ($P \leq 0.05$) than cattle grazed on fescue-clover (Larick and Turner, 1990). The researchers believed that these flavor differences were due to changes in phospholipid composition and the fatty acid profiles of the neutral lipids.

When Larick et al. (1987) grazed cattle on three different grasses the cattle grazed on tall fescue had lower hot carcass weights, smaller ribeyes, less backfat, lower quality grades, and lower numeric yield grade ($P < 0.05$). Levels of the volatiles heptanal, hexanoic acid, 2-tridecanone, phyt-1-ene, octadecane, phytane, neophytadiene, phyt-2-ene, diene isomer, and dihydrophytol were all higher in samples from the fescue treatment than other grass treatments ($P < 0.05$). In addition, all of those volatiles, except for hexanoic acid, were also positively correlated ($P < 0.05$) to grassy flavor in ground beef samples. Cattle were finished on the same diet for the same amount of time, so finishing diet should not have had an effect. It can be assumed that beef from cattle fed fescue had a much stronger grassy flavor than the beef from the other grazing treatments due to the increased amount of certain volatiles.

Jenschke et al. (2008) fed cattle from two different sources, South Dakota and Nebraska, several diets consisting of high or low levels of alfalfa hay, corn stalks, or corn silage. They found that adding corn silage or corn stalks to a finishing diet significantly ($P = 0.01$) lowered levels of C16:0 than the control (no roughage inclusion, 30% wet distillers grains with solubles (WDGS)) diets. When fed low amounts of alfalfa and corn stalks there were greater amounts of C18:1(n-9) and lower amounts of C18:2(n-6) ($P \leq 0.05$) compared to the control, high levels of alfalfa, high levels of corn stalks, and both levels of corn silage. Low alfalfa also caused lower levels of C20:4(n-6) and C22:5(n-3) compared to all other diets ($P \leq 0.05$). Diets low in alfalfa and/or corn stalks had the greatest amount of MUFA compared to the control, high levels of alfalfa, high levels of corn stalks, and all levels of corn silage. All levels of corn silage had the greatest amount

of PUFA and omega-6 FA ($P < 0.05$) compared to the control, both levels of alfalfa, and both levels of corn stalks.

When cattle were fed low amounts of corn stalks, three times as many sensory panelists noticed a liver-like off flavor, in comparison to all the other diets (Jenschke et al., 2008). They reported significant correlations between certain fatty acids and liver-like off flavor, with increased amounts of 20:1(n-9) and CLA 9c, 11t and decreased amounts of 18:2(n-6t) causing an increased amount of off flavor.

Conversely, beef from cattle fed high amounts of corn stalks received some of the lowest percentages of panelists that could identify a bloody off-flavor in the meat (Jenschke et al., 2008). Other diets that created some of lowest percentages of panelists that could identify a bloody off-flavor in the meat were the control diet (no roughage inclusion of any type), high amounts of alfalfa, and diets containing both high and low amounts of silage. Diets low in alfalfa had the highest percentage of panelists that could identify a bloody off-flavor in the meat compared to all other diets. In all three studies (Larick et al., 1987, Larick and Turner, 1990, and Jenschke et al., 2008) different grasses were fed but the results were the same: forage type affected flavor, some more than others. From this it is evident that forage type fed to cattle post-weaning can affect flavor desirability.

Supplementation

While grazing cattle on grass, it is common to supplement cattle so their energy and/or protein requirements are met. The effects of supplementing on beef flavor have not been thoroughly examined. Kiesling et al. (2011) grazed cattle on predominantly

fescue grass. Half of the cattle were offered soyhulls-dried distillers grains with solubles and the other half were offered ground corn-dried distillers grains with solubles as supplementation. Overall, there were no carcass differences (hot carcass weight, ribeye area, 12th rib fat thickness, kidney, pelvic, and heart fat, and marbling score) due to supplementation. Cattle that were offered soyhulls-dried distillers grains with solubles had a higher conjugated linoleic acid content and omega-3:omega-6 ratio though ($P < 0.0001$). This shift in FA profile may be sufficient to affect beef flavor.

Mandell et al. (1997) also found that supplementation can manipulate FA profiles. Their cattle were fed a high-moisture corn diet with protein supplementation. The supplementation included 0, 5, or 10% fish meal during the finishing phase. Total saturated FA, MUFA, and PUFA were unaffected by the addition of fish meal supplementation ($P > 0.10$) and 18:0 and 20:4(*n*-6) were decreased by it ($P \leq 0.04$). Earlier, it was discussed how these two FA can affect flavor, although conflicting reports of the effect of 18:0 on beef flavor have been reported (Larick and Turner, 1990, Melton et al., 1982). 20:4(*n*-6) decreased cooked beef fat flavor, so perhaps it is a good thing that fish meal supplementation decreased its content. They also found that omega-3 FA increased while omega-6 FA decreased when fish meal is supplemented at 10% ($P < 0.05$), which agrees with Kiesling et al. (2011). Some omega-6 FA can increase the occurrence of livery off-flavor (Jenschke et al., 2008).

Shand et al. (1998) experimented with supplementation and evaluated how it affected eating quality. In their study, cattle were supplemented either wet brewers grain or a wheat-based wet distillers grain during both the background and finishing phase.

The results for beef flavor intensity, flavor desirability, and off flavor intensity were not different between the different supplementation diets or the control diet ($P \geq 0.64$), which was absent of supplementation. None of the three diets significantly affected the FA profiles ($P \geq 0.05$) of the lean.

All three studies (Kiesling et al., 2011, Mandell et al., 1997, and Shand et al., 1998) showed how supplementing cattle can affect carcass traits and FA profiles. Today, distillers grains have become an economical feed source and are often used for supplementation during grazing. However, more research is needed to evaluate the effects of using distillers grains for supplementation on beef flavor.

Finishing diet

Diets fed in a finishing lot may also be a factor in determining beef quality and flavor. Even though a concentrate like corn has been fed for ages, feeding it in different ways can still cause changes in meat quality. When Larick and Turner (1989) fed corn in very different production systems, confinement vs. grain-on-grass, both marbling and USDA quality scores increased when cattle were fed grain-on-grass ($P < 0.05$). Within the neutral lipid layer, feeding grain-on-grass increased the content of 18:2, 18:3, and total PUFA ($P < 0.05$) compared to confinement feeding. There was no effect on FA in the phospholipid layer ($P > 0.05$). As discussed in previous sections, 18:3 has been found to both decrease cooked beef fat flavor and increase sour and fishy flavors in beef. By increasing its content, the flavor of beef could be negatively affected. The same study also compared corn to wheat as the grain component. There were no differences between the two diets for carcass characteristics or FA profiles in both the neutral and

phospholipid layers ($P > 0.05$). These findings are important because they show that wheat is just as good of a grain source in finishing diets as corn from the perspective of FA composition.

In many regions of the country barley is often chosen as a concentrate for finishing diets. Busboom et al. (2000), Jeremiah et al. (1998), and Miller et al. (1996) all reported that feeding barley created no differences in flavor intensity, flavor desirability, and off-flavors when compared to corn ($P > 0.05$). Miller et al. (1996) also reported no differences in FA profiles as well ($P > 0.05$). Both Busboom et al. (2000) and Jeremiah et al. (1998) did notice differences in specific flavor attributes. Beef from cattle fed barley had significantly more incidents of metallic (Busboom et al., 2000) and bloody (Jeremiah et al., 1998) aromas ($P < 0.05$). For aftertaste, barley diets created lower intensities of browned flavors (Busboom et al., 2000) and higher incidents ($P < 0.05$) of livery, bloody, and metallic flavors (Jeremiah et al., 1998), but all other aroma and flavor attributes were not different from each other ($P > 0.05$). Given that these are the only attributes affected out of 20 attributes studied, their significance does not seem great. It would appear that feeding barley during the finishing phase, just like wheat, is just as good for flavor as a finishing ration of corn.

Distillers grains (DG) are an economical choice for a finishing ration and are widely included in diets. When new feedstuffs are fed to cattle, their effect on carcass characteristics and palatability must be investigated. When DG are included in finishing diets differences in carcass composition begin to occur. Deppenbusch et al. (2009) fed dried corn distillers grains with solubles (DDGS) consisting of either a 0, 15, 30, 45, 60,

or 75% inclusion level for 148 days. The 12th-rib fat thickness decreased as DDGS inclusion levels increased ($P = 0.05$) but diet had no effect on marbling score or USDA yield grades ($P \geq 0.17$). They also recorded that the number of carcasses grading USDA Select increased as inclusion levels of DDGS increased ($P = 0.02$).

The SFA and MUFA concentrations were not different ($P \geq 0.30$, Depenbusch et al., 2009). As DDGS inclusion levels increased, levels of C15:0, C17:0, and C10:5n-3 linearly decreased ($P < 0.05$). Conversely, total CLA and CLA 10-*trans* 12-*cis* increased linearly ($P < 0.05$), but all other isomers of CLA remained the same. This shows that feeding DDGS to cattle increases levels of CLA. Linoleic acid (C18:2n-6*cis*), total n-6 FA, and total PUFA increased ($P = 0.01$). Ratios of PUFA:SFA and n-6:n-3 also increased with increasing levels of DDGS in the diet. Senaratne (2009) compared corn-based diets to diets including WDGS and also found that WDGS inclusion increases total PUFA concentrations as well as ratios of PUFA:SFA.

The increased levels of PUFA could lead to increased lipid oxidation rates which could also lead to an increased prevalence of off flavors. Beef flavor intensity was strongest in beef from cattle fed either 45 or 60% DDGS and was the least for cattle fed no DDGS ($P = 0.03$, quadratic). This would mean the feeding cattle diets containing DDGS could actually help improve a good beef flavor. Also, no differences were discovered in off-flavor intensity between dietary treatments ($P \geq 0.16$).

Senaratne (2009) compared an all corn-based diet to one including 40% wet distillers grains with solubles (WDGS). Senaratne (2009) found no differences in off-flavor, metallic, sour, oxidized, livery, bitter, or charred flavors due to WDGS inclusion

in the diet ($P > 0.05$). However, after 7 d of retail display, feeding WDGS caused a more livery flavor in the meat ($P = 0.05$) which was not present before. The formation of a livery flavor came about after the meat was allowed to oxidize for 7 d.

Jenschke et al. (2007b) performed a similar study and fed cattle diets with an inclusion of 0, 10, 20, 30, 40, or 50% WDGS, for 125 days. Samples of *M. rectus femoris* were collected, because of their use as an indicator of off flavor, and prepared for sensory analysis. Jenschke et al. (2007b) concluded that diet had no effect on off-flavor intensity, liver-like flavor, or metallic flavor ($P = 0.47, 0.07, \text{ and } 0.73$ respectively). It should be noted that liver-like off flavor due to treatment was approaching significance ($P = 0.07$). Also, Jenschke et al. (2007b) samples were stored under vacuum and were used immediately upon opening of the package. Since they were never part of a retail display and were constantly stored in air tight packages, oxidation was never allowed to occur. This could explain the lack of livery and off-flavors. Researchers did find that the 0 and 10% WDGS diets had the highest incidence of liver-like off flavor while the 30 and 50% diets had the lowest. These findings agree with those of Depenbusch et al. (2009) who also saw that certain off-flavors are not due to feeding DG.

The effects of feeding WDGS or DDGS compared to corn have been studied by many other researchers with similar results. Other researchers have continued the work to see how WDGS and DDGS compare to each other. Both Gill et al. (2008) and Kinman et al. (2011) fed cattle diets consisting of steam-flaked corn, steam-flaked corn with DDGS, and steam-flaked corn with WDGS. Similar to Depenbusch et al. (2009), Gill et al. (2008) found that there was an increase in the amount of PUFA to SFA when

any kind of DG with solubles were included in the diet ($P = 0.04$). In contrast, Kinman et al. (2011) saw no differences in FA profile due to DG type ($P \geq 0.23$).

Taste panelists for Gill et al. (2008) perceived no difference in sensory values for flavor due to diets, however there was a larger percent of panelists overall displeased with samples from both DG diets than the control steam-flaked corn diet ($P = 0.01$). The inclusion rate for both the DDGS and WDGS fraction was only 15%. Both Jenschke et al. (2007b) and Depenbusch et al. (2009) concluded that off-flavors are not due to DG, yet in this study researchers found that consumers do not care for beef from DG fed cattle. One explanation for the difference could be the fact that the DG used in this study was from both corn and barley. Both Jenschke et al. (2007b) and Depenbusch et al. (2009) used only corn DG so perhaps the addition of the barley DG could have been enough to change the consumers' perception of flavor acceptability.

Sensory evaluations for Kinman et al. (2011) showed that differences in livery flavor detectability between the different DG ($P = 0.34$). Even though Gill et al. (2008) did not study livery flavor directly, they did find that samples from DG treatments were less desirable. It could be hypothesized that the undesirability could be due to off flavors such as liver taste. Also, Kinman et al. (2011) fed WGDS at 10, 20, and 30% inclusion levels but only fed DDGS at the 10% level. Perhaps if they had also fed DDGS at 20 and 30% inclusion levels they would have had more differences in more of the attributes. Given the contrasts in all the studies, more research needs to be conducted to determine what the effect of feeding DG to cattle is on flavor acceptability, and what fatty acids and other compounds are responsible for those effects.

Aging

Aging of beef may also be responsible for some flavor differences. As meat ages, lipids (i.e. FA) are oxidized, creating unique flavors. In two studies, two very different types of aging were employed. Smith et al. (1978) dry aged whole right sides of beef carcasses for up to 28 days and Campo et al. (1999) wet aged strip loin steaks individually for up to 21 days. Smith et al. (1978) found that dry aging up to 11 d significantly ($P < 0.05$) increased flavor desirability. Campo et al. (1999) also observed that flavor intensity increased as aging increased ($P < 0.01$), but both studies agreed that after 11 d of aging flavor scores remained the same ($P > 0.05$).

Unfortunately, aging of meat does not always create only good flavors. In some cases, it can magnify the off flavors too. Senaratne et al. (2010) fed cattle 0 and 40% inclusion levels of WDGS with and without vitamin E supplementation. When steaks were not aged in a retail display there were no differences in off flavors between them. However, after steaks were aged in retail display for 7 d, steaks from cattle fed 40% WDGS had a significantly higher off/livery flavor ratings than steaks from cattle fed 0% WDGS, despite vitamin E supplementation ($P < 0.05$), as can be seen in Figure 1 (page 26). It is interesting that differences in flavor were observed after the meat had been aged in a retail display for 7 d.

As the meat ages, flavor compounds are being broken down and changed resulting in the different flavor profiles. One of the compounds being degraded is lipid. In some cases, lipid oxidation can create a very pleasing flavor profile and in other cases, like the one just studied, it can create off flavors. Similar to the previous study, Hodgen

et al. (2007) found that a liver-like flavor becomes more prevalent as lipids oxidize. They believed this was due to increased amounts of pentanol, hexanal, hexanol, 1-octen-3-ol, and nonal which are both associated with liver-like aromas and products of lipid oxidation.

Muscles

Different muscles are known to vary in flavor. Rhee et al. (2004) compared various attributes of eleven beef muscles, including flavor intensity. The *Longissimus dorsi* had the highest rating for beef flavor intensity, while the *Psoas major* had the lowest. Differences between muscles were not much, but they were still enough for consumer to tell a difference. McKeith et al. (1985) conducted a similar study and also ranked the *Longissimus dorsi* as one of the most desirable cuts on a flavor basis. In contrast, the *Psoas major* was also highly desirable, along with the *Infraspinatus* and the *Rectus femoris*. Of the eleven muscles they evaluated, the *Pectoral* and *Adductor* received the lowest flavor desirability scores. Similarly, Carmack et al. (1995) found that the *Psoas major* received some of the highest flavor intensity scores while the *Pectoralis profundis* and the *Infraspinatus* were ranked near the bottom. They also ranked the flavor intensity of the *Longissimus* muscle somewhere in the middle. This is completely different to what both Rhee et al. (2004) and McKeith et al. (1985) discovered in their studies.

Unlike all the previous studies, Sullivan and Calkins (2011) had completely differing results. Their evaluation of published research showed no significant flavor differences between the *Longissimus*, *Psoas major*, and *Infraspinatus* muscles ($P > 0.05$).

This means that, based solely on flavor, the *Longissimus* muscle is no more desirable than the *Psoas major* and the *Infraspinatus*.

Instead of looking at overall desirability, Meisinger et al. (2006) studied the intensity of off-flavor in shoulder clods and knuckles. The *Infraspinatus* had the lowest off-flavor intensity rating ($P < 0.05$) with one of the lowest in amounts of panelists that could detect a sour, metallic, or oxidized flavor and the highest for fatty flavor.

Conversely, the *Vastus lateralis* had the most off-flavor intensity ratings ($P < 0.05$) with one of the highest amounts of panelists that could detect a sour, charred, or oxidized flavor ($P < 0.05$). When one muscle from a carcass was determined to have an off-flavor, then most of the other muscles in the carcass were also found to have an off-flavor.

Certain off-flavors are unique to individual animals and do not appear to be caused by other elements in the muscle, such as pH and heme iron content.

As discussed earlier, both FA profile and amino acid content can determine how beef will taste. The flavor of all muscles is not affected the same way by FA changes. Dryden and Maechello (1970) fed cattle of similar age and breed the same diet until a final weight of 430 kg was attained. After harvesting, the *Triceps brachii*, *Longissimus dorsi* (between the 9th and 12th ribs), and posterior section of the *Semimembranosus* were collected and analyzed. The *Longissimus dorsi*'s flavor was enhanced most ($P < 0.05$) by oleic acid (18:1). Oleic acid (C18:1) was not significantly correlated to flavor, good or bad, in the other two muscles tested ($P > 0.05$). Similarly, *Iso18:0* favorably affected flavor ($P < 0.01$) in the *Triceps brachii*, but not the *Longissimus dorsi* or the *Semimembranosus* ($P > 0.05$). When looking at how diet and FA profiles can affect the

flavor desirability of beef, it is clearly also very wise to examine different muscles so one can truly comprehend the complete relationship.

Ma et al. (1961) evaluated the differences in free amino acids between nine different beef muscles. The *Longissimus dorsi* and the *Psoas major* had a larger content of leucine-isoleucine than the *Semitendinosus*. In addition, they found threonine in the *Longissimus dorsi* muscle but it appeared to be absent in the *Semitendinosus*.

Beef Lexicon

There are many ways to evaluate beef flavor, but one of the newest methods available to researchers is the beef lexicon. In simplicity, a beef lexicon is hundreds of words that are used to describe different beef flavor notes (Drake and Civille, 2002). Beef flavor is not made up of simply one flavor, but rather a mixing and merging of several different ones. The identification of flavor notes is usually determined through the use of trained panelists.

Several universities have developed their own beef flavor lexicons. Adhikari et al. (2011) described in detail how the lexicon at Texas A&M University in College Station, TX was developed. First, 176 beef samples varying in muscle, USDA Quality Grade, animal age, gender, meat age, diet, and packaging system were used. These samples were cooked to five different end temperatures using six different cooking methods. In some samples, they were even able to induce warmed-over flavor conditions as well as spoiled meat. For their panel, six panelists were chosen who underwent 120 hr of training as well as 1,200 hr of testing. Panelists were only told that the samples were

being used to develop a beef flavor lexicon. The panelists were uninformed about all other treatments; such as muscle, animal diet, or cooking method.

During sampling, panelists wrote a list of all the flavor notes they were able to detect. Then, the group engaged in a discussion that allowed the panelists to precisely define and agree on all the flavors and aromas present. Through their testing, their lexicon was able to identify 38 descriptors (Figure 2, page 27), including the five tastes (bitter, salty, sour, sweet, and umami), for beef flavor, as well as categorizing some of these descriptors into major attributes present in beef.

Other universities have created their own lexicons. Maughan et al. (2011) at Utah State developed a beef lexicon. Although they were able to identify the five tastes, their panel identified just 13 different descriptors. There were several differences in the researcher's methods that could account for the identification of fewer descriptors. First, all samples were from the same muscle and only one packaging system was employed. In addition, all steaks were cooked using the same cooking method and to the same endpoint temperature. Unlike Adhikari et al. (2011), panelists for Maughan et al. (2011) only received 50 hr of training and only received a total of six samples at the time of testing. Panelists for Adhikari et al. (2011) evaluated 176 samples over 36 sessions. The shorter training, fewer samples, and reduced variability between the samples could account for such a low number of descriptors being identified. Currently, the Adhikari et al. (2011) beef flavor lexicon is the most comprehensive one known.

Conclusion

As can be seen, not only do consumers base eating satisfaction on flavor, they are also willing-to-pay more for a product they deemed as having a desirable flavor. Few studies have investigated how the interaction of various post-weaning forage feeding strategies, with or without supplementation, and different finishing on flavor. Therefore, we propose to investigate how flavor and the constituents that influence flavor are affected in two different muscles from cattle fed two different forages post-weaning, with or without supplemental energy, and finished on either a corn or WDGS diet. Varying the diets in this way, along with a simulated retail display, allows study to identify flavor differences and evaluation of biochemical components responsible for these differences.

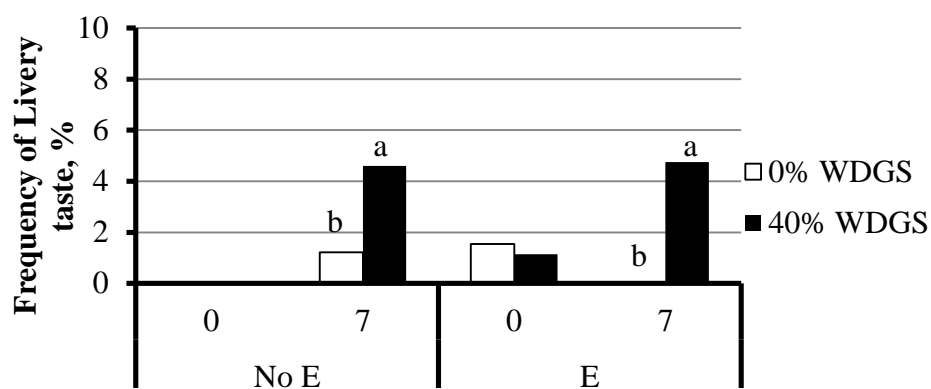


Figure 1. Frequency of livery-flavor identified by panelists of 7-days aged strip loins (*m. longissimus lumborum*) steaks from animals fed diets containing 0%, 40% WDGS with or without E supplementation during simulated retail display conditions (Senaratne et al., 2010).

^{a,b}Means in the same graph with different superscripts significantly differ ($P \leq 0.05$).

Figure 2. Definitions and References for beef flavor attributes (Adhikari et al., 2011).

Attribute	Definition	References
Animal hair	The aromatics perceived when raw wool is saturated with water.	Caproic acid (hexanoic acid) = 12.0 (aroma)
Beef identity*	Amount of beef flavor identity in the sample.	Swanson's beef broth = 5.0 (aroma and flavor) 80% lean ground beef = 7.0 (aroma and flavor) Beef brisket = 11.0 (aroma and flavor)
Bitter*	The fundamental taste factor associated with a caffeine solution.	0.01% caffeine solution = 2.0 (flavor) 0.02% caffeine solution = 3.5 (flavor)
Bloody/serumy*	The aromatics associated with blood on cooked meat products. Closely related to metallic aromatic.	USDA choice strip steak = 5.5 (aroma and flavor) Beef brisket = 6.0 (aroma and flavor)
Brown/roasted*	A round, full aromatic generally associated with beef suet that has been broiled.	Beef suet = 8.0 (aroma and flavor) 80% lean ground beef = 10.0 (aroma and flavor)
Burnt	The sharp/acrid flavor note associate with over-roasted beef muscle, something over-baked or excessively browned in oil.	Alf's red wheat Puffs = 5.0 (aroma and flavor)
Chemical	The aromatics associated with garden hose, hot Teflon pan, plastic packaging and petroleum based product such as charcoal liter fluid.	Zip-Loc sandwich bag = 13.0 (aroma) Clorox in water = 6.5 (flavor)
Cocoa	The aromatics associated with cocoa beans and powdered cocoa and chocolate bars. Brown, sweet, dusty, often bitter aromatics.	Hershey's cocoa powder in water = 3.0 (flavor) Hershey's chocolate kiss = 7.5 (aroma), 8.5 (flavor)
Cooked milk	A combination of sweet, brown flavor notes and aromatics associated with heated milk.	Mini Babybel original Swiss cheese = 2.5 (flavor) Dillon's whole milk = 4.5 (flavor)
Dairy	The aromatics associated with products made from cow's milk, such as cream, milk, sour cream or butter milk.	Dillon's reduced fat milk (2%) = 8.0 (flavor)
Fat-like*	The aromatics associated with cooked animal fat.	Hillshire farms Lit'l beef smokies = 7.0 (aroma and flavor) Beef suet = 12.0 (aroma and flavor)
Green	Sharp, slightly pungent aromatics associated with green/plant/vegetable matters such as parsley, spinach, pea pod, fresh cut grass, etc.	Hexanal in propylene glycol (5,000 ppm) = 6.5 (aroma) Fresh parsley water = 9.0 (flavor)
Green-hay	Brown/green dusty aromatics associated with dry grasses, hay, dry parsley and tea leaves	Dry parsley in medium snifter = 5.0 (aroma) Dry parsley in -30-mL cup = 6.0 (flavor)
Leather	Musty, old leather (like old book bindings)	2,3,4-Trimethoxybenzaldehyde = 3.0 (aroma)
Liver-like	The aromatics associated with cooked organ meat/liver	Beef liver = 7.5 (aroma and flavor) Braunschweiger liver sausage = 10.0 (aroma and flavor – must taste and swallow)
Metallic*	The impression of slightly oxidized metal, such as iron, copper and silver spoons.	0.10% potassium chloride solution = 1.5 (flavor) USDA choice strip steak = 4.0 (aroma and flavor) Dole canned pineapple juice = 6.0 (aroma and flavor)
Overall sweet*	A combination of sweet taste and sweet aromatics. The aromatics associated with the impression of sweet	Post-shredded wheat spoon size = 1.5 (flavor) Hillshire farms Lit'l beef smokies = 3.0 (flavor) SAFC ethyl maltol 99% = 4.5 (aroma)
Rancid	The aromatics commonly associated with oxidized fat and oils. These aromatics may include cardboard, painty, varnish and fishy.	Microwaved Wesson vegetable oil (3 min at high) = 7.0 (flavor) Microwaved Wesson vegetable oil (5 min at high) = 9.0 (flavor)
Salty*	The fundamental taste factor of which sodium chloride is typical.	0.15% sodium chloride solution = 1.5 (flavor) 0.25% sodium chloride solution = 3.5 (flavor)
Sour aromatics*	The aromatics associated with sour substances.	Dillon's buttermilk = 5.0 (flavor)
Sour dairy	Sour, fermented aromatics associated with dairy products such as buttermilk and sour cream.	Laughing cow light Swiss cheese = 3.0 (aroma), 7.0 (flavor) Dillon's buttermilk = 4.0 (aroma), 9.0 (flavor)
Sour*	The fundamental taste factor associated with citric acid.	0.015% citric acid solution = 1.5 (flavor) 0.050% citric acid solution = 3.5 (flavor)
Spoiled	The presence of inappropriate aromatics and flavors that is commonly associated with the products. It is a foul taste and/or smell that indicates the product is starting to decay and putrefy.	Dimethyl disulfide in propylene glycol (10,000 ppm) = 12.0 (aroma)
Sweet*	The fundamental taste factor associated with sucrose.	2.0% sucrose solution = 2.0 (flavor)
Umami*	Flat, salty, somewhat brothy. The taste of glutamate, salts of amino acids and other molecules called nucleotides.	0.035% accent flavor enhancer solution = 7.5 (flavor)
Warmed-over	Perception of a product that has been previously cooked and reheated.	80% lean ground beef (reheated) = 6.0 (flavor)
Other attributes	Smoky – charcoal, smoky – wood, buttery, refrigerator-stale, soapy, barnyard, heated oil, asparagus, cumin, floral, beet and petroleum-like	

* Major notes.

USDA, United States Department of Agriculture.

MATERIALS AND METHODS

STUDY 1

The role of post-weaning forage, energy supplementation, finishing diets, and aging on the color and biochemical constituents of beef

Diets

All protocols performed in this study were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. Cattle were fed in a 2x2x2 factorial design. Crossbred steers (n = 64) were backgrounded on either warm-season grasses (i.e. bluestem and switch grass) at the Barta Ranch in Western Nebraska or on cool-season (i.e. brome and bluegrass) pastures in Ithaca, NE for 177 d, shortly after weaning. Within each pasture, half of the cattle were supplemented (0.6 kg WDGS/kg body weight/ day) for energy using wet distillers grains with solubles (WDGS). After the grazing period was completed, all cattle were transported to the University of Nebraska-Lincoln's research feedlot in Ithaca, NE. While in the feedlot, half of each pasture and supplementation treatment was finished on an all corn diet while the other half were fed corn with WDGS at a 35% inclusion rate (DM basis). Cattle were on feed for 119 days and fed to an average live weight of 1,427 lbs.

Harvest

At the end of the feedlot period, cattle were harvested at the Greater Omaha Packing plant in Omaha, NE. Forty-eight carcasses grading either USDA Choice (n = 43) or USDA Select (n = 5), 6 from each treatment combination, were selected. Two carcasses that were grazed on warm-season grass without supplementation and were

finished on WDGS graded USDA Select, while one carcass from cattle that were grazed on warm-season grasses with supplementation and finished on corn, grazed on cool-season grasses without supplementation and finished on corn, and grazed on cool-season grass with supplementation and finished on WDGS graded USDA Select. All of the other carcasses from all dietary treatment combinations graded USDA Choice. Strip loins (*Longissimus dorsi*; IMPS #180, NAMP, 2007) and bottom round flats (*Biceps femoris*; IMPS #171B, NAMP, 2007) were collected from each side of the carcass (n = 12 muscles/treatment). Only ten *L. dorsi* muscles were collected from the cool-season grass, supplementation provided, and finished on WDGS treatment because two *L. dorsi* muscles (one from each side) were lost within Greater Omaha Packing Plant. Subprimals from the left side of the carcass were aged in a vacuum package for 7 d while subprimals from the right side were aged in a vacuum package 28 d at 2°C. Upon fabrication, 5 steaks were cut anterior to posterior from each subprimal.

Sample Collection

The first steak, cut 1.25 cm thick, was used for all lab analyses. The second steak, also 1.25 cm thick, was used as a back-up for lab analyses. Both steaks were vacuumed packaged and frozen at -20°C for approximately 2 months. Before any lab procedures were conducted, all lab steaks had any subcutaneous fat and epimysial tissue removed and were cut into cubes. The cubes were flash frozen in liquid nitrogen, powdered using a Waring blender (Waring Commercial, model 51BL32, Torrington, CT), and stored at -80°C for several weeks until further lab analyses. All lab analyses were conducted on powdered samples. Fat, protein, ash, amino acid, and mineral analysis were only

conducted on 7 d aged steaks while pH, moisture, non-heme iron, heme iron, and total carbohydrate analysis were conducted on both 7 and 28 d aged steaks.

The third steak, cut 2.54 cm thick, was placed on a Styrofoam tray, wrapped with PVC overwrap film, and placed under simulated retail display for 7 d for use by the beef lexicon panel. Objective color and subjective discoloration were measured daily while in the retail display. Steaks 4 and 5 were cut 2.54 cm thick, placed on a Styrofoam tray, wrapped with PVC overwrap film, and placed in a retail display case at 2°C for 7 d. Two steaks were used for consumer panels. Strip loin steaks were packaged as two steaks per tray. Steaks on the same tray were from animals that received identical feeding treatments so as to prevent any possible contamination or influence. At the end of retail display, steaks were vacuumed packaged and frozen at -20°C for two months until further use.

Retail Display

All of the trays were displayed on a table in a 2°C cooler and were constantly exposed to warm white fluorescence lighting (PHILIPS F32T8/TL741 ALTO 700 Series, 32 WATT B7, Royal Philips Electronics, Amsterdam, Netherlands) at 1000 to 1800 lux in order to simulate retail display conditions. Every day, packages were randomly relocated to minimize any effects due to location. After 7 days, steaks were vacuumed packaged and frozen until further analysis.

Color

Objective color was measured using a Minolta Chromometer CR-400 (Shanghai, China) set at a D65 light source and 2° observer with an 8 mm diameter measurement

area. The L^* , a^* , and b^* values were recorded using an average of six readings per steak. Readings were taken at 24 h intervals for 8 d. Subjective discoloration was evaluated based on percentage of surface discoloration (0% indicating no discoloration and 100% indicating complete discoloration of the entire steak) by five trained panelists.

On one of the retail display days, objective color was recorded as X, Y, and Z instead of L^* , a^* , and b^* . Values were converted to L^* , a^* , and b^* using a CIE color calculator (<http://www.brucelindbloom.com/index.html?ColorCalcHelp.html>). For the calculator Scale XYZ was selected, Ref. White was set as D65, RGB model was set as CIE RGB, and Adaptation was set as XYZ scaling. In order to verify the calculator, several readings on different solid colored pieces of paper in varying temperatures were recorded using the using a Minolta Chromometer CR-400 and both the L^* , a^* , and b^* and the X, Y, and Z scores from the Minolta were compared to the calculator's results.

pH

To determine ultimate pH, duplicate 10 g powdered samples from each steak were homogenized with 90 mL of double distilled water for 30 sec using a Polytron homogenizer (POLYTRON Kinimatica CH-6010, Switzerland) set at 10,800 rpm. A stir bar was added to the homogenized samples and placed on a stir plate so the sample would be continually mixed during the pH readings. The pH was determined using an Orion 4 STAR pH ISE Bench-top meter (Thermo Electron Corporation, Waltham, MA) calibrated using a 7.0 and 4.0 buffer. The pH probe was rinsed with double distilled water and wiped dry with a Kimwipe (Kimberly-Clark Professional, Roswell, GA) between every sample.

Proximate Analysis

Moisture and ash were determined using duplicate 2 g samples and analyzed on a LECO Thermogravimetric Analyzer (LECO Corporation, model 604-100-400, St. Joseph, MI). Below are the setting used:

Name	Covers	RampRate	RampTime	StartTemp	EndTemp
Moisture	Off	6 d/m	:17 min	25 C	130 C
Ash	Off	20 d/m	:30 min	130 C	600 C

Name	Atmosp	FlowRate	HoldTime	Const.Wt.	Const.Wt. Time
Moisture	N	High	00 min	0.05%	09 min
Ash	O	High	00 min	0.05%	09 min

Crucible density was set at 3.00, cover density was set at 3.00, and sample density was set at 1.00. The calculations used to determine moisture and ash content were as follows:

Equations

Initial Wt.	W [Initial]
Moisture	$((W[\text{Initial}] - W[\text{Moisture}])/W[\text{Initial}]) * 100$
Ash	$(W[\text{Ash}]/[\text{Initial}]) * 100$
Ash Dry Basis	$E[\text{Ash}] * (100/(100-E[\text{Moisture}]))$

Fat was measured using triplicate 2 g samples (inside of Whatman #2 filter paper packets) and extracted with anhydrous ether as described by the soxhlet procedure (AOAC, 1990). Samples were loaded into soxhlet tubes and the boiling flasks were filled with 400 mL of ether. The soxhlet tubes were next fitted onto the boiling flasks, the entire apparatus was fitted into the condenser, water supply to condenser turned on, and heating element turned on. After 48 hrs, burners were turned off and the ether was allowed to cool completely. After the soxhlet tubes and boiling flasks were disconnected, samples were allowed to dry under a fume hood for 2 hr in order to remove any remaining ether in the samples before being placed into a drying oven set at 105 ° C overnight. The following calculation was used to determine % fat in the samples:

$$\left(\frac{\text{Original weight including filter paper and paper clip} - \text{Fat extracted sample weight}}{\text{Sample Wt}} \right) * 100 - \% \text{ Moisture} = \% \text{ Fat}$$

Protein was determined by difference.

Total Carbohydrates

Samples were prepared by homogenizing 0.5 g of powdered meat with 20 mL of 80% ethanol in a 50 mL centrifuge tube in duplicates in order to extract the carbohydrates. Samples were then stored in a 2°C cooler until further testing, at least one hour later. Upon analysis, tubes were centrifuged at 783 RCF (g) for 5 min. A 1 mL aliquot of sample containing <0.1 mg/mL of total carbohydrates was removed and added to a new tube following the procedures of Dubois et al. (1956). To the new tubes, 50 µL of 80% phenol and 2.5 mL of concentrated sulfuric acid were added and vortexed

immediately. After 10 min, samples were moved to a cool water bath for 10 to 25 min. After samples were cooled, they were read on a spectrophotometer at 490 nm.

Sugar concentrations were estimated using a standard curve and then correcting for dilutions. The curve was prepared by mixing a stock solution of about 0.1 mg/ml glucose standard at varying concentrations (0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL, respectively) with varying amounts of double distilled water (1.0, 0.8, 0.6, 0.4, 0.2, and 0 mL, respectively). Standard samples were then prepared and read the same way as described above.

Non-heme Iron

The procedures described by Rhee and Ziprin (1987) were used to determine non-heme iron concentrations. Duplicate 5 g samples of powdered meat were mixed with a 0.2 mL of NaNO_2 solution (0.39% w/v) and 15 mL of a 40% TCA-HCL (1:1) acid solution, vortexed, and placed in a water shaker bath set at 65°C for 20 h. After incubation, samples were allowed to cool to room temperature for 1 h.

Approximately 1 mL aliquots of the liquid phase were removed and mixed with 5 mL of a color reagent (20:20:1 double distilled deionized water:saturated sodium acetate solution:bathophenathroline disulfonate reagent). To create a liquid phase without a color reagent blank, 1 mL aliquot of the liquid phase was mixed with 5 mL of a 21:20 double distilled deionized water:sodium acetate solution. Both a reagent blank and a liquid phase blank were created. The reagent blank was made by adding 5 mL of the same color reagent listed above to 1mL of the TCA-HCL acid mixture. The liquid phase blank was created by adding 5 mL of the 21:20 double distilled deionized water:sodium

acetate solution to 1 mL of the TCA-HCL acid solution. All 4 new mixtures were vortexed, centrifuged (Sorvall SE-12 rotor and Sorvall RC 5B centrifuge, Dupont Co., Wilmington, DE) at 7,045 RCF (g), and read at 540 nm using a Cary 100 Varian UV/Visual Spectrophotometer (Varian Instruments, Sugarland, TX).

Readings were compared against a standard curve. The curve was prepared using an iron stock standard (Sigma) and was mixed with the TCA-HCL acid solution at varying concentrations (0.5, 1.0, 1.5, 2.5, 3.5, and 4.5 $\mu\text{g/mL}$) to total 25 mL. Standard samples were then mixed with 5 mL of the color reagent, vortexed, centrifuged, and read at 540 nm.

To determine total non-heme iron, first final absorbance of each sample was calculated by subtracting the absorbance of the incubated liquid phase without color reagent from the absorbance of the incubated liquid phase with color reagent. Final concentration was calculated by subtracting the intercept of the standard curve from the final absorbance and dividing it all by the slope of the standard curve. Finally, non-heme iron concentration was calculated as follows:

$$\mu\text{g non-heme Fe/g meat} = \text{concentration } (\mu\text{g/mL}) \times \frac{(15+0.2+\text{moisture in 5g meat})}{5\text{g}} \times 1\text{mL}$$

Heme Iron

The procedures described by Hornsey (1956) and Lee et al. (1998) were used to determine heme iron concentration. Duplicate 2 g powdered meat samples were mixed with double distilled water, based on sample moisture percentage, so that the total volume of water in the sample was equal to 0.72 mL, or 72% moisture. About 8.1 mL of acetone and 0.2 mL of hydrochloric acid were next added. All tubes were kept in test

tube trays wrapped in aluminum foil to reduce light exposure. The sample was homogenized using a Polytron homogenizer at 10,800 rpm for 15 sec. Samples were immediately filtered through #2 Whatman filter paper (90 mm in diameter) and into a new tube which was also kept in a test tube rack wrapped in aluminum foil. The filtrate was immediately read on a spectrophotometer at 640 nm.

In order to determine total amount of heme iron, total pigment (mg/kg) was calculated by multiplying the absorbance of the sample by 680. Total heme iron (mg/kg) was then calculated by multiplying the total pigment by 8.82 and dividing it all by 100 (Lee et al., 1998).

Minerals and Amino Acids

About 5 g powdered samples in 50 mL centrifuge tubes were sent to Ward Laboratories, Inc. in Kearney, NE for mineral analysis. Atomic absorption spectroscopy was used to quantify the minerals following the procedures of Ward and Gray (1994).

Powdered 5 g samples in 50 mL centrifuge tubes were sent to AAA Service Laboratory, Inc. in Damascus, OR for amino acid analysis following the procedures of Moore and Stein (1949), Roach and Gehrke (1970), Simpson et al. (1976), Stanford (1963), and Keutmann and Potts (1969). After arrival at AAA Service Laboratories, samples were weighed, dried and hydrolyzed (1:2,000, v/v) in 6 N HCl/2% phenol at 110°C for 22 h. Next, the hydrolysate was dried and a sampling injected onto a Hitachi L8900 Amino Acid Analyzer with post-column-ninhydrin derivatization. Norleucine was added to the samples to act as an internal control.

Statistical Analysis

All data were analyzed as a 2x2x2x2 factorial arrangement split plot design within a randomized complete design using the Mixed procedure in SAS (Version 9.2, SAS Institute Inc., Cary, NC, 2006). The experimental unit was the individual animal and the model included grass, supplementation, finishing diet and age as fixed effects. The least square means procedure with the probability of difference (pdiff) option was used for mean separation with significance determined at $P \leq 0.05$ levels. Whenever there was a three- or four-way interaction, the LSmeans were reanalyzed using the GLIMMIX procedure with the slicediff option in order to more accurately study differences.

STUDY 2

The role of post-weaning forage, energy supplementation, finishing diets, and aging on the fatty acid profile of beef

The samples for this study were collected and prepared as described in the materials and methods for Study 1.

Fatty Acids

Fats were extracted following the procedures of Folch et al. (1957). Four g of powdered meat samples were mixed with 5 mL of 2:1 chloroform:methanol solution, vortexed, and allowed to sit at room temperature for 1 h. Homogenized samples were filtered through #2 Whatman paper into new tubes, mixed with 2 mL of 0.74% KCl solution, vortexed, purged with nitrogen gas, and kept in a -20°C freezer overnight. The

next day, the top aqueous phase was removed and 2 mL of the lower phase was collected and dried down by constant nitrogen gas purging at 60°C.

Samples were separated into neutral and phospholipid layers following the procedures described by Carr et al. (2005). Dried samples were plated onto aluminum thin layer chromatography plates (Silica Gel 60 w/o indicator, Catalog No.: M5547-7, Thermo Fisher Scientific Inc.), placed in a tank with a 75:25:2 hexane:diethyl ether:acetic acid solution, and allowed to run until the solution had travelled to the top of the plate, approximately 45 min. Upon completion, the plates were removed from the tanks and the solvent was allowed to evaporate. The dried plates were stained with a primulin dye (5mg of primulin in 100 mL of acetone\water (80\20)) and the neutral and phospholipid regions were identified and marked under a blacklight. The regions of interest were cut out, folded up, and placed in a glass tube. In order to extract the lipids off of the plates, the neutral lipid samples were submerged in 100% chloroform and the phospholipid samples were submerged in 100% methanol. Samples were placed in a 4°C cooler for 45 min to extract the fatty acids.

After extraction, the folded up plates were removed from the tube and the remaining solutions were dried at 60°C under constant nitrogen gas purging. Once samples were dried, lipids were saponified into fatty acid methyl esters following the procedures described by Morrison and Smith (1964) and Metcalfe et al. (1996). To prepare samples, 0.5 mL of 0.5 M NaOH in methanol was added, samples were vortexed, and then placed in a 100°C oven. After 5 min, samples were removed from the oven, mixed with 0.5 mL of 14% boron trifluoride in methanol, vortexed, and placed back into

the oven for an additional 5 min. After heating, 1 mL of hexane and 1 mL of a saturated salt solution were added. The samples were mixed on a vortex and centrifuged for 5 min at 1000 x g. The top phase was removed, about 1 mL of solution, and placed in a gas chromatography vial for analysis. Since the phospholipid samples contained less fatty acids, they were dried at 60°C under constant nitrogen gas purging and mixed with 100 µL of hexane to concentrate the sample. Gas chromatography (Hewlett-Packard Gas Chromatograph – Agilent Technologies, model 6890 series, Santa Clara, CA) was used to determine fatty acid content using a Chrompack CP-Sil 88 (0.25 mm x 100 m) column using Helium as the carrier gas with a flow rate of 1.1 mL/min. The injector temperature was held at 270°C and the detector temperature was 300°C. Fatty acids were identified by comparing retention times and peaks with known standards.

To get exact concentrations of each FA, additional thin layer chromatography plates were made separating the neutral and phospholipid layers using around 100 µL of sample. This time the plates were stained using iodine and scanned using a scanner and saved as a JPG file. In order to improve clarity, brightness and contrast of each plate JPG was adjusted (-60 and 60 respectively) and resaved as a TIFF.Bitmap uncompressed file. The areas on the plates were measured, as a percent, using Quantity One 1-D Analysis Software (Bio-Rad, Hercules, CA) and identified as neutral lipid, phospholipid, or other. To calculate the mg/100 g of meat for each FA in each layer, the total fat percentages attained for each sample from proximate analysis was converted to grams of fat per 100 g of meat. That value was multiplied by the percentage of the neutral and phospholipid layers, and converted to mg of neutral or phospholipid per 100 g of meat. From there the

percentage of each individual FA in each layer was multiplied by their respective value and the mg of each FA per 100 g of meat was attained.

Statistical Analysis

All data were analyzed as a 2x2x2x2 factorial arrangement split plot design within a randomized complete design using the Mixed procedure in SAS (Version 9.2, SAS Institute Inc., Cary, NC, 2006). The experimental unit was the individual animal and the model included grass, supplementation, and finishing diet as fixed effects. The least square means procedure with the probability of difference (pdiff) option was used for mean separation; with significance determined at $P \leq 0.05$ levels. Whenever there was a three-way interaction, the LSmeans were reanalyzed using the GLIMMIX procedure with the slicediff option in order to more accurately study differences.

STUDY 3

The role of post-weaning forage, energy supplementation, finishing diets, and aging on beef flavor and acceptability

The samples for this study were collected and prepared as described in the materials and methods for Study 1.

Consumer Panel

All consumer and lexicon panels were approved by the Institutional Review Board and all panelists signed a consent form. Consumer panels were conducted in Houston, Texas and Olathe, Kansas (n = 120 per location). Consumers were recruited using existing consumer data banks and random phone solicitation. Consumers were

selected that eat beef at least three times per week, range in age from 21 to 65, with an approximately equal balance of males and females, and a range in income.

In each city, consumer panels were conducted over two days, with the first day evaluating *Longissimus dorsi* steaks and the second day evaluating *Biceps femoris* steaks. Different consumers evaluated each muscle type. Steaks from each animal were evaluated at both locations. Panels were conducted with three sessions per day and 20 consumers per session. Five consumers evaluated each steak and treatment order was randomized and allocated to consumer using an incomplete block design. Each consumer evaluated eight steaks in a session.

Steaks were cooked on a Hamilton Beach Health Smart grill (model 31605A, Hamilton Beach/ Proctor-Silex, Inc., Southern Pines, NC) to an internal temperature of 70°C. Consumers evaluated each sample using 9-point hedonic (1=dislike extremely, 9=like extremely) and intensity scales (1=none or extremely bland, 9=extremely intense) for overall like, overall flavor like, beefy flavor like and intensity, and grilled flavor like and intensity.

Beef Flavor Lexicon

An expert, trained descriptive attribute sensory panel with over 23 cumulative years of experience in evaluating beef flavor and aromas was used. This panel was one of the three panels used to validate the Beef Lexicon at Texas A&M University (Philips et al., 2010; Miller, 2010). The panel underwent ballot development, training and validation sessions to assure consistent rating and identification of individual aroma and flavor attributes. Attributes were classified as major and minor notes. This provides a

standardized, defined reference guide for determining and measuring aroma and flavor in beef.

During training and testing, steaks were cooked the same way described for consumer panels. Aromas and flavor aromatics were evaluated using the Spectrum® Universal 16-point scale where 0 = none and 15 = extremely intense (Meilgaard et al., 2007). Traits evaluated were brown, bloody, fat, metal, liver, green hay, umami, overly sweet, sweet, sour, salty, bitter, sour aroma, barnyard, burnt, heated oil, chemical, apricot, asparagus, cumin, floral, beet, chocolate, green grass, musty, medicinal, petroleum, smoked/charred, smoked wood, spoiled, dairy, buttery, cooked milk, sour milk, refrigerator stale, warmed over, soapy, painty, fishy, and cardboardy.

Statistical Analysis

All data were analyzed as a 2x2x2x2 factorial arrangement split plot design within a randomized complete design using the Mixed procedure in SAS (Version 9.2, SAS Institute Inc., Cary, NC, 2006). The experimental unit was the individual animal and the model included grass, supplementation, finishing diet and age as fixed effects. The least square means procedure with the probability of difference (pdiff) option was used for mean separation; with significance determined at $P \leq 0.05$ levels. Whenever there was a three- or four-way interaction, the LSmeans were reanalyzed using the GLIMMIX procedure with the slicediff option in order to more accurately study differences. The demographic survey for the consumer panel was analyzed using the frequency procedure to determine frequencies.

STUDY 4

The use of biochemical constituents and beef flavor lexicon results to predict consumer flavor ratings

The samples analyzed and used in this study were collected, prepared, and analyzed in the materials and methods for Studies 1, 2, and 3.

Statistical Analysis

In order to determine regression coefficients, the biochemical constituents of the meat were separated into five distinct groupings: neutral lipid fatty acids, phospholipid fatty acids, minerals, amino acids, and composition (pH, moisture, fat, protein, ash, total carbohydrates, and cooking loss). Each individual grouping was analyzed using the principle component procedure in SAS. The first two principle components were identified and associations for each were determined. All of the newly identified principle components were merged into one file along with the consumer panel and lexicon data files. Three different analysis were conducted to determine the regression coefficients of the principle components of the biochemical constituents for the consumer panel results, the principle components of the biochemical constituents for the lexicon results, and the lexicon results for the consumer panel results using the regression procedure.

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Running Title: Diet and aging on beef composition

The role of post-weaning forage, energy supplementation, finishing diets, and aging on the color and biochemical constituents of beef¹

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Abstract

The objective of this study was to determine how color and biochemical constituents of meat are altered when diet and aging periods are varied. Crossbred steers (n = 64) were allowed to graze on warm or cool-season grasses, without or with energy supplementation of wet distillers grains with solubles (WDGS), and were finished on corn or 35% WDGS. Six carcasses from each treatment (n = 48) that graded USDA Choice or Select were identified and *Longissimus* and *Biceps femoris* (*B. femoris*) muscles from each side of each carcass were collected and aged under vacuum for 7 and 28 d. Samples were analyzed for proximate composition, pH, cooking loss, and heme and non-heme iron content, amino acid, and mineral content. Finishing on WDGS and aging 28 d caused the most amount of discoloration ($P = 0.02$) in *Longissimus dorsi* (*L. dorsi*) steaks (75%) at the end of retail display. No differences in discoloration for *B. femoris* steaks was observed until the end of retail display time where grazing on warm-season grasses and not supplementing caused higher ($P = 0.01$) scores than grazing on cool-season grasses and not supplementing for both 7 d (30% vs. 20%) and 28 d (75% vs. 52%) aged product. For both *L. dorsi* and *B. femoris* steaks, aging 28 d increased pH values ($P < 0.0001$) as compared to 7 d aged product. Warm-season grass increased ($P = 0.04$) moisture content (70.67% to 71.62%), decreased magnesium (0.033 vs. 0.029), and increased zinc concentration (37.46 vs. 42.21) in *L. dorsi* steaks ($P \leq 0.03$) as compared to cool-season grasses. Also in *L. dorsi* steaks, supplementation decreased ($P = 0.03$) protein (21.24% to 20.75%) and a three-way interaction between grass type, supplementation, and finishing diet ($P = 0.04$) was observed in ash content. Corn-

finished cattle had a higher heme iron content ($P = 0.003$) when grazed on warm-season versus cool-season grasses (7.98 vs. 7.11) in *L. dorsi* steaks. Carbohydrates increased ($P = 0.0003$) in *B. femoris* steaks when aged 28 d as compared to 7 d. A three-way interaction ($P = 0.05$) between grass type, supplementation, and aging period influenced non-heme iron content in *B. femoris* steaks. Glycine content was influenced ($P = 0.05$) by grass type, supplementation, and finishing diet interaction in *B. femoris* steaks. When not supplemented, phosphorous levels were higher ($P = 0.04$) when finished on WDGS instead of corn (0.23 vs. 0.21) in *B. femoris* steaks. The remaining components not discussed were unaffected by diet and aging period. Grass type caused the most biochemical changes in meat.

Keywords: Aging, beef, biochemical constituents, diet, forages, supplementation

Introduction

The diet of beef cattle can influence many of the biochemical constituents in meat. This influence begins with the type of forage cattle are grazed on post-weaning and before they enter a feedlot.

Larick and Turner (1990) fed three different grass types (tifleaf pearl millet, sorghum-sudangrass, or fescue-clover) to cattle. Even though they saw differences in in fat thickness and yield grade, no differences ($P \leq 0.05$) were found in moisture or fat content in meat from these animals. Grasses vary in both variety and composition and some tend to lack important nutrients. Because of this it is common to supplement with grain during the background phase. When Srinivasan et al. (1998) supplemented cattle

while on grass, protein, fat, and ash remained unchanged but moisture content decreased ($P \leq 0.05$). In addition, an increase ($P \leq 0.05$) in potassium content as well as a decrease in iron content was also reported.

Varying finishing diets also alters the composition of beef. Driskell et al. (2011) found that the flat iron steaks (*Supraspinatus*) from cattle fed wet distillers grains with solubles (40% DM basis) had an increased sodium content while in petite tenders (*Teres major*) thiamin content increased and manganese content decreased ($P \leq 0.03$) compared to animals fed an all corn diet. In addition, L* values decreased and a* and b* values decreased ($P \leq 0.04$) in steaks from cattle fed distillers grains (Gill et al., 2008).

The objective of this study was to identify changes in beef composition in two different muscles from cattle fed two different forages post-weaning, with or without supplemental energy, finished on either a corn or wet DGS (WDGS) diet, and aged for 7 or 28 d. By varying the diets in this way, along with a simulated retail display, compositional differences may be identified.

Materials and Methods

Diets

All protocols performed in this study were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. Cattle were fed in a 2x2x2 factorial design. Crossbred steers (n = 64) were backgrounded on either warm-season grasses (i.e. bluestem and switch grass) at the Barta Ranch in Western Nebraska or on cool-season (i.e. brome and bluegrass) pastures in Ithaca, NE for 177 d, shortly after weaning. Within each pasture, half of the cattle were supplemented with 0.6 kg

WDGS/kg body weight/ day for energy. At the end of the grazing period, all cattle were transported to the University of Nebraska-Lincoln's research feedlot in Ithaca, NE.

While in the feedlot, half of each pasture and supplementation treatments were finished on an all-corn diet while the other half were fed corn with WDGS at a 35% inclusion rate (DM basis). Cattle were on feed for 119 days and fed to an average live weight of 1,427 lbs.

Harvest

At the end of the feedlot period, cattle were transported and harvested at the Greater Omaha Packing (Omaha, NE). Forty-eight carcasses grading either USDA Choice (n = 43) or USDA Select (n = 5), 6 from each treatment combination, were selected. Strip loins (*Longissimus dorsi*; IMPS #180, NAMP, 2007) and bottom round flats (*Biceps femoris*; IMPS #171B, NAMP, 2007) were collected from each side of the carcass. Only ten *L. dorsi* muscles were collected from the cool-season grass, supplementation provided, and finished on WDGS treatment because two *L. dorsi* muscles (one from each side) were lost in the packing plant. All subprimals from the left side of the carcass were aged under vacuum for 7 d while subprimals from the right side were aged under vacuum 28 d at 2°C. After aging, 5 steaks were cut from each subprimal upon fabrication.

Sample collection

The first steak, cut 1.25 cm thick, was used for all lab analyses. The second steak, also 1.25 cm thick, was used as a back-up for lab analyses. Both steaks were vacuumed packaged and frozen at -20°C for approximately 2 months. Before any lab procedures

were conducted, all lab steaks had any subcutaneous fat removed and were cut into cubes. Next, the cubes were flash frozen in liquid nitrogen, powdered using a Waring blender (Waring Commercial, model 51BL32, Torrington, CT), and stored at -80°C until needed for further lab analyses. All lab analyses were conducted on powdered samples. Fat, protein, ash, amino acid, and mineral analysis were only conducted on 7 d aged steaks while pH, moisture, non-heme iron, heme iron, and total carbohydrate analysis were conducted on both 7 and 28 d aged steaks.

The third steak, cut 2.54 cm thick, was placed on a Styrofoam tray, wrapped with PVC overwrap film, and placed under simulated retail display for 7 d. Objective color and subjective discoloration scores were recorded daily while in the retail display. *L. dorsi* steaks were packaged as two steaks per tray and *B. femoris* steaks were one per tray. Steaks on the same tray were from animals that received identical feeding treatments so as to prevent any possible contamination or influence. At the end of retail display, steaks were vacuumed packaged and frozen until further use.

Retail display

All of the steaks were displayed on a table in a 2°C cooler and were constantly exposed to warm white fluorescence lighting (PHILIPS F32T8/TL741 ALTO 700 Series, 32 WATT B7, Royal Philips Electronics, Amsterdam, Netherlands) at 1000 to 1800 lux in order to simulate retail display conditions. Every day, packages were randomly relocated to minimize any effects due to location. After 7 d, steaks were vacuumed packaged and frozen (-20°C) until further analysis.

Color

Objective color was measured using a Minolta Chromometer CR-400 (Shanghai, China) set at a D65 light source and 2° observer with an 8 mm diameter measurement area. The L*, a*, and b* values were recorded using an average of six readings per steak. Readings were taken at 24 h intervals for 8 d. Subjective discoloration was evaluated based on percentage of surface discoloration (0% indicating no discoloration and 100% indicating complete discoloration of the entire steak) by five trained panelists.

pH

To determine ultimate pH, duplicate 10 g powdered samples from each steak were homogenized with 90 mL of double distilled water using a Polytron homogenizer (POLYTRON Kinimatica CH-6010, Switzerland). The pH was determined using an Orion 4 STAR pH ISE Bench-top meter (Thermo Electron Corporation, Waltham, MA) calibrated using a 7.0 and 4.0 buffer. The pH probe was rinsed with double distilled water and wiped dry with a Kimwipe between every sample.

Proximate Analysis

Moisture and ash were measured using a LECO Thermogravimetric Analyzer (LECO Corporation, model 604-100-400, St. Joseph, MI) and fat was measured by ether extraction using the Soxhlet procedure (AOAC, 1990). Protein was determined by difference.

Total Carbohydrates

Samples were prepared by homogenizing 0.5 g of powdered meat with 20 mL of 80% ethanol in a 50 mL centrifuge tube in duplicates. Samples were stored in a 2°C

cooler until further testing, at least one hour later. Upon analysis, tubes were centrifuged at 783 RCF (g) for 5 min. A 1 mL aliquot of sample containing <0.1 mg/mL of carbohydrate was removed and added to a new tube following the procedures of Dubois et al. (1956). To the new tubes, 50 μ L of 80% phenol and 2.5 mL of concentrated sulfuric acid were added and vortexed immediately. After 10 min, samples were moved to a cool water bath for 10 to 25 min. After samples were cooled, they were read on a Cary 100 Varian UV/Visual Spectrophotometer (Varian Instruments, Sugarland, TX) at 490 nm.

Carbohydrate concentrations were estimated using a standard curve and then correcting for dilutions. The curve was prepared by mixing a stock solution of about 0.1 mg/ml glucose standard at varying concentrations (0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL, respectively) with varying amounts of double distilled water (1.0, 0.8, 0.6, 0.4, 0.2, and 0 mL, respectively). Standard samples were prepared and read the same way as the samples above.

Non-heme Iron

The procedures described by Rhee and Ziprin (1987) were used to determine non-heme iron concentrations. Duplicate 5 g powdered samples were mixed with 0.2 mL of NaNO₂ solution (0.39% w/v) and 15 mL of 40% (1:1) trichloroacetic acid:hydrochloric acid (TCA-HCL) acid solution, vortexed, and placed in a water shaker bath set at 65°C for 20 h. After incubation, samples were allowed to cool to room temperature for 1 h.

Approximately 1 ml aliquots of the liquid phase were removed and mixed with 5 mL of a color reagent (20:20:1 double distilled deionized water:saturated sodium acetate

solution:bathophenathroline disulfonate reagent). To create a liquid phase without a color reagent blank, a 1mL aliquot of the liquid phase was mixed with 5 mL of a 21:20 double distilled deionized water:sodium acetate solution. Both a reagent blank and a liquid phase blank were also created. All 4 mixtures were vortexed, centrifuged (Sorvall SE-12 rotor and Sorvall RC 5B centrifuge, Dupont Co., Wilmington, DE), and read at 540 nm using the spectrophotometer.

Readings were compared against a standard curve created using an iron stock standard (Sigma) mixed with the TCA-HCL acid solution at varying concentrations (0.5, 1.0, 1.5, 2.5, 3.5, and 4.5 $\mu\text{g}/\text{mL}$) to total 25 mL. Standard samples were then mixed with 5 mL of the color reagent, vortexed, centrifuged, and read at 540 nm.

Final absorbance of each sample was calculated by subtracting the absorbance of the incubated liquid phase without color reagent from the absorbance of the incubated liquid phase with color reagent. Next, final concentration was calculated by subtracting the intercept of the standard curve from the final absorbance and dividing it all by the slope of the standard curve. Finally, non-heme iron was calculated as follows:

$$\mu\text{g non-heme Fe/g meat} = \text{concentration } (\mu\text{g}/\text{mL}) \times \frac{(15+0.2+\text{moisture in 5g meat})}{5\text{g}} \times 1\text{mL}$$

Heme Iron

Samples were prepared following the procedures described by Hornsey (1956) as modified by Lee et al. (1998). Duplicate 2 g samples of powdered meat were mixed with 8.1 mL of acetone and 0.2 mL of hydrochloric acid. All tubes were kept in test tube trays wrapped in aluminum foil to reduce light exposure. The sample was homogenized using a Polytron homogenizer at 10,800 rpm for 15 sec. Samples were immediately filtered

through #2 Whatman filter paper (90 mm in diameter) and into a new tube which was also kept in a test tube rack wrapped in aluminum foil. The filtrate was immediately read on a spectrophotometer at 640 nm.

In order to determine total amount of heme iron, total pigment (mg/kg) was calculated by multiplying the absorbance of the sample by 680. Total heme iron (mg/kg) was calculated by multiplying the total pigment by 8.82 and dividing it all by 100 (Hornsey, 1956 and Lee et al., 1998).

Minerals and Amino Acids

Mineral composition was determined by Ward Laboratories, Inc. in Kearney, NE. Atomic absorption spectroscopy was used to quantify the minerals following the procedures of Ward and Gray (1994).

Amino acid composition was determined by AAA Service Laboratory, Inc. in Damascus, OR. A Hitachi L8900 Amino Acid Analyzer with post-column-ninhydrin derivatization was used to quantify amino acids following the procedures of Moore and Stein (1949), Roach and Gehrke (1970), Simpson et al. (1976), Stanford (1963), and Keutmann and Potts (1969).

Statistical Analysis

All data were analyzed as a 2x2x2x2 factorial arrangement split plot design within a randomized complete design using the Mixed procedure in SAS (Version 9.2, SAS Institute Inc., Cary, NC, 2006). The experimental unit was the individual animal and the model included grass, supplementation, finishing diet and age as fixed effects. The least square means procedure with the probability of difference (pdiff) option was used for

mean separation; with significance determined at $P \leq 0.05$ levels. Whenever there was a three- or four-way interaction, the LSmeans were reanalyzed using the GLIMMIX procedure with the slicediff option in order to more accurately study differences.

Results and Discussion

The carcass characteristics within each dietary treatment combination can be seen in Table 1. Marbling scores were not significant ($P > 0.05$) from each other between treatment combinations. This was the intent so as to eliminate any differences that may have occurred due to marbling. Hot carcass weights, ribeye areas, 12th rib fat thicknesses, and calculated yield grades were also not different ($P > 0.05$) between dietary treatment combinations.

Color

L. dorsi Steaks

There were no differences in discoloration between treatments until day 6 of retail display. At day 6, finishing cattle on WDGS and aging the meat 28 d caused a higher level ($P = 0.02$) of discoloration, 54%, than any other finishing diet and aging period combination (Figure 1). At day 7, aging the beef for only 7 d caused lower discoloration than aging 28 d, regardless of finishing diet. Within aging for 28 d, finishing cattle on WDGS still caused a higher percent discoloration ($P = 0.02$) than finishing on corn (75% vs. 46%). Koger et al. (2010) showed that when DGS are added to the finishing diet total polyunsaturated fatty acids (PUFA) concentrations increased. The PUFA are known to be more susceptible to oxidation. Oxidation of the meat causes oxidation of the pigment, meaning an increased level of metmyoglobin so it is not unexpected for steaks

from animals finished on WDGS to have higher discoloration scores at the end of the retail display.

The L* scores were more influenced by grass type and finishing diet over the period of retail display ($P = 0.03$, Figure 2). Starting at day 0 of retail display, when cattle were finished on WDGS, grazing them on warm-season grass caused lower L* scores ($P = 0.03$) than if they were grazed on cool-season grass (41.42 vs. 42.48). This difference was only seen on day 0 of retail display though. Starting at day 3 of retail display, grazing on warm-season grasses caused lower scores ($P = 0.03$) than grazing on cool-season grass, but only when finished on corn (41.47 vs. 42.49). The difference remained for retail display day 4 (41.97 vs. 43.13), dissipated at days 5 (41.77 vs. 42.72) and 6 (42.72 vs. 43.51), and returned at day 7 (42.49 vs. 43.50). One reason for the differences at day 0 of retail display could be due to the steaks equilibrating to the environment. Before the steaks were fabricated they were aged in an oxygen free environment. At day 0 they are being exposed to oxygen for the first time which could have caused the differences. When there were differences, they were mostly due to grass type and finishing diet was less influential. Kinman et al. (2010) also found no differences in L* values due to finishing diets that compared corn and WDGS. The difference in elevation between the two different grass type pastures is about 3,000 feet. The cattle grazing on warm-season grasses were at the higher elevation and a different altitude. Those cattle on the warm-season grass may produce more blood cells in order to accommodate for the thinner air at that elevation. With increased red blood cell content there will also be a higher hemoglobin and myoglobin content. The higher myoglobin

content could explain why the meat from cattle grazed on warm-season grasses was darker.

There was a significant ($P = 0.04$) four-way interaction between retail display day, grass type, finishing diet, and aging period for a^* values. In order to better understand the data, a slicediff was used to separate the means based on aging period. Within the 7 d aging period, there were no differences ($P > 0.05$) between any grass type and finishing diet combination during any of the retail display days (Figure 3). After 28 d of aging, grazing cattle on warm-season grasses caused higher a^* values ($P = 0.04$) than grazing on cool-season grass when finished on WDGS (25.45 vs. 24.09, Figure 4) on day 1 of retail display. After day 1 of retail display, there were no differences between any grass type and finishing diet combinations until day 7 of retail display. On day 7 of retail display for product aged 28 d, finishing on WDGS after being grazed on cool-season grasses caused lower scores ($P = 0.04$) than when cattle were finished on corn after being grazed on both warm-season (18.04 vs. 19.63) and cool-season grasses (18.04 vs. 19.49). Over time the reducing agents in meat decrease, so meat that has been aged 28 d will have less reducing agents than meat aged 7 d. Since there are less reducing agents present, oxidation occurs at a faster rate and myoglobin will be converted to metmyoglobin faster, therefore changing a^* values.

Similarly, for b^* values there was a significant ($P = 0.01$) four-way interaction between retail display day, grass type, finishing diet, and aging period for a^* values. Again, a slicediff was used to separate the means based on aging period. Only days 0 and 1 of retail display showed any differences between dietary combinations for 7 d aged

product. On day 0, grazing on warm-season grasses caused higher b^* values ($P = 0.01$) than cool-season grasses (Figure 5) when finishing on WDGS (11.64 vs. 10.80). On day 1 these dietary combinations were no longer different from each other ($P > 0.05$), but grazing on cool-season grasses and finishing on WDGS caused significantly ($P \leq 0.05$) higher b^* scores than grazing on warm-season grasses and finishing on corn (11.73 vs. 10.94). After day 1, all differences dissipated. In contrast, Gill et al. (2008) saw higher b^* values when cattle were finished on corn compared to DGS ($P = 0.04$). Since in this study grass type was also part of the interaction, Gill et al.'s (2008) lack of variation in grass type could explain why their findings were different.

Product aged 28 d had a very similar pattern (Figure 6). Day 1 of retail display was the only day there were any differences between dietary combinations with both grazing on warm-season grasses and finishing on WDGS and grazing on cool-season grasses and finishing on corn both having higher b^* values ($P \leq 0.05$) than grazing on cool-season grasses and finishing on WDGS (11.77 and 11.83 vs. 10.90, respectively). Again, after day 1 of retail display all differences between dietary combinations dissipated. The differences at only day 1 and day 0 in the previous results could be due to the meat going from an oxygen free environment to an environment where oxygen is now available. Again, it may have just taken a couple of days for the meat to equilibrate to the environment. For both a^* and b^* values, even though there is a four-way interaction, grass type appears to have the most influence on the scores with warm-season grasses causing higher scores ($P \leq 0.05$).

B. femoris steaks

Discoloration scores for *B. femoris* steaks were significantly ($P = 0.01$) affected by a four-way interaction between retail display day, grass type, supplementation, and aging period. Means were separated by aging period in order to better understand the data. Within the 7 d aging period, scores were not different ($P > 0.05$) from each other until the last day of retail display (Figure 7). When no supplementation was provided, steaks from cattle grazed on warm-season grasses had a higher percent of discoloration ($P \leq 0.05$) than steaks from cattle grazed on cool-season grasses (29.78% vs. 19.52%) on day 7 of retail display.

Similarly, there were no differences between dietary combinations until days 6 and 7 of retail display for 28 d aged product. On day 6, grazing on warm-season grasses with no supplementation caused the highest amount of discoloration (51.13%) than any other grass type and supplementation combination ($P \leq 0.05$, Figure 8). On day 7, grazing on warm-season grasses without supplementation was not different ($P > 0.05$) from grazing on cool-season grasses with supplementation (74.77% vs. 69.30%), but both combinations had higher discoloration scores than any other grass type and supplementation combination. Clearly there are compounds in the grass that, in combination with supplementation type, are causing the myoglobin in the meat to oxidize and become metmyoglobin at different rates. One of these compounds could be vitamin A. Vitamin A is an antioxidant, so if the warm-season grass was lacking vitamin A, this could explain the higher discoloration scores.

On days 0, 1 and 3 of retail display aging product 7 d caused lower L* scores ($P < 0.0001$) then when steaks were aged 28 d (43.49 vs. 44.32, 42.57 vs. 43.31, and 41.16 vs. 41.78, respectively, Figure 9). After day 3, L* scores were not different between aging periods. Even though 7 d aged product had lower L* scores at first, meaning they were darker, after 3 days the 7 d aged product eventually lightened up and became were not different from 28 d aged product. Logically, it would have been expected for the meat to start off at the same L* values and then become different from each other, with the 28 d aged steaks having lower L* scores, as the end of the retail display neared. It may have taken the steaks 3 days to equilibrate to the new environment containing oxygen and that is why the differences in scores are first seen.

Three different two-way interactions significantly ($P \leq 0.05$) affected a* scores. First, during every day of retail display 7 d aged product had significantly higher ($P < 0.0001$) a* scores, i.e. were more red, than 28 d aged product (Figure 10). In addition, at days 5, 6, and 7 of retail display finishing cattle on corn caused higher a* scores ($P = 0.03$) than finishing on WDGS (Figure 11). Gill et al. (2008) also found that finishing cattle on DGS caused lower a* values compared to finishing on corn ($P = 0.01$). This may indicate that finishing cattle on WDGS with solubles causes the myoglobin to oxidize faster causing the lower redness values seen near the end of the retail display, as also seen in the *L. dorsi* steaks. The last interaction to influence a* values was between supplementation and finishing diet ($P = 0.03$) with no supplementation and finishing on WDGS causing lower scores than both no supplementation and finishing on corn (19.13

vs. 20.20) and receiving supplementation and finishing on WDGS (19.13 vs. 20.09, Figure 12).

A three-way interaction between retail display day, grass type, and aging period significantly ($P = 0.04$) affected b^* values. There were no differences on days 0 and 1, but on day 2 of retail display grazing on both warm- and cool-season grasses and aging the product for 7 d caused higher values than when cattle were grazed on warm-season grasses and the product was aged for 28 d (10.55 and 10.29 vs. 9.66, respectively, Figure 13). On days 3 and 4 all 7 d aged product had higher scores than all 28 d aged product regardless of grass type. On day 5 differences shifted again with 7 d aged product that was grazed on cool-season grasses having higher scores than 28 d aged product that was grazed on warm-season grasses (9.27 vs. 8.72). After day 5 of retail display there were no differences between any of the grass type and aging period combinations. Even though the interaction was between grass type and aging period, on days 2, 3, and 4 of retail display it appears that aging period is the major contributing factor with 7 d aged product always having higher scores, therefore being more yellow in pigment, than 28 d aged product. Again, this could be due to the myoglobin having more advanced oxidation than 7 d aged product.

pH

L. dorsi and *B. femoris* Steaks

The pH for *L. dorsi* steaks was unaffected by any of the feeding regimens (Table 2), but aging for 28 d increased pH values ($P < 0.0001$) when compared to 7 d aging (5.57 vs. 5.28, Table 3). *B. femoris* steaks were similar with pH values increasing ($P <$

0.0001) with longer aging periods (5.65 vs. 5.39). Shand et al. (1998) also reported that pH was unaffected by such dietary components as supplementation and finishing diets composed of DGS.

Proximate analysis

L. dorsi Steaks

For ash content there was a three-way interaction between grass-type, supplementation, and finishing diet so a slicediff was used to separate the means based on grass type. Within warm-season grass grazing, not supplementing and finishing on WDGS caused ash content to be the highest (2.20%) compared to any other supplementation and finishing diet combination ($P = 0.04$, Figure 14). Within cool-season grass grazing, supplementing and finishing on WDGS caused the ash content to be higher than if they weren't supplemented and finished on WDGS (2.22 vs. 1.65).

Table 4 shows how grazing cattle on a cool-season grass and finishing on WDGS caused the lowest moisture content (69.86%) compared to all other grass type and finishing diet combinations ($P = 0.02$). Larick and Turner (1990) and Mills et al. (1992) also grazed cattle on several different types of grasses and forages, but found that there were no differences in moisture content between them. The difference in finding could be due to the types of grasses fed, in that perhaps their grass types were not as different from each other as the ones used in this study. However, the difference was only seen when finishing on WDGS so perhaps that grass and finishing diet combination could have also caused the differences.

Supplementation caused protein content to decrease (20.75 vs. 21.24) as opposed to no supplementation ($P = 0.03$). Both fat content and cooking loss were not affected by any of the diet regimens. Shand et al. (1998) also showed that both supplementation and finishing diets, with or without DGS, also had no effect on fat or cooking loss. There was a tendency for the interactions between grass type and supplementation ($P = 0.07$) and the interaction of grass type and finishing diet ($P = 0.08$) to influence fat content.

B. femoris Steaks

Ash, fat, and protein content were all unaffected by main effects in the diet, as can be seen in Table 2. This is in contrast to Srinivasan et al. (1998) who found that when cattle are supplemented while on grass protein content decreased ($P \leq 0.05$) in *Semimembranosus* muscles, which are also located in the round. Instead of WDGS, Srinivasan et al. (1998) used cracked corn to supplement their cattle. The difference in composition between supplementation types could explain the differences in proximate analysis.

There was a three-way interaction between grass-type, supplementation, and finishing diet for moisture content (Figure 15). A slicediff was used to separate the means based on grass-type. Within warm-season grass grazing, finishing on WDGS caused a higher moisture content ($P = 0.03$) than finishing on corn when supplementation was not provided (72.28% vs. 70.98%). When supplementation was provided, there were no differences between finishing diets. When grazing on cool-season grasses there were no differences between any of the supplementation and finishing diet combinations. It can be perceived that the difference in moisture content may be mainly due to grass type

since there were no differences within cool-season grasses but there were within warm-season grass. Even though there were differences within warm-season grasses, the addition of supplementation was able to deter any differences.

The interaction between supplementation and finishing diet had a tendency to influence protein content of muscle ($P = 0.08$), with supplementing and finishing on corn appearing to decrease protein compared to all other supplementation and finishing diet combinations, 19.12 (supplemented and finished on corn) vs. 19.56 (not supplemented and finished on corn), 19.24 (not supplemented and finished on WDGS), and 19.46 (supplemented and finished on WDGS). In addition, the three-way interaction between grass type, supplementation, and finishing diet had a tendency to change fat content ($P = 0.08$). In this instance, it appeared that finishing on WDGS after being grazed on a warm-season grass with no supplementation drastically reduces fat content compared to all other diet combinations, 5.62 (warm-season grass grazing with no supplementation, WDGS finish) vs. 7.49 (warm-season grass grazing with no supplementation, corn finish), 6.78 (warm-season grass grazing with supplementation, corn finish), 6.79 (warm-season grass grazing with supplementation, WDGS finish), 6.13 (cool-season grass grazing with no supplementation, corn finish), 6.70 (cool-season grass grazing with no supplementation, WDGS finish), 7.36 (cool-season grass grazing with supplementation, corn finish), and 6.52 (cool-season grass grazing with supplementation, corn finish). Cooking loss was also unaffected by diet and aging, however there was a tendency for the interaction of supplementation and finishing diet to influence it ($P = 0.07$).

Total Carbohydrates

L. dorsi and *B. femoris* Steaks

Diet had no effect on total carbohydrate composition for both *L. dorsi* and *B. femoris* steaks ($P > 0.05$, Table 2). However, the interaction between grass type and finishing diet did have the tendency ($P = 0.06$) to influence carbohydrate concentration for *L. dorsi* steaks. Aging period did significantly ($P = 0.0003$) affect carbohydrate concentration in *B. femoris* steaks with 28 d aging periods causing a higher concentration than 7 d aging (1.00 vs. 0.81, Table 3). As the meat ages the moisture content decreases as can be seen in Table 3 for the *L. dorsi* steaks ($P = 0.02$) with a tendency for the same in the *B. femoris* steaks ($P = 0.08$). When the moisture content decreases other components, such as carbohydrate, will become more concentrated.

Minerals and Amino acids

L. dorsi Steaks

The amino acid profile was unaffected by any dietary components or their combinations (Table 5). The interaction between supplementation and finishing diet had a tendency ($P = 0.07$) to influence potassium concentration while grass type and diet tended to influence copper level ($P = 0.09$), but no dietary combinations significantly ($P \leq 0.05$) altered any other minerals, as can be seen in Table 5. Grazing on a warm-season grass decreased magnesium concentrations (291.67 vs. 326.67) and increased zinc concentrations (42.29 vs. 37.46) significantly ($P \leq 0.03$, Table 6). In addition, grazing on a warm-season grass had the tendency ($P = 0.06$) to decrease sulfur content. The warm-season grasses tend to have higher zinc and lower sulfur concentrations than cool-season

grasses (Muller, 2003 and Reid et al., 1988). This could explain some of the differences seen.

B. femoris Steaks

There was a three-way interaction between grass-type, supplementation, and finishing diet for glycine concentration ($P = 0.05$, Table 7). In order to better analyze the differences, a slicediff was used to separate out the means by grass-type. Within warm-season grass grazing there were no differences ($P > 0.05$). When cattle were grazed on cool-season grasses, providing supplementation and finishing on a WDGS diet caused the lowest glycine concentration (6.52) when compared to all other supplementation and finishing diet combinations (Figure 17).

As seen in Table 8, finishing cattle on corn increased ($P = 0.04$) histidine levels compared to WDGS (6.12 vs. 5.75) while providing supplementation caused proline levels to decrease (6.97 vs. 6.64, $P = 0.05$). Finishing on corn tended to increase both lysine and arginine levels when compared to WDGS ($P \leq 0.08$). All other amino acids were unaffected by any other dietary components or combinations (Table 7).

B. femoris steaks from cattle finished on WDGS had higher phosphorus content ($P = 0.04$) in the meat when not supplemented as opposed to supplemented (2,266.67 vs. 2,058.33, Table 9). In contrast, Driskell et al. (2011) did not see any differences in phosphorous levels in the meat when they compared corn and WDGS finishing diets. They could not have seen the same results though because they examined the *Infraspinatus* and *Teres major* muscles instead of the *B. femoris*.

There were tendencies for grass type and finishing diet to alter calcium ($P = 0.06$), supplementation and finishing diet to alter sodium ($P = 0.06$), and grass type to alter both magnesium and sulfur ($P = 0.07$). When comparing other research, it has been found that there are differences in mineral content between grasses. Specifically, warm-season grasses tend to have higher concentrations of magnesium and lower concentration of calcium and sulfur compared to cool-season grasses (Muller, 2003 and Reid et al., 1988). This could explain why grass type had the tendencies to alter the mineral content in the meat. All other minerals were unaffected by any other dietary components or combinations (Table 7). When supplementing, Srinivasan et al. (1998) saw increased levels of potassium and decreased levels of iron concentrations in *Semimembranosus* muscles. They used cracked corn to supplement, which could account for the differences.

Non- and heme iron

L. dorsi Steaks

No dietary regimen and aging period combinations influenced non-heme iron content (Table 2). When cattle were finished on corn, grazing them on a warm-season grass first caused higher heme iron concentration than grazing on cool-season grass ($P = 0.003$), 7.98 vs. 7.11 (Table 4). Even though no dietary regimens affected total iron concentrations, as seen above, apparently grass type and finishing diets were sufficient to still affect heme iron concentrations. As described previously, the warm-season grass pastures used in this study were at a higher altitude than the cool-season grass pastures. At the higher altitudes there will be more red blood cells and hemoglobin produced in order to compensate for the atmosphere. The increase in hemoglobin would then also

cause an increase in heme iron. Medeiros et al. (1988) also found that steaks from cattle grazed at higher altitudes contained more heme iron than steaks from cattle grazed at lower altitudes.

B. femoris Steaks

There was a three-way interaction between grass-type, supplementation, and age for non-heme iron content. A slicediff was used to separate the means based on grass-type. Within warm-season grass grazing, when the animals were supplemented, 28 d aged product had a higher non-heme iron concentration ($P = 0.05$) than 7 d aged product (3.36 vs. 2.13, Figure 16). Within cool-season grass grazing, 28 d aged product, from both not supplemented and supplemented cattle, had higher non-heme iron concentrations than 7 d not supplemented product (3.02 and 2.83 vs. 1.70, respectively). Aging steaks 28 d caused the concentration of heme iron to be significantly ($P = 0.0001$) higher than 7 d aged steaks (10.58 vs. 9.54, Table 3). Again, this is probably due to water being exuded from the meat and therefore other components become more concentrated.

In conclusion, grass-type was a major contributor in determining the biochemical composition of *L. dorsi* steaks, with warm-season grasses causing increased concentrations of moisture and zinc. In contrast, a longer aging period had an overwhelming effect on the biochemical components of *B. femoris* steaks. Aging 28 d instead of 7 d caused increased concentrations of pH, carbohydrates, and non-heme and heme iron most likely due to water being lost and other components becoming more concentrated.

Overall, grass type and aging were found to have the most effect on the biochemical constituents of meat. This shows that the grass type cattle grazed after weaning can still cause a residual effect on the meat composition even after finishing on a high concentrate diet. In most cases, the addition of supplementation was able to even out the effects and remove any differences due to grass type. The differences due to aging can most likely be attributed to moisture loss.

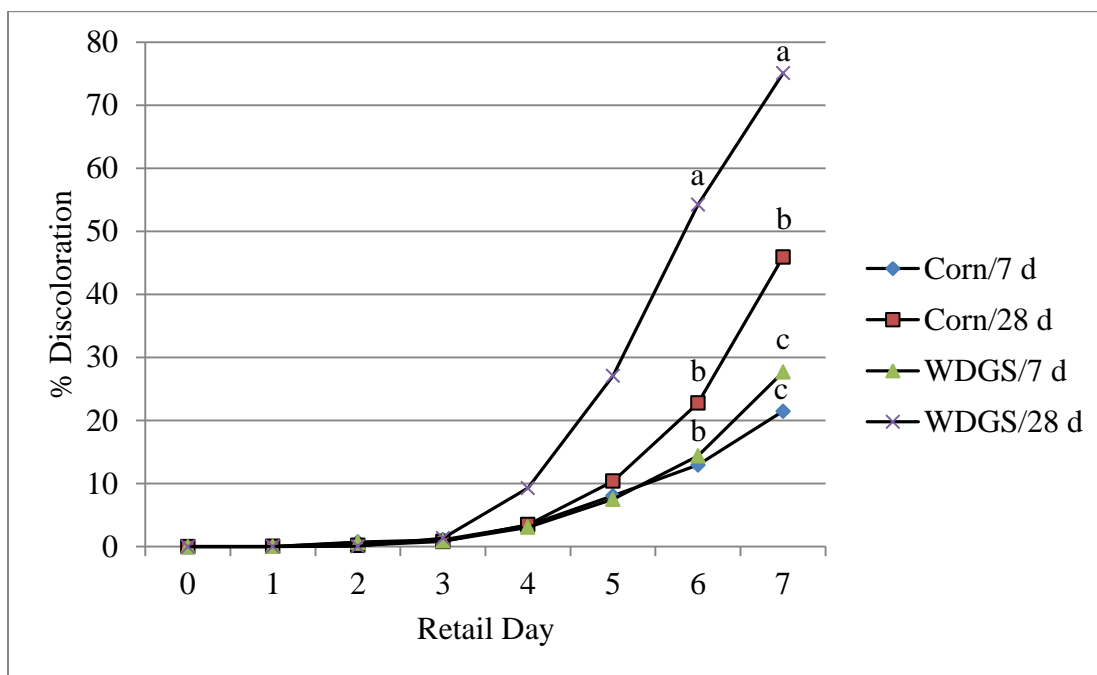
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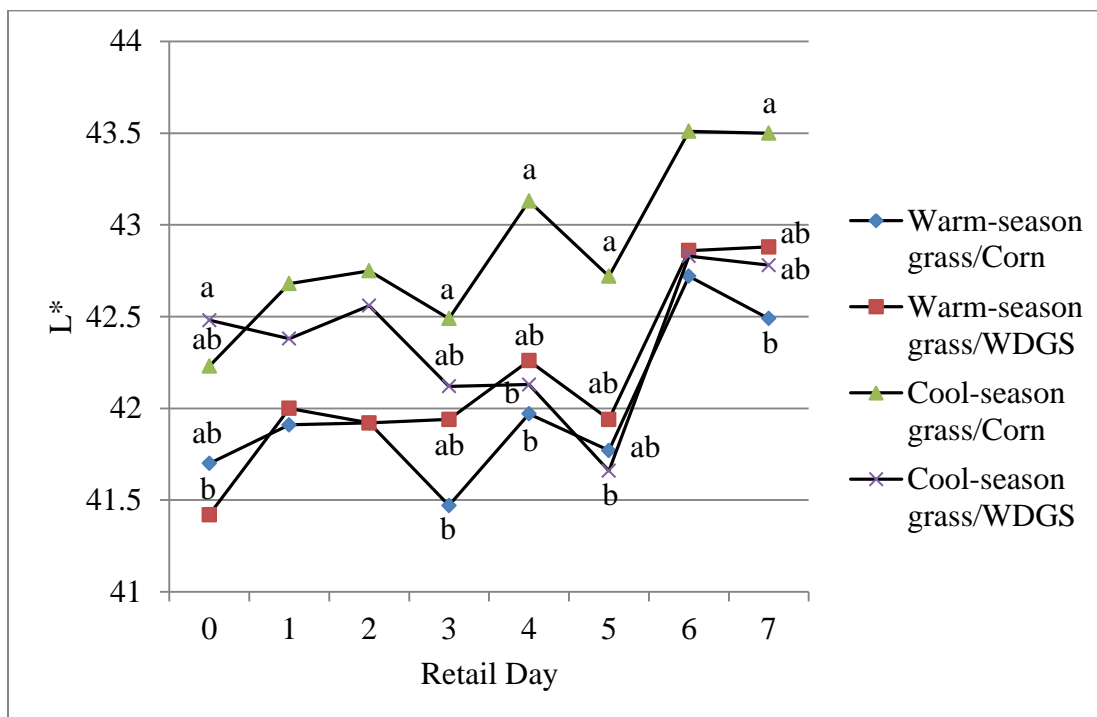
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Figures and Tables



^{abc} Means within the same retail day with different superscripts are significantly ($P \leq 0.05$) different

Figure 1. The effect of the interaction between day, finishing diet, and aging period on % discoloration for *L. dorsis* steaks ($P = 0.02$).



^{ab}Means within the same retail day with different superscripts are significantly ($P \leq 0.05$) different

Figure 2. The effect of the interaction between retail day, grass type, and finishing diet on L^* values of *L. dorsi* steaks ($P = 0.03$).

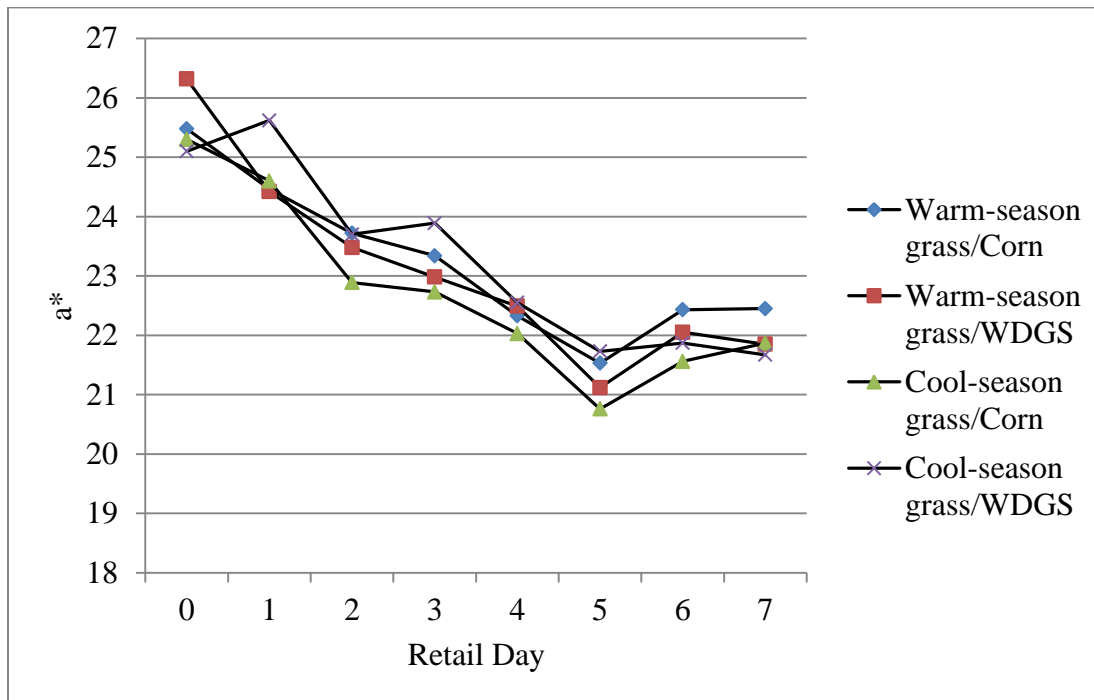
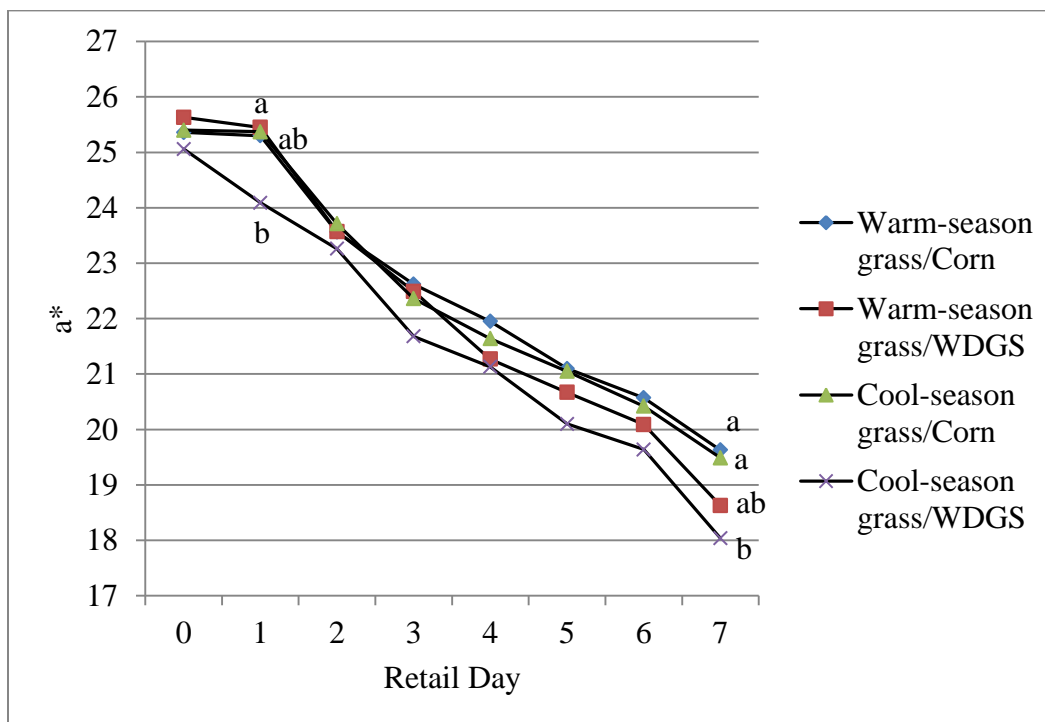
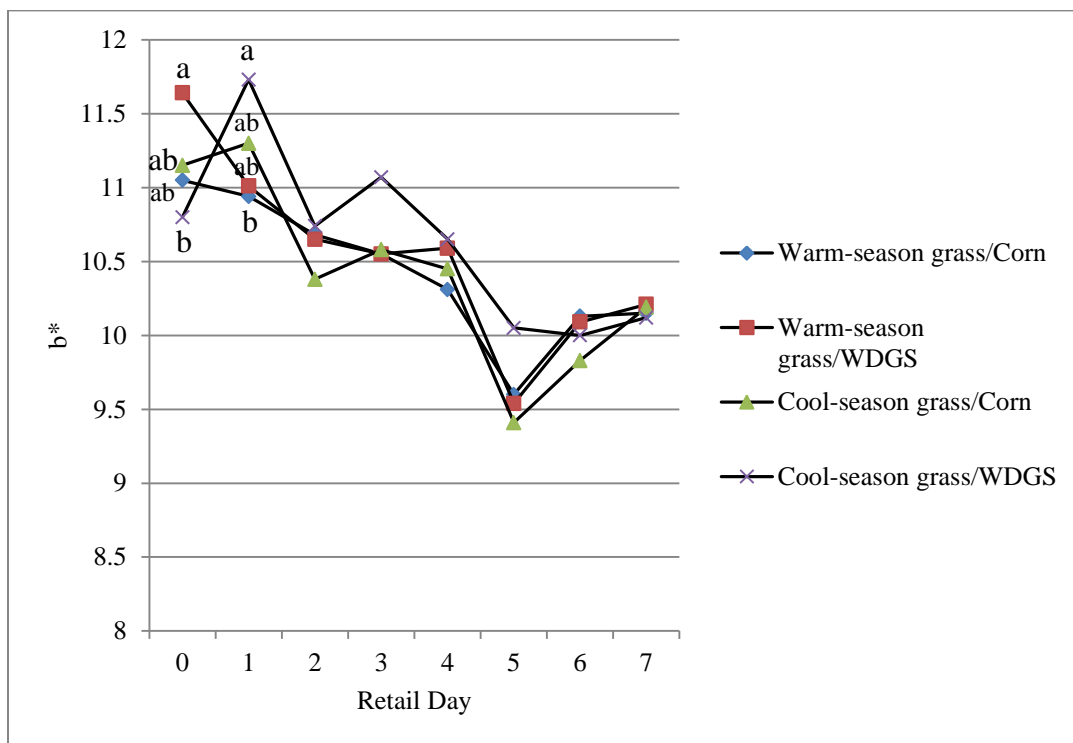


Figure 3. The effect of the interaction between retail display day, grass type, finishing diet, and aging period on a^* values within 7 d age for *L. dorsis* steaks ($P = 0.04$)



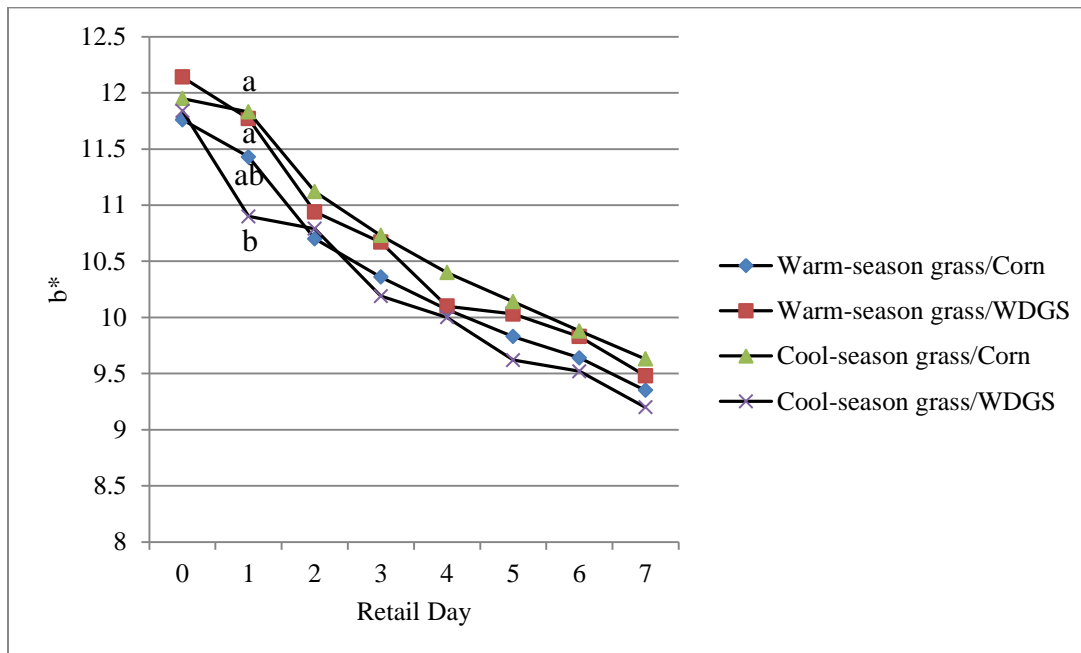
^{ab}Means within the same retail day with different superscripts are significantly ($P \leq 0.05$) different

Figure 4. The effect of the interaction between retail display day, grass type, finishing diet, and aging period on a^* values within 28 d age for *L. dorsis* steaks ($P = 0.04$)



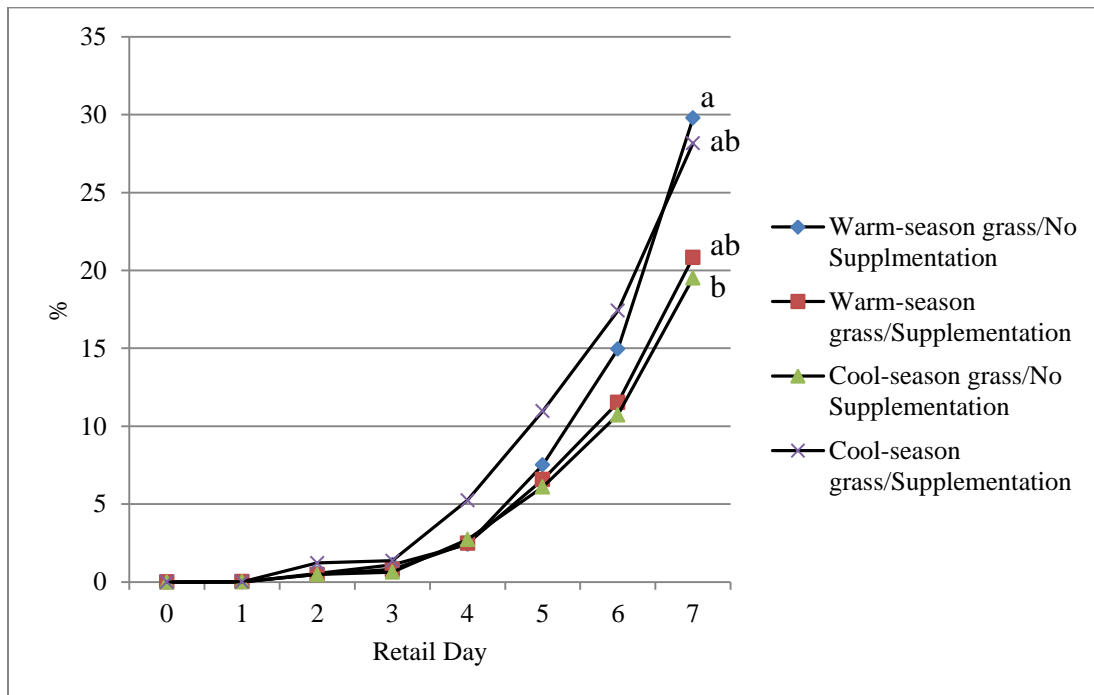
^{ab}Means within the same retail day with different superscripts are significantly ($P \leq 0.05$) different

Figure 5. The effect of the interaction between retail display day, grass type, finishing diet, and aging period on b^* values within 7 d age for *L. dors*i steaks ($P = 0.01$).



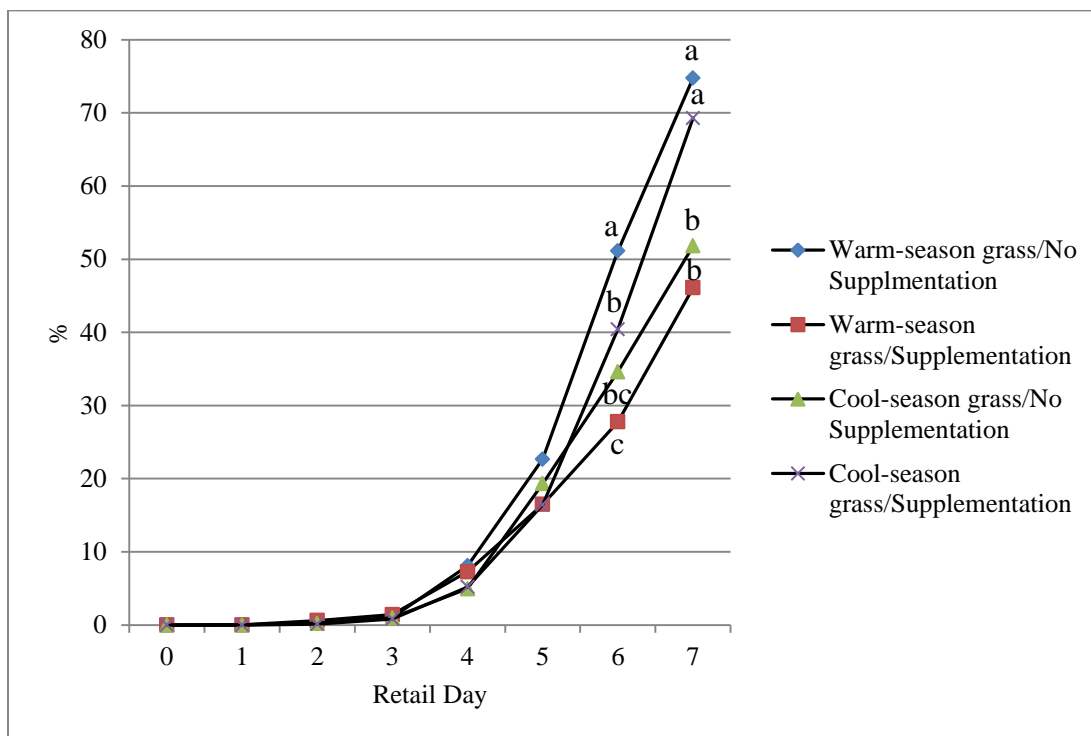
^{ab}Means within the same retail day with different superscripts are significantly ($P \leq 0.05$) different

Figure 6. The effect of the interaction between retail display day, grass type, finishing diet, and aging period on b^* values within 28 d age for *L. dorsi* steaks ($P = 0.01$).



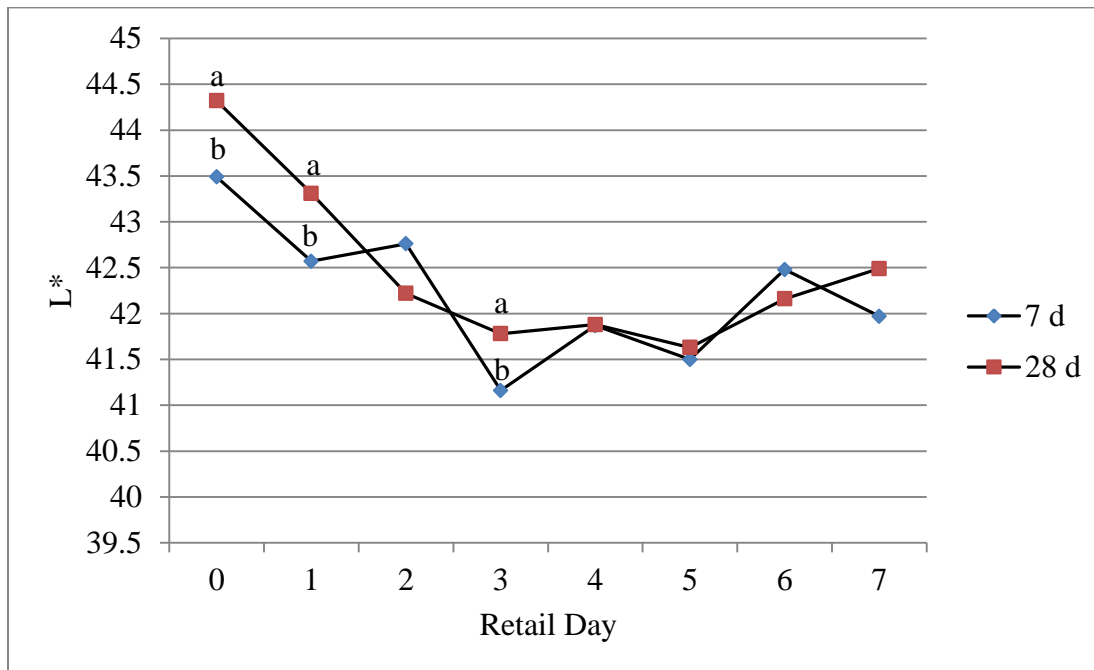
^{ab}Means within the same retail day with different superscripts are significantly ($P \leq 0.05$) different

Figure 7. The effect of the interaction between retail display day, grass type, supplementation, and aging period on % discoloration within 7 d age for *B. femoris* steaks ($P = 0.01$).



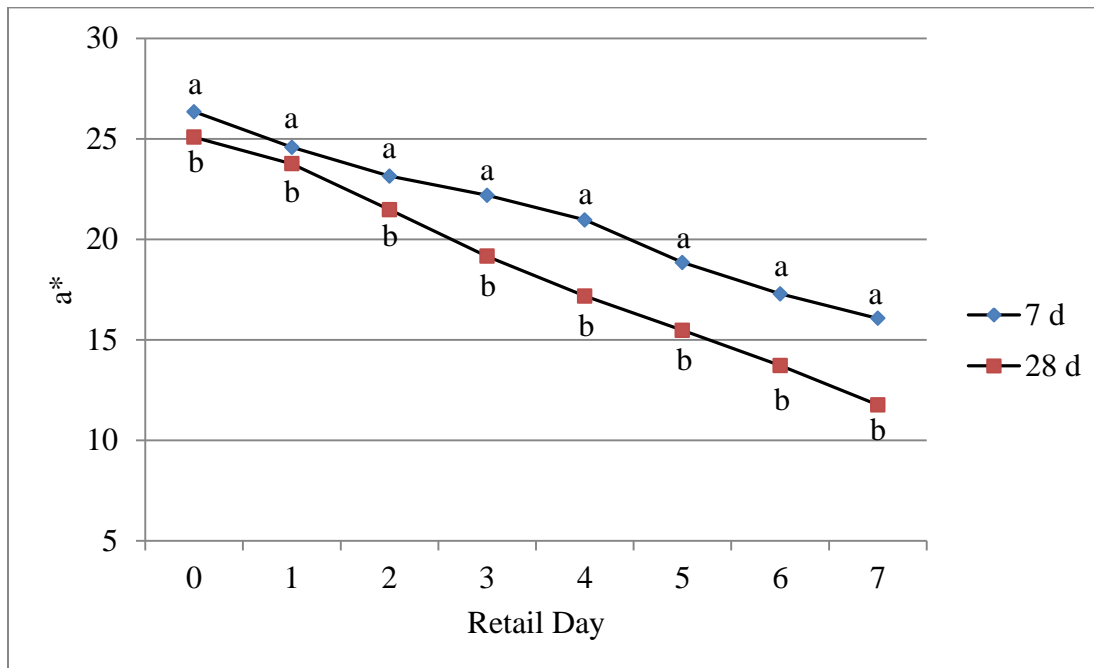
^{abc}Means within the same retail day with different superscripts are significantly ($P \leq 0.05$) different

Figure 8. The effect of the interaction between retail display day, grass type, supplementation, and aging period on % discoloration within 28 d age for *B. femoris* steaks ($P = 0.01$).



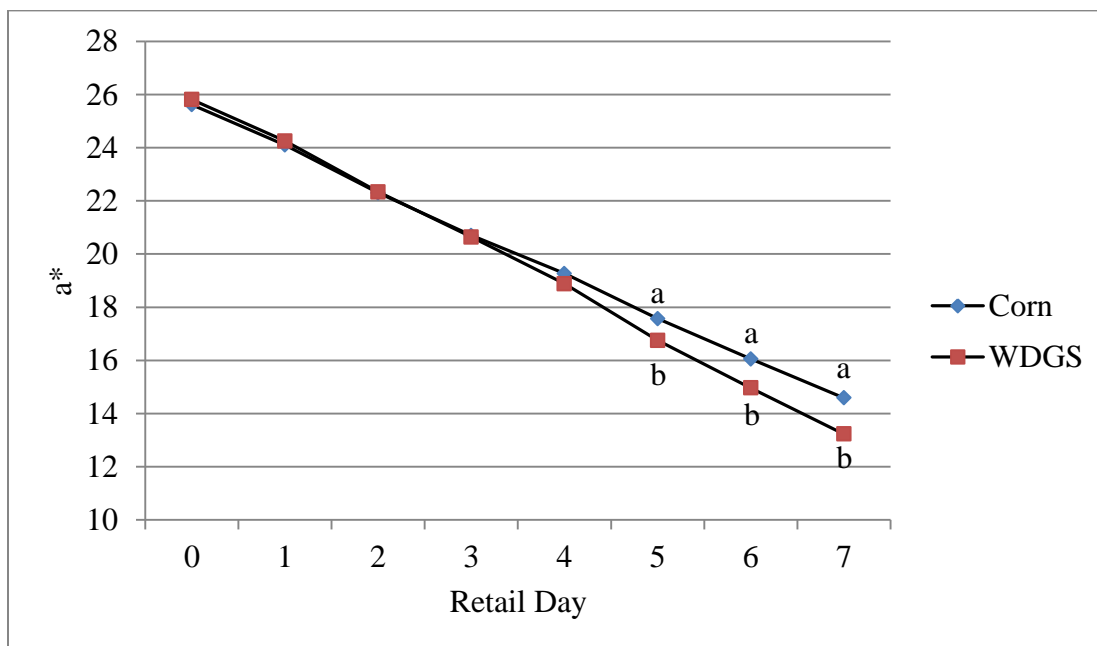
^{ab}Means within the same retail day with different superscripts are significantly ($P \leq 0.05$) different

Figure 9. The effect of the interaction between retail day and aging period on L^* values of *B. femoris* steaks ($P < 0.0001$).



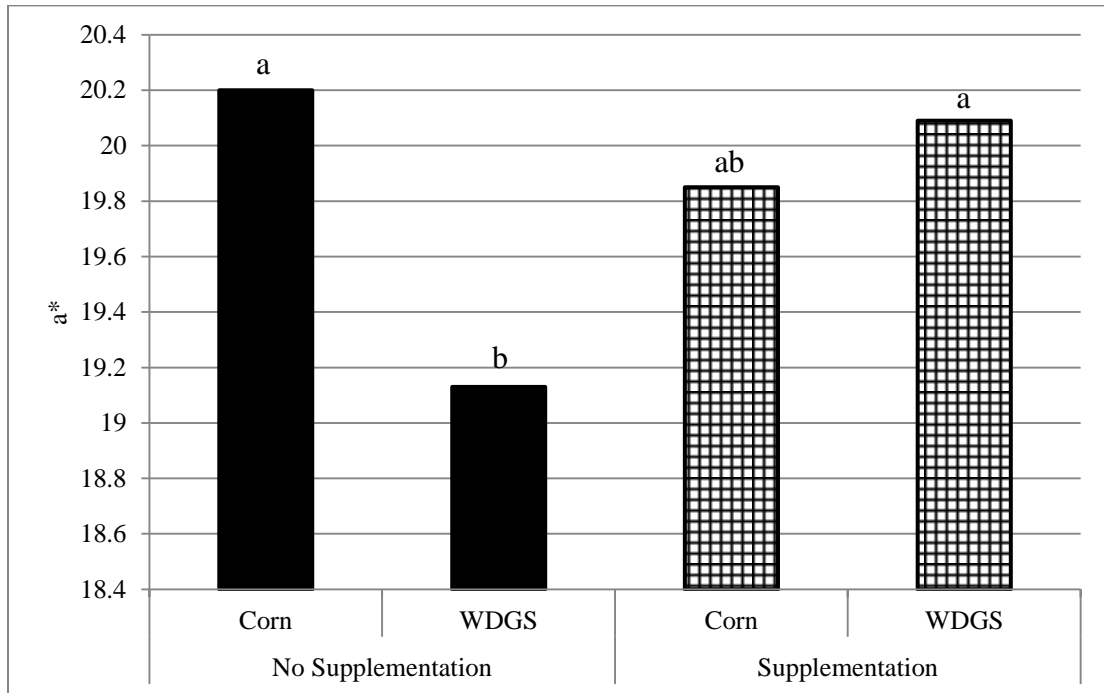
^{ab}Means within the same retail day with different superscripts are significantly ($P \leq 0.05$) different

Figure 10. The effect of the interaction between retail day and aging period on a^* values of *B. femoris* steaks ($P < 0.0001$).



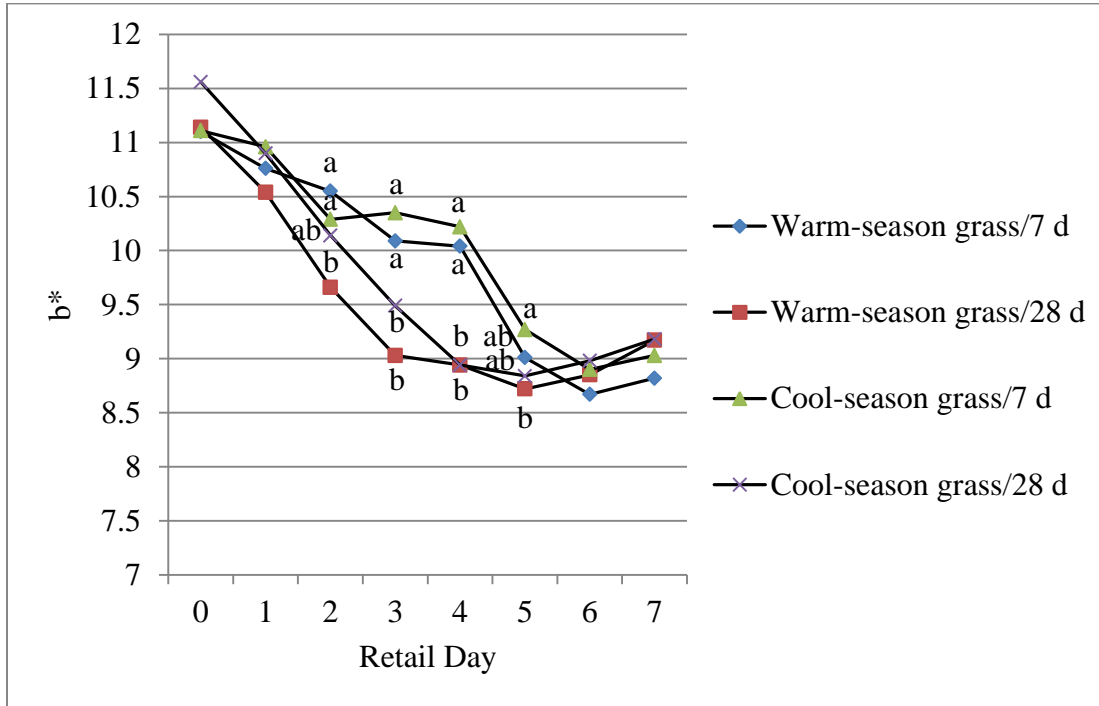
^{ab}Means within the same retail day with different superscripts are significantly ($P \leq 0.05$) different

Figure 11. The effect of the interaction between retail day and finishing diet on a* values of *B. femoris* steaks ($P = 0.03$).



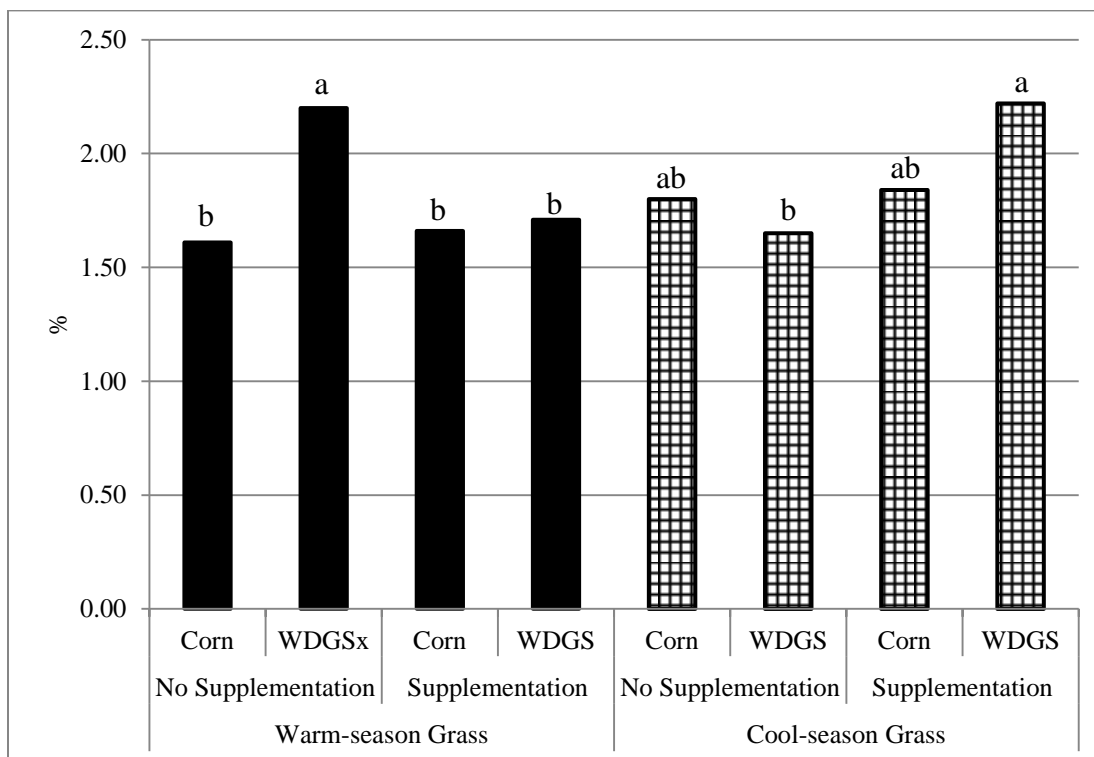
^{ab}Means with different superscripts are significantly ($P \leq 0.05$) different

Figure 12. The effect of the interaction between supplementation and finishing diet on a* values of *B. femoris* steaks ($P = 0.03$).



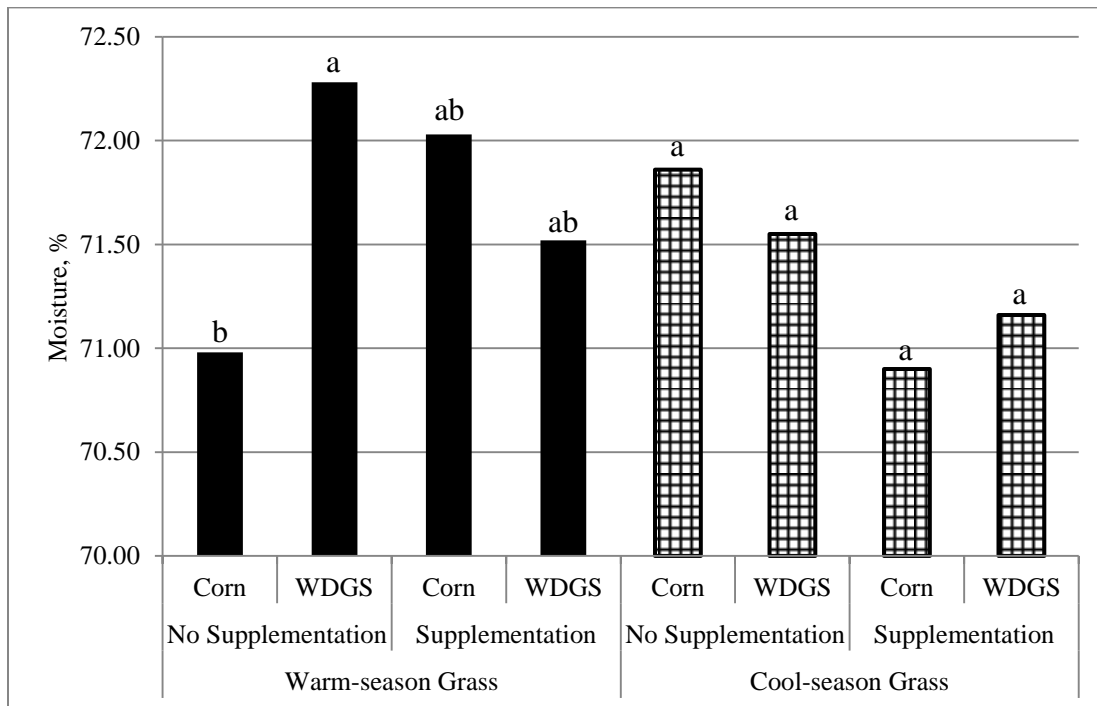
^{ab}Means within the same retail day with different superscripts are significantly ($P \leq 0.05$) different

Figure 13. The effect of the interaction between retail day, grass type, and aging period on b* values of *B. femoris* steaks ($P = 0.04$).



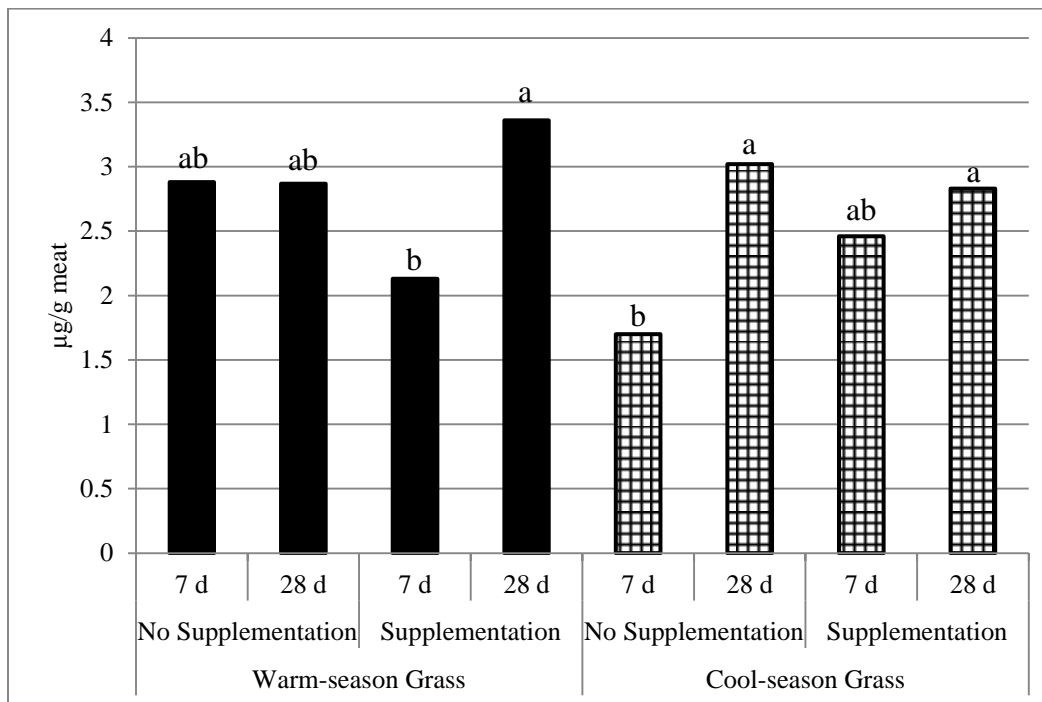
^{ab}Means within the same grass type with the different superscripts are significantly ($P \leq 0.05$) different

Figure 14. The effect of the interaction between grass type, supplementation, and finishing diet on the LS means of ash content when separated by grass type for *L. dorsi* steaks ($P = 0.04$).



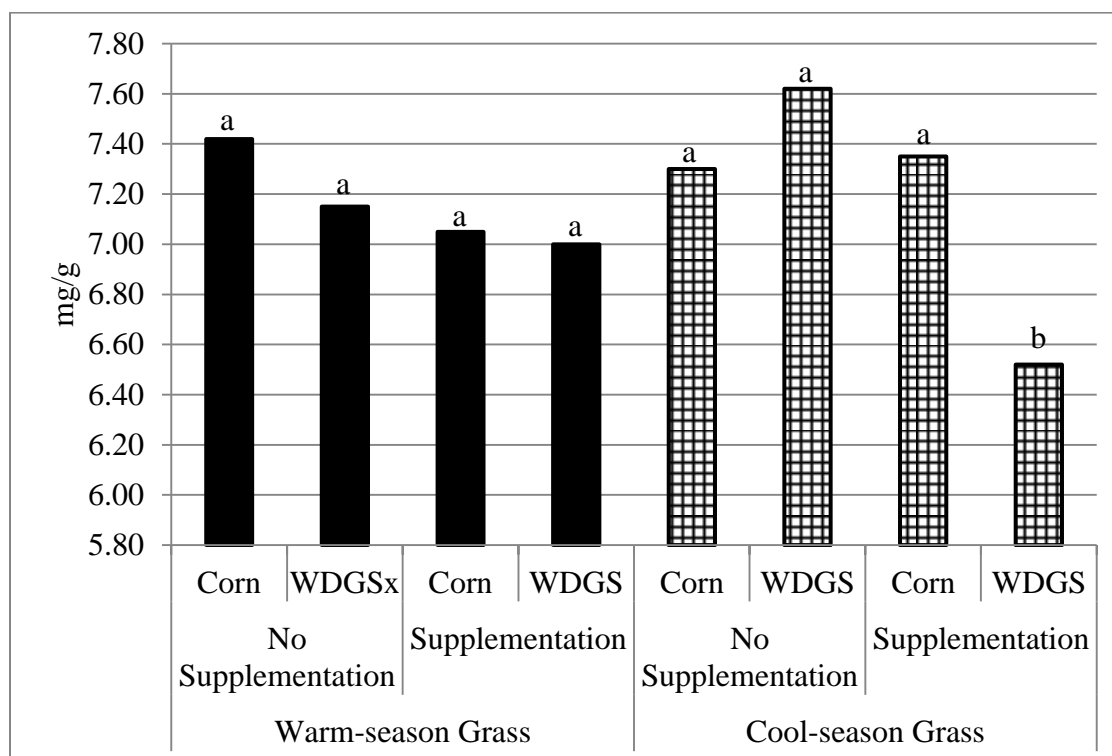
^{ab}Means within the same grass type with the different superscripts are significantly ($P \leq 0.05$) different

Figure 15. The effect of the interaction between grass type, supplementation, and finishing diet on the LS means of moisture content when separated by grass type for *B. femoris* steaks ($P = 0.04$).



^{ab}Means within the same grass type with the different superscripts are significantly ($P \leq 0.05$) different

Figure 16. The effect of the interaction between grass type, supplementation, and aging period on LS means of non-heme iron content when separated by grass type for *B. femoris* steaks ($P = 0.05$).



^{ab}Means within the same grass type with the different superscripts are significantly ($P \leq 0.05$) different

Figure 17. The effect of the interaction between grass type, supplementation, and finishing diet on the LS means of glycine content when separated by grass type for *B. femoris* steaks ($P = 0.05$).

Table 1. The effect of grass type, supplementation, and finishing diet on carcass characteristics

	Warm-Season Grass				Cool-Season Grass				SEM	P-Value
	No Supplementation		Supplementation		No Supplementation		Supplementation			
	Corn	WDGS ^x	Corn	WDGS	Corn	WDGS	Corn	WDGS		
HCW, lbs	370.15	382.80	401.67	421.82	405.83	413.26	433.49	437.20	8.54	0.65
Marbling ^y	605	517	585	592	577	597	617	627	32	0.25
REA, cm ²	88.26	90.97	88.26	97.94	87.61	88.58	93.68	91.61	3.23	0.28
12 th Rib Fat, cm	1.22	1.27	1.32	1.50	1.55	1.57	1.55	1.75	0.15	0.98
CYG ^z	2.92	2.93	3.28	3.13	3.56	3.61	3.50	3.82	0.23	0.53

^xWDGS = Wet distillers grains with solubles

^yMarbling score: 700 = MD, 600 = MT, 500 = SM

^zCalculated Yield Grade = $2.5 + (2.5 * 12^{\text{th}} \text{ rib fat, in}) - (0.32 * \text{REA, in}^2) + (0.2 * \text{estimated KPH, \%}) + 0.0038 * \text{HCW, lb}$

Table 2. The influence of diet and age on the *P*-values of the pH, proximate analysis, carbohydrate, cooking loss, non-heme iron, and heme iron content of *L. dorsi* and *B. femoris* steaks

Trait	<i>P</i> -Value														
	Grass ^x	Supp	Grass XSupp	Diet	Grass XDiet	SuppX Diet	Grass XSupp XDiet	Age	Grass XAge	SuppX Age	Grass XSupp XAge	DietX Age	Grass XDiet XAge	SuppX DietX Age	Grass XSupp XDiet XAge
<i>L. dorsi</i>															
pH	0.06	0.24	0.37	0.16	0.73	0.49	0.14	<0.0001	0.10	0.81	0.78	0.38	0.45	0.48	0.19
Moisture, %	0.01	0.13	0.11	0.59	0.02	0.39	0.52	0.02	0.75	0.76	0.52	0.61	0.83	0.85	0.54
Ash, %	0.53	0.74	0.04	0.09	0.41	0.99	0.04	NA ^y	NA	NA	NA	NA	NA	NA	NA
Fat, %	0.20	0.14	0.07	0.66	0.08	0.75	0.75	NA	NA	NA	NA	NA	NA	NA	NA
Protein, %	0.46	0.03	0.95	0.59	0.44	0.83	0.64	NA	NA	NA	NA	NA	NA	NA	NA
Total Carbohydrates, mg/mL	0.16	0.22	0.54	0.76	0.06	0.16	0.58	0.15	0.61	0.72	0.89	0.82	0.73	0.48	0.86
Cooking Loss, %	0.95	0.89	0.90	0.67	0.14	0.16	0.70	0.82	0.46	0.97	0.32	0.43	0.82	0.98	0.36
Non-Heme Iron, µg/g meat	0.41	0.80	0.64	0.92	0.82	0.65	0.69	0.57	0.65	0.51	0.15	0.95	0.50	0.89	0.17
Heme Iron, mg/kg	0.49	0.004	0.45	0.79	0.003	0.62	0.58	0.11	0.49	0.60	0.73	0.85	0.62	0.34	0.91
<i>B. femoris</i>															
pH	0.99	0.69	0.13	0.44	0.53	0.80	0.76	<0.0001	0.11	0.86	0.63	0.39	0.18	0.84	0.09
Moisture, %	0.22	0.32	0.13	0.50	0.43	0.25	0.03	0.08	0.73	0.74	0.18	0.37	0.59	0.98	0.70
Ash, %	0.78	0.52	0.31	0.51	0.24	0.33	0.53	NA	NA	NA	NA	NA	NA	NA	NA
Fat, %	0.98	0.41	0.75	0.25	0.38	0.80	0.08	NA	NA	NA	NA	NA	NA	NA	NA
Protein, %	0.30	0.58	0.18	0.96	0.26	0.08	0.20	NA	NA	NA	NA	NA	NA	NA	NA
Total Carbohydrates, mg/mL	0.99	0.84	0.62	0.84	0.72	0.98	0.91	0.0003	0.80	0.76	0.40	0.50	0.60	0.60	0.79
Cooking Loss, %	0.39	0.43	0.19	0.56	0.71	0.07	0.37	0.10	0.72	0.72	0.41	0.33	0.30	0.76	0.21
Non-Heme Iron, µg/g meat	0.28	0.77	0.47	0.30	0.06	0.26	0.94	0.01	0.68	0.79	0.05	0.92	0.81	0.21	0.99
Heme Iron, mg/kg	0.83	0.68	0.53	0.57	0.09	0.51	0.79	0.0001	0.43	0.64	0.34	0.87	0.26	0.27	0.47

^xGrass = Grass type, Supp = Supplementation, Diet = Finishing diet, Age = Aging period

^yNA = Not applicable, aging period was not tested for these factors

Table 3. The effect of grass type, supplementation, finishing diet, and aging period on the LS means scores of pH, proximate analysis, carbohydrate, cooking loss, non-heme iron, and heme iron content for *L. dorsi* and *B. femoris* steaks

Trait	Grass Type		SEM	Supplementation			Finishing Diets			Aging Period		
	Warm-season	Cool-season		No	Yes	SEM	Corn	WDGS ^x	SEM	7 d	28 d	SEM
<i>L. dorsi</i>												
pH	5.46	5.39	0.03	5.45	5.40	0.03	5.45	5.40	0.03	5.28 ^b	5.57 ^a	0.03
Moisture, %	71.19 ^a	70.34 ^b	0.23	71.02	70.52	0.23	70.86	70.68	0.23	71.14 ^a	70.39 ^b	0.23
Ash, %	1.80	1.88	0.090	1.82	1.86	0.09	1.73	1.95	0.09	NA ^y	NA	NA
Fat, %	5.67	6.38	0.400	5.60	6.45	0.40	5.90	6.15	0.40	NA	NA	NA
Protein, %	20.91	21.07	0.150	21.24 ^a	20.75 ^b	0.15	21.05	20.93	0.15	NA	NA	NA
Total Carbohydrates, mg/mL	0.81	0.75	0.040	0.81	0.76	0.04	0.78	0.79	0.04	0.75 ^b	0.81 ^a	0.03
Cooking Loss, %	14.73	14.78	0.630	14.69	14.82	0.63	14.95	14.56	0.63	14.86	14.65	0.63
Non-Heme Iron, µg/g meat	2.34	2.15	0.210	2.27	2.21	0.21	2.25	2.23	0.21	2.31	2.18	0.16
Heme Iron, mg/kg	7.66	7.50	0.220	7.23 ^b	7.92 ^a	0.22	7.55	7.61	0.22	7.39 ^b	7.77 ^a	0.16
<i>B. femoris</i>												
pH	5.52	5.52	0.03	5.53	5.52	0.03	5.54	5.51	0.03	5.39 ^b	5.65 ^a	0.03
Moisture, %	71.70	71.37	0.19	71.67	71.40	0.19	71.44	71.63	0.19	71.78	71.29	0.19
Ash, %	2.18	2.22	0.10	2.25	2.16	0.10	2.16	2.25	0.10	NA	NA	NA
Fat, %	6.67	6.68	0.32	6.49	6.86	0.32	6.94	6.41	0.32	NA	NA	NA
Protein, %	19.25	19.44	0.13	19.40	19.29	0.13	19.34	19.35	0.13	NA	NA	NA
Total Carbohydrates, mg/mL	0.91	0.91	0.04	0.91	0.90	0.04	0.91	0.90	0.04	0.81 ^b	1.00 ^a	0.04
Cooking Loss, %	20.02	20.83	0.67	20.05	20.80	0.67	20.15	20.70	0.67	21.21	19.64	0.67
Non-Heme Iron, µg/g meat	2.81	2.50	0.20	2.62	2.70	0.20	2.80	2.51	0.20	2.29 ^b	3.02 ^a	0.20
Heme Iron, mg/kg	10.09	10.03	0.19	10.00	10.11	0.19	9.98	10.13	0.19	9.54 ^b	10.58 ^a	0.19

^xWDGS = Wet distillers grains with solubles

^yNA = Not applicable, aging period was not tested for these factors

^{ab}Means within the same treatment and the same row with different superscripts are different ($P \leq 0.05$)

Table 4. The effect of grass type and finishing diet on the LS means of pH, proximate analysis, carbohydrate, cooking loss, non-heme iron, and heme iron content for *L. dorsi* steaks

Trait	Warm-season Grass		Cool-season Grass		SEM
	Corn	WDGS ^x	Corn	WDGS	
pH	5.48	5.44	5.42	5.36	0.04
Moisture, %	70.89 ^a	71.50 ^a	70.83 ^a	69.86 ^b	0.34
Ash, %	1.64	1.96	1.82	1.93	0.13
Fat, %	6.04	5.29	5.76	7.00	0.57
Protein, %	20.89	20.94	21.22	20.93	0.22
Total Carbohydrates, mg/mL	0.77	0.86	0.79	0.72	0.04
Cooking Loss, %	14.25	15.21	15.65	13.92	0.92
Non-Heme Iron, µg/g meat	2.38	2.3	2.13	2.16	0.24
Heme Iron, mg/kg	7.98 ^a	7.33 ^{ab}	7.11 ^b	7.88 ^{ab}	0.24

^xWDGS = Wet distillers grains with solubles

^{ab}Means within the same treatment combination and the same row with different superscripts are different ($P \leq 0.05$)

Table 5. The influence of diet and age on the *P*-values of the amino acids and minerals in *L. dorsi* steaks

Trait	<i>P</i> -Value						
	Grass ^x	Supp	GrassX Supp	Diet	GrassX Diet	SuppX Diet	GrassX SuppX Diet
<u>Amino Acids, mg/g</u>							
Aspartic Acid	0.11	0.51	0.83	0.13	0.33	0.95	0.72
Threonine	0.17	0.59	0.89	0.15	0.34	0.92	0.89
Serine	0.23	0.53	0.81	0.29	0.44	0.85	0.77
Glutamic Acid	0.27	0.88	0.93	0.23	0.33	0.79	0.84
Proline	0.19	0.76	0.45	0.39	0.39	0.59	0.45
Glycine	0.30	0.75	0.26	0.60	0.65	0.55	0.25
Alanine	0.15	0.69	0.68	0.25	0.35	0.86	0.81
Valine	0.11	0.57	0.81	0.13	0.33	1.00	0.62
Methionine	0.21	0.14	0.19	0.16	0.30	0.10	0.53
Isoleucine	0.11	0.71	0.80	0.13	0.32	0.93	0.77
Leucine	0.12	0.60	0.92	0.14	0.35	0.93	0.84
Tyrosine	0.12	0.60	0.80	0.10	0.44	0.97	0.83
Phenylalanine	0.13	0.61	0.92	0.16	0.40	0.92	0.82
Histidine	0.64	0.64	0.75	0.83	0.46	0.89	0.61
Lysine	0.13	0.69	0.84	0.13	0.36	0.95	0.93
Arginine	0.11	0.77	0.73	0.19	0.31	0.86	0.73
Total	0.12	0.80	0.69	0.14	0.42	0.71	0.89
<u>Minerals, mg/kg</u>							
Calcium	0.12	0.83	0.57	0.61	0.39	0.35	0.57
Phosphorous	0.11	0.54	0.37	0.95	0.15	0.73	0.61
Potassium	0.06	0.36	0.39	0.66	0.20	0.07	0.76
Magnesium	0.03	0.26	0.92	0.53	0.53	0.68	0.68
Zinc	0.002	0.22	0.74	0.91	0.67	0.35	0.95
Iron	0.79	0.80	0.58	0.83	0.40	0.12	0.27
Manganese	0.64	0.97	0.32	0.14	0.71	0.97	0.14
Copper	0.40	0.58	0.94	0.32	0.09	0.54	0.52
Sulfur	0.06	0.66	0.92	0.44	0.27	0.22	0.66
Sodium	0.28	0.33	0.33	0.14	0.64	0.64	0.57

^xGrass = Grass type, Supp = Supplementation, Diet = Finishing diet

Table 6. The effects of grass type, supplementation, and finishing diet on the LS means scores of minerals in *L. dorsi* steaks

Trait	Grass Type			Supplementation			Finishing Diets		
	Warm-season	Cool-season	SEM	No	Yes	SEM	Corn	WDGS ^x	SEM
<u>Minerals, mg/kg</u>									
Calcium	254.17	290.83	16.45	275.00	270.00	16.45	266.67	278.33	16.45
Phosphorous	2054.17	2102.50	20.97	2087.50	2069.17	20.97	2079.17	2077.50	20.97
Potassium	3220.83	3498.33	104.30	3291.67	3427.50	104.30	3391.67	3327.50	104.30
Magnesium	291.67 ^b	326.67 ^a	11.37	300.00	318.33	11.37	304.17	314.17	11.37
Zinc	42.29 ^a	37.46 ^b	1.01	39.00	40.75	1.01	39.95	39.80	1.01
Iron	120.33	130.08	26.11	120.63	129.78	26.11	129.04	121.37	26.11
Manganese	1.13	0.94	0.28	1.04	1.03	0.28	1.33	0.73	0.28
Copper	1.69	1.41	0.24	1.45	1.64	0.24	1.71	1.38	0.24
Sulfur	2012.50	2060.00	17.52	2041.67	2030.83	17.52	2045.83	2026.67	17.52
Sodium	533.33	550.83	11.42	550.00	534.17	11.42	554.17	530.00	11.42

^xWDGS = Wet distillers grains with solubles

^{ab}Means within the same treatment and the same row with different superscripts are different ($P \leq 0.05$)

Table 7. The influence of diet and age on the *P*-values of the amino acids and mineral in *B. femoris* steaks

Trait	<i>P</i> -Value						
	Grass ^x	Supp	GrassX Supp	Diet	GrassX Diet	SuppX Diet	GrassX SuppX Diet
<u>Amino Acids, mg/g</u>							
Aspartic Acid	0.62	0.19	0.25	0.12	0.54	0.85	0.40
Threonine	0.67	0.19	0.28	0.10	0.46	1.00	0.49
Serine	0.85	0.21	0.35	0.10	0.47	0.98	0.44
Glutamic Acid	0.96	0.14	0.36	0.11	0.45	0.99	0.45
Proline	0.86	0.05	0.55	0.16	0.70	0.34	0.10
Glycine	0.81	0.03	0.44	0.23	0.77	0.18	0.05
Alanine	0.78	0.12	0.36	0.11	0.57	0.72	0.23
Valine	0.59	0.21	0.24	0.14	0.70	0.84	0.25
Methionine	0.52	0.85	0.73	0.26	0.48	0.39	0.56
Isoleucine	0.54	0.17	0.23	0.11	0.62	0.95	0.33
Leucine	0.71	0.23	0.29	0.10	0.55	0.96	0.41
Tyrosine	0.68	0.17	0.17	0.11	0.68	0.88	0.44
Phenylalanine	0.78	0.24	0.26	0.11	0.59	0.91	0.45
Histidine	0.87	0.47	0.76	0.04	0.53	0.56	0.37
Lysine	0.68	0.22	0.29	0.09	0.55	0.93	0.45
Arginine	0.70	0.10	0.25	0.08	0.56	0.85	0.38
Total	0.80	0.18	0.34	0.10	0.53	0.93	0.39
<u>Minerals, mg/kg</u>							
Calcium	0.14	0.83	0.29	0.52	0.06	0.29	0.52
Phosphorous	0.18	0.32	0.39	0.71	0.80	0.04	0.62
Potassium	0.27	0.54	0.33	0.31	0.87	0.73	0.22
Magnesium	0.07	0.53	1.00	1.00	0.53	0.53	0.21
Zinc	0.52	0.18	0.33	0.67	0.38	0.22	0.53
Iron	0.30	0.07	0.73	0.43	0.31	0.90	0.36
Manganese	0.80	0.14	0.32	0.14	1.00	0.80	0.80
Copper	0.34	0.67	0.36	0.58	0.45	0.19	0.28
Sulfur	0.07	0.85	0.20	0.71	1.00	0.20	0.35
Sodium	0.43	1.00	1.00	0.69	0.69	0.06	0.24

^xGrass = Grass type, Supp = Supplementation, Diet = Finishing diet

Table 8. The effect of grass type, supplementation, and finishing diet on the LS means scores of amino acids and mineral content of *B. femoris* steaks

Trait	Grass Type		SEM	Supplementation			Finishing Diets		
	Warm-season	Cool-season		No	Yes	SEM	Corn	WDGS ^x	SEM
<u>Amino Acids, mg/g</u>									
Aspartic Acid	16.79	16.99	0.28	17.15	16.62	0.28	17.20	16.57	0.28
Threonine	7.83	7.92	0.14	8.00	7.75	0.14	8.04	7.71	0.14
Serine	6.43	6.45	0.11	6.54	6.34	0.11	6.57	6.31	0.11
Glutamic Acid	26.42	26.46	0.47	26.94	25.94	0.47	26.99	25.89	0.47
Proline	6.79	6.82	0.11	6.97 ^a	6.64 ^b	0.11	6.92	6.69	0.11
Glycine	7.15	7.20	0.12	7.37 ^a	6.98 ^b	0.12	7.28	7.07	0.12
Alanine	9.53	9.60	0.16	9.75	9.38	0.16	9.76	9.38	0.16
Valine	9.11	9.23	0.16	9.31	9.03	0.16	9.34	9.00	0.16
Methionine	4.57	4.30	0.29	4.40	4.48	0.29	4.67	4.20	0.29
Isoleucine	8.49	8.62	0.15	8.70	8.41	0.15	8.73	8.39	0.15
Leucine	14.88	15.02	0.26	15.18	14.73	0.26	15.26	14.64	0.26
Tyrosine	6.54	6.61	0.11	6.69	6.46	0.11	6.71	6.44	0.11
Phenylalanine	7.81	7.87	0.14	7.95	7.73	0.14	8.00	7.68	0.14
Histidine	5.92	5.95	0.13	6.00	5.87	0.13	6.12 ^a	5.75 ^b	0.13
Lysine	16.50	16.66	0.29	16.83	16.33	0.29	16.93	16.23	0.29
Arginine	12.27	12.38	0.20	12.57	12.08	0.20	12.58	12.06	0.20
Total	167.05	168.09	2.93	170.36	164.78	2.93	171.09	164.05	2.93

^xWDGS = Wet distillers grains with solubles

^{ab}Means within the same treatment and the same row with different superscripts are different ($P \leq 0.05$)

Table 9. The effects of the interaction between supplementation and finishing diet on the LS means of amino acids and minerals for *B. femoris* steaks

Trait	No Supplementation		Supplementation		SEM
	Corn	WDGS ^x	Corn	WDGS	
Amino Acids, mg/g					
Aspartic Acid	17.43	16.88	16.98	16.27	0.40
Threonine	8.17	7.84	7.91	7.58	0.19
Serine	6.67	6.41	6.48	6.21	0.15
Glutamic Acid	27.49	26.38	26.48	25.4	0.67
Proline	7.01	6.93	6.83	6.45	0.16
Glycine	7.36	7.38	7.20	6.76	0.17
Alanine	9.90	9.60	9.62	9.15	0.23
Valine	9.46	9.17	9.22	8.83	0.22
Methionine	4.81	3.98	4.53	4.42	0.41
Isoleucine	8.88	8.53	8.58	8.25	0.21
Leucine	15.08	14.68	15.44	14.60	0.37
Tyrosine	6.83	6.54	6.58	6.34	0.16
Phenylalanine	8.10	7.81	7.89	7.56	0.19
Histidine	6.13	5.87	6.11	5.63	0.18
Lysine	17.20	16.47	16.66	15.99	0.41
Arginine	12.80	12.33	12.37	11.79	0.29
Total	173.69	167.03	168.48	161.07	4.14
Minerals, mg/kg					
Calcium	283.33	291.67	300.00	266.67	19.58
Phosphorous	2100.00 ^{ab}	2266.67 ^a	2175.00 ^{ab}	2058.33 ^b	66.46
Potassium	3825.00	3675.00	4041.67	3733.33	225.04
Magnesium	308.33	316.67	308.33	300.00	13.18
Zinc	45.75	47.56	45.53	41.86	2.18
Iron	168.25	94.25	308.83	254.58	80.75
Manganese	1.08	0.67	1.67	1.08	0.33
Copper	1.38	1.54	1.74	1.36	0.20
Sulfur	1966.67	2041.67	2016.67	1975.00	44.49
Sodium	591.67	641.67	633.33	600.00	21.08

^xWDGS = Wet distillers grains with solubles

^{ab}Means within the same row with different superscripts are different ($P \leq 0.05$)

Running Title: Diet and aging on beef fatty acids

**The role of post-weaning forage, energy supplementation, finishing
diets, and aging on fatty acid profiles of beef¹**

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Abstract

The objective of this study was to determine how the fatty acid profiles of meat are altered when diet and aging periods are varied. Crossbred steers (n = 64) were grazed on warm or cool-season grasses, without or with energy supplementation from wet distillers grains with solubles (WDGS), and were finished on corn or 35% WDGS. Six carcasses from each treatment (n = 48) that graded USDA Choice or Select were identified and *Longissimus dorsi* and *Biceps femoris* muscles from each side of each carcass were collected and aged under vacuum 7 and 28 d. Samples were analyzed for fatty acid (FA) content (mg/100 g of meat) in the neutral and phospholipid layers. In the neutral layer of *L. dorsi* steaks, warm-season grass grazing without supplementation lowered C17:0, C17:1, C18:1, C18:2TT, total unsaturated FA, and total monounsaturated FA (MUFA) concentrations ($P \leq 0.05$) compared to cool-season grass when not receiving supplementation (29.03 vs. 44.05, 22.64 vs. 35.79, 848.22 vs. 1,261.53, 1.19 vs. 4.65, 1,045.30 vs. 1,607.48, and 1,013.53 vs. 1,545.75, respectively) and warm-season grass alone also lowered C18:1t, C18:1v, C18:2TT, C18:2, C20:1, and total polyunsaturated FA concentrations ($P \leq 0.006$). The phospholipid layer was only minimally affected by dietary components with supplementation causing lower concentrations of C18:3 and C20:5 ($P \leq 0.05$) compared not supplementing (7.57 vs. 10.88 and 13.09 vs. 21.19, respectively). In the neutral lipids layer of *B. femoris* steaks not supplementing and finishing on corn caused decreased concentrations ($P \leq 0.05$) of C14:0, C14:1, C15:0, C16:0, C17:0, C17:1, C18:1, total saturated FA, total unsaturated FA, and total MUFA were lower than when finished on WDGS (89.37 vs. 55.06, 33.30 vs. 19.48, 17.31 vs.

10.91, 867.17 vs. 517.94, 48.27 vs. 30.97, 53.33 vs. 30.94, 348.04 vs. 240.94, 1,558.94 vs. 1,060.05, 1,375.10 vs. 857.17, 1,952.46 vs. 1,330.93, and 1,905.19 vs. 1,285.39, respectively) within warm-season grass grazing. Similar to *L. dorsi* steaks, few dietary components had an effect on FA in the phospholipid layer. Finishing diet had the greatest effect with corn causing increased ($P \leq 0.05$) concentrations of C16:1, C18:1, C18:1v, C22:6, and total MUFA over WDGS (11.93 vs. 7.38, 110.82 vs. 78.05, 40.57 vs. 26.84, 2.84 vs. 0.73, and 233.07 vs. 185.12, respectively). Grazing on warm-season grasses also shifted the FA profile, but provision of a supplementation may prevent the changes.

Keywords: Beef, diet, fatty acids, forages, supplementation

Introduction

The diet of beef cattle can alter the fatty acid profile of meat. This alteration begins with the type of forage cattle are grazed on post-weaning and before they enter a feedlot. Jenschke et al. (2008) fed cattle different forage diets and found that fatty acid (FA) profiles were highly influenced by it.

While grazing, it is common to supplement cattle to ensure that they are getting enough energy and protein. Kiesling et al. (2011) and Mandell et al. (1997) discovered that certain types of supplementation can alter FA profiles, especially conjugated linoleic acid (CLA) content and omega-3:omega-6 ratios. Each researcher used different types of supplementation, including not supplementing at all, and both found that fatty acid profiles can be altered.

Diets fed in a finishing lot may also be a factor in determining beef composition. Distillers grains (DG) as a finishing ration are a more economical choice for producers and are often included. When Driskell et al. (2009) fed dried corn distillers grains with solubles (DDGS) FA profiles changed as inclusion levels of DDGS increased. Similarly, Gill et al. (2008) also saw that FA profiles could be affected by finishing cattle on DG instead of corn.

This research was conducted to investigate how fatty acids are affected in two different muscles from cattle fed two different forages post-weaning, with or without supplemental energy, and finished on either a corn or wet DGS (WDGS) diet.

Materials and Methods

Diets

All protocols performed in this study were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. Cattle were fed in a 2x2x2 factorial design. Crossbred steers (n = 64) were backgrounded on either warm-season grasses (i.e. bluestem and switch grass) at the Barta Ranch in Western Nebraska or on cool-season (i.e. brome and bluegrass) pastures in Ithaca, NE for 177 d, shortly after weaning. Within each pasture, half of the cattle were supplemented with 0.6 kg WDGS/kg body weight/ day for energy. At the end of the grazing period, all cattle were transported to the University of Nebraska-Lincoln's research feedlot in Ithaca, NE. While in the feedlot, half of each pasture and supplementation treatments were finished on an all-corn diet while the other half were fed corn with WDGS at a 35% inclusion rate

(DM basis). Cattle were on feed for 119 days and fed to an average live weight of 1,427 lbs.

Harvest

At the end of the feedlot period, cattle were transported and harvested at the Greater Omaha Packing (Omaha, NE). Forty-eight carcasses grading either USDA Choice (n = 43) or USDA Select (n = 5), 6 from each treatment combination, were selected. Strip loins (*Longissimus dorsi*; *IMPS #180*, NAMP, 2007) and bottom round flats (*Biceps femoris*; *IMPS #171B*, NAMP, 2007) were collected. Only five *L. dorsi* muscles were collected from the cool-season grass, supplementation provided, and finished on WDGS treatment because one *L. dorsi* muscle was lost within Greater Omaha Packing Plant. All subprimals were aged under vacuum for 7 d. Upon fabrication, one steak was cut from each subprimal.

Sample collection

One steak, cut 1.25 cm thick, was used for all lab analyses. Steaks were vacuumed packaged and frozen at -20°C for approximately 2 months. Before any lab procedures were conducted, all lab steaks had any subcutaneous fat removed and were cut into cubes. Next, the cubes were flash frozen in liquid nitrogen, powdered using a Waring blender (Waring Commercial, model 51BL32, Torrington, CT), and stored at -80°C until needed for further lab analyses. All lab analyses were conducted on powdered samples.

Fatty Acids

Fats were extracted following the procedures of Folch et al. (1957). Four gram powdered meat samples were mixed with 10 mL of 2:1 chloroform:methanol solution, vortexed, and allowed to sit at room temperature for 1 h. Homogenized samples were filtered into new tubes, brought to 15 mL with 2:1 chloroform:methanol solution, mixed with 2 mL of 0.74% KCl solution, vortexed, purged with nitrogen gas, and kept in a -20°C freezer overnight. The next day, the top aqueous phase was removed and 2 mL of the lower phase was collected and dried down at 60°C under constant nitrogen gas purging.

Samples were separated into neutral and phospholipid layers following the procedures described by Carr et al. (2005). The neutral and phospholipid regions of interest were isolated using thin layer chromatography plates (Silica Gel 60 w/o indicator, Catalog No.: M5547-7, Thermo Fisher Scientific Inc.) and isolated. The neutral lipid samples were submerged in chloroform and the phospholipid samples were submerged in methanol to extract the lipids. Samples were stored in a 2°C cooler for 45 min.

After incubation the solutions were dried at 60°C under constant nitrogen gas purging. Once dried, the fatty acid methyl esters were prepared following the procedures described by Morrison and Smith (1964) and Metcalfe et al. (1996). Gas chromatography (Hewlett-Packard Gas Chromatograph – Agilent Technologies, model 6890 series, Santa Clara, CA) was used to determine fatty acid content using a Chrompack CP-Sil 88 (0.25 mm x 100 m) column using Helium as the carrier gas with a flow rate of 1.1 mL/min. The injector temperature was held at 270°C and the detector

temperature was 300°C. Fatty acids were identified by comparing retention times and peaks with known standards.

To get exact concentrations of each FA, additional thin layer chromatography plates were made separating the neutral and phospholipid layers. This time the plates were stained using iodine, and the areas on the plates were measured, as a percent, using Quantity One 1-D Analysis Software (Bio-Rad, Hercules, CA). To calculate the mg/100 g of meat for each FA in each layer, the total fat percentages attained for each sample from proximate analysis was converted to grams of fat per 100 g of meat. That value was multiplied by the percentage of the neutral and phospholipid layers, and converted to mg of neutral or phospholipid per 100 g of meat. From there the percentage of each individual FA in each layer was multiplied by their respective value and the mg of each FA per 100 g of meat was attained.

Statistical Analysis

All data were analyzed as a 2x2x2x2 factorial arrangement split plot design within a randomized complete design using the Mixed procedure in SAS (Version 9.2, SAS Institute Inc., Cary, NC, 2006). The experimental unit was the individual animal and the model included grass, supplementation, and finishing diet as fixed effects. The least square means procedure with the probability of difference (pdiff) option was used for mean separation; with significance determined at $P \leq 0.05$ levels. Whenever there was a three-way interaction, the LSmeans were reanalyzed using the GLIMMIX procedure with the slicediff option in order to more accurately study differences.

Results and Discussion

L. dorsi Steaks

It was decided to separate the neutral and phospholipid layers because the composition between the lipid layers tends to be different. The phospholipid layer contains more unsaturated fatty acids (Bowyer et al., 1963) and therefore is more prone to oxidation than the neutral lipid layer. Also, the phospholipid layer tends to have a higher turnover rate. Due to these reasons it is important to investigate the effect of diet on each layer.

Tables 1 and 2 show the *P*-values for how dietary treatments affect FA in the neutral and phospholipid layer, respectively. The FA C14:1, C17:0, C17:1, C18:1, C18:2TT, total UFA, and total monounsaturated fatty acids (MUFA) were all affected by an interaction between grass type and supplementation in the neutral lipid layer. Grazing on warm-season grass and not supplementing created the lowest concentrations ($P \leq 0.05$) for all of the aforementioned FA. However, concentrations for most of the FA when supplementation was provided while grazing on warm-season grasses increased significantly ($P \leq 0.05$). Clearly, warm-season grasses caused a shift to occur in FA profiles that can easily be altered by the addition of supplementation with WDGS. This effect is echoed in the main effects with warm-season grass grazing causing significantly lower concentrations of C18:1t, C18:1v, C18:2TT, C18:2, C20:1, and total polyunsaturated fatty acids (PUFA) than cool-season grass grazing. All of the effects on FA in the phospholipid layer were minimal, granting, however that supplementation did decrease concentrations of C18:3 and C20:5 ($P \leq 0.05$).

Looking at all of the effects separately, when supplementation was not provided, grazing on a warm-season grass caused lower C17:0, C17:1, C18:1, C18:2TT, total UFA, and total MUFA ($P \leq 0.05$) concentrations (mg/100 g of meat) in the neutral lipid layer compared to grazing on cool-season grasses (29.03 vs. 44.05, 22.64 vs. 35.79, 848.22 vs. 1,261.53, 1.19 vs. 4.65, 1,045.30 vs. 1,607.48, and 1,013.53 vs. 1,545.75, respectively, Table 3). Within warm-season grass grazing, providing supplementation resulted in higher C14:1, 17:1, 18:1, 18:2TT, total UFA, and total MUFA concentrations ($P < 0.05$) than not supplementing (23.16 vs. 14.33, 33.48 vs. 22.64, 1,279.40 vs. 848.22, 3.61 vs. 1.19, 1,045.30 vs. 1,584.17, and 1,013.53 vs. 1,531.32, respectively).

When examining the interaction between supplementation and finishing diet the highest concentrations (5.67) of C18:3 occurred when cattle were supplemented and finished on a WDGS diet ($P = 0.02$, Table 5) as opposed to all other supplementation and finishing diet combinations. Mandell et al. (1997) reported a lot more differences when supplementation was provided while on a finishing diet. Although they did not report any differences in C18:3 concentrations, they did find that supplementing decreased concentrations of C18:0 and C20:4 and increased C20:5 and C22:6. Those differences were not observed in the present study. However, in this study supplementation was only provided during the grazing period. The difference of when supplementation was provided could explain the differences in FA profiles with the interaction of finishing diet and supplementation.

When examining dietary components individually, grass type had the most influence over FA concentrations (Tables 1 and 2). In the neutral lipid layer, grazing on a

cool-season grass instead of a warm-season grass increased concentrations of C10:0 (1.68 vs. 0.96), C18:1t (88.21 vs. 49.02), C18:1v (34.27 vs. 24.65), C18:2tt (4.45 vs. 2.40), C18:2 (52.88 vs. 36.68), C20:1 (18.05 vs. 11.33), and total PUFA (61.22 vs. 42.31) ($P \leq 0.05$, Table 6). Providing supplementation increased concentrations ($P = 0.05$) of C12:0 (2.03 vs. 1.28), but finishing diet had a greater effect. Finishing on an all-corn diet instead of a diet with WDGS caused lower concentrations ($P \leq 0.005$) of C18:1t (53.56 vs. 83.67), C18:2 (35.22 vs. 54.34), C18:3 (2.70 vs. 4.42), and total PUFA (40.67 vs. 62.86). As percentage inclusion of DGS in the finishing diet increased, so did concentrations of C18:2 (total) and total PUFA for Depenbusch et al. (2009), which was also found in this study. In contrast, Kinman et al. (2011) did not see any change in total PUFA between finishing on corn or corn with DGS. Depenbusch et al. (2009) did not report any differences in C18:1 and C18:3 concentrations, unlike what was reported in this study.

The phospholipid layer reacted quite differently and was not as affected by diet. Not providing supplementation while grazing on a cool-season grass caused the highest concentrations of C18:3 ($P = 0.007$) as opposed to providing supplementation (13.14 vs. 5.75) or grazing on warm-season grass without supplementation (13.14 vs. 8.61, Table 4). Grazing on a warm-season grass instead of a cool-season grass caused a lower concentration ($P = 0.03$) of C17:1 (3.42 vs. 7.27), but grass type affected no other FA (Table 7). Part of the reason for why the phospholipid layer was not as affected by diet could be due to the turnover rate. As stated previously, it is believed that the phospholipid layer has a faster turnover rate of fatty acids than the neutral lipid layer.

This means that any changes in fatty acid composition due to grass type or supplementation may be altered again by the end of the finishing lot period.

Supplementing cattle influenced a few more FA by decreasing concentrations ($P \leq 0.03$) of C18:3 (7.57 vs. 10.88) and C20:5 (13.09 vs. 21.19). This study showed that supplementation did not influence total saturated fatty acids (SFA), total MUFA, and total PUFA concentrations in either the neutral or phospholipid layer. In a study conducted by Kiesling et al. (2011), two different types of supplementation were provided to cattle while grazing. They reported that total MUFA concentrations were not different between the different types of supplementation, like in this study, but total SFA and total PUFA concentrations were. In their study, they did not have a control where no supplementation was provided, but the fact that FA profiles can vary based purely on the type of supplementation provided still has merit. Finishing on an all-corn diet instead of a diet with a WDGS inclusion caused a higher concentration ($P = 0.02$) of C16:1 (15.59 vs. 8.36) and a lower concentration ($P = 0.008$) of C18:2 (190.70 vs. 239.01).

B. femoris Steaks

A majority of the FA in the neutral lipid layer were significantly ($P \leq 0.05$) affected by a three-way interaction between grass type, supplementation, and finishing diet (Table 8). In order to better interpret the data, means were separated by grass type. Overall, within warm-season grass grazing, when cattle are not supplemented finishing on corn caused significantly higher ($P \leq 0.05$) concentrations than finishing on WDGS. In contrast, when supplementation was provided, there were very few differences in FA concentrations between finishing on corn or WDGS ($P > 0.05$). The lack of differences

could be because WDGS were used for supplementation. Even though half of the cattle were finished on corn, they were exposed to WDGS for the entire pre-finishing phase which could have influenced the FA profile from the beginning. The amount of time they were in the finishing lot on corn was insufficient to overcome the initial changes to the FA. In *L. dorsi* steaks, when cattle were grazed on warm-season grasses and not supplemented there was also a significant decrease ($P \leq 0.05$) in FA in the neutral lipid layer. This occurrence is mirrored in the *B. femoris* steaks. There were few differences between supplementation and finishing diet combinations within cool-season grass grazing.

For the three-way interaction between grass type, supplementation, and finishing diet (Table 8), within warm-season grazing, when the cattle were not supplemented finishing on an all corn diet caused the concentrations of C14:0, C14:1, C15:0, C18:0, C18:1, total SFA, total UFA, and total MUFA to be higher ($P \leq 0.05$) than when they are finished on WDGS (89.37 vs. 55.06, 33.30 vs. 19.48, 17.31 vs. 10.91, 348.04 vs. 240.94, 1,558.94 vs. 1,060.05, 1,375.10 vs. 857.17, 1,952.46 vs. 1,330.93, and 1,905.19 vs. 1,285.39, respectively, Table 10). Koger et al. (2010) saw no differences in total SFA, UFA, and MUFA when they included DGS into the diet. Their study only examined the *L. dorsi* muscle.

Cattle that were not supplemented and were finished on corn also had higher concentrations of C16:0 and C17:0 ($P \leq 0.04$) than when finished on WDGS, both without supplementation (867.17 vs. 517.49 and 48.27 vs. 30.97, respectively) and with supplementation (867.17 vs. 644.16 and 48.27 vs. 37.62, respectively). Finishing on corn

without supplementation caused the highest concentrations of C17:1 (53.33) compared to all other supplementation and finishing diet combinations ($P = 0.05$) but caused the concentration of C10:0 to only be higher ($P = 0.008$) when compared to supplementing and finishing on corn (1.55 vs. 0.23).

Not supplementing and finishing on corn as well as supplementing and finishing on WDGS caused concentrations of C12:0 to be higher ($P < 0.0001$) than when cattle were not supplemented and finished on WDGS (3.01 and 3.05 vs. 0.45, respectively) and when they were supplemented and finished on corn (3.01 and 3.05 vs. 1.45, respectively). Table 8 shows that C18:2tt was significantly ($P = 0.04$) affected by the three-way interaction, but when the means were separated and compared, no differences existed between the different supplementation and finishing diet combinations for either grass type (Table 10 and 11). This suggests that grass type is causing the differences and not so much the supplementation or finishing diet.

Similarly, when the means were separated within cool-season grass grazing and the different supplementation and finishing diet combinations were compared, the means for C14:0, C14:1, C15:0, C16:0, C17:0, C17:1, C18:0, C18:1, C18:2tt, total SFA, total UFA, and total MUFA were all not different ($P > 0.05$) from each other (Table 11). This shows that perhaps it is grass type that is the driving force behind this three-way interaction, with warm-season grasses causing the most differences. Within warm-season grasses, when supplementation was provided there were no differences between finishing diets. This shows that the addition of supplementation during the grazing period can deter any effects that grass type may cause on the fatty acid profile.

When finishing on corn, supplementing cattle caused the concentrations of C10:0 to be higher ($P = 0.008$) than when they were not supplementing (1.83 vs. 0.34). Also, not supplementing cattle and finishing on corn caused the concentrations of C12:0 to be the lowest (0.47, $P < 0.0001$) out of all the different supplementation and finishing diet combinations except for when cattle were supplemented and finished on WDGS, which was not different.

No other dietary regimen interactions significantly influenced any other FA in the neutral layer. Grass type did influence both C18:1t, C18:2tt, and C20:1 concentrations ($P \leq 0.05$), with cool-season grasses creating higher concentration than warm-season grasses (70.29 vs. 50.12, 4.03 vs. 3.09, and 21.48 vs. 17.46, respectively, Table 12), similar to what was observed in the *L. dorsi* steaks. In contrast, concentrations of C17:1 were lower ($P = 0.05$) when cattle were grazed on cool-season grasses instead of warm-season grasses (32.68 vs. 39.54). Jenschke et al. (2008) also fed various roughages to cattle and in doing so was also able to influence C18:1 concentrations in *R. femoris* muscles, which are also from the round. They did not report any differences in C17:1 and C20:1 concentrations, perhaps because the roughages they fed were different from the grasses grazed in this study.

Unlike in the neutral layer, there were no significant ($P \leq 0.05$) three-way interactions between grass type, supplementation, and finishing diets for any of the FA in the phospholipid layer (Table 9). The FA in the phospholipid layer were mostly affected by finishing diet with corn causing increased concentrations over WDGS ($P \leq 0.05$).

There were two two-way interactions, both involving grass-type, which influenced FA. Not supplementing while grazing on a cool-season grass caused the concentration of C20:5 to be the highest (26.93, $P = 0.01$), when compared to all other grass type and supplementation combinations (Table 14). When cattle were grazed on a cool-season grass, finishing on WDGS instead of corn (Table 15) caused higher concentrations ($P = 0.05$) of C14:1 (8.29 vs. 2.41). In both instances, grass type was involved corroborating that the type of forage cattle grazed can have an effect on the composition of the final product.

Grass type alone did not have any significant influence ($P \leq 0.05$) over any of the FA concentrations, however supplementing did cause higher concentrations ($P \leq 0.04$) of C20:4 and C24:1 as opposed to not providing supplementation (185.43 vs. 159.90 and 16.32 vs. 12.97, respectively, Table 13). In contrast, concentrations of C20:5 were higher when cattle were not supplemented (22.97 vs. 14.63, $P = 0.03$). Srinivasan et al. (1998) did not see any differences in C20:5 concentrations in *Semimembranosus* muscles when comparing supplementing and not supplementing. The grain type used for supplementing was different from the ones used in this study, which could account for the differences. When the cattle were finished on an all corn diet, concentrations of C16:1, C18:1, C18:1v, C22:6, and total MUFA were higher ($P \leq 0.03$) than when they were finished on WDGS (11.93 vs. 7.38, 110.82 vs. 78.05, 40.57 vs. 26.84, 2.84 vs. 0.73, and 233.07 vs. 185.12, respectively). In contrast, the concentration of C18:2 decreased ($P = 0.0007$) on a corn finishing diet (205.67 vs. 269.96).

In conclusion, FA in neutral lipids are more easily manipulated by diet than those in the phospholipid layer. Again, this could be attributed to the phospholipid layer having a faster turnover rate than the neutral lipid layer. Since the phospholipids have a faster turnover rate, any changes in composition due to diet, especially the grass type and supplementation which were fed at a young age, could become negligible by the end of the finishing period.

Overall, grass type had the biggest effect on the fatty acid profile with warm-season grasses causing decreased concentrations in a majority of the FA, especially in the neutral lipid layer. Even though grass type had such a major effect, the provision of WDGS as a supplemental energy source was able to minimize, if not deter, a majority of the changes. This would mean that if a producer was concerned about the effect of the grass their cattle are being grazed on, they could provide an energy supplementation to their cattle and effectively negate any effects.

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Figures and Tables

Table 1. The influence of grass-type, supplementation, and finishing diet on the *P*-values of fatty acids in the neutral lipid layers in *L. dorsi* steaks

Neutral Lipids, mg/100 g of meat	<i>P</i> -Value						
	Grass ^x	Supp	GrassX Supp	Diet	GrassX Diet	SuppX Diet	GrassX SuppX Diet
C10:0	0.04	0.46	0.85	0.93	0.10	0.13	0.73
C12:0	0.08	0.05	0.27	0.85	0.09	0.29	0.92
C14:0	0.08	0.18	0.10	0.72	0.18	0.71	0.43
C14:1	0.61	0.21	0.05	0.64	0.15	0.77	0.52
C15:0	0.19	0.57	0.08	0.75	0.36	0.79	0.51
C16:0	0.08	0.20	0.14	0.98	0.22	0.90	0.38
C16:1	0.44	0.24	0.06	0.43	0.15	0.98	0.52
C17:0	0.23	0.70	0.03	0.95	0.30	0.58	0.58
C17:1	0.34	0.74	0.01	0.36	0.28	0.50	0.73
C18:0	0.10	0.11	0.14	0.18	0.39	0.67	0.31
C18:1t	0.0003	0.49	0.27	0.005	0.22	0.85	0.36
C18:1	0.23	0.18	0.03	0.74	0.30	0.68	0.44
C18:1v	0.05	0.08	0.51	0.67	0.34	0.25	0.44
C18:2TT	0.006	0.16	0.05	0.06	0.29	0.64	0.11
C18:2	0.008	0.18	0.10	0.002	0.25	0.68	0.78
C20:0	0.51	0.95	0.78	0.30	0.68	0.91	0.98
C18:3	0.27	0.06	0.80	0.005	0.83	0.02	0.88
C20:1	0.002	0.06	0.19	0.31	0.09	0.68	0.45
C22:0	0.11	0.17	0.08	0.14	0.27	0.17	0.36
Others	0.10	0.59	0.05	0.49	0.27	0.67	0.37
Total SFA ^y	0.08	0.17	0.13	0.64	0.26	0.79	0.37
Total UFA	0.14	0.18	0.04	0.59	0.26	0.69	0.47
Total MUFA	0.15	0.18	0.04	0.68	0.26	0.69	0.46
Total PUFA	0.006	0.13	0.10	0.002	0.27	0.53	0.68

^xGrass = Grass type, Supp = Supplementation, Diet = Finishing diet

^ySFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids

Table 2. The influence of grass-type, supplementation, and finishing diet on the *P*-values of fatty acids in the phospholipid layers in *B. femoris* steaks

Phospholipids, mg/100 g of meat	<i>P</i> -Value						
	Grass ^x	Supp	GrassX Supp	Diet	GrassX Diet	SuppX Diet	GrassX SuppX Diet
C12:0	0.84	0.70	0.34	0.30	0.39	0.09	0.64
C13:0	0.44	0.70	0.75	0.62	0.14	0.20	0.52
C14:0	0.60	0.14	0.68	0.94	0.93	0.67	0.85
C14:1	0.30	0.81	0.39	0.46	0.12	0.06	0.91
C15:0	0.17	0.57	0.93	0.57	0.10	0.30	0.77
C16:0	0.46	0.67	0.59	0.60	0.89	0.82	0.62
C16:1	0.78	0.13	0.42	0.02	0.96	0.37	0.91
C17:0	0.15	0.31	0.89	0.61	0.16	0.28	0.36
C17:1	0.03	0.88	0.98	0.48	0.71	0.26	0.50
C18:0	0.29	0.48	0.74	0.76	0.50	0.24	0.56
C18:1t	0.80	0.69	0.55	0.64	0.23	0.73	0.84
C18:1	0.73	0.41	0.63	0.11	0.89	0.67	0.59
C18:1v	0.49	0.67	0.72	0.14	0.73	0.47	0.57
C18:2	0.07	0.56	0.49	0.008	0.18	0.38	0.09
C20:0	0.59	0.34	0.46	0.13	0.32	0.72	0.76
C18:3	0.76	0.03	0.007	0.06	0.23	0.73	0.67
C22:0	0.57	0.37	0.24	0.32	0.20	0.18	0.17
C20:4	0.34	0.51	0.69	0.48	0.59	0.34	0.39
C20:5	0.51	0.0008	0.07	0.32	0.85	0.37	0.90
C24:1	0.17	0.71	0.74	0.95	0.25	0.29	0.65
C22:6	0.25	0.10	0.11	0.72	0.70	0.91	0.40
Others	0.06	0.02	0.13	0.33	0.83	0.54	0.93
Total SFA ^y	0.26	0.39	0.63	0.66	0.40	0.45	0.80
Total UFA	0.25	0.61	0.67	0.96	0.57	0.64	0.57
Total MUFA	0.62	0.47	0.80	0.13	0.93	0.80	0.61
Total PUFA	0.13	0.82	0.63	0.23	0.30	0.32	0.17

^xGrass = Grass type, Supp = Supplementation, Diet = Finishing diet

^ySFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids

Table 3. The effect of grass type and supplementation on the LS means of fatty acids in the neutral lipid layers in *L. dorsi* steaks

Neutral Lipids, mg/100 g of meat	Warm-season Grass		Cool-season Grass		SEM
	No Supplementation	Supplementation	No Supplementation	Supplementation	
C10:0	0.80	1.12	1.58	1.77	0.35
C12:0	0.72	1.90	1.84	2.16	0.39
C14:0	51.65	81.72	86.35	83.06	10.23
C14:1	14.33 ^b	23.16 ^a	21.13 ^{ab}	19.09 ^{ab}	2.76
C15:0	9.95	13.65	14.83	12.95	1.63
C16:0	503.46	723.12	764.72	747.74	81.16
C16:1	61.65	91.97	87.97	81.04	10.11
C17:0	29.03 ^b	40.52 ^{ab}	44.05 ^a	35.87 ^{ab}	4.41
C17:1	22.64 ^b	33.48 ^a	35.79 ^a	27.35 ^{ab}	3.79
C18:0	257.21	385.49	387.67	393.92	42.41
C18:1t	39.95	58.09	90.39	86.04	10.35
C18:1	848.22 ^b	1279.40 ^a	1261.53 ^a	1159.56 ^{ab}	125.10
C18:1v	18.75	30.56	31.54	37.00	4.91
C18:2TT	1.19 ^b	3.61 ^a	4.65 ^a	4.25 ^a	0.73
C18:2	27.85	45.51	53.83	51.93	5.97
C20:0	1.85	1.73	2.08	2.27	0.59
C18:3	2.74	3.73	3.24	4.53	0.60
C20:1	7.99	14.67	17.41	18.69	2.09
C22:0	0.23	1.50	1.60	1.44	0.42
Others	17.32 ^b	25.21 ^{ab}	28.63 ^a	24.09 ^{ab}	3.16
Total SFA ^x	854.90	1250.75	1304.72	1281.18	138.88
Total UFA	1045.3 ^b	1584.17 ^a	1607.48 ^a	1489.49 ^a	159.18
Total MUFA	1013.53 ^b	1531.32 ^a	1545.75 ^a	1428.78 ^{ab}	153.35
Total PUFA	31.78	52.85	61.73	60.71	6.73

^xSFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids

^{ab}Means within the same row with different superscripts are different ($P \leq 0.05$)

Table 4. The effect of grass type and supplementation on the LS means of fatty acids in the phospholipid layers in *L. dorsi* steaks

Phospholipids, mg/100 g of meat	Warm-season Grass		Cool-season Grass		SEM
	No Supplementation	Supplementation	No Supplementation	Supplementation	
C12:0	4.52	5.34	5.61	3.65	1.50
C13:0	2.65	2.73	1.39	2.21	1.18
C14:0	7.21	10.14	7.54	12.71	2.80
C14:1	4.29	3.45	1.69	3.19	1.40
C15:0	23.69	26.30	30.84	34.41	5.62
C16:0	131.14	147.46	152.55	150.87	17.38
C16:1	8.16	14.96	11.33	13.43	2.98
C17:0	17.97	23.18	25.08	29.02	4.60
C17:1	3.26	3.57	7.16	7.37	1.79
C18:0	75.39	85.83	89.47	93.18	10.37
C18:1t	36.61	30.80	31.65	32.80	2.95
C18:1	98.44	121.09	112.82	118.68	17.70
C18:1v	23.82	27.34	28.53	28.87	4.62
C18:2	187.27	209.65	232.10	230.40	17.99
C20:0	1.29	1.45	0.42	1.59	0.71
C18:3	8.61 ^b	9.40 ^{ab}	13.14 ^a	5.75 ^b	1.49
C22:0	21.24	30.15	28.75	27.54	4.39
C20:4	123.11	126.83	131.05	146.22	14.73
C20:5	18.40	14.42	23.99	11.75	2.31
C24:1	14.27	14.34	16.43	17.88	2.10
C22:6	0.29	0.28	1.95	0.00	0.61
Others	33.11	28.77	49.29	30.62	4.80
Total SFA ^x	285.10	332.58	341.65	355.18	35.97
Total UFA	526.53	576.14	611.84	616.34	55.18
Total MUFA	188.85	215.55	209.61	222.22	28.10
Total PUFA	337.68	360.58	402.23	394.12	32.94

^xSFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids

^{ab}Means within the same row with different superscripts are different ($P \leq 0.05$)

Table 5. The effect of supplementation and finishing diet on the LS means of fatty acids in the neutral lipid layers in *L. dorsi* steaks

Neutral Lipids, mg/100 g of meat	No Supplementation		Supplementation		SEM
	Corn	WDGS ^x	Corn	WDGS	
C10:0	1.44	0.94	1.16	1.72	0.35
C12:0	1.45	1.11	1.79	2.27	0.39
C14:0	69.11	68.90	78.77	86.01	10.23
C14:1	18.76	16.71	21.35	20.90	2.76
C15:0	12.34	12.44	12.84	13.76	1.63
C16:0	638.20	629.98	729.28	741.58	81.16
C16:1	78.82	70.81	90.28	82.73	10.11
C17:0	37.59	35.48	36.88	39.51	4.41
C17:1	32.14	26.29	30.87	29.96	3.79
C18:0	303.21	341.68	353.09	426.32	42.41
C18:1t	49.13	81.20	57.99	86.14	10.35
C18:1	1060.17	1049.57	1173.98	1264.99	125.10
C18:1v	26.88	23.41	30.02	37.54	4.91
C18:2TT	2.40	3.43	3.08	4.78	0.73
C18:2	32.47	49.21	37.97	59.47	5.97
C20:0	1.70	2.23	1.67	2.33	0.59
C18:3	2.82 ^b	3.17 ^b	2.59 ^b	5.67 ^a	0.60
C20:1	12.07	13.33	15.23	18.14	2.09
C22:0	0.89	0.94	0.88	2.06	0.42
Others	22.57	23.38	22.95	26.36	3.16
Total SFA ^y	1065.93	1093.69	1216.36	1315.57	138.88
Total UFA	1315.66	1337.13	1463.35	1610.30	159.18
Total MUFA	1277.97	1281.32	1419.72	1540.39	153.35
Total PUFA	37.69	55.81	43.64	69.92	6.73

^xWDGS = Wet distillers grains with solubles

^ySFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids

^{ab}Means within the same row with different superscripts are different ($P \leq 0.05$)

Table 6. The effect of grass type, supplementation, and finishing diet on the LS means scores of fatty acids in the neutral lipid layers in *L. dorsi* steaks

Neutral Lipids, mg/100 g of meat	Grass Type		SEM	Supplementation			Finishing Diets		
	Warm- season	Cool- season		No	Yes	SEM	Corn	WDGS ^x	SEM
C10:0	0.96 ^b	1.68 ^a	0.24	1.19	1.44	0.24	1.30	1.33	0.24
C12:0	1.31	2.00	0.27	1.28 ^b	2.03 ^a	0.27	1.62	1.69	0.27
C14:0	66.69	84.71	7.07	69.00	82.39	7.07	73.94	77.45	7.07
C14:1	18.74	20.11	1.90	17.73	21.12	1.90	20.05	18.80	1.90
C15:0	11.80	13.89	1.13	12.39	13.30	1.13	12.59	13.10	1.13
C16:0	613.29	756.23	56.07	634.09	735.43	56.07	683.74	685.78	56.07
C16:1	76.81	84.51	6.99	74.81	86.51	6.99	84.55	76.77	6.99
C17:0	34.77	39.96	3.05	36.54	38.20	3.05	37.24	37.50	3.05
C17:1	28.06	31.57	2.62	29.22	30.42	2.62	31.50	28.13	2.62
C18:0	321.35	390.8	29.30	322.44	389.71	29.30	328.15	384.00	29.30
C18:1t	49.02 ^b	88.21 ^a	7.15	65.17	72.07	7.15	53.56 ^b	83.67 ^a	7.15
C18:1	1063.81	1210.54	86.42	1054.87	1219.48	86.42	1117.07	1157.28	86.42
C18:1v	24.65 ^b	34.27 ^a	3.39	25.14	33.78	3.39	28.45	30.47	3.39
C18:2TT	2.40 ^b	4.45 ^a	0.51	2.92	3.93	0.51	2.74	4.11	0.51
C18:2	36.68 ^b	52.88 ^a	4.12	40.84	48.72	4.12	35.22 ^b	54.34 ^a	4.12
C20:0	1.79	2.17	0.41	1.97	2.00	0.41	1.68	2.28	0.41
C18:3	3.24	3.89	0.42	2.99	4.13	0.42	2.70 ^b	4.42 ^a	0.42
C20:1	11.33 ^b	18.05 ^a	1.44	12.70	16.68	1.44	13.65	15.73	1.44
C22:0	0.86	1.52	0.29	0.91	1.47	0.29	0.89	1.50	0.29
Others	21.27	26.36	2.18	22.98	24.65	2.18	22.76	24.87	2.18
Total SFA ^y	1052.82	1292.95	95.95	1079.81	1266.00	95.95	1141.14	1204.63	95.95
Total UFA	1314.74	1548.48	109.97	1326.39	1536.80	109.97	1389.51	1473.72	109.97
Total MUFA	1272.43	1487.27	105.95	1279.64	1480.10	105.95	1348.84	1410.85	105.95
Total PUFA	42.31 ^b	61.22 ^a	4.65	46.75	56.78	4.65	40.67 ^b	62.86 ^a	4.65

^xWDGS = Wet distillers grains with solubles

^ySFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids

^{ab}Means within the same treatment and the same row with different superscripts are different ($P \leq 0.05$)

Table 7. The effect of grass type, supplementation, and finishing diet on the LS means scores of fatty acids in the phospholipid layers in *L. dorsi* steaks

Phospholipids, mg/100 g of meat	Grass Type		SEM	Supplementation			Finishing Diets		
	Warm- season	Cool- season		No	Yes	SEM	Corn	WDGS ^x	SEM
C12:0	4.93	4.63	1.04	5.06	4.50	1.04	4.01	5.55	1.04
C13:0	2.69	1.80	0.81	2.02	2.47	0.81	2.53	1.97	0.81
C14:0	8.68	10.13	1.94	7.38	11.43	1.94	9.50	9.30	1.94
C14:1	3.87	2.44	0.97	2.99	3.32	0.97	3.66	2.65	0.97
C15:0	25.00	32.63	3.89	27.27	30.36	3.89	30.36	27.27	3.89
C16:0	139.30	151.71	12.01	141.84	149.17	12.01	149.90	141.11	12.01
C16:1	11.56	12.38	2.06	9.75	14.19	2.06	15.59 ^a	8.36 ^b	2.06
C17:0	20.57	27.05	3.18	21.52	26.10	3.18	24.95	22.68	3.18
C17:1	3.42 ^b	7.27 ^a	1.23	5.21	5.47	1.23	5.96	4.72	1.23
C18:0	80.61	91.33	7.17	82.43	89.50	7.17	84.43	87.50	7.17
C18:1t	33.70	32.22	4.11	34.13	31.80	4.11	31.62	34.30	4.11
C18:1	109.77	115.75	12.23	105.63	119.88	12.23	126.64	98.88	12.23
C18:1v	25.58	28.70	3.19	26.17	28.11	3.19	30.53	23.74	3.19
C18:2	198.46	231.25	12.43	209.69	220.02	14.43	190.70 ^b	239.01 ^a	12.43
C20:0	1.37	1.00	0.49	0.86	1.52	0.49	1.72	0.66	0.49
C18:3	9.00	9.44	1.03	10.88 ^a	7.57 ^b	1.03	7.86	10.59	1.03
C22:0	25.69	28.14	3.03	24.99	28.84	3.03	29.04	24.80	3.03
C20:4	124.97	138.64	10.18	127.08	136.53	10.18	136.83	126.78	10.18
C20:5	16.41	17.87	1.59	21.19 ^a	13.09 ^b	1.59	18.25	16.03	1.59
C24:1	14.31	17.15	1.45	15.35	16.11	1.45	15.80	15.66	1.45
C22:6	0.28	0.97	0.42	1.12	0.14	0.42	0.73	0.52	0.42
Others	30.94	39.96	3.31	41.20 ^a	29.69 ^b	3.31	37.71	33.18	3.31
Total SFA ^y	308.84	348.42	24.85	313.38	343.88	24.85	336.44	320.82	24.85
Total UFA	551.33	614.09	38.12	569.19	596.24	38.12	584.18	581.24	38.12
Total MUFA	202.20	215.91	19.41	199.23	218.89	19.41	229.80	188.32	19.41
Total PUFA	349.13	398.18	22.76	369.96	377.35	22.76	354.38	392.93	22.76

^xWDGS = Wet distillers grains with solubles

^ySFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids

^{ab}Means within the same treatment and the same row with different superscripts are different ($P \leq 0.05$)

Table 8. The influence of grass-type, supplementation, and finishing diet on the *P*-values of fatty acids in the neutral lipid layers in *B. femoris* steaks

Neutral Lipids, mg/100 g of meat							GrassX
	Grass ^x	Supp	GrassX Supp	Diet	GrassX Diet	SuppX Diet	SuppX Diet
C10:0	0.37	0.52	0.13	0.91	0.76	0.71	0.008
C12:0	0.25	0.26	0.84	0.90	0.28	0.39	<0.0001
C14:0	0.63	0.92	0.64	0.36	0.20	0.38	0.02
C14:1	0.09	0.57	0.72	0.21	0.15	0.47	0.04
C15:0	0.22	0.80	0.56	0.09	0.30	0.46	0.05
C16:0	0.76	0.88	0.39	0.13	0.15	0.32	0.02
C16:1	0.26	0.85	0.55	0.06	0.08	0.48	0.06
C17:0	0.08	0.22	0.70	0.13	0.39	0.47	0.04
C17:1	0.05	0.14	0.97	0.02	0.23	0.33	0.05
C18:0	0.60	0.41	0.60	0.49	0.65	0.56	0.03
C18:1t	0.007	0.48	0.65	0.19	0.50	0.64	0.28
C18:1	0.27	0.60	0.65	0.24	0.29	0.54	0.03
C18:1v	0.99	0.82	0.66	0.62	0.14	0.17	0.28
C18:2TT	0.05	0.56	0.91	0.49	0.80	0.47	0.04
C18:2	0.36	0.37	0.50	0.09	0.65	0.94	0.26
C20:0	0.58	0.07	0.58	0.64	0.81	0.64	0.81
C18:3	0.63	0.99	0.36	0.98	0.52	0.50	0.45
C20:1	0.05	0.14	0.40	0.50	0.72	0.56	0.10
C22:0	0.93	0.77	0.38	0.14	0.90	0.35	0.06
Others	0.14	0.35	0.40	0.58	0.65	0.66	0.12
Total SFA ^y	0.65	0.91	0.47	0.20	0.24	0.38	0.02
Total UFA	0.42	0.70	0.63	0.29	0.26	0.55	0.03
Total MUFA	0.38	0.67	0.63	0.25	0.25	0.54	0.03
Total PUFA	0.30	0.47	0.65	0.13	0.62	0.92	0.20

^xGrass = Grass type, Supp = Supplementation, Diet = Finishing diet

^ySFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids

Table 9. The influence of grass-type, supplementation, and finishing diet on the *P*-values of fatty acids in the phospholipid layers in *B. femoris* steaks

Phospholipids, mg/100 g of meat			GrassX		SuppX		GrassX
	Grass ^x	Supp	Supp	Diet	Diet	Diet	SuppX Diet
C12:0	0.53	0.40	0.08	0.10	0.93	0.56	0.30
C13:0	0.46	0.10	0.12	0.92	0.15	0.38	0.11
C14:0	0.55	0.64	0.48	0.31	0.15	0.91	0.93
C14:1	0.99	0.07	0.50	0.08	0.05	0.19	0.99
C15:0	0.56	0.68	0.32	0.82	0.51	0.63	0.87
C16:0	0.62	0.39	0.13	0.41	0.25	0.95	0.24
C16:1	0.63	0.78	0.50	0.003	0.62	0.09	0.12
C17:0	0.74	0.50	0.29	0.18	0.82	0.49	0.59
C17:1	0.46	0.51	0.35	0.46	0.91	0.62	0.35
C18:0	0.90	0.36	0.58	0.34	0.57	0.81	0.74
C18:1t	0.82	0.75	0.15	0.79	0.71	0.52	0.25
C18:1	0.54	0.57	0.21	0.003	0.22	0.71	0.18
C18:1v	0.36	0.20	0.41	0.03	0.46	0.25	0.18
C18:2	0.06	0.15	0.54	0.0007	0.23	0.89	0.17
C20:0	0.50	0.40	0.48	0.20	0.12	0.09	0.85
C18:3	0.06	0.13	0.10	1.00	0.76	0.15	0.13
C22:0	0.19	0.14	0.63	0.63	0.58	0.38	0.16
C20:4	0.69	0.04	0.66	0.66	0.70	0.65	0.34
C20:5	0.37	0.0006	0.01	0.13	0.96	0.87	0.32
C24:1	0.35	0.006	0.62	0.87	0.59	0.72	0.46
C22:6	0.69	0.41	0.33	0.03	0.54	0.28	0.47
Others	0.16	0.18	0.25	0.02	0.65	0.67	0.10
Total SFA ^y	0.47	0.11	0.63	0.95	0.20	0.88	0.29
Total UFA	0.43	0.14	0.53	0.90	0.42	0.60	0.08
Total MUFA	0.95	0.21	0.43	0.02	0.65	0.36	0.06
Total PUFA	0.29	0.19	0.69	0.09	0.40	0.87	0.19

^xGrass = Grass type, Supp = Supplementation, Diet = Finishing diet

^ySFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids

Table 10. The effect of grass type, supplementation, and finishing diet on the LS means of fatty acid concentrations within in warm-season grass type in the neutral lipid layer when separated by grass type for *B. femoris* steaks

Warm-Season Grass Neutral Lipids (mg/100 g of meat)	No Supplementation		Supplementation		SEM
	Corn	WDGS ^x	Corn	WDGS	
C10:0	1.55 ^a	0.38 ^{ab}	0.23 ^b	1.12 ^{ab}	0.46
C12:0	3.01 ^a	0.45 ^b	1.45 ^b	3.05 ^a	0.55
C14:0	89.37 ^a	55.06 ^b	65.32 ^{ab}	71.93 ^{ab}	8.82
C14:1	33.30 ^a	19.48 ^b	23.95 ^{ab}	24.28 ^{ab}	3.47
C15:0	17.31 ^a	10.91 ^b	13.15 ^{ab}	13.14 ^{ab}	1.63
C16:0	867.17 ^a	517.94 ^b	628.56 ^b	644.16 ^b	77.41
C16:1	146.67	82.95	112.36	101.3	14.09
C17:0	48.27 ^a	30.97 ^b	36.07 ^{ab}	37.62 ^{ab}	4.61
C17:1	53.33 ^a	30.94 ^b	37.92 ^b	35.96 ^b	4.77
C18:0	348.04 ^a	240.94 ^b	280.02 ^{ab}	325.43 ^{ab}	37.89
C18:1t	49.07	49.33	46.55	55.51	10.07
C18:1	1558.94 ^a	1060.05 ^b	1183.94 ^{ab}	1245.22 ^{ab}	136.71
C18:1v	43.88	28.99	33.45	35.02	4.66
C18:2TT	3.77 ^a	2.63 ^a	2.19 ^a	3.75 ^a	0.67
C18:2	39.57	40.44	35.77	46.26	6.30
C20:0	0.00	0.00	0.77	0.42	0.35
C18:3	3.94	2.47	3.60	4.12	0.98
C20:1	20.00	13.64	16.93	19.26	2.74
C22:0	0.38	0.52	0.44	1.29	0.50
Others	33.16	28.02	26.16	26.50	3.33
Total SFA ^y	1375.10 ^a	857.17 ^b	1026.02 ^{ab}	1098.15 ^{ab}	125.99
Total UFA	1952.46 ^a	1330.93 ^b	1496.66 ^{ab}	1570.67 ^{ab}	174.3
Total MUFA	1905.19 ^a	1285.39 ^b	1455.10 ^{ab}	1516.54 ^{ab}	168.35
Total PUFA	47.27	45.55	41.56	54.13	7.27

^xWDGS = Wet distillers grains with solubles

^ySFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids

^{ab}Means within the same row with different superscripts are different ($P \leq 0.05$)

Table 11. The effect of grass type, supplementation, and finishing diet on the LS means of fatty acid concentrations within cool-season grass type in the neutral lipid layer when separated by grass type for *B. femoris* steaks

Cool-Season Grass Neutral Lipids (mg/100 g of meat)	No Supplementation		Supplementation		SEM
	Corn	WDGS ^x	Corn	WDGS	
C10:0	0.34 ^b	1.18 ^{ab}	1.83 ^a	1.11 ^{ab}	0.46
C12:0	0.47 ^b	2.25 ^a	2.23 ^a	1.21 ^{ab}	0.55
C14:0	60.45 ^a	72.13 ^a	72.11 ^a	64.95 ^a	8.82
C14:1	19.21 ^a	23.24 ^a	22.20 ^a	19.23 ^a	3.47
C15:0	11.63 ^a	12.33 ^a	13.48 ^a	11.26 ^a	1.63
C16:0	593.70 ^a	662.40 ^a	706.67 ^a	628.92 ^a	77.41
C16:1	91.88	102.87	107.99	94.99	14.09
C17:0	33.89 ^a	36.37 ^a	33.24 ^a	26.37 ^a	4.61
C17:1	35.36 ^a	34.97 ^a	33.89 ^a	26.50 ^a	4.77
C18:0	246.91 ^a	285.51 ^a	328.20 ^a	276.96 ^a	37.89
C18:1t	53.37	78.85	72.86	76.08	10.07
C18:1	1082.62 ^a	1230.83 ^a	1238.18 ^a	1063.27 ^a	136.71
C18:1v	33.76	36.11	33.46	37.79	4.66
C18:2TT	3.64 ^a	4.76 ^a	3.97 ^a	3.76 ^a	0.67
C18:2	33.50	48.67	45.99	50.26	6.30
C20:0	0.00	0.00	0.38	0.26	0.35
C18:3	3.94	4.43	3.36	3.74	0.98
C20:1	18.46	19.91	25.15	22.41	2.74
C22:0	0.00	1.59	0.81	0.37	0.50
Others	22.77	27.37	27.38	22.28	3.33
Total SFA ^y	947.37 ^a	1073.76 ^a	1158.95 ^a	1011.42 ^a	125.99
Total UFA	1375.74 ^a	1584.63 ^a	1587.05 ^a	1398.04 ^a	174.30
Total MUFA	1334.67 ^a	1526.77 ^a	1533.73 ^a	1340.28 ^a	168.35
Total PUFA	41.07	57.85	53.32	57.76	7.27

^xWDGS = Wet distillers grains with solubles

^ySFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids

^{ab}Means within the same row with different superscripts are different ($P \leq 0.05$)

Table 12. The effect of grass type, supplementation, and finishing diet on the LS means scores of fatty acids in the neutral lipid layers in *B. femoris* steaks

Neutral Lipids, mg/100 g of meat	Grass Type		SEM	Finishing Diets		
	Warm- season	Cool- season		Corn	WDGS ^x	SEM
C10:0	0.82	1.12	0.23	0.99	0.95	0.23
C12:0	1.99	1.54	0.28	1.79	1.74	0.28
C14:0	70.42	67.41	4.41	71.81	66.02	4.41
C14:1	25.25	20.97	1.74	24.67	21.56	1.74
C15:0	13.63	12.18	0.82	13.89	11.91	0.82
C16:0	664.46	647.92	38.71	699.02	613.35	38.71
C16:1	110.82	99.43	7.05	114.72	95.53	7.05
C17:0	38.23	32.47	2.31	37.87	32.83	2.31
C17:1	39.54 ^a	32.68 ^b	2.39	40.13 ^a	32.09 ^b	2.39
C18:0	298.60	284.39	18.95	300.79	282.21	18.95
C18:1t	50.12 ^b	70.29 ^a	5.03	55.46	64.95	5.03
C18:1	1262.04	1153.72	68.35	1265.92	1149.84	68.35
C18:1v	35.34	35.28	2.33	36.14	34.48	2.33
C18:2TT	3.09 ^b	4.03 ^a	0.34	3.39	3.72	0.34
C18:2	40.51	44.61	3.15	38.71	46.41	3.15
C20:0	0.30	0.16	0.18	0.29	0.17	0.18
C18:3	3.53	3.86	0.49	3.71	3.69	0.49
C20:1	17.46 ^b	21.48 ^a	1.37	20.13	18.80	1.37
C22:0	0.66	0.69	0.25	0.41	0.94	0.25
Others	28.46	24.95	1.67	27.37	26.04	1.67
Total SFA ^y	1089.11	1047.88	63.00	1126.86	1010.13	63.00
Total UFA	1587.68	1486.36	87.15	1602.98	1471.07	87.15
Total MUFA	1540.56	1433.86	84.17	1557.17	1417.24	84.17
Total PUFA	47.13	52.50	3.64	45.81	53.82	3.64

^xWDGS = Wet distillers grains with solubles

^ySFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids

^{ab}Means within the same treatment and the same row with different superscripts are different ($P \leq 0.05$)

Table 13. The effect of grass type, supplementation, and finishing diet on the LS means scores of fatty acids in the phospholipid layers in *B. femoris* steaks

Phospholipids, mg/100 g of meat	Supplementation			Finishing Diets		
	No	Yes	SEM	Corn	WDGS ^x	SEM
C12:0	1.31	0.75	0.47	1.59	0.48	0.47
C13:0	3.09	5.31	0.92	4.27	4.13	0.92
C14:0	5.10	5.78	1.02	6.18	4.70	1.02
C14:1	3.91	6.81	1.09	3.96	6.76	1.09
C15:0	29.32	31.87	4.31	29.90	31.29	4.31
C16:0	136.37	147.88	9.42	147.66	136.59	9.42
C16:1	9.46	9.85	1.00	11.93 ^a	7.38 ^b	1.00
C17:0	22.34	25.89	3.70	20.59	27.64	3.70
C17:1	6.18	7.69	1.60	7.79	6.09	1.60
C18:0	88.87	95.76	5.27	88.70	95.93	5.27
C18:1t	43.28	45.44	4.81	43.45	45.26	4.81
C18:1	91.46	97.41	7.38	110.82 ^a	78.05 ^b	7.38
C18:1v	29.63	37.78	4.43	40.57 ^a	26.84 ^b	4.43
C18:2	224.93	250.70	12.40	205.67 ^b	269.96 ^a	12.40
C20:0	1.17	1.82	0.54	1.99	1.00	0.54
C18:3	7.66	6.05	0.73	6.86	6.85	0.73
C22:0	27.33	34.50	3.33	32.05	29.78	3.33
C20:4	159.90 ^b	185.43 ^a	8.71	175.42	169.90	8.71
C20:5	22.97 ^a	14.63 ^b	1.58	20.51	17.09	1.58
C24:1	12.97 ^b	16.32 ^a	0.82	14.55	14.74	0.82
C22:6	2.19	1.39	0.68	2.84 ^a	0.73 ^b	0.68
Others	43.28	38.57	2.46	45.33 ^a	36.52 ^b	2.46
Total SFA ^y	314.90	349.57	15.03	332.94	331.53	15.03
Total UFA	614.54	679.51	30.14	644.39	649.65	30.14
Total MUFA	196.89	221.30	13.40	233.07 ^a	185.12 ^b	13.40
Total PUFA	417.64	458.21	21.62	411.31	464.54	21.62

^xWDGS = Wet distillers grains with solubles^ySFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids^{ab}Means within the same treatment and the same row with different superscripts are different ($P \leq 0.05$)

Table 14. The effect of grass type and supplementation on the LS means of fatty acids in the phospholipid layers in *B. femoris* steaks

Phospholipids, mg/100 g of meat	Warm-season Grass		Cool-season Grass		SEM
	No Supplementation	Supplementation	No Supplementation	Supplementation	
C12:0	0.51	1.13	2.12	0.37	0.66
C13:0	3.64	3.78	2.54	6.83	1.31
C14:0	4.15	5.86	6.04	5.71	1.44
C14:1	4.44	6.29	3.37	7.32	1.54
C15:0	30.62	27.00	28.02	36.74	6.09
C16:0	122.73	154.81	150.01	140.96	13.32
C16:1	8.63	9.99	10.29	9.71	1.42
C17:0	26.02	23.98	18.67	27.80	5.23
C17:1	6.40	5.78	5.96	9.60	2.27
C18:0	87.25	98.33	90.50	93.19	7.46
C18:1t	39.11	51.21	47.45	39.67	6.80
C18:1	88.09	107.20	94.83	87.63	10.44
C18:1v	29.35	32.30	29.91	43.26	6.27
C18:2	202.41	239.15	247.44	262.26	17.54
C20:0	1.71	1.81	0.64	1.83	0.76
C18:3	5.76	5.92	9.56	6.18	1.04
C22:0	25.34	30.22	29.32	38.78	4.71
C20:4	165.10	185.19	154.70	185.67	12.32
C20:5	19.01 ^b	16.56 ^{bc}	26.93 ^a	12.70 ^c	2.24
C24:1	12.72	15.49	13.22	17.15	1.15
C22:6	2.86	1.10	1.52	1.67	0.96
Others	38.72	38.10	47.85	39.04	3.48
Total SFA ^y	301.96	346.92	327.84	352.22	21.26
Total UFA	583.89	676.19	645.18	682.82	42.63
Total MUFA	188.75	228.26	205.03	214.34	18.96
Total PUFA	395.14	447.92	440.15	468.49	30.58

^xWDGS = Wet distillers grains with solubles

^ySFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids

^{abc}Means within the same treatment combination and the same row with different superscripts are different ($P \leq 0.05$)

Table 15. The effects of the interaction between grass type and finishing diet on the LS means of fatty acids in the phospholipid layers in *B. femoris* steaks

Phospholipids, mg/100 g of meat	Warm-season Grass		Cool-season Grass		SEM
	Corn	WDGS ^x	Corn	WDGS	
C12:0	1.41	0.24	1.77	0.72	0.66
C13:0	4.75	2.67	3.79	5.59	1.31
C14:0	6.81	3.20	5.55	6.20	1.44
C14:1	5.51 ^{ab}	5.22 ^{ab}	2.41 ^b	8.29 ^a	1.54
C15:0	30.15	27.47	29.66	35.10	6.09
C16:0	152.10	125.43	143.21	147.75	13.32
C16:1	11.94	6.68	11.93	8.07	1.42
C17:0	22.07	27.93	19.10	27.36	5.23
C17:1	6.81	5.38	8.76	6.80	2.27
C18:0	91.33	94.25	86.08	97.61	7.46
C18:1t	42.98	47.34	43.93	43.19	6.80
C18:1	120.49	74.80	101.15	81.30	10.44
C18:1v	35.36	26.29	45.78	27.39	6.27
C18:2	199.33	242.23	212.01	297.69	17.54
C20:0	2.86	0.65	1.12	1.34	0.76
C18:3	6.00	5.68	7.71	8.03	1.04
C22:0	27.60	27.97	36.51	31.58	4.71
C20:4	180.33	169.96	170.52	169.84	12.32
C20:5	19.55	16.02	21.48	18.16	2.24
C24:1	13.70	14.51	15.40	14.97	1.15
C22:6	2.74	1.22	2.95	0.25	0.96
Others	42.01	34.81	48.65	38.23	3.48
Total SFA ^y	339.07	309.80	326.80	353.26	21.26
Total UFA	644.75	615.33	644.03	683.97	42.63
Total MUFA	236.79	180.23	229.36	190.01	18.96
Total PUFA	407.96	435.10	414.67	493.97	30.58

^xWDGS = Wet distillers grains with solubles

^ySFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids

^{abc}Means within the same treatment combination and the same row with different superscripts are different ($P \leq 0.05$)

Running Title: Diet and aging on beef flavor

**The role of post-weaning forage, energy supplementation, finishing
diets, and aging on beef flavor and acceptability¹**

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Abstract

The objective of this study was to determine diet and aging combinations that generate desirable beef flavor. Crossbred steers ($n = 64$) were grazed on warm or cool-season grasses, without or with energy supplementation from wet distillers grains with solubles (WDGS), and were finished on corn or 35% WDGS. Six carcasses from each treatment ($n = 48$) that graded USDA Choice or Select were identified and *Longissimus dorsi* and *Biceps femoris* muscles from each side of each carcass were collected and aged under vacuum 7 or 28 d. Steaks displayed under retail conditions for 7 d were used for consumer taste panels in Olathe, KS and Houston, TX. Panelists ($n=120$ per city) rated cooked steaks for overall acceptability, overall flavor acceptability, and beefy flavor and intensity (1 = extremely dislike or extremely bland and 9 = extremely like or extremely intense). When supplementing on pasture with WDGS, finishing on corn caused higher ($P \leq 0.04$) scores for overall like, overall flavor like, and beefy flavor like of *L. dorsi* steaks than finishing on WDGS (6.52 vs. 5.98, 6.34 vs. 5.84, and 6.43 vs. 5.91, respectively). Although beefy flavor intensity scores of *L. dorsi* steaks were influenced by an interaction between grass type, supplementation, and aging period ($P = 0.05$), no discernible pattern existed. For *B. femoris* steaks, the highest (most desirable) ratings for overall like, overall flavor like, beefy flavor like, and beefy flavor intensity scores occurred with cool season grasses and shorter aging times ($P \leq 0.05$). Lexicon scores for *L. dorsi* steaks had 2 significant ($P \leq 0.04$) three-way interactions; grass type, finishing diet, and aging period for fat scores and supplementation, diet, and aging period for bloody scores. Within the 7 d aging period, finishing on WDGS caused low scores for fat

flavor and high scores for bloody flavor. The highest intensities of liver flavor ($P \leq 0.04$) occurred in beef from cattle grazed on warm-season grass without supplement or aged for 28 d. Warmed-over flavors were four times worse when cattle were finished on WDGS instead of corn ($P = 0.002$). For *B. femoris* steaks, the least desirable flavor notes were associated with warm-season grasses (liver, bloody, metallic, and sour), most of which were improved with supplementation. Aging increased painty, sour milk, bitter, and (when corn was fed) fishy flavors. These data suggest that beef flavor is best established with cool season grasses, feeding WDGS as an energy supplement during grazing and finishing on corn. Shorter aging periods appear to reduce off-flavor development.

Keywords: Aging, beef, diet, flavor, forages, supplementation

Introduction

Flavor is often the most important attribute people rely on for a pleasurable beef eating experience. Umberger et al. (2002) found that consumers were willing to pay considerably more for cuts they had identified as having a desirable flavor. The type of forage on which an animal is grazed post-weaning can influence the flavor profile of the meat. When Larick et al. (1987) fed cattle fescue grass, the beef had a much more prevalent grassy flavor. Jenschke et al. (2008) also reported that feeding low levels of alfalfa caused a higher prevalence of bloody flavor notes than feeding high levels of alfalfa, high levels of corn stalks, and low and high levels of corn silage.

More flavor changes have been found with finishing cattle on distillers grains with solubles (DGS). Taste panelists in a study conducted by Gill et al. (2008) perceived no difference in beef flavor due to diets, however, consumers were more displeased with

samples from beef fed DGS than samples from beef fed steam-flaked corn. Jenscke et al. (2007) fed cattle diets with an inclusion of 0, 10, 20, 30, 40, or 50% WDGS, for 125 days and found no differences in off-flavor intensity, liver-like flavor, or metallic flavor between samples. In the previous study, samples were stored under a constant vacuum and were never allowed to oxidize. In contrast, Depenbusch et al. (2009) found that beef from cattle fed dried DGS at higher rates had greater beef flavor intensity.

As meat ages, lipid oxidation creates unique flavors. When Smith et al. (1978) dry aged meat up to 11 d, flavor desirability was significantly increased. Campo et al. (1999) also found that flavor intensity increased as the length of wet aging increased up to 10 d. Seneratne et al. (2010) showed a higher degree of liver and/or off-flavor in meat from cattle fed wet distillers grains with solubles as opposed to corn. These differences were only found after the meat had been aged in a retail display for 7 d.

This research was conducted to investigate how beef flavor is affected in two different muscles from cattle fed two different forages post-weaning, with or without supplemental energy, finished on either a corn or wet DGS (WDGS) diet, and aged for 7 or 28 d.

Materials and Methods

Diets

All protocols performed in this study were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. Cattle were fed in a 2x2x2 factorial design. Crossbred steers (n = 64) were backgrounded on either warm-season grasses (i.e. bluestem and switch grass) at the Barta Ranch in Western Nebraska

or on cool-season (i.e. brome and bluegrass) pastures in Ithaca, NE for 177 d, shortly after weaning. Within each pasture, half of the cattle were supplemented with 0.6 kg WDGS/kg body weight/ day for energy. At the end of the grazing period, all cattle were transported to the University of Nebraska-Lincoln's research feedlot in Ithaca, NE.

While in the feedlot, half of each pasture and supplementation treatments were finished on an all-corn diet while the other half were fed corn with WDGS at a 35% inclusion rate (DM basis). Cattle were on feed for 119 days and fed to an average live weight of 1,427 lbs.

Harvest

At the end of the feedlot period, cattle were transported and harvested at the Greater Omaha Packing (Omaha, NE). Forty-eight carcasses grading either USDA Choice (n = 43) or USDA Select (n = 5), 6 from each treatment combination, were selected. Strip loins (*Longissimus dorsi*; IMPS #180, NAMP, 2007) and bottom round flats (*Biceps femoris*; IMPS #171B, NAMP, 2007) were collected from each side of the carcass. Only ten *L. dorsi* muscles were collected from the cool-season grass, supplementation provided, and finished on WDGS treatment because two *L. dorsi* muscles (one from each side) were lost within Greater Omaha Packing Plant. Subprimals from the left side of the carcass were aged under vacuum for 7 d while subprimals from the right side were aged under vacuum 28 d at 2°C. Upon fabrication, 5 steaks were cut from each subprimal.

Sample collection

Three steaks were cut 2.54 cm thick, placed on Styrofoam trays, wrapped with PVC overwrap film, and placed under simulated retail display for 7 d. Two of the steaks were used for consumer panels and the third steak was used for a beef lexicon panel. *L. dorsi* steaks were packaged as two steaks per tray and *B. femoris* steaks were one per tray. At the end of retail display, steaks were vacuumed packaged and frozen until further use.

Retail display

All of the steaks were displayed on a table in a 2°C cooler and were constantly exposed to warm white fluorescence lighting (PHILIPS F32T8/TL741 ALTO 700 Series, 32 WATT B7, Royal Philips Electronics, Amsterdam, Netherlands) at 1000 to 1800 lux in order to simulate retail display conditions. Every day, packages were randomly relocated to minimize any effects due to location. After 7 d, steaks were vacuumed packaged and frozen until further analysis.

Consumer Panel

All consumer and lexicon panels were approved by the Institutional Review Board and all panelists signed a consent form. Consumer panels were conducted in Houston, Texas and Olathe, Kansas (n = 120 per location). Consumers were recruited using existing consumer data banks and random phone solicitation. Consumers were selected who eat beef at least three times per week, range in age from 21 to 65, with an approximately equal balance of males and females, and a range in income.

In each city, consumer panels were conducted over two days, with the first day evaluating *Longissimus dorsi* steaks and the second day evaluating *Biceps femoris* steaks. Different consumers evaluated each muscle type. Steaks from each animal were evaluated at both locations. Panels were conducted with three sessions per day and 20 consumers per session. Five consumers evaluated each steak. Treatment order was randomized and allocated to consumers using an incomplete block design. Each consumer evaluated eight steaks in a session.

Steaks were cooked on a Hamilton Beach Health Smart grill (model 31605A, Hamilton Beach/ Proctor-Silex, Inc., Southern Pines, NC) to an internal temperature of 70°C. Consumers evaluated each sample using 9-point hedonic (1=dislike extremely, 9=like extremely) and intensity scales (1=none or extremely bland, 9=extremely intense) for overall like, overall flavor like, beefy flavor like and intensity, and grilled flavor like and intensity.

Beef Flavor Lexicon

An expert, trained descriptive attribute sensory panel with over 23 cumulative years of experience in evaluating beef flavor and aromas was used. This panel was one of the three panels used to validate the Beef Lexicon at Texas A&M University (Philips et al., 2010; Miller, 2010). The panel underwent ballot development, training and validation sessions to assure consistent rating and identification of individual aroma and flavor attributes. Attributes were classified as major and minor notes. This provides a standardized, defined reference guide for determining and measuring aroma and flavor in beef.

During training and testing, steaks were cooked the same way as described for consumer panels. Aromas and flavor aromatics were evaluated using the Spectrum® Universal 16-point scale where 0 = none and 15 = extremely intense (Meilgaard et al., 2007). Traits evaluated were browned, bloody, fat, metal, liver, green hay, umami, overly sweet, sweet, sour, salty, bitter, sour aroma, barnyard, burnt, heated oil, chemical, apricot, asparagus, cumin, floral, beet, chocolate, green grass, musty, medicinal, petroleum, smoked/charred, smoked wood, spoiled, dairy, buttery, cooked milk, sour milk, refrigerator stale, warmed over, soapy, painty, fishy, and cardboardy.

Statistical Analysis

All data were analyzed as a 2x2x2x2 factorial arrangement split plot design within a randomized complete design using the Mixed procedure in SAS (Version 9.2, SAS Institute Inc., Cary, NC, 2006). The experimental unit was the individual animal and the model included grass, supplementation, finishing diet, and age as fixed effects. The least square means procedure with the probability of difference (pdiff) option was used for mean separation; with significance determined at $P \leq 0.05$ levels. Whenever there was a three- or four-way interaction, the LSmeans were reanalyzed using the GLIMMIX procedure with the slicediff option in order to more accurately study differences.

Results and Discussion

Consumer Panel

L. dorsi Steaks

Overall like, flavor like, beefy flavor like, and beefy flavor intensity were all influenced by finishing diet (Table 1). The first three of these traits had an interaction

between diet and supplementation ($P \leq 0.04$) while beefy flavor intensity was influenced by the interaction of finishing diet and aging period ($P = 0.05$). When cattle had been supplemented with WDGS while grazing, finishing on WDGS caused a significant decline ($P \leq 0.04$) in scores of overall like, flavor like, and beefy flavor like. Beefy flavor intensity was significantly ($P = 0.04$) affected by a three-way interaction between grass-type, supplementation, and aging period. When the means were separated out by aging period (Figure 1) there were no differences between the means. This would show that it is the aging period that is causing the interaction to be significant. Shand et al. (1998) also showed no differences in beefy flavor intensity, flavor desirability, and off flavor intensity due to a distillers grain supplementation.

Also, when supplementing cattle, finishing on corn caused higher ($P \leq 0.04$) overall like, overall flavor like, and beefy flavor like scores than if they were finished on WDGS (6.52 vs. 5.98, 6.34 vs. 5.84, and 6.43 vs. 5.91, respectively, Table 2), suggesting that long-term exposure to WDGS may be detrimental to flavor. In contrast, Gill et al. (2008) found that when cattle were finished on steam flaked corn and various types of distillers grains, the panelists could not determine a difference in taste between the two samples. However, Gill et al. (2008) also found that the number of panelists overall displeased with the flavor was higher with samples from cattle fed distillers grains. Miller et al. (1996) and Busboom et al. (2000) also reported that finishing diet had no effect on flavor intensity, flavor desirability, and off-flavors in *L. dorsi* steaks when comparing corn to barley.

When cattle were finished on WDGS, aging the beef to 28 d caused the beefy intensity to decrease ($P = 0.05$) when compared to aging for only 7 d (5.67 vs 6.06, Table 3). In addition, at 28 d age, finishing on WDGS decreased ($P = 0.05$) beefy intensity from 6.05 to a 5.67 when compared to finishing on corn. For Depenbusch et al. (2009), beefy flavor intensity was strongest in beef from cattle fed either 45 or 60% dried DGS and was the least for cattle fed no dried DGS. Their findings differ from the results in this study perhaps because the meat in the Depenbusch et al. study was only aged for 14 d. The difference could be due to the much shorter aging period, especially since this study shows that aging period has an influence on flavor. Neither grill flavor like nor grill flavor intensity scores were affected by any combinations of feeding regimens and aging.

B. femoris Steaks

All consumer panel scores, except for grill flavor like and grill flavor intensity, were significantly ($P \leq 0.05$) influenced by the four-way interaction of grass type, supplementation, finishing diet, and aging (Table 1). In order to better analyze the data, means were separated by aging period (Table 4). Within the 7 d aging period, grazing on warm-season grasses without supplementation and finishing on WDGS caused the greatest amount of differences. That diet combination caused lower overall like, overall flavor like, beefy flavor like, beefy flavor intensity, grill flavor like and grill flavor intensity scores (although not all were significantly different) than all other diet regimen combinations. In contrast, Jeremiah et al. (1998) found that finishing diet did not affect overall flavor desirability and intensity. Their study only looked at finishing diet and all

animals came from various background diets, which could account for the difference. In addition, when supplementation was given, not only were the finishing diets no longer different from each other, but they were not different from any of the dietary combination within cool-season grass grazing either. This would show that the addition of supplementation is able to deter any differences in consumer scores due to grass type.

When aging for 28 d, grazing on warm-season grasses with supplementation and finishing on corn caused higher ($P \leq 0.05$) overall like (6.28), overall flavor like (6.38), beefy flavor like (6.50), and beefy flavor intensity (6.36) scores than all other supplementation and finishing diet combinations within warm-season grass grazing. The only exception was for beefy flavor intensity scores, where within warm-season grass grazing, supplementing and finishing on corn was not significantly ($P < 0.05$) different from not supplementing and finishing on WDGS (6.36 vs. 5.73). Most scores from within cool-season grass grazing were not different from the scores within warm-season grass grazing. Perhaps the longer aging period was able to dissipate the negative influences that warm-season grass grazing seemed to have on the consumer scores that were seen when the meat was only aged for 7 d. However, the differences in scores between supplementation and finishing diet were only within warm-season grass grazing, so its effects are not completely dismissed. None of the diet regimen and aging combinations influenced grill flavor like or grill flavor intensity scores ($P > 0.05$).

*Lexicon Scores**L. dorsi* Steaks

Grass type appeared to be the main component in flavor note development. Overall, grazing on warm-season grass caused higher levels of undesirable flavor notes, such as bloody, liver, barnyard, and burnt. Finishing diets also altered flavors notes. Finishing on WDGS not only increased the prevalence of warmed over flavors, but it also decreased sweet flavors which may be considered a desirable flavor. When aging and supplementation were taken into effect, differences became more prevalent. The highest intensities of liver flavor occurred in beef from cattle grazed on warm-season grass without supplement or aged for 28 d. Warmed-over flavors were four times worse when cattle were finished on WDGS instead of corn

Table 5 shows the *P*-values for all dietary and aging period interactions for lexicon flavor notes in *L. dorsi* steaks. At 7 d aging, the prevalence of fat flavor was weaker ($P = 0.02$) when cattle were grazed on cool-season grasses and finished on WDGS than when they were grazed on cool-season grasses and finished on corn (1.49 vs. 1.76) and grazed on warm-season grasses and finished on WDGS (1.49 vs. 1.77, Figure 2). Miller et al. (1996) found no differences in cooked beef flavor due to finishing diet, but they did not have varying grass types and aging periods in their study. Conversely, when the meat was aged for 28 d, meat from cattle grazed on warm-season grasses had a stronger fat flavor when finished on corn instead of WDGS (1.84 vs. 1.59).

For bloody flavor, within 7 d aged product, not supplementing and finishing on WDGS caused the highest scores (1.85, $P = 0.04$) than all other supplementation and

finishing diet combination (Figure 3). Within the 28 d aging periods, there were no differences in bloody flavor scores between all supplementation and finishing diet combinations. It would appear that the longer aging period is able to dissipate differences caused supplementation and finishing diet.

The intensity of browned flavor ($P = 0.04$) was higher when cattle were grazed on cool-season grasses and the steaks were aged for 28 d instead of 7 d, 1.95 vs. 1.65 (Table 6). Metal and livery flavors were also influenced by a grass type and aging interaction, but in a much different manner. For metal flavor, grazing on a cool-season grass and aging for 28 d caused the beef to have the lowest intensity scores (1.62) compared to all other grass type and aging period combinations except for grazing on a warm-season grass and only aging 7 d ($P = 0.02$). Grazing on a warm-season grass and aging for 28 d caused the steaks to have a significantly ($P = 0.04$) higher liver flavor (0.28) than all other grass type and aging period combinations (Table 6). Not supplementing and aging *L. dorsi* steaks 28 d caused higher intensities of cardboardy flavor ($P = 0.03$) when compared to both aging for 7 d (0.23 vs. 0.10) and supplementing and aging 28 d (0.23 vs. 0.11, Table 6). In all instances, aging tended to be the main influence on flavor notes.

Not supplementing while grazing on warm-season grasses caused the highest liver flavor scores (0.30, $P = 0.03$) compared to all other grass type and supplementation combinations (Table 7). Grazing on warm-season grass and aging to 28 d also caused higher liver scores. Clearly grass type is a key factor in the development of liver flavor in beef. Similarly, not supplementing while grazing on cool-season grasses created the lowest barnyard flavor scores (0.00, $P = 0.05$) compared to all other grass type and

supplementation combinations. Supplementing while grazing on cool-season grasses also caused the lowest burnt flavor scores (0.04, $P = 0.05$) compared to all other grass type and supplementation combinations except for when cattle were not supplemented while grazing warm-season grasses, which was not different.

Finishing diet, in combination with grass type, had the most influence over several *L. dorsi* steak flavors. When finishing cattle on an all-corn diet, grazing them on a warm-season grass first instead of a cool-season grass significantly ($P = 0.02$) increased the metal flavor intensity (1.77 vs. 1.63, Table 8). However, when grazed on cool-season grasses, finishing on WDGS caused higher scores of metal flavor than finishing on corn (1.77 vs. 1.63). When not providing supplementation, finishing on corn instead of WDGS significantly increased ($P = 0.02$) soured milk intensity from a 0.00 to a 0.11 (Table 8). In contrast, when not supplementing, refrigerator stale scores were higher ($P = 0.04$) when cattle were finished on WDGS instead of corn (0.047 vs. 0.00).

When finishing diet alone is examined, finishing on corn significantly increases ($P = 0.04$) the sweet flavor intensity (1.07 vs. 0.94) but decreases ($P = 0.002$) the warmed over flavor (0.06 vs. 0.24, Table 9). This would show that corn tended to promote desirable flavors while dissipating undesirable flavors. Liver flavor was not influenced by finishing diet ($P = 0.56$). Kinman et al. (2011) also compared finishing on corn and WDGS and also reported no difference in liver flavor due to finishing diet. Providing supplementation caused higher smoked wood scores than not supplementing (0.02 vs. 0.00, Table 9). Larick et al. (1987) found that grassy flavor in *L. dorsi* steaks was influenced by grass type, with the highest scores caused by fescue grass. In this study

grass type, or any other dietary component, had no influence ($P > 0.05$) on green grass flavor.

B. femoris Steaks

For *B. femoris* steaks, the least desirable flavor notes were associated with warm season grasses (liver, bloody, metallic, sour, and salty), most of which were improved with supplementation. In fact, when supplementation was not provided, liver, sour, sour milk, and warmed over lexicon flavor notes were higher, regardless of grass type. Increasing aging from 7 d to 28 d also increased painty, sour milk, bitter, and (when corn was fed) fishy flavors.

For both chemical and fishy flavors there were significant ($P \leq 0.05$) three-way interactions (Table 10). In order to better understand the data, the means were separated by aging period. When aged for 7 d, not supplementing while grazing on cool-season grasses caused more intense ($P = 0.05$) chemical flavors than supplementing (0.22 vs. 0.07, Figure 4). Again showing that supplementation may help to dissipate undesirable flavors. When aging for 28 d, there were no differences between the different grass type and supplementation combinations. Similarly, for fishy flavors, within 7 d aging there were no differences between any grass type and finishing diet combinations (Figure 5). When aged 28 d, grazing on warm-season grass followed by a finishing diet of corn caused the highest ($P = 0.02$) fishy flavor scores (0.150) out of all the different grass type and finishing diet combinations, except for when cattle were grazed on cool-season grasses and finished on WDGS, which was not different.

Aging also was a heavy influence in several other flavor intensities.

Supplementing caused metal flavor intensities to be higher ($P = 0.03$) than not supplementing (2.08 vs. 1.88, Table 11) after aging 28 d. There was a significant interaction between finishing diet and aging period for metal flavor ($P = 0.05$), but when the means were separated and compared there were no differences between any of the combinations (Table 11). This could mean that it is aging that is driving the interaction and causing the differences. Busboom et al. (2000) found that finishing diet did alter the incidences of metallic flavors, but that was comparing corn to barley finishing diets. Jenschke et al. (2007) finished cattle on WDGS and reported that finishing diet had no effect on metallic flavor. Finishing on corn and aging for 7 d caused the beef to have the most intense ($P = 0.003$) bloody flavor (1.83) compared to all other finishing diet and aging period combinations except for when cattle were finished on WDGS and the steaks were aged for 28 d, which was not different.

In addition, finishing on a WDGS diet and aging the beef for 28 d caused the green hay flavor to be the most intense ($P = 0.02$) when compared to all other finishing diet and aging period combination. Aging 28 d significantly increased bitter ($P < 0.0001$), sour milk ($P = 0.05$), and painty ($P = 0.01$) flavor intensities when compared to the 7 d aging period (1.52 vs. 1.30, 0.13 vs. 0.05, and 0.07 vs. 0.01, respectively), regardless of any diet regimen (Table 13).

When examining diet regimen only, grazing on warm-season grass without supplementation caused the beef to have the most intense metal ($P = 0.03$) and liver ($P = 0.005$) scores (2.11 and 0.53, respectively) compared to all other grass type and

supplementation combinations (Table 12). This is similar to what was observed in the *L. dorsi* steaks as well. Especially with the addition of supplementation being able to dissipate the intensities of all the undesirable flavors. In contrast, cool-season grass grazing without supplementation significantly increased ($P = 0.03$) salty flavor intensities, which could be considered a desirable flavor, from 1.32 to 1.47 when compared to warm-season grass grazing without supplementation, but no other grass type and supplementation combination were significantly ($P \leq 0.05$) different from each other.

Bloody ($P = 0.02$), liver ($P = 0.0004$), and sour ($P = 0.02$) flavors were all highest when cattle were grazed on warm-season grasses instead of cool-season grasses (1.77 vs. 1.62, 0.38 vs. 0.17, and 1.53 vs. 1.37, respectively, Table 13). Larick and Turner (1990) also found that sour flavor intensity can change when cattle are fed different types of forages, however, bloodlike flavor was not affected by grass type. Their grass pastures were all in the same area so their grass was all grown on the same soil type and the grass type could have all been the same season type, which may explain why they saw no differences in bloodlike flavor due to grass. Jenschke et al. (2008) did see a change in bloody flavor notes in *R. femoris* muscles when roughage type was changed, which is similar to the findings in this study.

In contrast, cool-season grass grazing caused higher browned flavor scores ($P = 0.04$) than warm-season grass grazing (1.83 vs. 1.67). Not supplementing while grazing caused more intense liver ($P = 0.03$), sour ($P = 0.03$), sour milk ($P = 0.005$) and warmed over ($P = 0.05$) flavors than providing supplementation (0.34 vs. .021, 1.51 vs. 1.39, 0.15 vs. 0.04, and 0.65 vs. 0.48, respectively, Table 13).

In conclusion, finishing diet had the most influence on flavor scores in *L. dorsi* steaks with finishing on WDGS, especially after being supplemented with WDGS, causing declines in several scores. Conversely, grass type appeared to be more important for consumer panel scores in *B. femoris* steaks with warm-season grasses being the most detrimental. For both *L. dorsi* and *B. femoris* steaks, the least desirable flavor notes were associated with warm-season grasses most of which were improved with supplementation. Aging also changed the prevalence of specific flavor notes.

Overall, even though finishing diet had the most effect on consumer panel scores in *L. dorsi* steaks, grass type had a much larger effect on both consumer panel and lexicon flavor scores. Clearly, the type of grass cattle graze plays a vital role in both flavor development and consumer preference. Unfortunately warm-season grasses tended to create unfavorable scores. Luckily, the addition of supplementation was able to remove any differences in consumer panel scores as well as promote desirable flavors and lessen the intensities of undesirable flavors. Due to these results, it is highly recommended that if producers are grazing cattle on warm-season grasses that they supplement in order to remove any negative effects.

As previously stated, aging also played a key role in flavor development. For the most part, longer aging periods caused an increase in desirable flavor intensities and a decrease in undesirable flavor intensities. Due to these facts, a longer aging period of beef is recommended.

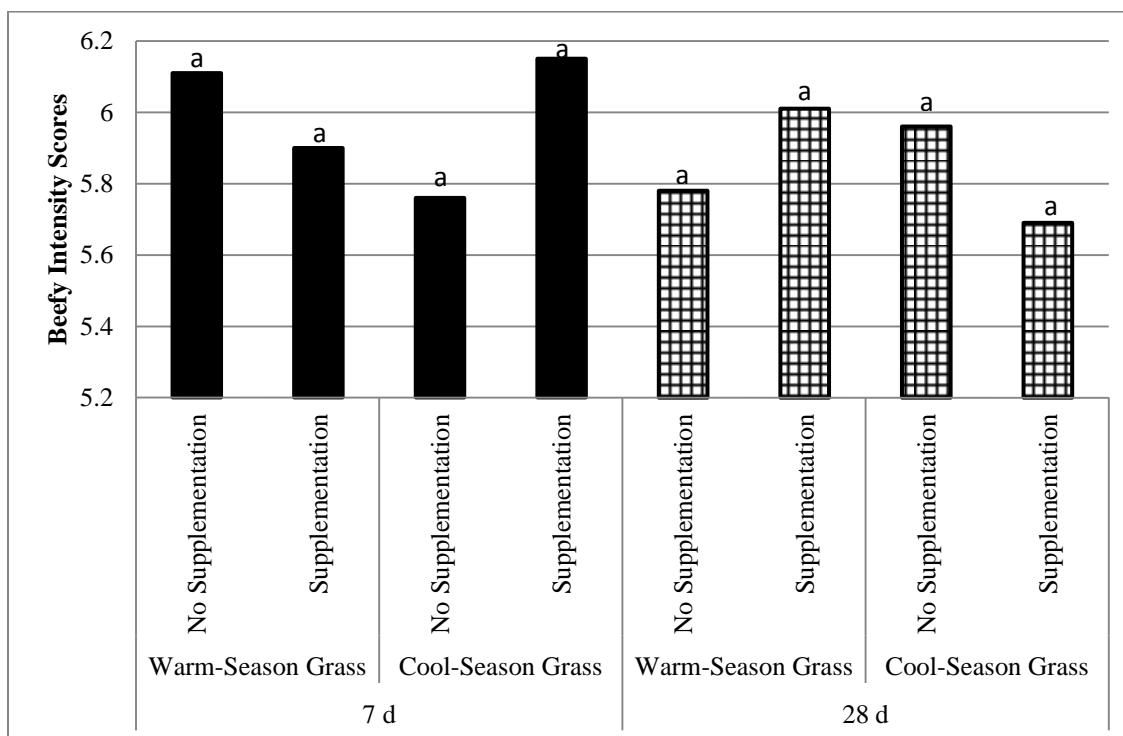
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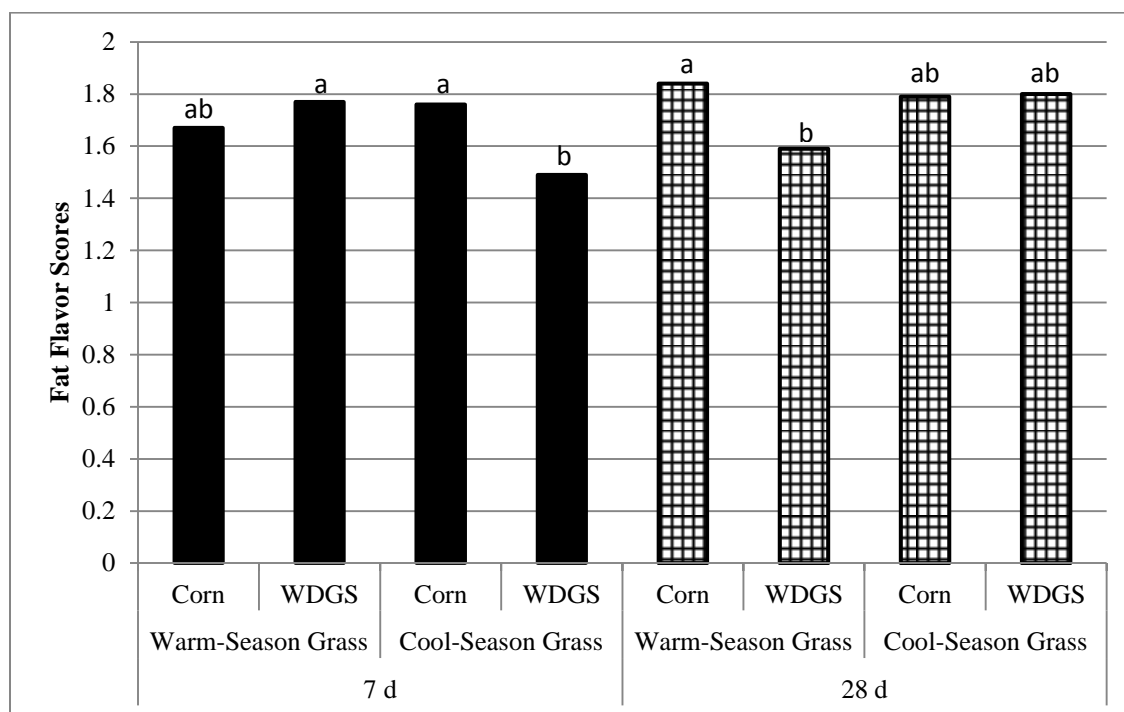
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Figures and Tables



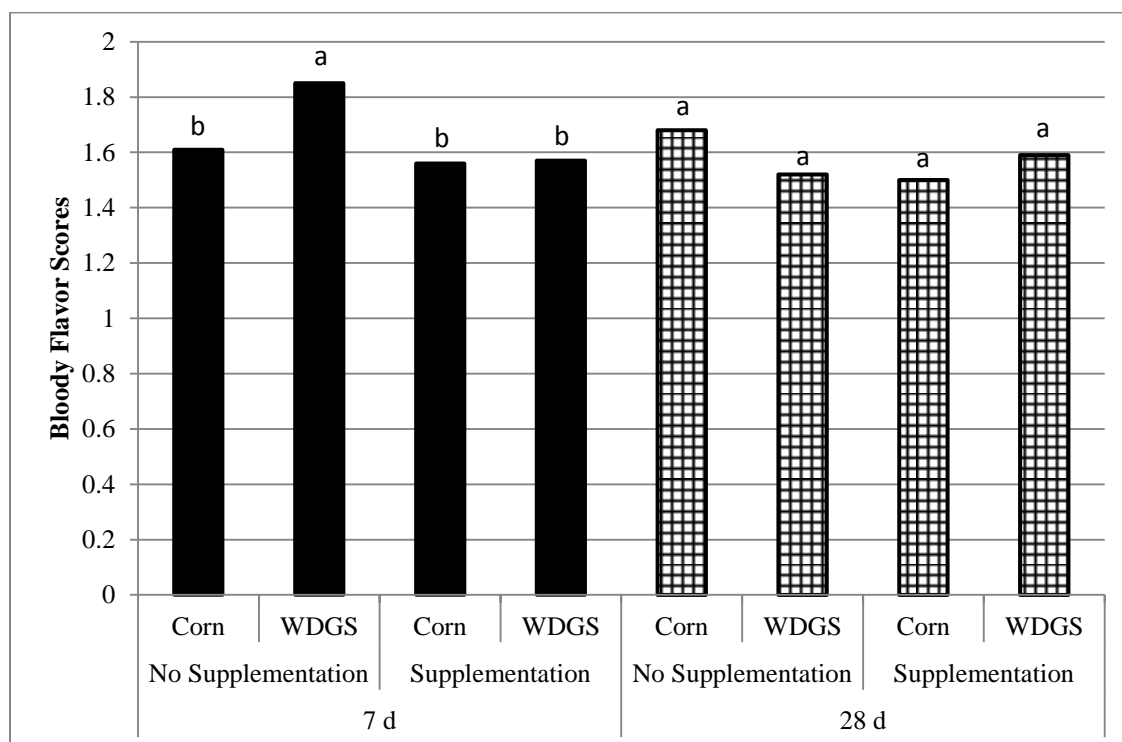
^aMeans within the same aging period with the same superscript are not significantly ($P > 0.05$) different

Figure 1. The effect of grass type, supplementation, and aging period on the LS means of beefy flavor intensity consumer panel scores when separated by aging period in *L. dorsi* steaks ($P = 0.05$).



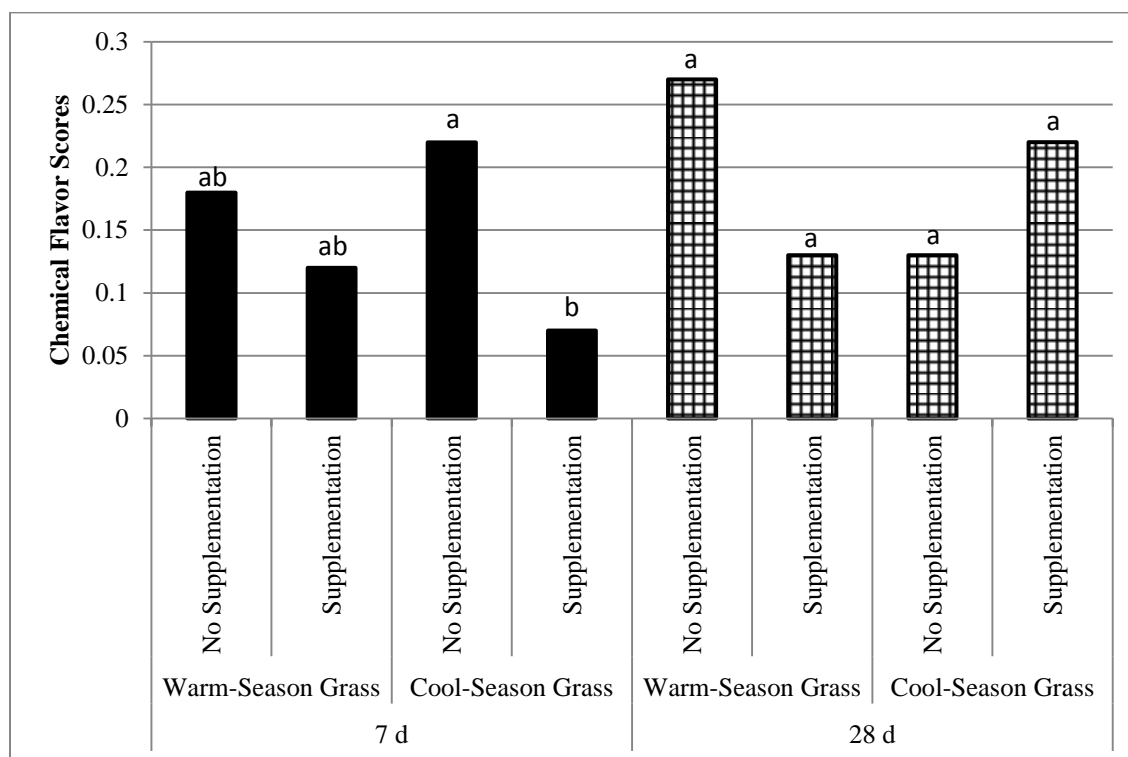
^{ab}Means within the same aging period with the different superscripts are significantly ($P \leq 0.05$) different

Figure 2. The effect of grass type, finishing diet, and aging period on the LS means of fat flavor scores when separated by aging period in *L. dorsi* steaks ($P = 0.02$).



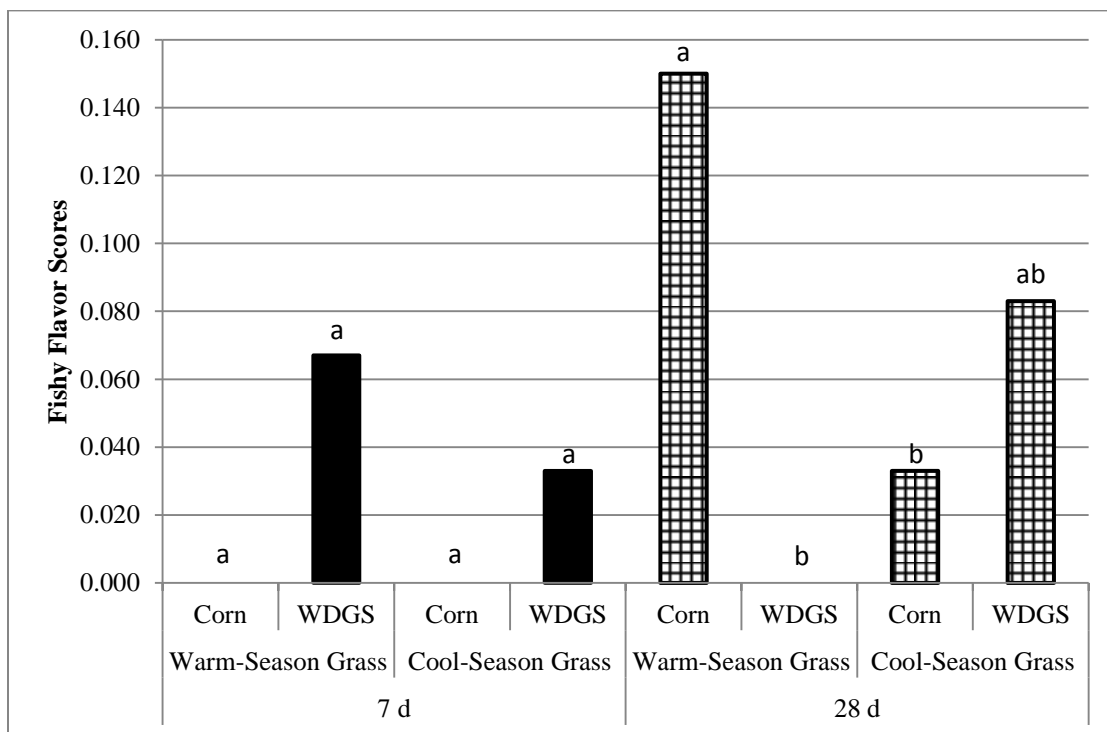
^{ab}Means within the same aging period with the different superscripts are significantly ($P \leq 0.05$) different

Figure 3. The effect of supplementation, finishing diet, and aging period on the LS means of bloody flavor scores when separated by aging period in *L. dorsi* steaks ($P = 0.04$).



^{ab}Means within the same aging period with the different superscripts are significantly ($P \leq 0.05$) different

Figure 4. The effect of grass type, supplementation, and aging period on the LS means of chemical flavor scores when separated by aging period in *B. femoris* steaks ($P = 0.05$).



^{ab}Means within the same aging period with the different superscripts are significantly ($P \leq 0.05$) different

Figure 5. The effect of grass type, finishing diet, and aging period on the LS means of fishy flavor scores when separated by aging period in *B. femoris* steaks ($P = 0.02$).

Table 1. The influence of diet and age on the *P*-values of consumer panel scores for *L. dorsi* and *B. femoris* steaks

Trait	<i>P</i> -Value														
	Grass ^x	Supp	Grass XSupp	Diet	Grass XDiet	Supp XDiet	Grass XSupp XDiet	Age	Grass XAge	Supp XAge	Grass XSupp XAge	DietX Age	Grass XDiet XAge	Supp XDiet XAge	Grass XSupp XDiet XAge
<i>L. dorsi</i>															
Overall															
Like ^y	0.55	0.50	0.29	0.05	0.94	0.03	0.62	0.99	0.94	0.76	0.09	0.67	0.38	0.43	0.31
Flavor															
Like	0.70	0.90	0.19	0.08	0.91	0.04	0.90	0.99	1.00	0.25	0.12	0.47	0.33	0.39	0.32
Beefy															
Like	0.35	0.82	0.36	0.10	0.63	0.02	0.80	0.57	0.80	0.97	0.11	0.39	0.16	0.28	0.35
Beefy															
Intensity	0.64	0.79	0.86	0.41	0.26	0.11	0.58	0.37	0.94	0.70	0.04	0.05	0.15	0.38	0.25
Grill Like	0.87	0.74	0.38	0.43	0.43	0.13	0.15	0.25	0.92	0.94	0.14	0.34	0.52	0.38	0.17
Grill															
Intensity	0.76	0.64	0.87	0.36	0.30	0.50	0.39	0.31	0.41	0.59	0.29	0.73	0.17	0.50	0.13
<i>B. femoris</i>															
Overall															
Like	0.01	0.31	0.39	0.17	0.01	0.66	0.45	0.08	0.99	0.96	0.96	0.95	0.73	0.06	0.005
Flavor															
Like	0.04	0.10	0.29	0.15	0.02	0.16	0.62	0.09	0.70	0.64	0.63	0.39	0.89	0.20	0.01
Beefy															
Like	0.08	0.21	0.13	0.41	0.08	0.31	0.91	0.04	0.31	0.99	0.49	0.85	0.63	0.24	0.01
Beefy															
Intensity	0.18	0.27	0.32	0.96	0.21	0.12	0.43	0.03	0.45	0.23	0.28	0.09	0.65	0.07	0.05
Grill Like	0.12	0.17	0.44	0.10	0.24	0.06	0.39	0.39	0.54	0.93	0.41	0.45	0.65	0.17	0.23
Grill															
Intensity	0.67	0.14	0.09	0.06	0.21	0.55	0.61	0.71	0.24	0.82	0.35	0.31	0.87	0.79	0.06

^xGrass = Grass type, Supp = Supplementation, Diet = Finishing diet, Age = Aging period

^y1=dislike extremely, none, or extremely bland, 9=like extremely or extremely intense

Table 2. The effects supplementation and finishing diet on the LS means of consumer panel scores for *L. dorsi* and *B. femoris* steaks

Trait	No Supplementation		Supplementation		SEM
	Corn	WDGS ^x	Corn	WDGS	
<i>L. dorsi</i>					
Overall Like ^y	6.14 ^b	6.18 ^{ab}	6.52 ^a	5.98 ^b	0.13
Overall Flavor Like	6.06 ^{ab}	6.10 ^{ab}	6.34 ^a	5.84 ^b	0.14
Beefy Flavor Like	6.15 ^{ab}	6.24 ^{ab}	6.43 ^a	5.91 ^b	0.13
Beefy Flavor Intensity	5.85	5.96	6.10	5.77	0.14
Grill Flavor Like	5.78	5.87	5.93	5.64	0.13
Grill Flavor Intensity	5.33	5.30	5.49	5.27	0.14
<i>B. femoris</i>					
Overall Like	5.77	5.64	5.97	5.72	0.14
Overall Flavor Like	5.65	5.65	6.08	5.69	0.14
Beefy Flavor Like	5.85	5.87	6.15	5.90	0.14
Beefy Flavor Intensity	5.63	5.84	6.00	5.77	0.14
Grill Flavor Like	5.51	5.54	5.95	5.48	0.14
Grill Flavor Intensity	5.16	4.97	5.46	5.10	0.15

^xWDGS = Wet distillers grains with solubles

^y1=dislike extremely, none, or extremely bland, 9=like extremely or extremely intense

^{ab}Means within the same row with different superscripts are different ($P \leq 0.05$)

Table 3. The effects of finishing diet and aging period on the LS means of consumer panel scores for *L. dorsi* and *B. femoris* steaks

Trait	Corn		WDGS ^x		SEM
	7 d	28 d	7 d	28 d	
<i>L. dorsi</i>					
Overall Like ^y	6.30	6.35	6.10	6.05	0.13
Overall Flavor Like	6.15	6.25	6.02	5.92	0.13
Beefy Flavor Like	6.27	6.31	6.17	5.98	0.13
Beefy Flavor Intensity	5.90 ^{ab}	6.05 ^a	6.06 ^a	5.67 ^b	0.13
Grill Flavor Like	5.84	5.87	5.63	5.89	0.12
Grill Flavor Intensity	5.37	5.46	5.19	5.38	0.14
<i>B. femoris</i>					
Overall Like	5.99	5.75	5.81	5.55	0.14
Overall Flavor Like	6.04	5.69	5.73	5.61	0.14
Beefy Flavor Like	6.15	5.85	6.02	5.76	0.14
Beefy Flavor Intensity	6.08	5.54	5.84	5.77	0.14
Grill Flavor Like	5.84	5.62	5.52	5.50	0.14
Grill Flavor Intensity	5.36	5.26	4.94	5.14	0.15

^xWDGS = Wet distillers grains with solubles

^y1=dislike extremely, none, or extremely bland, 9=like extremely or extremely intense

^{ab}Means within the same row with different superscripts are different ($P \leq 0.05$)

Table 4. The effect of grass type, supplementation, finishing diet, and aging period on the LS means of consumer panel scores when separated by aging period for *B. femoris* steaks

Trait	Warm-Season Grass				Cool-Season Grass				SEM
	No Supplementation		Supplementation		No Supplementation		Supplementation		
	Corn	WDGS ^x	Corn	WDGS	Corn	WDGS	Corn	WDGS	
7 d									
Overall Like ^y	6.12 ^a	5.02 ^b	5.89 ^a	5.78 ^{ab}	5.92 ^a	6.25 ^a	6.02 ^a	6.17 ^a	0.29
Overall Flavor Like	6.06 ^a	5.13 ^b	6.04 ^a	5.64 ^{ab}	5.83 ^{ab}	6.19 ^a	6.24 ^a	5.96 ^a	0.31
Beefy Flavor Like	6.08 ^{ab}	5.44 ^b	6.02 ^{ab}	6.05 ^{ab}	6.08 ^{ab}	6.41 ^a	6.43 ^a	6.17 ^{ab}	0.29
Beefy Flavor Intensity	6.11 ^a	5.55 ^a	5.88 ^a	5.72 ^a	6.11 ^a	6.10 ^a	6.24 ^a	5.98 ^a	0.32
Grill Flavor Like	5.85	5.22	5.81	5.59	5.59	5.71	6.12	5.55	0.28
Grill Flavor Intensity	5.38	4.65	5.60	5.16	5.03	5.02	5.41	4.91	0.31
28 d									
Overall Like	5.18 ^{cd}	5.48 ^{bcd}	6.28 ^a	4.91 ^d	5.85 ^{abc}	5.80 ^{abc}	5.71 ^{abc}	6.02 ^{ab}	0.29
Overall Flavor Like	5.08 ^c	5.48 ^{bc}	6.38 ^a	5.19 ^c	5.64 ^{abc}	5.81 ^{abc}	5.66 ^{abc}	5.95 ^{ab}	0.31
Beefy Flavor Like	5.39 ^b	5.65 ^b	6.50 ^a	5.47 ^b	5.84 ^{ab}	6.00 ^{ab}	5.66 ^b	5.92 ^{ab}	0.29
Beefy Flavor Intensity	4.88 ^c	5.73 ^{ab}	6.36 ^a	5.49 ^{bc}	5.40 ^{bc}	5.96 ^{ab}	5.52 ^{bc}	5.90 ^{ab}	0.32
Grill Flavor Like	5.14	5.28	6.03	5.21	5.47	5.96	5.85	5.56	0.28
Grill Flavor Intensity	4.73	4.87	5.77	4.96	5.49	5.35	5.06	5.37	0.31

^xWDGS = Wet distillers grains with solubles

^y1=dislike extremely, none, or extremely bland, 9=like extremely or extremely intense

^{abcd}Means within the same treatment combination and the same row with different superscripts are different ($P \leq 0.05$)

Table 5. The influence of diet and age on the *P*-values of lexicon scores for *L. dorsi* steaks

Flavor	<i>P</i> -Value														
	Grass ^x	Supp	GrassX Supp	Diet	GrassX Diet	SuppX Diet	Grass XSupp XDiet	Age	GrassX Age	SuppX Age	GrassX SuppX Age	DietX Age	Grass XDiet XAge	SuppX DietX Age	Grass XSupp XDiet XAge
Browned	0.75	0.96	0.17	0.20	0.37	0.32	0.40	0.26	0.04	0.53	0.96	0.09	0.35	0.83	0.65
Bloody	0.05	0.05	0.04	0.43	0.19	0.92	0.28	0.19	0.11	0.32	0.98	0.16	0.87	0.04	0.07
Fat	0.88	0.59	0.20	0.10	0.69	0.51	0.70	0.21	0.17	0.34	0.92	0.79	0.02	0.41	0.58
Metal	0.47	0.18	0.39	0.59	0.02	0.64	0.18	0.38	0.02	0.71	0.67	0.20	0.84	0.51	0.21
Liver Green	0.03	0.19	0.03	0.56	0.27	0.42	0.80	0.33	0.04	0.88	0.18	0.10	0.71	0.82	0.84
Hay	0.58	0.95	0.60	0.98	0.54	0.27	0.50	0.63	0.28	0.08	1.00	0.59	0.22	0.56	0.24
Umami Overly	0.26	0.96	0.13	0.17	0.45	0.74	0.08	0.30	0.21	0.97	0.30	0.92	0.32	0.56	0.50
Sweet	0.27	0.81	0.57	0.18	0.77	0.75	0.47	0.95	0.86	0.50	0.89	0.92	0.14	0.72	0.88
Sweet	0.83	0.28	0.34	0.04	0.64	0.37	0.18	0.49	0.55	0.67	0.57	0.78	0.30	0.39	0.47
Sour	0.84	0.21	0.30	0.25	0.65	0.88	0.16	0.71	0.82	0.47	0.17	0.20	0.40	0.92	0.24
Salty	0.85	0.70	0.92	0.37	0.63	0.82	0.13	0.08	0.11	0.89	0.71	0.78	0.69	0.81	0.20
Bitter Sour	0.39	0.85	0.56	0.99	0.68	0.90	0.92	0.29	0.62	0.26	0.79	0.27	0.56	0.39	0.51
Aroma	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Barnyard	0.90	0.44	0.05	0.88	0.16	0.45	0.42	0.50	0.50	0.97	0.18	0.48	0.58	0.92	0.96
Burnt Heated	0.34	0.27	0.05	0.52	0.77	0.86	0.19	0.13	0.13	0.33	0.94	0.25	0.81	0.22	0.25
Oil	0.46	0.91	0.09	0.67	0.60	0.64	0.19	0.88	0.88	0.70	0.91	0.84	0.18	0.90	0.40

^xGrass = Grass type, Supp = Supplementation, Diet = Finishing diet, Age = Aging period

Table 5 cont. The influence of diet and age on the *P*-values of lexicon scores for L. dorsi steaks

Flavor	<i>P</i> -Value														
	Grass ^x	Supp	Grass XSupp	Diet	Grass XDiet	Supp XDiet	Grass XSupp XDiet	Age	Grass XAge	Supp XAge	Grass XSupp XAge	DietX Age	Grass XDiet XAge	Supp XDiet XAge	Grass XSupp XDiet XAge
Chemical	0.67	0.33	0.64	0.98	0.94	0.97	0.17	0.29	0.54	0.54	0.74	0.55	0.36	0.71	0.79
Floral	0.79	0.32	0.72	0.72	0.32	0.79	0.10	0.79	0.10	0.72	0.32	0.32	0.72	0.10	0.79
Green Grass	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31
Musty	0.83	0.94	0.11	0.25	0.70	0.25	0.99	0.23	0.19	0.13	0.62	0.35	0.91	0.18	0.77
Medicinal	0.97	0.99	0.16	0.99	0.95	0.15	0.97	0.15	0.97	0.99	0.95	0.17	0.95	0.15	0.16
Smoked/ Charred	0.55	0.61	0.55	0.55	0.09	0.55	0.61	0.09	0.55	0.61	0.55	0.55	0.09	0.55	0.61
Smoked Wood	0.36	0.04	0.36	0.98	0.26	0.98	0.26	0.92	0.29	0.92	0.29	0.92	0.29	0.92	0.29
Spoiled	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Buttery Cooked	0.94	0.48	0.36	0.91	0.89	0.74	0.64	0.74	0.06	0.97	0.36	0.47	0.38	0.13	0.19
Milk	0.67	0.67	0.20	0.20	0.67	0.67	0.20	0.67	0.20	0.20	0.67	0.67	0.20	0.20	0.67
Sour Milk Refrigerator	0.74	0.81	0.80	0.28	0.28	0.02	0.62	0.62	0.59	0.52	0.94	0.14	0.36	0.74	0.78
Stale Warmed	0.34	0.57	0.08	0.29	0.10	0.04	0.39	0.55	0.08	0.54	0.30	0.60	0.54	0.61	0.93
over	0.71	0.60	0.77	0.002	0.19	0.71	0.25	0.06	0.80	0.83	0.26	0.31	0.67	0.44	0.79
Painty	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Fishy	0.14	0.14	0.81	0.13	0.75	0.15	0.82	0.36	0.37	0.41	0.42	0.37	0.38	0.72	0.71
Cardboardy	0.97	0.39	0.84	0.49	0.64	0.70	0.72	0.26	0.92	0.03	0.70	0.24	0.37	0.60	0.64

^xGrass = Grass type, Supp = Supplementation, Diet = Finishing diet, Age = Aging period

Table 6. The effect of grass type and aging period and the interaction between supplementation and aging period on the LS means of lexicon scores for *L. dorsi* steaks

Trait	Warm-Season Grass		Cool-Season Grass		SEM	No Supplementation		Supplementation		SEM
	7 d	28 d	7 d	28 d		7 d	28 d	7 d	28 d	
Browned	1.88 ^{ab}	1.79 ^{ab}	1.65 ^b	1.95 ^a	0.09	1.74	1.90	1.79	1.84	0.09
Bloody	1.66	1.68	1.64	1.47	0.06	1.73	1.60	1.56	1.55	0.06
Fat	1.72	1.71	1.62	1.79	0.07	1.69	1.70	1.66	1.80	0.07
Metal	1.69 ^{ab}	1.77 ^a	1.77 ^a	1.62 ^b	0.05	1.76	1.73	1.71	1.65	0.05
Liver	0.13 ^b	0.28 ^a	0.13 ^b	0.07 ^b	0.05	0.16	0.21	0.10	0.14	0.05
Green Hay	0.02	0.01	0.01	0.03	0.02	0.00	0.04	0.03	0.01	0.02
Umami	0.99	0.97	0.98	1.22	0.11	0.99	1.10	0.99	1.09	0.11
Overly Sweet	1.04	1.02	0.95	0.96	0.07	1.01	0.96	0.98	1.02	0.07
Sweet	1.04	0.96	1.01	1.01	0.06	0.98	0.96	1.07	1.00	0.06
Sour	1.37	1.34	1.37	1.36	0.05	1.38	1.40	1.36	1.30	0.05
Salty	1.37	1.38	1.30	1.46	0.05	1.33	1.41	1.34	1.43	0.05
Bitter	1.10	1.17	1.08	1.11	0.04	1.06	1.16	1.12	1.12	0.04
Sour Aroma	0.000	0.010	0.000	0.000	0.005	0.000	0.000	0.000	0.100	0.005
Barnyard	0.02	0.02	0.03	0.01	0.01	0.02	0.01	0.03	0.02	0.01
Burnt	0.17	0.07	0.09	0.09	0.03	0.13	0.11	0.13	0.05	0.03
Heated Oil	0.04	0.05	0.07	0.07	0.03	0.05	0.07	0.06	0.06	0.03

^{ab}Means within the same treatment combination and the same row with different superscripts are different ($P < 0.05$)

Table 6 cont. The effect of grass type and aging period and the interaction between supplementation and aging period on the LS means of lexicon scores for *L. dorsi* steaks

Trait	Warm-Season Grass		Cool-Season Grass		SEM	No Supplementation		Supplementation		SEM
	7 d	28 d	7 d	28 d		7 d	28 d	7 d	28 d	
Chemical	0.12	0.13	0.11	0.17	0.04	0.11	0.12	0.12	0.18	0.04
Floral	0.00	0.03	0.02	0.00	0.01	0.02	0.02	0.00	0.01	0.01
Green Grass	0.00	0.00	0.01	0.00	0.005	0.01	0.00	0.00	0.00	0.005
Musty	0.06	0.15	0.10	0.10	0.04	0.05	0.15	0.11	0.10	0.04
Medicinal	0.03	0.01	0.03	0.01	0.01	0.03	0.01	0.03	0.01	0.01
Smoked/Charred	0.00	0.02	0.00	0.01	0.01	0.00	0.01	0.00	0.02	0.01
Smoked Wood	0.01	0.02	0.01	0.00	0.01	0.00	0.00	0.02	0.02	0.01
Spoiled	0.00	0.02	0.00	0.00	0.01	0.00	0.02	0.00	0.00	0.01
Buttery	0.06	0.11	0.12	0.06	0.03	0.08	0.07	0.10	0.09	0.03
Cooked Milk	0.00	0.02	0.01	0.00	0.01	0.00	0.02	0.01	0.00	0.01
Sour Milk	0.04	0.04	0.04	0.07	0.03	0.04	0.07	0.05	0.04	0.03
Refrigerator Stale	0.04	0.02	0.03	0.08	0.02	0.04	0.02	0.03	0.08	0.02
Warmed over	0.10	0.18	0.10	0.21	0.05	0.11	0.21	0.09	0.18	0.05
Painty	0.00	0.00	0.00	0.01	0.005	0.00	0.01	0.00	0.00	0.005
Fishy	0.01	0.01	0.02	0.04	0.02	0.02	0.04	0.01	0.01	0.02
Cardboardy	0.13	0.17	0.12	0.17	0.04	0.10 ^b	0.23 ^a	0.15 ^{ab}	0.11 ^b	0.04

^{ab}Means within the same treatment combination and the same row with different superscripts are different ($P < 0.05$)

Table 7. The effect of grass type and supplementation on the LS means of lexicon scores for *L. dorsi* steaks

Trait	Warm-season Grass		Cool-season Grass		SEM
	No Supplementation	Supplementation	No Supplementation	Supplementation	
Browned	1.77	1.90	1.87	1.74	0.10
Bloody	1.78 ^a	1.55 ^b	1.55 ^b	1.56 ^b	0.06
Fat	1.66	1.78	1.73	1.68	0.07
Metal	1.79	1.68	1.71	1.68	0.05
Liver	0.30 ^a	0.12 ^b	0.08 ^b	0.12 ^b	0.05
Green					
Hay	0.01	0.02	0.03	0.02	0.02
Umami	0.90	1.06	1.18	1.02	0.11
Overly					
Sweet	1.00	1.06	0.96	0.94	0.07
Sweet	0.94	1.06	1.01	1.02	0.06
Sour	1.41	1.30	1.37	1.36	0.05
Salty	1.37	1.38	1.37	1.39	0.05
Bitter	1.14	1.13	1.08	1.11	0.04
Sour					
Aroma	0.00	0.009	0.000	0.000	0.005
Barnyard	0.03 ^a	0.01 ^a	0.00 ^b	0.04 ^a	0.01
Burnt	0.11 ^{ab}	0.14 ^a	0.14 ^a	0.04 ^b	0.02
Heated					
Oil	0.03	0.07	0.10	0.04	0.03

^{ab}Means within the same row with different superscripts are different ($P \leq 0.05$)

Table 7 cont. The effect of grass type and supplementation on the LS means of lexicon scores for *L. dors*i steaks

Trait	Warm-season Grass		Cool-season Grass		SEM
	No		No		
	Supplementation	Supplementation	Supplementation	Supplementation	
Chemical	0.12	0.13	0.11	0.17	0.04
Floral	0.02	0.01	0.02	0.00	0.01
Green Grass	0.000	0.000	0.009	0.000	0.005
Musty	0.14	0.08	0.07	0.13	0.04
Medicinal	0.01	0.03	0.03	0.01	0.01
Smoked/Charred	0.01	0.01	0.00	0.01	0.01
Smoked Wood	0.00	0.03	0.00	0.01	0.01
Spoiled	0.02	0.00	0.00	0.00	0.01
Buttery	0.06	0.11	0.09	0.08	0.03
Cooked Milk	0.02	0.00	0.00	0.01	0.01
Sour Milk	0.04	0.04	0.06	0.05	0.03
Refrigerator					
Stale	0.05	0.02	0.03	0.08	0.02
Warmed over	0.14	0.13	0.18	0.14	0.05
Painty	0.000	0.000	0.009	0.000	0.005
Fishy	0.02	0.00	0.04	0.02	0.02
Cardboardy	0.16	0.13	0.17	0.12	0.04

^{ab}Means within the same row with different superscripts are different ($P \leq 0.05$)

Table 8. The effect of grass type and finishing diet and the interaction between supplementation and finishing diet on the LS means of lexicon scores for *L. dorsi* steaks

Trait	Warm-season Grass		Cool-season Grass		SEM	No Supplementation		Supplementation		SEM
	Corn	WDGS ^x	Corn	WDGS		Corn	WDGS ^x	Corn	WDGS	
Browned	1.85	1.81	1.91	1.70	0.10	1.83	1.81	1.92	1.71	0.10
Bloody	1.68	1.65	1.50	1.62	0.06	1.65	1.69	1.53	1.58	0.06
Fat	1.76	1.68	1.77	1.64	0.07	1.73	1.66	1.81	1.66	0.07
Metal	1.77 ^a	1.69 ^{ab}	1.63 ^b	1.77 ^a	0.05	1.74	1.75	1.65	1.70	0.05
Liver	0.17	0.25	0.11	0.09	0.05	0.19	0.18	0.09	0.16	0.05
Green Hay	0.02	0.01	0.02	0.03	0.02	0.01	0.03	0.03	0.01	0.02
Umami	1.01	0.95	1.21	0.99	0.11	1.13	0.95	1.09	0.98	0.11
Overly Sweet	1.06	0.99	1.01	0.89	0.07	1.02	0.95	1.06	0.94	0.07
Sweet	1.08	0.92	1.06	0.96	0.06	1.01	0.94	1.13	0.95	0.06
Sour	1.31	1.40	1.35	1.38	0.05	1.36	1.42	1.30	1.35	0.05
Salty	1.41	1.34	1.39	1.37	0.05	1.40	1.34	1.40	1.37	0.05
Bitter	1.14	1.12	1.09	1.11	0.05	1.11	1.11	1.12	1.12	0.05
Sour Aroma	0.009	0.000	0.000	0.000	0.005	0.000	0.000	0.009	0.000	0.005
Barnyard	0.03	0.01	0.01	0.03	0.01	0.02	0.01	0.02	0.03	0.01
Burnt	0.13	0.12	0.11	0.07	0.03	0.13	0.12	0.10	0.07	0.03
Heated Oil	0.06	0.04	0.07	0.07	0.03	0.06	0.06	0.07	0.05	0.03

^xWDGS = Wet distillers grains with solubles

^{ab}Means within the same treatment combination and the same row with different superscripts are different ($P < 0.05$)

Table 8 cont. The effect of grass type and finishing diet and the interaction between supplementation and finishing diet on the LS means of lexicon scores for *L. dorsi* steaks

Trait	Warm-season Grass		Cool-season Grass		SEM	No Supplementation		Supplementation		SEM
	Corn	WDGS ^x	Corn	WDGS		Corn	WDGS ^x	Corn	WDGS	
Chemical	0.13	0.12	0.14	0.14	0.04	0.11	0.12	0.15	0.15	0.04
Floral	0.02	0.01	0.00	0.02	0.01	0.02	0.02	0.00	0.01	0.01
Green Grass	0.000	0.000	0.000	0.009	0.005	0.000	0.009	0.000	0.000	0.005
Musty	0.08	0.14	3.09	0.11	0.04	0.06	0.14	0.11	0.11	0.04
Medicinal	0.02	0.02	0.02	0.02	0.01	0.01	0.03	0.03	0.01	0.01
Smoked/Charred	0.00	0.02	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.01
Smoked Wood	0.02	0.01	0.00	0.01	0.01	0.00	0.00	0.02	0.02	0.01
Spoiled	0.02	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.01
Buttery	0.08	0.09	0.09	0.09	0.03	0.08	0.07	0.09	0.10	0.03
Cooked Milk	0.02	0.00	0.01	0.00	0.01	0.02	0.00	0.01	0.00	0.01
Sour Milk Refrigerator	0.04	0.04	0.09	0.02	0.03	0.11 ^a	0.00 ^b	0.03 ^{ab}	0.06 ^{ab}	0.03
Stale	0.00	0.06	0.06	0.05	0.02	0.00 ^b	0.07 ^a	0.06 ^{ab}	0.04 ^{ab}	0.02
Warmed over	0.08	0.20	0.03	0.28	0.05	0.06	0.26	0.05	0.22	0.05
Painty	0.000	0.000	0.009	0.000	0.005	0.009	0.000	0.000	0.000	0.005
Fishy	0.00	0.02	0.02	0.05	0.02	0.01	0.05	0.01	0.01	0.02
Cardboardy	0.14	0.15	0.12	0.17	0.04	0.16	0.17	0.11	0.15	0.04

^xWDGS = Wet distillers grains with solubles

^{ab}Means within the same treatment combination and the same row with different superscripts are different ($P < 0.05$)

Table 9. The effect of grass type, supplementation, finishing diet, and aging period on the LS means for lexicon scores for *L. dorsi* steaks

Trait	Grass Type			Supplementation			Finishing Diets			Aging Period		
	Warm-season	Cool-season	SEM	No	Yes	SEM	Corn	WDGS ^x	SEM	7 d	28 d	SEM
Browned	1.83	1.80	0.07	1.82	1.82	0.07	1.88	1.76	0.07	1.76	1.87	0.07
Bloody	1.67 ^a	1.56 ^b	0.04	1.67 ^a	1.56 ^b	0.04	1.59	1.63	0.04	1.65	1.57	0.04
Fat	1.72	1.71	0.05	1.70	1.73	0.05	1.77	1.66	0.05	1.67	1.75	0.05
Metal	1.73	1.70	0.04	1.75	1.68	0.04	1.70	1.73	0.04	1.73	1.69	0.04
Liver	0.21 ^a	0.10 ^b	0.03	0.19	0.12	0.04	0.14	0.17	0.04	0.13	0.18	0.03
Green												
Hay	0.01	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.01	0.02	0.01
Umami	0.98	1.10	0.07	1.04	1.04	0.07	1.11	0.97	0.08	0.99	1.09	0.07
Overly												
Sweet	1.03	0.95	0.05	0.98	1.00	0.05	1.04	0.94	0.05	0.99	0.99	0.05
Sweet	1.00	1.01	0.04	0.97	1.04	0.04	1.07 ^a	0.94 ^b	0.04	1.03	0.98	0.04
Sour	1.35	1.37	0.04	1.39	1.33	0.04	1.33	1.39	0.04	1.37	1.35	0.04
Salty	1.37	1.38	0.03	1.37	1.39	0.03	1.40	1.36	0.03	1.34	1.42	0.03
Bitter	1.13	1.10	0.03	1.11	1.12	0.03	1.12	1.11	0.03	1.09	1.14	0.03
Sour												
Aroma	0.005	0.000	0.003	0.000	0.005	0.003	0.005	0.000	0.003	0.000	0.005	0.003
Barnyard	0.02	0.02	0.01	0.01	0.02	0.01	0.02	0.02	0.01	0.02	0.01	0.01
Burnt	0.12	0.09	0.02	0.12	0.09	0.02	0.12	0.09	0.02	0.13	0.08	0.02
Heated												
Oil	0.05	0.07	0.02	0.06	0.06	0.02	0.07	0.05	0.02	0.06	0.06	0.02

^xWDGS = Wet distillers grains with solubles

^{ab}Means within the same treatment and the same row with different superscripts are different ($P < 0.05$)

Table 9 cont. The effect of grass type, supplementation, finishing diet, and aging period on the LS means for lexicon scores for *L. dorsi* steaks

Trait	Grass Type		SEM	Supplementation			Finishing Diets			Aging Period		
	Warm-season	Cool-season		No	Yes	SEM	Corn	WDGS ^x	SEM	7 d	28 d	SEM
Chemical	0.13	0.14	0.03	0.12	0.15	0.03	0.13	0.13	0.03	0.11	0.15	0.03
Floral	0.010	0.010	0.010	0.020	0.004	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Green Grass	0.000	0.005	0.003	0.005	0.000	0.003	0.000	0.005	0.003	0.005	0.000	0.003
Musty	0.11	0.10	0.03	0.10	0.11	0.03	0.08	0.12	0.03	0.08	0.13	0.03
Medicinal	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.03	0.01	0.01
Smoked/Charred	0.009	0.004	0.006	0.005	0.009	0.006	0.004	0.009	0.006	0.00	0.013	0.006
Smoked Wood	0.01	0.01	0.01	0.00 ^b	0.02 ^a	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Spoiled	0.01	0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.00	0.01	0.01
Buttery	0.08	0.09	0.02	0.08	0.10	0.02	0.08	0.09	0.02	0.09	0.08	0.02
Cooked Milk	0.009	0.004	0.007	0.009	0.004	0.007	0.013	0.000	0.007	0.004	0.009	0.007
Sour Milk	0.04	0.05	0.02	0.05	0.05	0.02	0.07	0.03	0.02	0.04	0.06	0.02
Refrigerator												
Stale	0.03	0.05	0.02	0.04	0.05	0.02	0.03	0.05	0.02	0.04	0.05	0.02
Warmed over	0.14	0.16	0.03	0.16	0.14	0.03	0.06 ^b	0.24 ^a	0.04	0.10	0.19	0.04
Painty	0.000	0.004	0.003	0.004	0.000	0.003	0.004	0.000	0.003	0.000	0.004	0.003
Fishy	0.01	0.03	0.01	0.03	0.01	0.01	0.01	0.03	0.01	0.01	0.03	0.01
Cardboardy	0.15	0.15	0.03	0.16	0.13	0.03	0.13	0.16	0.03	0.12	0.17	0.03

^xWDGS = Wet distillers grains with solubles

^{ab}Means within the same treatment and the same row with different superscripts are different ($P < 0.05$)

Table 10. The influence of diet and age on the *P*-values of lexicon scores for *B. femoris* steaks

Flavor	<i>P</i> -Value														
	Grass ^x	Supp	GrassX Supp	Diet	Grass XDiet	Supp XDiet	Grass XSupp XDiet	Age	Grass XAge	Supp XAge	Grass XSupp XAge	DietX Age	Grass XDiet XAge	Supp XDiet XAge	Grass XSupp XDiet XAge
Browned	0.04	0.16	0.43	0.49	0.79	0.87	0.19	0.19	0.32	0.79	0.16	0.16	0.71	0.13	0.56
Bloody	0.02	0.21	0.08	0.31	0.61	0.80	0.80	0.80	0.45	0.08	0.08	0.003	1.00	0.80	0.45
Fat	1.00	0.72	0.72	0.28	0.28	0.63	1.00	0.55	0.90	0.63	0.15	0.81	0.09	0.28	0.72
Metal	0.09	0.26	0.03	0.89	0.39	0.16	0.89	0.57	0.67	0.03	0.39	0.05	0.89	0.89	1.00
Liver	0.0004	0.03	0.005	0.32	1.00	1.00	0.07	0.16	0.20	0.32	0.26	0.89	0.16	0.78	0.89
Green															
Hay	0.43	0.19	0.79	0.07	0.19	0.43	0.79	0.07	0.19	0.43	0.79	0.02	0.43	0.19	0.79
Umami	0.27	0.89	0.36	0.36	0.19	0.67	0.31	0.60	0.67	0.74	0.47	0.81	0.81	0.81	0.74
Overly															
Sweet	0.95	0.67	0.50	0.24	0.95	0.95	0.42	0.19	0.95	0.19	0.29	0.67	0.76	0.35	0.76
Sweet	0.23	0.29	0.23	0.18	0.36	0.72	0.43	0.23	0.94	0.36	0.43	0.72	0.72	0.10	0.94
Sour	0.01	0.03	0.60	0.82	0.26	0.94	0.41	0.09	0.20	0.20	0.60	0.71	0.71	0.71	0.41
Salty	0.34	0.93	0.03	0.34	0.26	0.93	0.43	0.10	0.93	0.79	0.19	0.79	0.93	0.79	0.43
Bitter	0.94	0.32	0.70	0.94	0.19	0.82	0.25	<0.0001	0.70	0.49	0.70	0.49	0.82	0.49	0.94
Sour															
Aroma	0.21	0.21	0.21	0.53	0.53	0.53	0.53	0.21	0.21	0.21	0.21	0.53	0.53	0.53	0.53
Barnyard	0.62	0.32	0.62	0.62	0.32	0.13	0.32	0.62	0.32	0.62	1.00	0.32	0.62	1.00	0.62
Burnt	0.27	0.46	0.85	0.46	0.20	0.85	0.46	0.10	0.27	0.46	0.58	0.27	0.85	0.85	0.71
Heated															
Oil	0.52	0.20	0.52	0.28	0.20	0.52	0.09	0.28	1.00	0.83	0.20	0.39	0.83	1.00	0.28

^xGrass = Grass type, Supp = Supplementation, Diet = Finishing diet, Age = Aging period

Table 10 cont. The influence of diet and age on the *P*-values of lexicon scores for *B. femoris* steaks

Flavor	<i>P</i> -Value															
	Grass ^x	Supp	Grass XSupp	Diet	Grass XDiet	Supp XDiet	GrassX SuppX	Diet	Age	Grass XAge	Supp XAge	Grass XSupp XAge	DietX Age	Grass XDiet XAge	Supp XDiet XAge	Grass XSupp XDiet XAge
Chemical	0.66	0.08	0.38	0.66	1.00	0.38	0.38	0.27	0.83	0.27	0.05	0.12	0.83	0.83	0.83	0.83
Beet	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Green Grass	0.16	0.48	0.16	0.16	0.48	1.00	0.48	1.00	0.48	1.00	0.48	0.48	1.00	0.48	0.16	0.16
Musty	0.17	0.61	0.31	0.61	0.73	0.23	0.09	0.06	0.09	0.61	0.50	0.61	0.17	0.87	0.50	0.50
Medicinal	1.00	0.19	0.08	0.67	1.00	0.67	1.00	0.39	0.19	0.39	0.67	1.00	0.67	1.00	0.67	0.67
Petroleum	0.06	0.21	0.21	1.00	1.00	0.23	0.23	0.21	0.21	0.53	0.53	0.53	0.53	1.00	1.00	1.00
Smoked/ Charred	0.87	0.27	0.43	0.27	0.87	0.87	0.63	0.63	0.15	0.43	0.63	0.63	0.87	0.43	0.63	0.63
Smoked Wood	0.16	0.16	0.16	1.00	1.00	1.00	1.00	0.16	0.16	0.16	0.16	1.00	1.00	1.00	1.00	1.00
Dairy	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Buttery Cooked	0.84	0.16	0.54	0.54	0.16	0.07	0.31	0.84	0.84	0.84	0.84	0.54	0.31	0.31	0.31	0.31
Milk	0.65	0.37	0.65	1.00	0.65	1.00	0.18	0.65	0.37	0.65	0.37	0.18	1.00	0.65	0.37	0.37
Sour Milk	1.00	0.005	0.51	0.13	0.51	0.66	0.66	0.05	0.83	0.08	0.66	0.66	0.19	0.28	0.28	0.28
Refrigerator Stale	0.16	0.84	0.54	0.84	0.31	0.84	0.54	0.07	0.54	0.31	0.84	0.31	0.16	0.31	0.84	0.84
Warmed over	0.53	0.05	0.89	0.67	0.74	0.81	0.17	0.23	0.89	0.74	0.36	0.96	0.67	0.89	0.60	0.60
Soapy	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Painty	0.07	0.71	0.27	0.46	0.46	0.46	1.00	0.01	0.07	0.71	0.71	0.46	1.00	1.00	1.00	1.00
Fishy	0.50	0.17	0.31	1.00	0.09	0.31	1.00	0.09	1.00	1.00	0.31	0.04	0.02	0.73	1.00	1.00
Cardboardy	0.15	0.52	0.52	0.42	0.87	0.75	0.20	0.33	0.75	0.87	0.08	0.52	0.33	0.87	0.87	0.87

^xGrass = Grass type, Supp = Supplementation, Diet = Finishing diet, Age = Aging period

Table 11. The effect of supplementation and aging period and the interaction between finishing diet and aging period on the LS means for lexicon scores for *B. femoris* steaks

Trait	No		Supplementation		SEM	Corn		WDGS ^x		SEM
	Supplementation		Supplementation			7 d	28 d	7 d	28 d	
Browned	1.63	1.76	1.77	1.85	0.08	1.62	1.83	1.78	1.78	0.08
Bloody	1.68	1.78	1.72	1.58	0.07	1.83 ^a	1.62 ^{bc}	1.57 ^c	1.75 ^{ab}	0.07
Fat	1.70	1.71	1.69	1.77	0.07	1.74	1.77	1.65	1.71	0.07
Metal	1.92 ^{ab}	2.08 ^a	1.98 ^{ab}	1.88 ^b	0.06	2.00 ^a	1.92 ^a	1.89 ^a	2.04 ^a	0.06
Liver	0.27	0.41	0.20	0.23	0.06	0.21	0.28	0.26	0.35	0.06
Green										
Hay	0.008	0.050	0.000	0.017	0.016	0.008 ^b	0.000 ^b	0.000 ^b	0.067 ^a	0.016
Umami	0.79	0.72	0.78	0.76	0.09	0.83	0.77	0.73	0.71	0.09
Overly										
Sweet	0.98	0.81	0.93	0.93	0.07	1.01	0.89	0.90	0.84	0.07
Sweet	0.90	0.78	0.91	0.89	0.06	0.93	0.88	0.88	0.78	0.06
Sour	1.43	1.59	1.38	1.40	0.06	1.42	1.49	1.38	1.50	0.06
Salty	1.43	1.36	1.43	1.34	0.05	1.46	1.37	1.40	1.33	0.05
Bitter	1.31	1.57	1.29	1.48	0.05	1.28	1.54	1.32	1.50	0.05
Sour										
Aroma	0.000	0.000	0.000	0.033	0.013	0.000	0.025	0.000	0.008	0.013
Barnyard	0.02	0.02	0.03	0.04	0.02	0.01	0.03	0.03	0.03	0.02
Burnt	0.14	0.18	0.14	0.25	0.05	0.13	0.26	0.15	0.18	0.05
Heated										
Oil	0.07	0.10	0.11	0.16	0.04	0.09	0.17	0.08	0.09	0.04

^xWDGS = Wet distillers grains with solubles

^{abc}Means within the same treatment combination and the same row with different superscripts are different ($P < 0.05$)

Table 11 cont. The effect of supplementation and aging period and the interaction between finishing diet and aging period on the LS means for lexicon scores for *B. femoris* steaks

Trait	No Supplementation		Supplementation		SEM	Corn		WDGS ^x		SEM
	7 d	28 d	7 d	28 d		7 d	28 d	7 d	28 d	
Chemical	0.20	0.20	0.09	0.18	0.04	0.17	0.15	0.13	0.23	0.04
Beet	0.000	0.000	0.000	0.008	0.004	0.000	0.000	0.000	0.008	0.004
Green Grass	0.017	0.017	0.008	0.008	0.012	0.000	0.008	0.025	0.017	0.012
Musty	0.18	0.30	0.18	0.25	0.05	0.18	0.30	0.18	0.25	0.05
Medicinal	0.03	0.03	0.04	0.08	0.02	0.04	0.06	0.03	0.05	0.02
Petroleum	0.000	0.008	0.008	0.033	0.013	0.000	0.025	0.008	0.017	0.013
Smoked/ Charred Smoked	0.05	0.06	0.10	0.07	0.03	0.07	0.04	0.08	0.08	0.03
Wood	0.000	0.000	0.017	0.000	0.006	0.008	0.000	0.008	0.000	0.006
Dairy	0.000	0.000	0.008	0.000	0.004	0.008	0.000	0.000	0.000	0.004
Buttery Cooked	0.03	0.03	0.06	0.06	0.02	0.04	0.06	0.04	0.03	0.02
Milk	0.03	0.02	0.04	0.04	0.02	0.05	0.02	0.03	0.04	0.02
Sour Milk	0.08	0.22	0.03	0.04	0.04	0.03	0.09	0.08	0.17	0.04
Refrigerator Stale Warmed over	0.03	0.05	0.02	0.08	0.02	0.03	0.05	0.02	0.08	0.02
Soapy	0.58	0.72	0.44	0.52	0.09	0.49	0.60	0.53	0.63	0.09
Painty	0.000	0.017	0.000	0.000	0.008	0.000	0.000	0.000	0.017	0.008
Fishy	0.01	0.08	0.01	0.06	0.02	0.01	0.05	0.01	0.08	0.02
Cardboardy	0.04	0.08	0.01	0.05	0.02	0.00 ^b	0.09 ^a	0.05 ^{ab}	0.04 ^{ab}	0.02
	0.17	0.23	0.21	0.25	0.05	0.15	0.23	0.23	0.24	0.05

^xWDGS = Wet distillers grains with solubles

^{abc}Means within the same treatment combination and the same row with different superscripts are different ($P < 0.05$)

Table 12. The effect of grass type and supplementation on the LS means of lexicon scores for *B. femoris* steaks

Trait	Warm-season Grass		Cool-season Grass		SEM
	No Supplementation	Supplementation	No Supplementation	Supplementation	
Browned	1.58	1.76	1.81	1.86	0.08
Bloody	1.58	1.76	1.81	1.86	0.08
Fat	1.69	1.74	1.72	1.72	0.07
Metal	2.11 ^a	1.92 ^b	1.88 ^b	1.94 ^b	0.06
Liver	0.53 ^a	0.23 ^b	0.15 ^b	0.19 ^b	0.06
Green					
Hay	0.03	0.00	0.03	0.02	0.02
Umami	0.67	0.76	0.84	0.78	0.09
Overly					
Sweet	0.88	0.95	0.92	0.90	0.07
Sweet	0.77	0.90	0.91	0.90	0.06
Sour	1.60	1.45	1.42	1.33	0.06
Salty	1.32 ^b	1.42 ^{ab}	1.47 ^a	1.36 ^{ab}	0.05
Bitter	1.43	1.39	1.45	1.38	0.05
Sour					
Aroma	0.00	0.03	0.00	0.00	0.01
Barnyard	0.03	0.03	0.01	0.03	0.02
Burnt	0.13	0.18	0.19	0.22	0.05
Heated					
Oil	0.06	0.13	0.11	0.13	0.04

^xWDGS = Wet distillers grains with solubles

^{ab}Means within the same row with different superscripts are different ($P \leq 0.05$)

Table 12 cont. The effect of grass type and supplementation on the LS means of lexicon scores for *B. femoris* steaks

Trait	Warm-season Grass		Cool-season Grass		SEM
	No		No		
	Supplementation	Supplementation	Supplementation	Supplementation	
Chemical	0.23	0.13	0.18	0.14	0.04
Beet	0.000	0.008	0.000	0.000	0.004
Green Grass	0.000	0.008	0.033	0.008	0.012
Musty	0.30	0.23	0.18	0.21	0.05
Medicinal	0.02	0.08	0.05	0.04	0.02
Petroleum	0.00	0.00	0.01	0.04	0.01
Smoked/Charred	0.07	0.08	0.04	0.09	0.03
Smoked Wood	0.00	0.02	0.00	0.00	0.01
Dairy	0.000	0.008	0.000	0.000	0.004
Buttery	0.03	0.07	0.03	0.05	0.02
Cooked Milk	0.03	0.05	0.03	0.03	0.02
Sour Milk	0.13	0.05	0.16	0.03	0.04
Refrigerator					
Stale	0.03	0.03	0.05	0.07	0.02
Warmed over	0.62	0.46	0.68	0.50	0.09
Soapy	0.02	0.00	0.00	0.00	0.01
Painty	0.01	0.03	0.08	0.04	0.02
Fishy	0.06	0.05	0.07	0.01	0.02
Cardboardy	0.22	0.28	0.18	0.18	0.05

^xWDGS = Wet distillers grains with solubles

Table 13. The effect of grass type, supplementation, finishing diet, and ageing period on the LS means for lexicon scores for *B. femoris* steaks

Trait	Grass Type			Supplementation			Finishing Diets			Ageing Period		
	Warm-season	Cool-season	SEM	No	Yes	SEM	Corn	WDGS ^x	SEM	7 d	28 d	SEM
Browned	1.67 ^b	1.83 ^a	0.06	1.70	1.81	0.06	1.73	1.78	0.06	1.70	1.80	0.06
Bloody	1.77 ^a	1.62 ^b	0.05	1.73	1.65	0.05	1.73	1.66	0.05	1.7	1.68	0.05
Fat	1.72	1.72	0.05	1.70	1.73	0.05	1.75	1.68	0.05	1.70	1.74	0.05
Metal	2.01	1.91	0.04	2.00	1.93	0.04	1.96	1.97	0.04	1.95	1.98	0.04
Liver	0.38 ^a	0.17 ^b	0.05	0.34 ^a	0.21 ^b	0.05	0.25	0.30	0.04	0.23	0.32	0.04
Green												
Hay	0.013	0.025	0.011	0.029	0.008	0.011	0.004	0.033	0.011	0.004	0.033	0.011
Umami	0.71	0.81	0.06	0.75	0.77	0.06	0.80	0.72	0.06	0.78	0.74	0.06
Overly												
Sweet	0.91	0.91	0.05	0.90	0.93	0.05	0.95	0.87	0.05	0.95	0.87	0.05
Sweet	0.83	0.90	0.04	0.84	0.90	0.04	0.91	0.83	0.04	0.90	0.83	0.04
Sour	1.53 ^a	1.37 ^b	0.04	1.51 ^a	1.39 ^b	0.04	1.45	1.44	0.04	1.40	1.50	0.04
Salty	1.37	1.41	0.03	1.39	1.39	0.03	1.41	1.37	0.03	1.43	1.35	0.03
Bitter	1.41	1.41	0.04	1.44	1.38	0.04	1.41	1.41	0.04	1.30 ^b	1.52 ^a	0.04
Sour												
Aroma	0.017	0.000	0.009	0.000	0.017	0.009	0.013	0.004	0.009	0.000	0.017	0.009
Barnyard	0.03	0.02	0.01	0.02	0.03	0.01	0.02	0.03	0.01	0.02	0.03	0.01
Burnt	0.15	0.20	0.03	0.16	0.20	0.03	0.20	0.16	0.03	0.14	0.22	0.03
Heated												
Oil	0.10	0.12	0.03	0.08	0.13	0.03	0.13	0.09	0.03	0.09	0.13	0.03

^xWDGS = Wet distillers grains with solubles

^{ab}Means within the same treatment and the same row with different superscripts are different ($P < 0.05$)

Table 13 cont. The effect of grass type, supplementation, finishing diet, and ageing period on the LS means for lexicon scores for *B. femoris* steaks

Trait	Grass Type			Supplementation			Finishing Diets			Aging Period		
	Warm-season	Cool-season	SEM	No	Yes	SEM	Corn	WDGS ^x	SEM	7 d	28 d	SEM
Chemical	0.18	0.16	0.03	0.20	0.13	0.03	0.16	0.18	0.03	0.15	0.19	0.03
Beet	0.004	0.000	0.003	0.000	0.004	0.003	0.000	0.004	0.003	0.000	0.004	0.003
Green Grass	0.004	0.020	0.008	0.017	0.008	0.008	0.004	0.021	0.008	0.013	0.013	0.008
Musty	0.26	0.20	0.03	0.24	0.22	0.03	0.24	0.22	0.03	0.18	0.28	0.03
Medicinal	0.05	0.05	0.01	0.03	0.06	0.01	0.05	0.04	0.01	0.04	0.05	0.01
Petroleum	0.000	0.025	0.009	0.004	0.021	0.009	0.013	0.013	0.009	0.004	0.021	0.009
Smoked/ Charred Smoked	0.07	0.07	0.02	0.05	0.08	0.02	0.05	0.08	0.02	0.08	0.06	0.02
Wood	0.008	0.000	0.004	0.00	0.008	0.004	0.004	0.004	0.004	0.008	0.000	0.004
Dairy	0.004	0.000	0.003	0.000	0.004	0.003	0.004	0.000	0.003	0.004	0.000	0.003
Buttery	0.05	0.04	0.01	0.03	0.06	0.01	0.05	0.04	0.01	0.04	0.05	0.01
Cooked												
Milk	0.04	0.03	0.01	0.03	0.04	0.01	0.030	0.03	0.01	0.04	0.03	0.01
Sour Milk	0.09	0.09	0.03	0.15 ^a	0.04 ^b	0.03	0.06	0.12	0.03	0.05 ^b	0.13 ^a	0.03
Refrigerator												
Stale	0.03	0.06	0.01	0.04	0.05	0.01	0.04	0.05	0.01	0.03	0.06	0.01
Warmed												
over	0.54	0.59	0.06	0.65 ^a	0.48 ^b	0.06	0.55	0.58	0.06	0.51	0.62	0.06
Soapy	0.008	0.000	0.006	0.008	0.000	0.006	0.000	0.008	0.006	0.000	0.008	0.006
Painty	0.02	0.06	0.02	0.04	0.03	0.02	0.03	0.05	0.02	0.01 ^b	0.07 ^a	0.02
Fishy	0.05	0.04	0.02	0.06	0.03	0.02	0.05	0.05	0.02	0.03	0.07	0.02
Cardboardy	0.25	0.18	0.04	0.20	0.23	0.04	0.19	0.23	0.04	0.19	0.24	0.04

^xWDGS = Wet distillers grains with solubles

^{ab}Means within the same treatment and the same row with different superscripts are different ($P < 0.05$)

Running Title: Beef constituents impacting flavor ratings

**The use of biochemical constituents and beef flavor lexicon results to
predict consumer flavor ratings¹**

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Abstract

The objective of this study was to investigate the relationships of biochemical constituents to consumer flavor like ratings and specific flavor notes in two different muscles from cattle fed different diets. Biochemical constituents of the meat were separated into five distinct groupings- neutral lipid fatty acids, phospholipid fatty acids, minerals, amino acids, and composition (pH, moisture, fat, protein, ash, total carbohydrates, and cooking loss)- and analyzed using the principle component procedure. The first two principle components were used to determine the regression coefficients of the biochemical constituents for consumer flavor ratings and flavor notes. In *L. dorsi* steaks phospholipid principle component (PC) 1, mineral PC 2, and amino acid PC1 were related ($P < 0.15$) to overall flavor like, beefy flavor like, and grill flavor like scores while neutral lipid PC 1 was related ($P < 0.15$) to overall like, overall flavor like, grill flavor like, and grill flavor intensity scores. Mineral PC 2 and meat aging duration greatly influenced ($P < 0.15$) overall like, overall flavor like, beefy flavor like, and beefy flavor intensity in *B. femoris* steaks. City contributed ($P < 0.15$) to beefy flavor like, beefy flavor intensity, and grill flavor like. In *L. dorsi* steaks, lexicon traits browned (positive) and floral (negative) were significant ($P < 0.15$) factors influencing overall like, overall flavor like, beefy flavor like, beefy intensity like, grill flavor like, and grill flavor intensity. The lexicon trait burnt was a significant ($P < 0.15$) factor in all of the consumer sensory trait equations for *B. femoris* steaks with refrigerator stale and buttery contributing ($P < 0.15$) to overall like, overall flavor like, beefy flavor like, beefy flavor intensity, and grill flavor intensity. Lexicon traits listed were also related to the meat

components. For *L. dorsi* steaks, amino acid PC 1 influenced ($P < 0.15$) browned, liver, umami, overly sweet, salty, bitter, barnyard, heated oil, floral, and buttery. Interestingly, amino acid PC 1 was also a significant influence ($P < 0.15$) on a majority of the consumer panel traits. In contrast, mineral PC 2 of *B. femoris* steaks contributed to a majority of consumer traits but had no effect ($P > 0.15$) on any of the lexicon traits that were found to influence consumer scores. Neutral lipid PC 1, phospholipid PC 1, mineral PC 1, amino acid PC 1, and composition PC 1 did not influence ($P > 0.15$) any of the lexicon traits that related to consumer ratings. While biochemical constituents could significantly influence consumer flavor ratings and flavor notes, flavor notes had the strongest relationships with consumer flavor ratings.

Keywords: beef, beef lexicon, consumer scores, diet, regression coefficients

Introduction

Flavor and tenderness are the major determinants in eating pleasure when it comes to beef. Even the smallest change in sensory ratings, including flavor and tenderness, could greatly change consumers overall acceptance (Platter et al., 2003). Consumers' acceptance can then translate into increased profit. Feuz and Umberger (2001) found that consumers were willing to pay at least an additional \$1.30 per pound for a steak they thought had a good flavor compared to a less desirable steak.

There are many different biochemical constituents of meat that influence flavor. One way cooked meat gets its unique flavor is through the maillard reaction, which occurs when amino acid compounds react with carbonyl groups of reducing sugars in the

presence of heat, along with the degradation of fats while cooking (Mottram, 1998, Calkins and Hodgen, 2007). When cysteine, an amino acid, and ribose, a sugar/carbohydrate, react with each other under heating many aromatic volatiles are formed (Farmer et al., 1989) creating unique flavor and aromas. Larick and Turner (1990) and Melton et al. (1982) were able to identify specific FA that also promoted a desirable cooked beef flavor when their presence was increased, such as palmitoleic acid (C16:1) and oleic acid (C18:1).

Contrasting findings have occurred when minerals are studied. Some report an increase in iron content also increased livery off-flavor (Yancey et al., 2006), while others found the exact opposite (Jenschke et al., 2007). In addition, Meynier and Mottram (1995) reported different flavor notes were observed when pH changed. Aging of beef may also be responsible for some flavor differences. As meat ages, lipids (i.e. fatty acids (FA)) are oxidized, creating unique flavors. Smith et al. (1978) and Campo et al. (1999) reported that aging meat up to 11 d significantly ($P < 0.05$) increased flavor desirability.

This study investigated the relationship of biochemical constituents to consumer flavor ratings and specific flavor notes in two different muscles from cattle fed different diets. Following a simulated retail display, flavor differences were identified and the various biochemical components and flavor notes responsible for these differences were studied.

Materials and Methods

Diets

All protocols performed in this study were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. Cattle were fed in a 2x2x2 factorial design. Crossbred steers (n = 64) were backgrounded on either warm-season grasses (i.e. bluestem and switch grass) at the Barta Ranch in Western Nebraska or on cool-season (i.e. brome and bluegrass) pastures in Ithaca, NE for 177 d, shortly after weaning. Within each pasture, half of the cattle were supplemented with 0.6 kg WDGS/kg body weight/ day for energy. At the end of the grazing period, all cattle were transported to the University of Nebraska-Lincoln's research feedlot in Ithaca, NE. While in the feedlot, half of each pasture and supplementation treatments were finished on an all-corn diet while the other half were fed corn with WDGS at a 35% inclusion rate (DM basis). Cattle were on feed for 119 days and fed to an average live weight of 1,427 lbs.

Harvest

At the end of the feedlot period, cattle were transported and harvested at the Greater Omaha Packing (Omaha, NE). Forty-eight carcasses grading either USDA Choice (n = 43) or USDA Select (n = 5), 6 from each treatment combination, were selected. Strip loins (*Longissimus dorsi*; IMPS #180, NAMP, 2007) and bottom round flats (*Biceps femoris*; IMPS #171B, NAMP, 2007) were collected from each side of the carcass. Only ten *L. dorsi* muscles were collected from the cool-season grass, supplementation provided, and finished on WDGS treatment because two *L. dorsi*

muscles (one from each side) were lost within Greater Omaha Packing Plant. Subprimals from the left side of the carcass were aged under vacuum for 7 d while subprimals from the right side were aged under vacuum 28 d at 2°C. Upon fabrication, five steaks were cut from each subprimal.

Sample collection

The first steak, cut 1.25 cm thick, was used for all lab analyses. The second steak, also 1.25 cm thick, was used as a back-up for lab analyses. Both steaks were vacuumed packaged and frozen at -20°C for approximately 2 months. Before any lab procedures were conducted, all lab steaks had any subcutaneous fat and epimysial tissue removed and were cut into cubes. The cubes were flash frozen in liquid nitrogen, powdered using a Waring blender (Waring Commercial, model 51BL32, Torrington, CT), and stored at -80°C for several weeks until further lab analyses. All lab analyses were conducted on powdered samples. Fat, protein, ash, amino acid, mineral, and fatty acid analysis were only conducted on 7 d aged steaks while pH, moisture, non-heme iron, heme iron, and total carbohydrate analysis were conducted on both 7 and 28 d aged steaks.

The third steak, cut 2.54 cm thick, was placed on a Styrofoam tray, wrapped with PVC overwrap film, and placed under simulated retail display for 7 d for use by a beef lexicon panel. Steaks 4 and 5 were cut 2.54 cm thick, placed on a Styrofoam tray, wrapped with PVC overwrap film, and placed under simulated retail display at 2°C for 7 d. These two steaks were used for consumer panels. Strip loin steaks were packaged as two steaks per tray. Steaks on the same tray were from animals that received identical feeding treatments so as to prevent any possible contamination or influence. At the end

of retail display, steaks were vacuumed packaged and frozen at -20°C for two months until further use.

Retail Display

All of the trays were displayed on a table in a 2°C cooler and were constantly exposed to warm white fluorescence lighting (PHILIPS F32T8/TL741 ALTO 700 Series, 32 WATT B7, Royal Philips Electronics, Amsterdam, Netherlands) at 1000 to 1800 lux in order to simulate retail display conditions. Every day, packages were randomly relocated to minimize any effects due to location. After 7 days, steaks were vacuumed packaged and frozen until further analysis.

pH

To determine ultimate pH, duplicate 10 g powdered samples from each steak were homogenized with 90 mL of double distilled water using a Polytron homogenizer (POLYTRON Kinimatica CH-6010, Switzerland). The pH was determined using an Orion 4 STAR pH ISE Bench-top meter (Thermo Electron Corporation, Waltham, MA) calibrated using a 7.0 and 4.0 buffer. The pH probe was rinsed with double distilled water and wiped dry with a Kimwipe (Kimberly-Clark Professional, Roswell, GA) between every sample.

Proximate Analysis

Moisture and ash were measured using a LECO Thermogravimetric Analyzer (LECO Corporation, model 604-100-400, St. Joseph, MI) and fat was measured by ether extraction using the Soxhlet procedure (AOAC, 1990). Protein was determined by difference.

Total Carbohydrates

Samples were prepared by homogenizing 0.5 g of powdered meat with 20 mL of 80% ethanol in a 50 mL centrifuge tube in duplicates. Samples were stored in a 2°C cooler until further testing, at least one hour later. Upon analysis, tubes were centrifuged at 783 RCF (g) for 5 min. A 1 mL aliquot of sample containing <0.1 mg/mL of carbohydrate was removed and added to a new tube following the procedures of Dubois et al. (1956). To the new tubes, 50 µL of 80% phenol and 2.5 mL of concentrated sulfuric acid were added and vortexed immediately. After 10 min, samples were moved to a cool water bath for 10 to 25 min and read on a Cary 100 Varian UV/Visual Spectrophotometer (Varian Instruments, Sugarland, TX) at 490 nm.

Sugar concentrations were estimated using a standard curve. The curve was prepared by mixing a stock solution of 0.1 mg/mL glucose standard at varying concentrations (0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL, respectively) with varying amounts of double distilled water (1.0, 0.8, 0.6, 0.4, 0.2, and 0 mL, respectively). Standard samples were then prepared and read the same way as described above.

Non-heme Iron

The procedures described by Rhee and Ziprin (1987) were used to determine non-heme iron concentrations. Duplicate 5 g powdered samples were mixed with 0.2 mL of NaNO₂ solution (0.39% w/v) and 15 mL of 40% TCA-HCL (1:1) acid solution, vortexed, and placed in a water shaker bath set at 65°C for 20 h. After incubation, samples were allowed to cool to room temperature for 1 h.

Approximately 1 mL aliquots of the liquid phase were removed and mixed with 5 mL of a color reagent (20:20:1 double distilled deionized water:saturated sodium acetate solution:bathophenanthroline disulfonate reagent). To create a liquid phase without a color reagent blank, a 1mL aliquot of the liquid phase was mixed with 5 mL of a 21:20 double distilled deionized water:sodium acetate solution. Both a reagent blank and a liquid phase blank were created. All 4 mixtures were vortexed, centrifuged (Sorvall SE-12 rotor and Sorvall RC 5B centrifuge, Dupont Co., Wilmington, DE), and read at 540 nm using the spectrophotometer.

Readings were compared against a standard curve created using an iron stock standard (Sigma) mixed with the TCA-HCL acid solution at varying concentrations (0.5, 1.0, 1.5, 2.5, 3.5, and 4.5 $\mu\text{g/mL}$) to total 25 mL. Standard samples were then mixed with 5 mL of the color reagent, vortexed, centrifuged, and read at 540 nm.

Final absorbance of each sample was calculated by subtracting the absorbance of the incubated liquid phase without color reagent from the absorbance of the incubated liquid phase with color reagent. Next, final concentration was calculated by subtracting the intercept of the standard curve from the final absorbance and dividing it all by the slope of the standard curve. Finally, non-heme iron was calculated as follows:

$$\mu\text{g non-heme Fe/g meat} = \text{concentration } (\mu\text{g/mL}) \times \frac{(15+0.2+\text{moisture in 5g meat})}{5\text{g}} \times 1\text{mL}$$

Heme Iron

Samples were prepared following the procedures described by Hornsey (1956) as modified by Lee et al. (1998). Duplicate 2 g samples of powdered meat were mixed with 8.1 mL of acetone and 0.2 mL of hydrochloric acid. All tubes were kept in test tube trays

wrapped in aluminum foil to reduce light exposure. The sample was homogenized using a Polytron homogenizer at 10,800 rpm for 15 sec. Samples were immediately filtered through #2 Whatman filter paper (90 mm in diameter) and into a new tube which was also kept in a test tube rack wrapped in aluminum foil. The filtrate was immediately read on a spectrophotometer at 640 nm.

In order to determine total amount of heme iron, total pigment (mg/kg) was calculated by multiplying the absorbance of the sample by 680. Total heme iron (mg/kg) was calculated by multiplying the total pigment by 8.82 and dividing it all by 100.

Minerals and Amino Acids

Mineral composition was determined by Ward Laboratories, Inc. in Kearney, NE. Atomic absorption spectroscopy was used to quantify the minerals following the procedures of Ward and Gray (1994).

Amino acid composition was determined by AAA Service Laboratory, Inc. in Damascus, OR. A Hitachi L8900 Amino Acid Analyzer with post-column-ninhydrin derivatization was used to quantify amino acids following the procedures of Moore and Stein (1949), Roach and Gehrke (1970), Simpson et al. (1976), Stanford (1963), and Keutmann and Potts (1969).

Fatty Acids

Fats were extracted following the procedures of Folch et al. (1957). Four gram powdered meat samples were mixed with 10 mL of 2:1 chloroform:methanol solution, vortexed, and allowed to sit at room temperature for 1 h. Homogenized samples were filtered into new tubes, brought to 15 mL with 2:1 chloroform:methanol solution, mixed

with 2 mL of 0.74% KCl solution, vortexed, purged with nitrogen gas, and kept in a -20°C freezer overnight. The next day, the top aqueous phase was removed and 2 mL of the lower phase was collected and dried down at 60°C under constant nitrogen gas purging.

Samples were separated into neutral and phospholipid layers following the procedures described by Carr et al. (2005). The neutral and phospholipid regions of interest were isolated using thin layer chromatography plates (Silica Gel 60 w/o indicator, Catalog No.: M5547-7, Thermo Fisher Scientific Inc.) and isolated. The neutral lipid samples were submerged in chloroform and the phospholipid samples were submerged in methanol to extract the lipids. Samples were stored in a 2°C cooler for 45 min.

After incubation the solutions were dried at 60°C under constant nitrogen gas purging. Once dried, the fatty acid methyl esters were prepared following the procedures described by Morrison and Smith (1964) and Metcalfe et al. (1996). Gas chromatography (Hewlett-Packard Gas Chromatograph – Agilent Technologies, model 6890 series, Santa Clara, CA) was used to determine fatty acid content using a Chrompack CP-Sil 88 (0.25 mm x 100 m) column using Helium as the carrier gas with a flow rate of 1.1 mL/min. The injector temperature was held at 270°C and the detector temperature was 300°C. Fatty acids were identified by comparing retention times and peaks with known standards.

To get exact concentrations of each FA, additional thin layer chromatography plates were made separating the neutral and phospholipid layers. This time the plates were stained using iodine, and the areas on the plates were measured, as a percent, using

Quantity One 1-D Analysis Software (Bio-Rad, Hercules, CA). To calculate the mg/100 g of meat for each FA in each layer, the total fat percentages attained for each sample from proximate analysis was converted to grams of fat per 100 g of meat. That value was multiplied by the percentage of the neutral and phospholipid layers, and converted to mg of neutral or phospholipid per 100 g of meat. From there the percentage of each individual FA in each layer was multiplied by their respective value and the mg of each FA per 100 g of meat was attained.

Consumer Panel

All consumer and lexicon panels were approved by the Institutional Review Board and all panelists signed a consent form. Consumer panels were conducted in Houston, Texas and Olathe, Kansas (n = 120 per location). Consumers were recruited using existing consumer data banks and random phone solicitation. Consumers were selected that eat beef at least three times per week, range in age from 21 to 65, with an approximately equal balance of males and females, and a range in income.

In each city, consumer panels were conducted over two days, with the first day evaluating *Longissimus dorsi* steaks and the second day evaluating *Biceps femoris* steaks. Different consumers evaluated each muscle type. Steaks from each animal were evaluated at both locations. Panels were conducted with three sessions per day and 20 consumers per session. Five consumers evaluated each steak and treatment order was randomized and allocated to consumer using an incomplete block design. Each consumer evaluated eight steaks in a session.

Steaks were cooked on a Hamilton Beach Health Smart grill (model 31605A, Hamilton Beach/ Proctor-Silex, Inc., Southern Pines, NC) to an internal temperature of 70°C. Consumers evaluated each sample using 9-point hedonic (1=dislike extremely, 9=like extremely) and intensity scales (1=none or extremely bland, 9=extremely intense) for overall like, overall flavor like, beefy flavor like and intensity, and grilled flavor like and intensity.

Beef Flavor Lexicon

An expert, trained descriptive attribute sensory panel with over 23 cumulative years of experience in evaluating beef flavor and aromas was used. This panel was one of the three panels used to validate the Beef Lexicon at Texas A&M University (Philips et al., 2010; Miller, 2010). The panel underwent ballot development, training and validation sessions to assure consistent rating and identification of individual aroma and flavor attributes. Attributes were classified as major and minor notes. This provides a standardized, defined reference guide for determining and measuring aroma and flavor in beef.

During training and testing, steaks were cooked the same way as described for consumer panels. Aromas and flavor aromatics were evaluated using the Spectrum® Universal 16-point scale where 0 = none and 15 = extremely intense (Meilgaard et al., 2007). Traits evaluated were browned, bloody, fat, metal, liver, green hay, umami, overly sweet, sweet, sour, salty, bitter, sour aroma, barnyard, burnt, heated oil, chemical, apricot, asparagus, cumin, floral, beet, chocolate, green grass, musty, medicinal,

petroleum, smoked/charred, smoked wood, spoiled, dairy, buttery, cooked milk, sour milk, refrigerator stale, warmed over, soapy, painty, fishy, and cardboardy.

Statistical Analysis

All data were analyzed as a 2x2x2x2 factorial arrangement split plot design within a randomized complete design using the Mixed procedure in SAS (Version 9.2, SAS Institute Inc., Cary, NC, 2006). The experimental unit was the individual animal and the model included grass, supplementation, finishing diet, and age as fixed effects. The least square means procedure with the probability of difference (pdiff) option was used for mean separation; with significance determined at $P \leq 0.05$ levels. Whenever there was a three- or four-way interaction, the LSmeans were reanalyzed using the GLIMMIX procedure with the slicediff option in order to more accurately study differences.

In order to determine regression coefficients, the biochemical components of the meat were separated into five distinct groupings: neutral lipid fatty acids, phospholipid fatty acids, minerals, amino acids, and composition (pH, moisture, fat, protein, ash, total carbohydrates, and cooking loss). Each individual grouping was analyzed using the principle component procedure in SAS. The first two principle components were identified and associations for each were determined. Three different analysis were conducted to determine the regression coefficients of the principle components of the biochemical constituents for the consumer panel results, the principle components of the biochemical constituents for the lexicon results, and the lexicon results for the consumer panel results using the regression procedure.

Results and Discussion

Principle component identification

L. dorsi Steaks

For all principle components (PC), no identifiable associations or trends could be found for the third PC for any of the attributes. The first two PC were selected for the model, however, in some instances no identifiable associations or trends could be established for the second PC. In those instances, the second PC was not included. The first PC for both the neutral lipids (neutral lipid PC 1) and phospholipids (phospholipid PC 1) represented the weights/dimensions of each fatty acid (Appendix 1 and 2). No association could be determined for the second PC so they were not included in the regression models. The first PC accounted for 68% of the variation of neutral lipids and 36% of the variation for phospholipids.

Both PC for minerals had identifiable associations (Appendix 3). The first PC (mineral PC 1) represented the weights/dimensions of each mineral. All of the positive values in the second PC (mineral PC 2) tended to be associated with trace minerals while the negative values were minerals that were the most abundant in the human body. Both PC accounted for 41% of the variation.

The first PC for amino acids (amino acid PC 1) was associated with the weights/dimensions of each amino acid (Appendix 4). The second PC had an overwhelming association with methionine and no other amino acid. For this reason, methionine was used in the regression model instead of the second PC to avoid any unnecessary variance. The first two PC explained 93% of the variation.

The PC for the composition in *L. dorsis* steaks behaved a little differently (Appendix 5). The first PC (composition PC 1) was associated with the general composition of meat with moisture and fat having the largest values. The second PC (composition PC 2) was mostly associated with carbohydrates and pH. Water holding capacity, which is related to cook loss, and protein denaturation are affected by pH and their association with pH may explain why their values are negative just like pH values. Both of the PC's explain 44% of the variation.

B. femoris steaks

The PC's in *B. femoris* steaks behaved similarly to the PC in *L. dorsis* steaks. For neutral lipids, the first PC (neutral lipid PC 1) was associated with the general weights/dimensions of each fatty acid (Appendix 6). All of the positive values in the second PC (neutral lipid PC 2) were associated with long chain fatty acids, 18 carbons or more. The first two PC accounted for 65% of the variation.

Similar to the neutral lipids, the first PC's for phospholipids (phospholipid PC 1) was associated with the weights/dimensions of each fatty acid (Appendix 7). For the second PC (phospholipid PC 2), the largest values tended to be associated with odd chain fatty acids. About 46% of the variation was accounted for by the first two PC.

The first PC for minerals (mineral PC 1) explained the variation in most of the minerals except for iron (Appendix 8). In concurrence, the highest values in the second PC (mineral PC 2) were mostly for iron and its parts (heme and non-heme iron). The first two PC accounted for 50% of the variation.

Similar to *L. dorsi* steaks, the first two PC for amino acids in *B. femoris* steaks (Appendix 9) were associated with the weights/dimensions of each amino acids (amino acid PC 1) and methionine (amino acid PC 2). The effect of methionine in the second PC was not as strong as it was in *L. dorsi* steaks though, so the entire PC was still included in the model. These two PC explained 96% of the variation.

The first PC for composition (composition PC 1) was associated with moisture, ash, and protein (Appendix 10). The second PC (composition PC 2) was associated with the other constituents of meat, pH and carbohydrates. The first two PC explained 96% of the variation.

Biochemical factors influencing consumer panel traits

L. dorsi steaks

Prediction models were created for each consumer panel sensory trait using the principle components of meat. Table 1 shows the variables and coefficients included in each of the models for *L. dorsi* steaks. City had a large effect on several of the equations. This was not surprising as personal preferences vary greatly across different geographical locations. Phospholipid PC 1, mineral PC 2, and amino acid PC1 were related to overall flavor like, beefy flavor like, and grill flavor like scores. Also, neutral lipid PC 1 was related to overall like, overall flavor like, grill flavor like, and grill flavor intensity scores. In contrast, mineral PC 1, methionine, composition PC 2, and aging period did not significantly ($P > 0.15$) contribute to any of the equations.

The equation for overall flavor like was found to be significant ($P = 0.01$) with neutral lipid PC 1, mineral PC 2, amino acid PC 1 negatively ($P \leq 0.10$) influencing the

equation and phospholipid PC 1 positively ($P \leq 0.10$) influencing the equation. City was also associated ($P \leq 0.10$) with overall flavor like scores. Both neutral lipid PC 1 and amino acid PC 1 represented the general weights of all the variables in those components. The general neutral lipid and amino acid composition in the meat negatively affects overall like. Varnold (2013) previously showed that when cattle are supplemented, finishing on corn instead of WDGS increased overall flavor like scores (6.34 vs. 5.84, $P = 0.04$). In addition, finishing on corn lowered some neutral lipid concentrations, both when supplemented and not ($P \leq 0.05$). Since finishing on corn both decreased neutral lipid concentrations and increased overall flavor like scores, the regression coefficient was expected.

Phospholipid PC 1 positively ($P \leq 0.10$) influenced the equation for beefy flavor like while mineral PC 1 and amino acid PC 1 negatively ($P \leq 0.15$) influenced it. City was also found to influence ($P \leq 0.10$) beefy flavor like ratings.

Similar to overall flavor like, the equation for grill flavor like was significant ($P = 0.04$), with many of the same factors contributing to the equation. Neutral lipid PC 1, mineral PC 2, and amino acid PC 1 negatively ($P \leq 0.10$) influenced the equation for grill flavor like while phospholipid PC 1 and methionine positively ($P \leq 0.10$) influenced it. Here, mineral PC 2 became a contributor. Mineral PC 2 was associated with essential trace minerals. This means that grill flavor like is influenced by the presence and concentrations of trace minerals in the meat. Grill flavor like ratings were not affected by grass type (data not shown), but were affected by neutral lipid concentration. Grazing on warm-season grass caused significant ($P \leq 0.05$) decreases in many neutral lipids. Since

neutral lipids were such a major contributing factor in grill flavor like, grass type may have an underlying effect after all.

The equation for predicting overall like ratings was not significant ($P = 0.19$), but several variables contributing to the equation were. Neutral lipid PC 1, mineral PC2, and amino acid PC1 were all found to significantly ($P \leq 0.15$) contribute to the equation, however their contribution was minimal. It is interesting to note that all of the significant prediction coefficients negatively influence scores. The equations for beefy flavor intensity and grill flavor intensity were also not significant ($P \geq 0.34$), perhaps because city was the only significant ($P \leq 0.05$) prediction coefficient in the entire model. Grill flavor intensity had more significant ($P \leq 0.15$) prediction coefficients (neutral lipid PC 1, mineral PC 2, amino acid PC 1, and composition PC 1) but their contributions for the most part were negatively related to scores. It appears that grill flavor intensity can be influenced by moisture and fat content in meat.

B. femoris steaks

Unlike in *L. dorsi* steaks, none of the prediction equations for consumer scores for *B. femoris* steaks (Table 2) were found to be significant ($P > 0.05$), but beefy flavor like and grill flavor like had a tendency to be significant ($P = 0.07$ and 0.06). Mineral PC 2 and age greatly influenced ($P \leq 0.15$) overall like, overall flavor like, beefy flavor like, and beefy flavor intensity ratings. In *L. dorsi* steaks, aging did not contribute at all but now in *B. femoris* steaks it is one of the most important factors. City was found to contribute ($P \leq 0.15$) to beefy flavor like, beefy flavor intensity, and grill flavor like ratings. Also, amino acid PC 2 greatly influenced ($P \leq 0.15$) overall like, beefy flavor

like, and grill flavor like. In contrast, neutral lipid PC 1, neutral lipid PC 2, phospholipid PC 1, phospholipid PC 2, mineral PC 1, composition PC 1, and composition PC 2 had no effect ($P \geq 0.15$) on any of the equations.

For overall like ($P = 0.27$), mineral PC 2 and age positively ($P \leq 0.15$) influenced the equation while only amino acid PC 2 negatively influenced ($P \leq 0.10$) it. Similarly the equation for overall flavor like ($P = 0.46$) was positively influenced ($P \leq 0.05$) by both mineral PC 2 and age. Varnold (2013) previously reported that 28 d aged product had a higher heme and non-heme iron content ($P \leq 0.05$). Earlier it was shown that mineral PC 2 was associated with iron content.

Both of the equations for beefy flavor like ($P = 0.07$) and beefy flavor intensity ($P = 0.29$) ratings were positively ($P \leq 0.15$) influenced by mineral PC 2, and age while amino acid PC 2 negatively influenced ($P \leq 0.05$) the equation for beefy flavor like. City also influenced ($P \leq 0.15$) beefy flavor like and beefy flavor intensity ratings. Mineral PC 2 was associated with iron content while amino acid PC 2 was associated with methionine. In turn, beefy flavor like ratings are positively related to iron content and negatively related to methionine content. Again, both of those factors are influenced by aging, further justifying the importance of aging in consumer traits.

The equation for grill flavor like ratings had several contributing factors affecting it including positively influences ($P \leq 0.05$) due to mineral PC 2 and negative influences ($P \leq 0.10$) from amino acid PC 1 and 2. City was also found to influence ($P \leq 0.05$) grill flavor like ratings. In contrast, the equation for grill flavor intensity ($P = 0.95$) had no factors that significantly ($P \geq 0.15$) contributed to it.

*Lexicon traits and consumer panel traits**L. dorsi* steaks

The beef lexicon panel at Texas A&M University in College Station, TX was used to characterize the flavor profile in both *L. dorsi* and *B. femoris* steaks. Those results were then used to create models that could be used to predict consumer panel scores. Table 3 shows the lexicon flavor traits and their coefficients that can be used to create the models for *L. dorsi* steaks. The lexicon traits browned and floral appear to be the major significant ($P \leq 0.15$) factors influencing overall like, overall flavor like, beefy flavor like, beefy intensity like, grill flavor like, and grill flavor intensity ratings. Also, overly sweet, and spoiled were found to influence ($P \leq 0.15$) overall flavor like, beefy flavor like, beefy intensity like ratings. City also influenced ($P \leq 0.15$) overall flavor like, beefy flavor like, beefy intensity like ratings. While developing a beef lexicon, Maughan (2011) also found browned flavor to be highly correlated to consumer like ratings. In contrast, the lexicon traits metal, sour, salty, barnyard, burnt, heated oil, chemical, cooked milk, sour milk, painty, and cardboard did not significantly ($P \geq 0.15$) influence any of the equations.

The equation for overall flavor like ratings ($P = 0.01$) was found to be positively influenced ($P \leq 0.15$) by browned, green hay, sweet, green grass, and buttery lexicon traits and was negatively influenced ($P \leq 0.15$) by overly sweet, floral, smoked/charred, spoiled, and warmed over lexicon traits. Overall flavor like ratings were also influenced by city ($P \leq 0.15$). The lexicon traits green hay, overly sweet, floral, green grass, smoked/charred, spoiled, and buttery appeared to be the strongest contributors to the

equation, with prediction coefficients greater than or equal to 1 for all of them. Browned, sweet, and buttery are often associated as being desirable flavors, which corresponds to their positive influence on overall flavor like scores. In congruence, overly sweet, floral, smoked/charred, spoiled, and warmed over were negatively associated with overall flavor like scores and they are also known to be undesirable flavors in meat.

The equation for beefy flavor like ratings was also significant ($P = 0.005$) with the lexicon traits browned, sour aroma, green grass, and musty all positively influencing ($P \leq 0.15$) the equation while the traits overly sweet, floral, smoked/charred, spoiled, refrigerator stale, and warmed over, all of which are undesirable flavors in beef, negatively influenced ($P \leq 0.05$) it. Beefy flavor like ratings were also influenced by city ($P \leq 0.15$). Again, overly sweet, sour aroma, floral, green grass, smoked/charred, spoiled, and refrigerator stale all had prediction coefficients greater than or equal to 1. Varnold (2013) showed that when cattle are supplemented, finishing on corn will cause higher consumer ratings for beefy flavor like than when cattle were finished on WDGS (6.43 vs. 5.91, $P \leq 0.05$). Refrigerator stale, one of the factors that had a large prediction coefficient, was also affected by an interaction between supplementation and diet ($P \leq 0.05$) with finishing on corn creating lower concentrations than finishing on WDGS when no supplementation is provided (0.00 vs. 0.07). Since finishing on corn lowered levels of refrigerator stale flavor, so finishing on corn would also increase beefy flavor like scores, hence the negative association. Melton (1990) also found a difference in flavor liking ratings between finishing cattle on corn and corn silage, which would be similar to WDGS.

All three of the equations described above accounted for 28% or more of the differences found between scores. The equations for overall like, beefy flavor intensity, grill flavor like, and grill flavor intensity were not significant ($P \geq 0.09$). Several of the equations were influenced by the green grass trait.

B. femoris steaks

Table 4 shows the lexicon flavor traits and their coefficients that can be used to create the models for *B. femoris* steaks. The lexicon traits burnt was a significant ($P \leq 0.15$) factor in all of the consumer sensory trait equations with refrigerator stale and buttery contributing ($P \leq 0.15$) to overall like, overall flavor like, beefy flavor like, beefy flavor intensity, and grill flavor intensity ratings. Umami and overly sweet influenced ($P \leq 0.15$) overall like, overall flavor like, beefy flavor like, and grill flavor like scores while browned and sweet contributed ($P \leq 0.15$) to overall like, overall flavor like, and grill flavor intensity. In addition, city significantly ($P \leq 0.10$) influenced beefy flavor like, beefy flavor intensity, and grill flavor like. In contrast, the lexicon traits bloody, fat, metal, liver, sour, salty, bitter, sour aroma, heated oil, chemical, beet, green grass, petroleum, smoked/charred, cooked milk, sour milk, warmed over, soapy, fishy, and cardboardy as well as the factor of age did not significantly ($P \geq 0.15$) influence any of the equations.

The equations for overall like, beefy flavor like, beefy flavor intensity, grill flavor like, and grill flavor intensity ratings were all not significant ($P > 0.05$). Only the equation for overall flavor like was significant ($P = 0.04$) with the traits umami, sweet, burnt, smoked wood, buttery, and refrigerator stale positively influencing ($P \leq 0.05$) the

equation and browned, overly sweet, musty, and medicinal negatively influencing ($P \leq 0.15$) it. Even though aging was not a contributing factor in any of the equations, previous research by Varnold (2013) showed that it can affect both consumer and lexicon traits. In the four-way interaction between grass type, supplementation, finishing diet, and aging period ($P = 0.01$), aging the meat 28 d caused numerically lower overall flavor like scores in most of the dietary combinations. In addition, aging had a tendency ($P = 0.06$) to increase the prevalence of a musty flavor (0.13 vs 0.08), which was a negative contributor to overall flavor like scores in this study. Clearly, aging plays a role in desirability of beef.

The traits overly sweet, sweet, burnt, medicinal, smoked wood, buttery, and refrigerator stale all had large regression coefficients (> 1.00), which suggests these traits may be the most important in predicting consumer overall flavor like ratings. Of those traits, sweet, smoked wood, and buttery are often associated with desirable flavors so it makes sense that they positively influenced consumer traits. Overly sweet and medicinal had large coefficients showing that they greatly negatively influence consumer traits.

Biochemical constituents influence on the flavor notes that determine consumer acceptance

L. dorsi steaks

All of the lexicon traits can also be related to the meat components, just like the consumer panel traits (Table 5). Overall, amino acid PC 1 influenced ($P \leq 0.15$) many of the lexicon traits. It was previously noted that amino acid PC 1 was a significant influence ($P \leq 0.15$) on 5 of the 7 consumer panel traits. Clearly there is a link between

amino acid content and flavor. Also, amino acid PC 1 was the only meat component to influence several lexicon traits including having a positive influence ($P \leq 0.15$) on floral (0.004) and a negative influence ($P \leq 0.15$) on both browned and buttery.

Neutral lipid PC 1 was found to positively influence ($P \leq 0.10$) umami, smoked wood, warmed over, and fishy lexicon traits (0.04, 0.005, 0.02, and 0.01, respectively) while smoked/charred was negatively influenced ($P \leq 0.15$) by it (-0.003). For consumer panel scores neutral lipid PC 1 was found to influence four of the scores, perhaps that influence is due to these lexicon traits. In connection, feeding WDGS was found to increase the prevalence of warmed over flavor as well as increase several neutral lipid fatty acids (Varnold, 2013). Since neutral lipid PC 1, which represented a general distribution of fatty acids, was negatively associated with most consumer traits, it can be concluded that finishing diet can have an effect on flavor. Mandell et al. (1998) also found that increased concentrations of fatty acids, specifically C18:3 and C20:4, could cause undesirable flavors.

Phospholipid PC 1 was found previously to influence three of the consumer scores, and it also positively influences ($P \leq 0.05$) fat, umami, and overly sweet traits (0.04, 0.05, and 0.02, respectively) while negatively influencing ($P \leq 0.15$) bitter and smoked/charred (-0.02 and -0.003).

Mineral PC 1 only influenced ($P \leq 0.15$) liver notes. Mineral PC 1 did not have any influence over any of the consumer traits. Mineral PC 2 however was a significant contributor ($P \leq 0.15$) to four of the consumer traits discussed previously, and likewise it contributed to several lexicon traits as well. Mineral PC 2 positively influenced ($P \leq$

0.15) liver, smoked wood, and warmed over traits (0.03, 0.01, and 0.06, respectively) and negatively influenced ($P \leq 0.10$) green hay and smoked/charred (-0.01 for both). Varnold (2013) found that not only did grass type affect liver flavor prevalence, it also altered several of the minerals in the meat. Even though grass type did not influence any of the consumer scores, it clearly has an influence over the factors that do. Melton et al. (1982) found that ground beef samples from grass-fed or limit grain fed steers had a noticeable undesirable flavor notes. In addition, Larick and Turner (1990) also observed that grazing on different types of grasses increased and decreased the prevalence of specific flavor notes. Perhaps samples in the present study were affected by undesirable flavor notes because of their grazing background.

Amino acid PC 1 positively influenced ($P \leq 0.05$) liver (0.02) and negatively influences bloody, umami, overly sweet, and bitter lexicon traits (-0.02, -0.02, -0.01, and -0.01, respectively). In addition, the amino acid methionine positively influenced ($P \leq 0.05$) medicinal and warmed over lexicon traits (0.01 and 0.07, respectively) and negatively influenced ($P \leq 0.05$) smoked/charred flavors (-0.01). Methionine contributed to grill flavor like ratings.

Composition PC 1 only contributed to grill flavor intensity scores and composition PC 2 had no contribution in any of the consumer trait equations, but both components had fairly large prediction coefficients for several lexicon traits. This may mean that its effects have a stronger background contribution than primary contribution to flavor. Composition PC 1 positively influenced ($P \leq 0.15$) bloody, liver, warmed over, and fishy lexicon traits (0.12, 0.07, 0.06, and 0.01, respectively) and negatively affected

($P \leq 0.10$) smoked/charred traits (-0.01). Similarly, composition PC 2 positively influenced ($P \leq 0.10$) the lexicon traits musty, medicinal, and warmed over (0.04, 0.01, and 0.04, respectively) but negatively influenced ($P \leq 0.05$) bloody (-0.13). Grass type was found to greatly change ($P \leq 0.05$) the prevalence of both liver and bloody flavors in meat, as well as the moisture content (Varnold, 2013). Since one of the main factors associated with composition PC 1 was moisture, it can be concluded that the composition of meat as affected by grass type can influence flavor. In this study, neither neutral lipid PC 1 nor phospholipid PC 1 influenced liver flavors. This is in contrast to Calkins and Hodgen (2007) who found that several fatty acids were strongly correlated to livery flavors in beef.

Even though aging was found to positively influence ($P \leq 0.15$) medicinal traits (0.02) and negatively influence ($P \leq 0.10$) both smoked/charred and warmed over (-0.02 and -0.09, respectively) it had no effect on any of the consumer trait prediction equations discussed previously. Green grass, spoiled, and refrigerator stale were unaffected ($P \geq 0.15$) by any of the meat components or the factor age.

B. femoris steaks

The results for *B. femoris* steaks (Table 6) were completely different from those found in *L. dorsi* steaks. Mineral PC 2 was previously found to be a major contributor in 5 of the 7 consumer trait prediction equations discussed earlier. It has no effect ($P \geq 0.15$) on any of the lexicon traits that were previously found to influence consumer scores. Similarly, neutral lipid PC 1, phospholipid PC 1, mineral PC 1, amino acid PC 1, and composition PC 1 did not influence ($P \geq 0.15$) any of the lexicon traits that were

previously found to influence consumer scores. In contrast, both neutral lipid PC 1 and phospholipid PC 1 were significantly associated ($P \leq 0.05$) with the green grass trait. Larick et al. (1987) also found that several fatty acids were strongly correlated to grassy flavor in ground beef.

Neutral lipid PC 2 was found to positively influence ($P \leq 0.10$) the lexicon trait medicinal (0.01) and negatively influence ($P \leq 0.15$) overly sweet, smoked wood, and buttery (-0.03, -0.005, and -0.01, respectively). Even though their contributions were significant, they were still minimal as can be seen in their small prediction coefficients. Phospholipid PC 2 positively influenced ($P \leq 0.10$) browned and umami characteristics (0.05 and 0.04, respectively) and negatively influenced ($P \leq 0.10$) medicinal (-0.01). It is interesting to note that even though both the neutral lipid PC 2 and phospholipid PC 2 contributed to several lexicon traits, they had no effect on any of the consumer traits. Grass type altered a majority of the long chain fatty acids (up to 18 C) in the neutral lipid layer, with warm-season grass causing significant decreases in concentration ($P \leq 0.05$).

Amino acid PC 2 only negatively influenced ($P \leq 0.10$) the lexicon trait browned (-0.12) and three of the consumer traits. Composition PC 2 also negatively influenced ($P \leq 0.10$) both browned and umami (-0.11 and -0.06, respectively). Since the regression coefficient for browned (-0.11) is so large, it can be assumed that composition PC 2 plays a large role in its occurrence. Grass type has been found to affect ($P \leq 0.05$) the prevalence of a browned flavor (Varnold, 2013).

Aging positively influenced ($P \leq 0.15$) overly sweet and sweet characteristics (0.09 and 0.07, respectively) and negatively influenced ($P \leq 0.10$) burnt, musty, and

refrigerator stale prevalence (-0.08, -0.09, and -0.04, respectively). Aging also contributed to 5 of the 7 consumer trait prediction equations discussed earlier.

In *L. dorsi* steaks neutral and phospholipids, mineral, and amino acid content greatly determined consumer panel scores. Specific lexicon traits such as browned and warmed over can also greatly affect consumer panel traits. In addition, several lexicon traits, i.e. umami and warmed over, and biochemical components, i.e. neutral lipids and amino acids, were also related to each other. All three different traits (components, consumer panel, and lexicon) were all found to be influenced by diet, specifically grass type. Aging was also a major contributing factor in several of the different consumer flavor scores and flavor notes.

Mineral and amino acid content, as well as aging greatly contributed to several consumer panel traits in *B. femoris* steaks. Specific lexicon traits such as browned and umami also influenced consumer panel traits. In addition, lexicon traits like browned and umami, and biochemical components like phospholipids and amino acids, were also related to each other. All three different traits (components, consumer panel, and lexicon) were all found to be influenced by production factors such as aging.

Even though significant influences were found between the meat PC and consumer panel scores, the regression coefficients were small. The significant regression coefficients were also small for the meat PC and lexicon flavor notes. Several regression coefficients between the lexicon traits and the consumer panel scores were not only significant, but also large. Clearly, individual flavor notes are good predictors of consumer acceptability. A majority of the lexicon flavor notes were shown to be altered

by both diet and aging. Through diet formulations and proper aging periods the flavor notes can be altered to such a degree that they can create a product that is highly acceptable to consumers.

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Tables

Table 1. Regression coefficients for the influence of meat principle components on consumer panel scores for *L. dorsi* steaks.

Principle Components	Consumer Panel Traits					
	Overall Like	Overall Flavor Like	Beefy Flavor Like	Beefy Flavor Intensity	Grill Flavor Like	Grill Flavor Intensity
Intercept	5.94***	5.72***	5.73***	5.66***	5.52***	5.30***
Neutral Lipid PC 1	-0.06*	-0.07**	-0.05	-0.05	-0.07**	-0.09***
Phospholipid PC 1	0.03	0.08***	0.05**	0.02	0.06**	-0.002
Mineral PC 1	0.04	0.02	0.05	0.03	0.01	0.04
Mineral PC 2	-0.09**	-0.09**	-0.12***	-0.07	-0.10***	-0.09*
Amino Acid PC 1	-0.04***	-0.05***	-0.03*	-0.02	-0.04***	-0.03*
Methionine	0.06	0.07	0.06	0.02	0.09**	0.05
Composition PC 1	-0.11	-0.08	-0.09	-0.10	-0.08	-0.19**
Composition PC 2	0.07	0.07	0.07	0.03	0.07	0.05
Age	-0.004	0.01	0.06	0.10	-0.14	-0.11
City	0.13	0.24**	0.38***	0.31***	0.13	-0.09
<i>P</i> -value	0.19	0.01	0.003	0.46	0.04	0.34
R^2	0.07	0.12	0.14	0.05	0.10	0.06

*** $P \leq 0.05$

** $P \leq 0.10$

* $P \leq 0.15$

Table 2. Regression coefficients for the influence of meat principle components on consumer panel scores for *B. femoris* steaks.

Principle Components	Consumer Panel Traits					
	Overall Like	Overall Flavor Like	Beefy Flavor Like	Beefy Flavor Intensity	Grill Flavor Like	Grill Flavor Intensity
Intercept	5.51***	5.53***	5.61***	5.50***	5.33***	5.06***
Neutral Lipid PC 1	-0.01	-0.03	0.02	-0.04	0.06	0.03
Neutral Lipid PC 2	0.03	0.03	0.08	0.02	0.04	0.01
Phospholipid PC 1	0.01	-0.002	-0.004	-0.06	0.07	0.06
Phospholipid PC 2	0.03	0.01	0.04	0.005	0.03	-0.04
Mineral PC 1	0.01	0.01	0.004	0.04	-0.02	0.003
Mineral PC 2	0.15***	0.13***	0.16***	0.10*	0.16***	0.05
Amino Acid PC 1	-0.03	-0.03	-0.01	0.002	-0.04**	-0.02
Amino Acid PC 2	-0.21**	-0.12	-0.23***	-0.15	-0.20**	0.004
Composition PC 1	-0.07	-0.11	0.01	-0.19	0.15	0.08
Composition PC 2	0.01	0.02	-0.02	-0.01	0.11	0.09
Age	0.28*	0.25*	0.30**	0.34***	0.10	-0.05
City	0.22	0.18	0.34***	0.29**	0.41***	0.24
<i>P</i> -value	0.27	0.46	0.07	0.29	0.06	0.95
<i>R</i> ²	0.08	0.06	0.10	0.07	0.11	0.03

*** $P \leq 0.05$ ** $P \leq 0.10$ * $P \leq 0.15$

Table 3. Regression coefficients for the influence of beef lexicon traits on consumer panel scores for *L. dorsi* steaks.

Beef Lexicon Traits	Consumer Panel Trait						Mean Score
	Overall Like	Overall Flavor Like	Beefy Flavor Like	Beefy Flavor Intensity	Grill Flavor Like	Grill Flavor Intensity	
Intercept	6.80***	5.96***	6.96***	6.54***	5.48***	5.70***	
Brown	0.40*	0.62***	0.59***	0.46*	0.70***	0.71***	1.82
Bloody	0.16	0.31	0.23	0.37	0.56***	0.55**	1.62
Fat	0.51**	0.41	0.30	0.15	-0.13	-0.18	1.71
Metal	-0.60	-0.41	-0.55	-0.31	-0.03	-0.16	1.71
Liver	0.06	0.03	0.15	0.07	-0.31	-0.14	0.16
Green Hay	1.49*	1.70**	1.06	0.76	-0.08	-0.10	0.02
Umami	-0.48**	-0.34	-0.22	-0.32	-0.14	-0.41	1.04
Overly Sweet	-0.79	-1.00**	-1.00**	-0.95*	-0.75	-1.23***	1.00
Sweet	0.49	0.97***	0.55	1.10***	0.79**	1.32***	1.01
Sour	-0.25	-0.30	-0.39	-0.55	-0.27	-0.53	1.35
Salty	-0.49	-0.66	-0.60	-0.49	-0.36	-0.001	1.38
Bitter	0.43	0.17	0.20	-0.08	-0.24	-0.38	1.11
Sour Aroma	7.49***	4.50	5.67*	3.31	2.07	2.29	0.002
Barnyard	-1.09	-1.73	-1.67	-1.66	-1.88*	-1.96	0.02
Burnt	0.20	-0.17	-0.08	0.22	-0.08	0.18	0.11
Heated Oil	0.39	0.09	0.03	-0.67	-0.06	0.10	0.06
Chemical	-0.15	0.06	-0.20	-0.35	-0.18	-0.39	0.13
Floral	-2.89***	-2.89***	-2.85***	-3.69***	-2.04**	-2.36***	0.01
Green Grass	6.14*	5.75*	6.35*	5.50	-0.44	1.26	0.002
Musty	0.63*	0.43	0.62*	0.23	0.43	0.58	0.10
Medicinal	0.09	1.21	-0.12	-0.58	1.30	0.99	0.02
Smoked/Charred	-4.38***	-4.79***	-3.67**	-2.09	-0.55	-0.46	0.01
Smoked Wood	-0.23	-0.94	-0.89	0.90	-1.84	-2.57	0.01
Spoiled	-3.07**	-2.68*	-3.42***	-2.75*	-2.27	-2.46	0.004
Buttery	0.47	1.08**	0.42	0.41	0.50	-0.21	0.09
Cooked Milk	-1.54	-0.78	-0.92	-0.58	-0.80	-0.03	0.06
Sour Milk	-0.13	0.04	-0.41	-0.13	-0.61	-0.04	0.05
Refrigerator							
Stale	-0.94	-0.89	-1.34***	-0.89	-0.73	-0.98	0.04
Warmed over	-0.58**	-0.53*	-0.71***	-0.31	-0.53*	-0.39	0.14
Painty	0.48	3.42	1.67	1.49	0.95	1.72	0.002
Fishy	-0.74	-1.77	-1.60	-1.28	-1.05	-2.45*	0.02
Cardboardy	-0.13	0.30	0.45	0.10	-0.01	0.02	0.14
Age	-0.15	-0.18	-0.07	-0.10	-0.33***	-0.32**	
City	0.11	0.22**	0.35***	0.30***	0.12	-0.10	
Mean	6.20	6.09	6.19	5.93	5.82	5.36	
P-Value	0.09	0.01	0.005	0.14	0.09	0.18	
R ²	0.24	0.28	0.30	0.23	0.24	0.22	

*** $P \leq 0.05$ ** $P \leq 0.10$ * $P \leq 0.15$

Table 4. Regression coefficients for the influence of beef lexicon traits on consumer panel scores for *B. femoris* steaks.

Beef Lexicon Traits	Consumer Panel Trait						Mean Score
	Overall Like	Overall Flavor Like	Beefy Flavor Like	Beefy Flavor Intensity	Grill Flavor Like	Grill Flavor Intensity	
Intercept	8.22***	8.39***	7.44***	7.08***	5.31***	5.86***	
Brown	-0.72*	-0.99***	-0.73**	-0.34	-0.41	-0.88***	1.75
Bloody	-0.12	-0.06	0.09	0.21	0.20	-0.12	1.69
Fat	-0.21	-0.28	-0.05	-0.17	0.19	-0.28	1.72
Metal	-0.18	-0.23	-0.44	0.04	0.08	0.16	1.96
Liver	0.33	0.42	0.11	-0.11	-0.03	0.21	0.28
Green Hay	0.04	0.06	0.57	0.74	-1.52	-2.16**	0.02
Umami	0.77*	0.93***	0.75*	0.49	0.67*	1.01***	0.76
Overly Sweet	-0.93*	-1.05**	-0.86*	-0.77	-0.78*	-0.73	0.91
Sweet	1.17**	1.33***	0.78	0.43	1.04**	1.38***	0.87
Sour	-0.68	-0.49	-0.14	-0.08	-0.10	0.13	1.45
Salty	-0.36	-0.31	-0.15	-0.45	-0.63	-0.92	1.39
Bitter	-0.10	-0.12	-0.0005	-0.37	-0.10	0.27	1.41
Sour Aroma	-1.29	-0.23	1.08	0.83	0.80	1.30	0.008
Barnyard	1.06	1.51	1.38	0.96	1.04	2.09*	0.03
Burnt	1.10*	1.37***	0.98*	1.14**	1.22***	1.44***	0.18
Heated Oil	0.37	0.19	-0.18	0.29	-0.16	-0.53	0.11
Chemical	-0.27	-0.41	-0.59	-0.12	-0.26	-0.12	0.17
Beet	-1.87	-1.17	0.58	2.68	0.75	-1.33	0.002
Green Grass	0.52	0.25	0.14	-0.44	1.10	0.56	0.01
Musty	-0.45	-0.80**	-0.68*	-0.54	-0.38	-1.02***	0.23
Medicinal	-1.44	-1.80*	-1.55*	-0.70	-1.16	-0.48	0.05
Petroleum	-0.63	-0.10	-0.52	-1.85	-0.19	0.89	0.01
Smoked/Charred	0.46	0.57	0.03	-0.63	0.30	1.18	0.07
Smoked Wood	3.69	5.80**	3.40	3.70	2.20	6.31**	0.004
Dairy	7.68*	6.72	4.86	-0.96	4.05	8.06*	0.002
Buttery	2.36**	2.47***	2.13**	2.33**	1.44	1.91*	0.04
Cooked Milk	0.62	0.29	0.84	0.78	-0.17	0.35	0.03
Sour Milk	-0.37	-0.47	-0.62	-0.08	0.19	0.72	0.09
Refrigerator							
Stale	1.73*	2.23***	2.38***	1.84**	1.56*	2.35***	0.04
Warmed over	0.04	0.13	0.17	-0.13	0.25	0.17	0.56
Soapy	-2.91	-2.43	-2.01	-3.03	-2.02	-1.63	0.004
Painty	1.06	0.87	0.32	0.58	1.19	2.16***	0.04
Fishy	0.18	0.06	0.14	0.39	-0.01	-0.57	0.05
Cardboardy	-0.06	-0.07	0.08	-0.38	-0.10	-0.36	0.21
Age	0.13	0.08	0.22	0.34	0.16	0.04	
City	0.22	0.18	0.34***	0.29**	0.41***	0.24	
Mean	5.75	5.74	5.92	5.78	5.59	5.15	
P-Value	0.19	0.04	0.08	0.22	0.25	0.15	
R ²	0.22	0.26	0.25	0.22	0.21	0.23	

*** $P \leq 0.05$ ** $P \leq 0.10$ * $P \leq 0.15$

Table 5. Regression coefficients for the influence of principle components on beef lexicon traits for *L. dorsi* steaks.

Beef Lexicon Trait	Principle Components										P-value	R ²
	Intercept	Neutral Lipid PC 1	Phospholipid PC 1	Mineral PC 1	Mineral PC 2	Amino Acid PC 1	Methionine	Other PC 1	Other PC 2	Age		
Brown	1.88***	0.01	0.02	0.03	-0.01	-0.02**	-0.001	-0.07	0.06	-0.10	0.004	0.25
Bloody	1.52***	0.02	0.02	0.004	-0.02	-0.02*	0.01	0.12***	-0.13***	0.10	0.001	0.29
Fat	1.79***	0.01	0.04***	0.0001	0.01	-0.01	-0.01	0.004	-0.01	-0.05	0.09	0.16
Metal	1.64***	0.01	0.004	-0.01	0.02	-0.01	0.01	0.06**	-0.05***	0.05	0.04	0.19
Liver	0.29***	0.01	0.004	0.03*	0.03*	0.02***	-0.03	0.07**	-0.02	-0.05	0.03	0.19
Green Hay	0.03	-0.002	-0.004	0.002	-0.01**	-0.002	-0.0004	-0.01	-0.01	-0.01	0.69	0.07
Umami	1.13***	0.04***	0.05***	-0.03	-0.03	-0.02**	-0.01	0.03	-0.01	-0.10	0.0002	0.31
Overly												
Sweet	1.04***	-0.003	0.02***	-0.001	-0.01	-0.01**	-0.01	-0.02	0.02	0.02	0.02	0.21
Sweet	1.04***	0.002	0.01	0.01	-0.02	-0.004	-0.01	-0.05	0.01	0.05	0.03	0.20
Sour	1.31***	-0.01	-0.001	-0.01	-0.02	0.002	0.01	0.03	-0.03*	0.02	0.005	0.24
Salty	1.42***	0.01	0.02**	-0.02**	0.01	-0.01***	-0.001	-0.02	-0.02	-0.08**	0.0002	0.31
Bitter	1.13***	-0.0002	-0.02*	-0.02	0.01	-0.01*	0.005	0.01	-0.02	-0.06	0.08	0.17
Sour Aroma	0.005	-0.001	0.00002	0.0001	0.001	0.0001	-0.0001	-0.001	0.001	-0.004	0.99	0.03
Barnyard	0.02	-0.004	-0.001	0.005	-0.001	-0.004***	-0.002	-0.01	0.002	0.01	0.49	0.09
Burnt	0.08*	0.003	-0.004	-0.002	-0.02	-0.002	0.0004	-0.01	0.03*	0.05	0.41	0.10
Heated Oil	0.03	-0.001	0.003	-0.01	0.01	-0.01**	0.01	-0.003	0.02	-0.002	0.67	0.08

*** $P \leq 0.05$

** $P \leq 0.10$

* $P \leq 0.15$

Table 5 cont. Regression coefficients for the influence of principle components on beef lexicon traits for *L. dorsi* steaks.

Beef Lexicon Trait	Principle Components										P-value	R ²
	Intercept	Neutral Lipid PC 1	Phospholipid PC 1	Mineral PC 1	Mineral PC 2	Amino Acid PC 1	Methionine	Other PC 1	Other PC 2	Age		
Chemical	0.10***	0.01	-0.01*	-0.01	0.02*	0.004	0.01	0.01	0.005	-0.03	0.44	0.10
Floral	0.02	-0.001	0.004	0.001	0.01	0.004*	-0.0004	0.01	0.0001	-0.01	0.83	0.06
Green Grass	-0.01	0.0002	-0.0004	0.001	0.001	0.0001	0.002	0.0003	0.002	0.004	0.86	0.05
Musty	0.06	-0.01	-0.01	-0.002	0.01	-0.01	0.02	-0.003	0.04**	-0.05	0.35	0.11
Medicinal	-0.03*	0.002	0.0001	-0.002	0.01	-0.001	0.01***	0.01	0.01***	0.02*	0.18	0.14
Smoked/Charred	0.04***	-0.003*	-0.003**	0.002	-0.01**	0.001	-0.01***	-0.01**	0.001	-0.02**	0.04	0.18
Smoked Wood	0.01	0.005***	0.002	-0.003	0.01***	0.002	-0.001	0.002	-0.002	-0.001	0.001	0.28
Spoiled	0.02*	0.001	-0.001	-0.001	0.003	0.0003	-0.003	-0.00003	-0.004	-0.01	0.81	0.06
Buttery	0.11***	0.01	0.01	-0.01	0.002	-0.01*	-0.01	0.0003	-0.0002	0.01	0.20	0.13
Cooked Milk	0.01	-0.002	-0.0004	-0.01**	0.001	-0.001	-0.001	-0.004	-0.01	-0.004	0.49	0.09
Sour Milk	0.13***	-0.003	0.01	-0.01*	-0.02***	-0.004	-0.02***	-0.002	-0.04***	-0.02	0.05	0.18
Refrigerator												
Stale	0.03	0.005	-0.01	-0.003	0.004	0.002	0.01	0.01	0.005	-0.02	0.91	0.05
Warmed over	-0.05	0.02**	-0.01	-0.01	0.06***	0.001	0.07***	0.06**	0.04***	-0.09***	0.001	0.29
Painty	-0.001	0.0004	0.001	-0.001	-0.001	-0.001	0.001	0.003	-0.001	-0.004	0.79	0.06
Fishy	0.004	0.01***	-0.005	0.001	0.003	0.001	0.01	0.01*	0.004	-0.02	0.10	0.16
Cardboardy	0.06	0.01	-0.01	0.003	-0.01	0.0005	0.03***	-0.005	0.02	-0.04	0.31	0.12

*** $P \leq 0.05$

** $P \leq 0.10$

* $P \leq 0.15$

Table 6. Regression coefficients for the influence of principle components on beef lexicon traits for *B. femoris* steaks.

Beef Lexicon Trait	Principle Components												Age	P-value	R ²
	Intercept	Neutral Lipid PC 1	Neutral Lipid PC 2	Phospholipid PC 1	Phospholipid PC 2	Mineral PC 1	Mineral PC 2	Amino Acid PC 1	Amino Acid PC 2	Other PC 1	Other PC 2				
Brown	1.80***	-0.05	0.002	0.002	0.05**	-0.01	-0.02	-0.01	-0.12**	-0.07	-0.11***	-0.10	0.16	0.16	
Bloody	1.68***	0.10***	0.02	0.05	-0.02	-0.04**	0.08***	0.02***	0.13***	0.22***	0.19***	0.02	0.001	0.29	
Fat	1.74***	0.03	-0.06***	0.02	0.02	-0.01	0.02	-0.02***	-0.001	0.02	0.0005	-0.04	0.002	0.29	
Metal	1.98***	0.01	0.01	-0.01	-0.04***	0.02	0.01	0.003	0.11***	-0.004	0.06***	-0.03	0.20	0.15	
Liver Green	0.32***	0.02	-0.07***	-0.02	-0.003	0.01	-0.04*	0.01	0.08**	0.001	0.07**	-0.08	0.03	0.22	
Hay	0.03***	-0.003	0.003	-0.004	0.002	0.01	-0.001**	0.0002	-0.002	-0.02	-0.01	-0.03*	0.50	0.11	
Umami	0.74***	0.01	-0.03	0.02	0.04**	0.01	-0.02	-0.01	-0.01	0.001	-0.06**	0.05	0.02	0.22	
Overly Sweet	0.87***	0.02	-0.03*	0.02	0.01	0.01	0.01	-0.01	0.01	0.06	0.01	0.09**	0.24	0.15	
Sweet	0.83***	0.004	-0.02	0.01	0.01	0.01	0.02	-0.01	0.02	-0.003	-0.02	0.07*	0.24	0.15	
Sour	1.50***	0.04**	-0.03*	-0.01	-0.02	0.002	-0.04**	0.01*	0.02	0.07	0.07***	-0.10**	0.001	0.31	
Salty	1.35***	0.002	-0.001	0.008	0.03***	-0.01	-0.001	0.004	-0.03	-0.001	-0.01	0.08**	0.31	0.13	
Bitter Sour	1.52***	0.02	-0.02	0.01	-0.01	0.02	-0.03**	-0.004	-0.06***	0.07	0.01	-0.22***	<0.0001	0.36	
Aroma	0.02**	-0.01	-0.01**	-0.01	0.0002	0.002	-0.001	-0.001	0.002	-0.02	-0.01	-0.02	0.76	0.08	
Barnyard	0.03***	0.01	0.01	0.01	0.01	-0.01**	0.01***	-0.0003	0.003	0.01	0.01	-0.01	0.15	0.16	
Burnt Heated	0.22***	0.005	0.02	0.02	-0.00003	0.02	-0.002	-0.01	-0.02	0.06	0.01	-0.08**	0.36	0.13	
Oil	0.13***	-0.003	-0.02	-0.002	-0.002	-0.01	0.003	-0.01***	-0.01	-0.02	-0.02	-0.04	0.18	0.16	
Chemical	0.19***	-0.01	-0.02	-0.01	0.004	-0.002	-0.01	0.0003	-0.02	-0.01	-0.02	-0.04	0.66	0.09	

*** $P \leq 0.05$

** $P \leq 0.10$

* $P \leq 0.15$

Table 6 cont. Regression coefficients for the influence of principle components on beef lexicon traits for *B. femoris* steaks.

Beef Lexicon Trait	Principle Components											P- value	R ²	
	Intercept	Neutral Lipid PC 1	Neutral Lipid PC 2	Phospholipid PC 1	Phospholipid PC 2	Mineral PC 1	Mineral PC 2	Amino Acid PC 1	Amino Acid PC 2	Other PC 1	Other PC 2			Age
Beet	0.004	0.001	0.001	-0.0005	-0.0003	-0.0001	0.001	-0.001	0.0005	0.002	-0.0005	-0.004	0.93	0.05
Green Grass	0.01*	0.01***	0.01	0.01***	0.002	-0.003	-0.002	-0.002	-0.02***	0.05***	-0.002	0.00	0.02	0.23
Musty	0.28***	0.003	-0.02	-0.03	0.01	0.02	-0.02	0.01	-0.02	-0.04	-0.003	-0.09***	0.20	0.15
Medicinal	0.05***	-0.005	0.01**	0.001	-0.01**	0.001	-0.01	-0.001	0.01	-0.01	-0.005	-0.02	0.25	0.14
Petroleum	0.02***	-0.00001	0.01**	-0.002	0.0005	-0.003	0.002	-0.005***	0.01	-0.004	-0.003	-0.02	0.14	0.17
Smoked/Charred	0.06***	-0.0004	0.005	-0.01	-0.004	0.003	0.004	-0.003	-0.02	-0.004	-0.01	0.01	0.74	0.08
Smoked Wood	0.00	-0.001	-0.005**	-0.001	0.001	0.0001	-0.003	0.00002	0.004	-0.003	0.0003	0.01	0.74	0.08
Dairy	0.00	-0.001	0.003*	0.0001	-0.003***	0.001	-0.0001	0.0001	0.01***	-0.002	0.003	0.004	0.15	0.16
Buttery	0.05***	-0.01	-0.01*	-0.01	-0.01	0.01	-0.01	-0.001	-0.001	-0.02	-0.01	-0.004	0.86	0.07
Cooked Milk	0.03***	-0.003	-0.01	-0.01	0.004	0.001	-0.001	0.002	-0.02	-0.02	-0.01	0.01	0.93	0.06
Sour Milk	0.13***	0.03*	-0.02	0.004	0.01	0.002	-0.03**	0.01	0.01	0.06	0.02	-0.08***	0.07	0.19
Refrigerator Stale	0.06***	-0.01	0.004	-0.01	0.003	-0.01	-0.0003	0.003	-0.01	-0.03	-0.02	-0.04***	0.60	0.10
Warmed over	0.62***	0.03	0.03	0.02	0.001	-0.003	0.02	0.003	-0.09*	0.09	-0.04	-0.10	0.62	0.10
Soapy	0.01	0.001	-0.003	0.002	-0.003	0.001	-0.0004	-0.001	0.01	0.01	0.01	-0.01	0.59	0.10
Painty	0.07***	-0.004	0.01	-0.004	-0.004	-0.01**	-0.0001	0.001	0.002	-0.02	-0.01	-0.06***	0.14	0.17
Fishy	0.07***	0.01	0.01	-0.01	-0.01	-0.01	0.01	0.003	0.01	-0.01	0.02**	-0.04**	0.28	0.14
Cardboardy	0.24***	0.01	0.002	0.01	0.02	-0.01	-0.01	-0.01	0.01	0.02	-0.004	-0.05	0.73	0.08

*** $P \leq 0.05$

** $P \leq 0.10$

* $P \leq 0.15$

RECOMMENDATIONS FOR FUTURE RESEARCH

This study determined that grass type cattle graze during the background phase plays a key role in determining the biochemical make-up of the meat and the flavors associated with it. Warm-season grasses specifically were found to be the most detrimental in consumer flavor acceptability and also caused most of the changes in the biochemical constituents. A complete analysis of the nutrient composition of each grass type would be helpful in determining why the changes in both the beef composition and flavor were seen. In addition, all of the cattle were grazed in the summer time. It would be interesting to see if the same observations that occurred in this study are seen during different seasons when the grass is at different maturities, such as spring and fall. A second study grazing the cattle on the same pastures would also show if the findings in this study are repeatable.

Even though warm-season grasses were detrimental, this study found that if the cattle were supplemented for energy during the background phase most of the detriments were mitigated. A future study comparing supplementing for energy vs. protein may show different results since protein would require a higher level of supplementation.

A majority of the biochemical constituents (moisture, ash, fat, protein, minerals, amino acids, and fatty acids) were measured on steaks that were never part of a retail display. It is known that during retail display the fat oxidizes and changes composition. It would be interesting to see how the other biochemical constituents listed above may also be affected by a retail display period.

All the steaks analyzed in both the consumer and beef lexicon panels were aged under vacuum either 7 or 28 d and then placed in a retail display for 7 d. A study comparing 0 and 7 d retail display could not only evaluate the differences caused by oxidation, but the data from biochemical constituents at both 0 and 7 d retail display could also be used to give more insight into how the different meat constituents effect flavor acceptability and notes.

In this study all of the meat constituents were placed into groupings and analyzed as a whole using principle components. Within the principle components it was seen that certain factors within a grouping had a little more influence than others. An analysis evaluating each biochemical constituent may give more insight into how each affected flavor acceptability and notes.

APPENDIX I: Principle components of the neutral lipids in *L. dors* steaks

Eigenvalues of the Correlation Matrix

	Eigenvalue	Difference	Proportion	Cumulative
1	13.5855211	11.8019051	0.6793	0.6793
2	1.7836160	0.4987043	0.0892	0.7685
3	1.2849117	0.4563723	0.0642	0.8327
4	0.8285394	0.2321844	0.0414	0.8741
5	0.5963549	0.1003876	0.0298	0.9039
6	0.4959673	0.1629688	0.0248	0.9287
7	0.3329985	0.0225565	0.0166	0.9454
8	0.3104420	0.0877001	0.0155	0.9609
9	0.2227419	0.0845362	0.0111	0.9721
10	0.1382057	0.0333825	0.0069	0.9790
11	0.1048232	0.0192387	0.0052	0.9842
12	0.0855844	0.0128491	0.0043	0.9885
13	0.0727354	0.0172203	0.0036	0.9921
14	0.0555151	0.0145889	0.0028	0.9949
15	0.0409262	0.0109414	0.0020	0.9969
16	0.0299849	0.0156108	0.0015	0.9984
17	0.0143741	0.0046485	0.0007	0.9992
18	0.0097256	0.0051523	0.0005	0.9996
19	0.0045734	0.0021144	0.0002	0.9999
20	0.0024589		0.0001	1.0000

Eigenvectors

		Prin1	Prin2	Prin3	Prin4	Prin5	Prin6
n10	n10	0.171541	0.484178	-.142420	0.014848	-.099385	-.355220
n12	n12	0.182388	0.443595	-.112615	-.162180	-.258666	-.345591
n14	n14	0.263157	-.060786	-.047813	-.009111	-.111699	-.090003
n141	n141	0.238226	-.054860	-.237255	-.217702	0.010561	-.103686
n15	n15	0.250310	-.177138	-.117188	0.062266	0.157820	-.108688
n16	n16	0.262894	-.089017	-.063909	0.043610	0.000806	-.077752
n161	n161	0.248521	-.109582	-.185290	-.172409	-.014341	-.081588
n17	n17	0.245859	-.107641	-.157203	0.085947	0.245978	0.032353
n171	n171	0.238790	-.121504	-.283395	-.068263	0.214088	0.078551
n18	n18	0.257564	-.086053	0.060835	0.151653	0.049237	0.072725
n181t	n181t	0.205587	-.029185	0.445865	0.264987	-.198955	-.068181
n181	n181	0.260948	-.062638	-.069662	-.078783	0.069409	0.103794
n181v	n181v	0.223978	0.071022	0.006666	-.092173	0.185746	0.402302
n182tt	n182tt	0.229253	-.071859	0.239914	0.048143	-.357123	-.075436
n182	n182	0.229333	-.016668	0.364075	0.135934	-.128546	0.241196
n20	n20	0.114708	0.385739	-.152989	0.738825	0.211069	0.095518
n183	n183	0.093981	0.278976	0.541799	-.310058	0.647996	-.209316
n201	n201	0.243046	-.165390	0.184399	-.113477	-.205337	-.058027
n22	n22	0.150888	0.438151	-.033996	-.302068	-.204167	0.629112
Tot0thern	Tot0thern	0.253894	-.125745	0.040870	0.060112	0.074102	-.072748

APPENDIX II: Principle components of the phospholipids in *L. dors* steaks

Eigenvalues of the Correlation Matrix				
	Eigenvalue	Difference	Proportion	Cumulative
1	7.80951950	4.73471002	0.3550	0.3550
2	3.07480949	1.04484664	0.1398	0.4947
3	2.02996285	0.21713075	0.0923	0.5870
4	1.81283210	0.43560657	0.0824	0.6694
5	1.37722553	0.25038231	0.0626	0.7320
6	1.12684322	0.12570081	0.0512	0.7832
7	1.00114241	0.19740404	0.0455	0.8287
8	0.80373837	0.19245762	0.0365	0.8653
9	0.61128075	0.06932618	0.0278	0.8931
10	0.54195457	0.09504723	0.0246	0.9177
11	0.44690734	0.08657039	0.0203	0.9380
12	0.36033696	0.07911907	0.0164	0.9544
13	0.28121789	0.11166256	0.0128	0.9672
14	0.16955532	0.01609020	0.0077	0.9749
15	0.15346513	0.04924665	0.0070	0.9819
16	0.10421848	0.02727913	0.0047	0.9866
17	0.07693935	0.00744276	0.0035	0.9901
18	0.06949659	0.01789355	0.0032	0.9932
19	0.05160304	0.01151891	0.0023	0.9956
20	0.04008413	0.00913242	0.0018	0.9974
21	0.03095171	0.00503644	0.0014	0.9988
22	0.02591527		0.0012	1.0000

Eigenvectors								
		Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7
p12	p12	0.084375	0.127295	0.231277	0.241133	0.543820	-.157723	-.285632
p13	p13	0.038466	0.424414	0.040376	0.337760	-.289284	-.039450	-.147260
p14	p14	0.255825	-.041276	-.217343	0.221731	0.311889	-.156680	-.045261
p141	p141	0.039673	0.352360	0.002865	0.445926	-.267421	-.165886	-.184120
p15	p15	0.206455	0.360637	-.178628	-.258670	0.100662	0.061383	-.045764
p16	p16	0.310273	-.187968	0.018390	0.171814	0.024800	-.013941	0.012980
p161	p161	0.272058	-.149365	-.222500	0.155289	0.116442	-.078093	0.114140
p17	p17	0.230300	0.299100	-.207529	-.279912	0.087604	0.104328	-.109236
p171	p171	0.113490	0.307674	-.109809	-.033268	-.293962	-.138965	0.484271
p18	p18	0.310712	-.057881	-.040296	-.145168	-.058222	0.055734	-.105481
p181t	p181t	0.102779	-.413918	0.151073	0.198592	-.354200	-.052534	-.092130
p181	p181	0.286108	-.132851	-.115817	0.211893	0.213972	-.071054	0.141191
p181v	p181v	0.282516	-.077310	-.195672	0.056269	-.207246	-.153699	0.125195
p182	p182	0.225685	0.085498	0.219215	-.169341	-.101123	0.181423	-.394508
p20	p20	-.083897	0.145630	-.222384	0.338415	0.122357	0.353458	0.224952
p183	p183	0.061590	0.173878	0.511344	0.153225	0.095075	0.301957	0.108315
p22	p22	0.199844	-.004800	0.037947	0.081275	0.052145	0.595613	0.211181
p204	p204	0.303448	-.010994	-.024635	-.174747	-.143473	-.043936	-.282353
p205	p205	0.199536	-.088537	0.410777	-.055456	-.059056	0.001383	0.273161
p241	p241	0.285820	-.007661	0.037926	-.114473	-.070487	0.155991	0.072617
p226	p226	0.000835	0.190512	0.227438	-.225516	0.221402	-.392210	0.328751
Tot0therp	Tot0therp	0.247957	0.067745	0.318865	0.017889	-.028182	-.250663	0.116424

APPENDIX III: Principle components for minerals in *L. dorsis* steaks

Eigenvalues of the Correlation Matrix

	Eigenvalue	Difference	Proportion	Cumulative
1	3.40780674	1.11715601	0.2434	0.2434
2	2.29065073	0.22625803	0.1636	0.4070
3	2.06439270	0.70520192	0.1475	0.5545
4	1.35919078	0.18491117	0.0971	0.6516
5	1.17427961	0.28215984	0.0839	0.7355
6	0.89211977	0.19050905	0.0637	0.7992
7	0.70161072	0.10737133	0.0501	0.8493
8	0.59423939	0.07997821	0.0424	0.8917
9	0.51426118	0.20785697	0.0367	0.9285
10	0.30640421	0.07134943	0.0219	0.9504
11	0.23505478	0.04747747	0.0168	0.9671
12	0.18757731	0.03675759	0.0134	0.9805
13	0.15081973	0.02922738	0.0108	0.9913
14	0.12159235		0.0087	1.0000

Eigenvectors

		Prin1	Prin2	Prin3	Prin4
ConvC	ConvC	0.408322	-.005055	-.263719	0.108734
ConvP	ConvP	0.314860	-.271185	0.057828	0.456702
ConvK	ConvK	0.077536	-.220848	-.192853	-.180658
ConvMg	ConvMg	0.421005	0.033296	-.221695	0.028289
ConvZn	ConvZn	0.150842	0.430005	0.195141	-.031091
ConvFe	ConvFe	0.407322	0.033023	-.073456	-.235696
ConvMn	ConvMn	0.323833	0.185390	0.128314	-.321214
ConvCu	ConvCu	0.239855	0.133099	0.210790	-.443811
ConvS	ConvS	0.282234	-.153924	0.210267	0.331531
ConvNa	ConvNa	0.306486	-.021055	-.233454	0.059104
NonHeme7	NonHeme7	0.094634	0.093626	0.560203	-.104054
NonHeme28	NonHeme28	0.114679	-.042192	0.525917	0.340678
Heme7	Heme7	0.004621	0.531314	-.196718	0.278618
Heme28	Heme28	-.064019	0.567005	-.092560	0.267532

APPENDIX IV: Principle components for amino acids in *L. dors* steaks

Eigenvalues of the Correlation Matrix

	Eigenvalue	Difference	Proportion	Cumulative
1	13.9271322	12.9209415	0.8704	0.8704
2	1.0061907	0.4134999	0.0629	0.9333
3	0.5926908	0.3150411	0.0370	0.9704
4	0.2776497	0.1718712	0.0174	0.9877
5	0.1057785	0.0660565	0.0066	0.9943
6	0.0397220	0.0229557	0.0025	0.9968
7	0.0167663	0.0071565	0.0010	0.9979
8	0.0096098	0.0018918	0.0006	0.9985
9	0.0077180	0.0027637	0.0005	0.9990
10	0.0049544	0.0010461	0.0003	0.9993
11	0.0039083	0.0009542	0.0002	0.9995
12	0.0029541	0.0006452	0.0002	0.9997
13	0.0023089	0.0009496	0.0001	0.9998
14	0.0013594	0.0005921	0.0001	0.9999
15	0.0007672	0.0002775	0.0000	1.0000
16	0.0004898	0.0004898	0.0000	1.0000
17	0.0000000	0.0000000	0.0000	1.0000
18	0.0000000	0.0000000	0.0000	1.0000
19	0.0000000	0.0000000	0.0000	1.0000
20	0.0000000	0.0000000	0.0000	1.0000

Eigenvectors

		Prin1	Prin2	Prin3	Prin4	Prin5	Prin6
Cys	Cys	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
Hyp	Hyp	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
Asp	Asp	0.266309	-.010992	-.054237	-.047419	-.196391	-.292619
Thr	Thr	0.265614	-.001983	-.114855	-.072841	0.177829	-.226938
Ser	Ser	0.261761	-.012007	-.031107	0.035564	0.541562	-.547284
Glu	Glu	0.259802	-.010326	-.094466	-.140159	0.551604	0.623634
Pro	Pro	0.247896	0.007561	0.466446	0.140845	0.064844	0.227668
Gly	Gly	0.216239	-.003997	0.746614	0.217723	-.109015	-.089919
Ala	Ala	0.266758	0.016252	0.082542	0.033368	0.098901	0.045861
Val	Val	0.264183	0.017218	-.081081	-.073306	-.415317	0.095442
Met	Met	-.009024	0.995602	0.026090	-.061551	0.017632	-.009735
Ile	Ile	0.264030	-.007162	-.112324	-.162375	-.293758	0.247862
Leu	Leu	0.266518	-.004871	-.098053	-.103445	-.070082	-.092289
Nle	Nle	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
Tyr	Tyr	0.264024	0.001307	-.092818	-.211413	-.180603	-.124153
Phe	Phe	0.266389	-.005007	-.083568	-.071634	-.015934	-.039103
His	His	0.224268	0.065685	-.363832	0.881411	-.070645	0.093232
Hlys	Hlys	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
Lys	Lys	0.265873	0.036651	-.109970	-.129270	-.054068	0.012628
Arg	Arg	0.266209	-.044807	0.051041	-.116323	-.039894	0.086380

APPENDIX V: Principle components for other biochemical constituents in *L. dorsi* steaks

Eigenvalue	Difference	Proportion	Cumulative	
1	2.56820437	0.77733816	0.2568	0.2568
2	1.79086621	0.30365943	0.1791	0.4359
3	1.48720678	0.44599198	0.1487	0.5846
4	1.04121480	0.11267470	0.1041	0.6887
5	0.92854010	0.17002978	0.0929	0.7816
6	0.75851032	0.10688033	0.0759	0.8575
7	0.65162999	0.04117274	0.0652	0.9226
8	0.61045724	0.44708704	0.0610	0.9837
9	0.16337021	0.16337021	0.0163	1.0000
10	0.00000000		0.0000	1.0000

		Eigenvectors				
		Prin1	Prin2	Prin3	Prin4	Prin5
ph7	ph7	0.231696	-.250045	0.161699	0.422653	0.415915
ph28	ph28	0.092300	-.371848	-.099309	-.031781	0.681120
Moisture	Moisture	0.533641	-.169618	0.061509	0.010063	-.176046
Ash	Ash	0.167271	0.067441	0.570149	-.275627	-.168381
Fat	Fat	-.579235	0.168032	0.023406	-.011341	0.271912
Protein	Protein	0.270882	-.117068	-.544951	0.176553	-.234969
CkLoss7	CkLoss7	-.141683	0.298157	-.164240	0.656198	-.144761
CkLoss28	CkLoss28	0.099751	0.188096	0.523559	0.469091	0.041712
PerCarb7	PerCarb7	0.361537	0.526351	-.084112	-.010360	0.241660
PerCarb28	PerCarb28	0.235417	0.568001	-.171621	-.249467	0.305785

APPENDIX VI: Principle components of the neutral lipids in *B. femoris* steaks

Eigenvalues of the Correlation Matrix

	Eigenvalue	Difference	Proportion	Cumulative
1	11.2568315	9.4325428	0.5628	0.5628
2	1.8242887	0.4486691	0.0912	0.6541
3	1.3756196	0.0780059	0.0688	0.7228
4	1.2976136	0.3245444	0.0649	0.7877
5	0.9730692	0.3041009	0.0487	0.8364
6	0.6689683	0.1636528	0.0334	0.8698
7	0.5053155	0.0318749	0.0253	0.8951
8	0.4734405	0.1032893	0.0237	0.9188
9	0.3701513	0.0557763	0.0185	0.9373
10	0.3143749	0.0557277	0.0157	0.9530
11	0.2586473	0.0678921	0.0129	0.9659
12	0.1907552	0.0266015	0.0095	0.9755
13	0.1641537	0.0549651	0.0082	0.9837
14	0.1091886	0.0081034	0.0055	0.9891
15	0.1010852	0.0539023	0.0051	0.9942
16	0.0471828	0.0164030	0.0024	0.9965
17	0.0307798	0.0134603	0.0015	0.9981
18	0.0173195	0.0047045	0.0009	0.9989
19	0.0126150	0.0040154	0.0006	0.9996
20	0.0085997		0.0004	1.0000

Eigenvectors

		Prin1	Prin2	Prin3	Prin4	Prin5	Prin6
n10	n10	0.184110	0.142369	-.357774	0.352059	-.050325	-.215089
n12	n12	0.183087	-.024190	-.327518	0.440228	0.091615	0.253258
n14	n14	0.283681	-.045149	-.044887	0.010119	0.043164	0.123139
n141	n141	0.236299	-.267354	-.153073	-.040396	0.112471	0.403518
n15	n15	0.268200	-.144422	0.169778	-.029319	0.028524	-.041380
n16	n16	0.287967	-.021058	0.075148	-.019195	-.102842	0.049071
n161	n161	0.254777	-.244794	-.082197	-.128298	-.059012	0.220505
n17	n17	0.244920	-.147965	0.296584	0.170503	-.023749	-.346296
n171	n171	0.232298	-.334900	0.203371	0.087492	-.032317	-.285518
n18	n18	0.257704	0.177036	0.217607	0.167140	-.103628	-.141431
n181t	n181t	0.193936	0.412254	-.166946	-.236209	0.018678	-.141443
n181	n181	0.282177	-.083675	0.085493	0.055639	-.086107	-.059772
n181v	n181v	0.238594	-.024202	0.110994	-.251300	-.153916	-.176770
n182tt	n182tt	0.175715	0.373046	0.006115	-.030185	-.276710	0.202572
n182	n182	0.223506	0.317133	-.113546	-.269033	0.121313	-.152868
n20	n20	-.032384	0.343557	0.463994	0.404281	-.316945	0.349040
n183	n183	0.151370	0.135428	0.365966	-.362839	0.353601	0.348604
n201	n201	0.233875	0.179121	-.303441	-.097901	-.166678	-.045199
n22	n22	0.103702	0.230288	0.103470	0.304204	0.755330	-.105836
Tot0thern	Tot0thern	0.227459	-.142281	-.094585	0.074945	0.028446	0.248177

APPENDIX VI: Principle components of the phospholipids in *B. femoris* steaks

Eigenvalues of the Correlation Matrix

	Eigenvalue	Difference	Proportion	Cumulative
1	5.96463428	1.77633465	0.2711	0.2711
2	4.18829963	1.34470180	0.1904	0.4615
3	2.84359783	0.58689115	0.1293	0.5908
4	2.25670668	0.84278119	0.1026	0.6933
5	1.41392549	0.21607242	0.0643	0.7576
6	1.19785307	0.14990934	0.0544	0.8120
7	1.04794373	0.44014487	0.0476	0.8597
8	0.60779886	0.04770799	0.0276	0.8873
9	0.56009087	0.06059805	0.0255	0.9128
10	0.49949282	0.16978298	0.0227	0.9355
11	0.32970984	0.08987098	0.0150	0.9505
12	0.23983886	0.04129140	0.0109	0.9614
13	0.19854746	0.02881219	0.0090	0.9704
14	0.16973527	0.02420958	0.0077	0.9781
15	0.14552570	0.04535979	0.0066	0.9847
16	0.10016590	0.03149277	0.0046	0.9893
17	0.06867313	0.00907864	0.0031	0.9924
18	0.05959449	0.01008340	0.0027	0.9951
19	0.04951110	0.02055670	0.0023	0.9973
20	0.02895439	0.01273657	0.0013	0.9987
21	0.01621782	0.00303505	0.0007	0.9994
22	0.01318277		0.0006	1.0000

Eigenvectors

		Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7
p12	p12	0.139500	0.180824	0.053339	-.419163	0.052547	0.048520	0.168430
p13	p13	0.081951	0.305397	0.114000	0.275054	0.347133	-.177608	0.048148
p14	p14	0.152631	0.030012	0.379360	-.048073	0.077352	0.182551	0.425996
p141	p141	0.102876	0.262376	-.107371	0.261241	0.352407	0.027748	0.381190
p15	p15	0.048496	0.409426	-.227218	-.100171	-.004509	0.193117	-.017506
p16	p16	0.315242	-.241925	0.012982	0.080852	0.112228	-.023640	0.218285
p161	p161	0.260637	-.106814	0.234092	-.125301	0.051530	0.350666	-.003323
p17	p17	0.012055	0.371319	-.251173	-.050325	-.127139	0.349182	-.025063
p171	p171	0.042052	0.406887	0.252503	0.061301	-.175271	0.028735	-.104441
p18	p18	0.290123	-.034567	-.212853	0.144964	-.140649	0.286949	0.140640
p181t	p181t	0.217419	-.341599	-.014908	0.171150	-.102067	-.158487	-.017470
p181	p181	0.297316	-.215648	0.130793	-.054547	0.189112	0.253889	0.052342
p181v	p181v	0.107968	0.090545	0.489330	0.214264	-.159808	0.086079	-.148794
p182	p182	0.227250	0.044478	-.278557	0.281785	-.136606	-.261263	0.200934
p20	p20	0.092284	0.043414	-.019306	-.124315	0.642927	-.242719	-.263939
p183	p183	0.243403	0.158270	-.091354	-.134978	-.140175	-.385909	0.248019
p22	p22	0.121571	0.211155	0.349078	0.294658	-.165112	-.232560	-.186357
p204	p204	0.313560	0.027144	-.217002	0.177130	-.056438	0.126005	-.246602
p205	p205	0.245911	0.013692	-.000365	-.389848	-.234794	-.293641	0.054100
p241	p241	0.263540	-.063362	-.189807	0.227261	-.004251	0.105456	-.319380
p226	p226	0.249456	0.056107	-.012283	-.220845	0.193343	0.026526	-.384076
Tot0therp	Tot0therp	0.334987	0.095502	0.041971	-.238034	-.135149	-.158109	-.136128

APPENDIX VIII: Principle components for minerals in *B. femoris* steaks

Eigenvalues of the Correlation Matrix

	Eigenvalue	Difference	Proportion	Cumulative
1	4.13629300	1.25051165	0.2954	0.2954
2	2.88578135	1.28397365	0.2061	0.5016
3	1.60180770	0.43717772	0.1144	0.6160
4	1.16462998	0.24172370	0.0832	0.6992
5	0.92290629	0.17371958	0.0659	0.7651
6	0.74918671	0.07085115	0.0535	0.8186
7	0.67833556	0.12246870	0.0485	0.8671
8	0.55586686	0.10395318	0.0397	0.9068
9	0.45191368	0.14525850	0.0323	0.9391
10	0.30665518	0.07377257	0.0219	0.9610
11	0.23288262	0.09426788	0.0166	0.9776
12	0.13861473	0.03375830	0.0099	0.9875
13	0.10485643	0.03458653	0.0075	0.9950
14	0.07026990		0.0050	1.0000

Eigenvectors

		Prin1	Prin2	Prin3	Prin4
ConvC	ConvC	0.364846	0.097270	-.220057	-.258159
ConvP	ConvP	0.396121	-.188906	0.017254	-.003397
ConvK	ConvK	0.251653	0.245576	0.215048	-.301344
ConvMg	ConvMg	0.343502	0.201734	-.084440	-.439977
ConvZn	ConvZn	0.264612	-.172420	0.330971	0.208212
ConvFe	ConvFe	0.012623	0.405338	-.263567	0.454020
ConvMn	ConvMn	0.263406	0.318163	-.202215	0.454296
ConvCu	ConvCu	0.334039	0.063474	0.014944	0.313742
ConvS	ConvS	0.396818	-.102125	0.087748	-.060395
ConvNa	ConvNa	0.332425	-.290607	-.018846	0.163451
NonHeme7	NonHeme7	-.046551	0.367853	0.328541	-.074763
NonHeme28	NonHeme28	0.042364	0.480031	0.218875	-.042832
Heme7	Heme7	-.048922	0.145501	0.620971	0.127004
Heme28	Heme28	0.056419	-.269753	0.358152	0.201817

APPENDIX IX: Principle components for amino acids in *B. femoris* steaks

Eigenvalues of the Correlation Matrix

	Eigenvalue	Difference	Proportion	Cumulative
1	14.5033492	13.6941499	0.9065	0.9065
2	0.8091994	0.3574986	0.0506	0.9570
3	0.4517008	0.3091645	0.0282	0.9853
4	0.1425363	0.1061475	0.0089	0.9942
5	0.0363888	0.0212125	0.0023	0.9964
6	0.0151763	0.0034843	0.0009	0.9974
7	0.0116920	0.0030797	0.0007	0.9981
8	0.0086122	0.0018172	0.0005	0.9987
9	0.0067950	0.0026225	0.0004	0.9991
10	0.0041725	0.0007549	0.0003	0.9994
11	0.0034176	0.0011036	0.0002	0.9996
12	0.0023139	0.0006296	0.0001	0.9997
13	0.0016843	0.0001436	0.0001	0.9998
14	0.0015408	0.0006244	0.0001	0.9999
15	0.0009164	0.0004118	0.0001	1.0000
16	0.0005045	0.0005045	0.0000	1.0000
17	0.0000000	0.0000000	0.0000	1.0000
18	0.0000000	0.0000000	0.0000	1.0000
19	0.0000000	0.0000000	0.0000	1.0000
20	0.0000000		0.0000	1.0000

Eigenvectors

		Prin1	Prin2	Prin3	Prin4	Prin5	Prin6
Cys	Cys	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
Hyp	Hyp	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
Asp	Asp	0.261413	-.052993	-.064555	-.003341	0.154893	-.343892
Thr	Thr	0.261044	-.006499	-.134184	0.010275	-.091309	-.241303
Ser	Ser	0.260635	0.033629	-.069261	0.023882	-.309001	-.574967
Glu	Glu	0.257828	-.012979	-.142208	0.224011	-.637675	0.506542
Pro	Pro	0.247302	-.034942	0.478817	0.113422	-.279981	-.033777
Gly	Gly	0.223305	-.099376	0.767059	0.038387	0.189288	0.085097
Ala	Ala	0.261297	-.025998	0.111322	-.035996	-.005687	0.079277
Val	Val	0.260018	-.093548	-.061597	-.013097	0.432872	0.104829
Met	Met	0.127624	0.968516	0.050697	0.146301	0.109228	0.043016
Ile	Ile	0.259221	-.107907	-.148451	0.053509	0.167585	0.298218
Leu	Leu	0.260976	-.040223	-.144078	0.000704	0.073547	-.027601
Nle	Nle	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
Tyr	Tyr	0.258374	-.061686	-.192786	0.179532	0.280772	0.257181
Phe	Phe	0.261042	-.002024	-.110175	0.054838	0.151521	-.075546
His	His	0.244506	0.113148	-.002507	-.923189	-.115339	0.145368
Hlys	Hlys	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
Lys	Lys	0.260874	-.007200	-.153287	0.018232	-.004225	-.008690
Arg	Arg	0.260528	-.085890	-.028279	0.145633	-.058250	-.161493

APPENDIX X: Principle components for other biochemical constituents in *B. femoris* steaks

Eigenvalues of the Correlation Matrix

	Eigenvalue	Difference	Proportion	Cumulative
1	2.36062136	0.60281618	0.2361	0.2361
2	1.75780518	0.47628352	0.1758	0.4118
3	1.28152166	0.07837431	0.1282	0.5400
4	1.20314735	0.17843995	0.1203	0.6603
5	1.02470740	0.06749533	0.1025	0.7628
6	0.95721207	0.27521558	0.0957	0.8585
7	0.68199648	0.22409425	0.0682	0.9267
8	0.45790224	0.18281596	0.0458	0.9725
9	0.27508628	0.27508628	0.0275	1.0000
10	0.00000000		0.0000	1.0000

Eigenvectors

		Prin1	Prin2	Prin3	Prin4	Prin5
ph7	ph7	-.081344	0.264864	-.119125	0.368851	0.711958
ph28	ph28	-.047423	0.125427	0.632616	-.320584	0.165780
Moisture	Moisture	0.588104	0.098057	0.084079	0.026039	0.043556
Ash	Ash	0.024927	0.581925	0.309227	-.111598	-.251288
Fat	Fat	-.635864	-.123457	-.089023	-.009370	0.017813
Protein	Protein	0.484612	-.309003	-.163256	0.061050	0.071003
CkLoss7	CkLoss7	-.045882	-.197151	0.239999	0.532252	-.540939
CkLoss28	CkLoss28	-.038600	-.335172	0.467363	0.445897	0.217091
PerCarb7	PerCarb7	0.023393	-.398567	0.384346	-.295342	0.232086
PerCarb28	PerCarb28	0.036507	0.380887	0.156810	0.418192	0.040305

APPENDIX XI: pH

1. Weigh out 10 g of raw sample in duplicate into small beakers.
2. Add 90 mL of distilled, deionized water to each.
3. Homogenize the solution for 30 seconds using a Polytron at 10,800 rpm.
4. Place stir bar in the solution after homogenizing and while stirring the solution, read the pH using a pH meter which has been calibrated using 7.0 buffer and 4.0 buffer.
5. Be sure to keep electrode clean by rinsing well between samples.

APPENDIX XII: Proximate analysis

University of Nebraska-Lincoln

Meat Science and Muscle Biology Research Lab

A.O.A.C. Methods-Serial Sample Analysis

Moisture and Ash by LECO

(This portion of the proximate analysis will be done on a Thermogravimetric Analyzer (TGA-601) Leco Corp., St. Jos. MO.)

1. Powdered samples are removed from the ultra-low freezer.
2. Samples are stored in a small styro-foam cooler containing liquid nitrogen to keep from thawing while loading subsamples into the TGA-601. Samples are kept in their original plastic bags so they do not come in direct contact with the liquid nitrogen.
3. Sample identification numbers are entered into the computer.

4. The method of operation is selected (User defined).

Name	Covers	RampRate	RampTime	StartTemp	EndTemp
Moisture	Off	6 d/m	:17 min	25 C	130 C
Ash	Off	20 d/m	:30 min	130 C	600 C

Name	Atmosp	FlowRate	HoldTime	Const.Wt.	Const.Wt. Time
Moisture	N	High	00 min	0.05%	09 min
Ash	O	High	00 min	0.05%	09 min

General Setting

Crucible Density 3.00

Cover Density 3.00

Sample Density 1.00

Equations:

Initial Wt. W [Initial]

Moisture ((W[Initial] - W[Moisture])/W[Initial])*100

Ash (W[Ash]/[Initial]) * 100

Ash Dry Basis E[Ash] * (100/(100-E[Moisture]))

5. Select “Analysis” and click on “collect”.
6. Select furnace to be used.
7. Load empty crucibles into selected furnace.
8. TGA-601 will weigh all crucibles to obtain a tare weight.
9. After tare is obtained the machine will call to load each sample (1g).
10. Return samples to ultra-low freezer.
11. After all samples are loaded the machine will automatically start.
12. When analysis is finished click the “save” icon on toolbar and print a hard copy of results.
13. Remove crucibles after they have cooled down for 30 minutes. Wash them in soapy water and allow to dry in drying oven for at least 1 ½ hours.
14. Remove dry crucibles and transfer to desiccator for future use.
15. Before doing another run the machine must cool down to 25 C.

APPENDIX XIII: Fat extraction – Soxhlet method

WARNING: ETHER IS EXTREMELY FLAMMABLE AND PRODUCES EXPLOSIVE PEROXIDES. NEVER BRING A RADIO OR ANY OTHER POTENTIALLY SPARK-PRODUCING ITEM INTO THE FAT EXTRACTION ROOM.

1. Check ground glass connections. They should be wiped clean with a dry paper towel and given a thin coating of stopcock grease.
2. Each boiling flask must contain boiling stones. This helps prevent violent boiling of the solvent which could be dangerous.
3. Load samples into soxhlet tubes, arranging them so that no samples are above the level of the top bend in the narrower tubing on the outside of the soxhlet. (The soxhlet will only fill with the solvent up to this point before cycling back down into the boiling flask.) In general, the large soxhlets will hold about 20 two-gram samples and the small soxhlets from 4-6.
4. Fill the large (500ml) boiling flasks with @ 400ml of solvent and the small (125ml) flasks with 100ml of solvent. DO THIS UNDER THE FUME HOOD!
5. Fit the soxhlet onto the boiling flask. Very carefully, bring the assembly into the extraction room and fit it onto the condenser. Make sure all ground glass connections are snug and each boiling flask is resting on the heating element. The ceramic fiber sheet should be covering the bare metal surfaces of the burners completely.
6. Turn on the water supply to the condensers (usually a quarter turn). Check later

to make sure condensers are cool enough - if not, increase water flow.

7. Turn heating element control dials to 2 way between three and four. Each burner has its own dial. NEVER TURN THE BURNER BEYOND FIVE. Ether has a very low boiling point and violent boiling is dangerous. Double check fittings, boiling stones, etc.
8. Fat extraction will take from 24 to 72 hours depending on the sample. (Beef-48 hours, Bacon-72 hours). Check extractions twice daily to see that everything is alright while they are running.
9. When done, turn off the burners and let solvent cool completely before removing samples.
10. After it has cooled down, slowly uncouple the flask and soxhlet tube from the condenser. Cover the top of the soxhlet with one palm so as to reduce ether vapors while transporting it to the fume hood. Air dry samples in the fume hood for two hours to get rid of the remaining ether in the samples. Pour ether back slowly into an approved container for reuse or discarding. DO NOT LEAVE ETHER OUT OF THE HOOD OR THE FLAMMABLE CABINET.
11. Place samples in drying oven (105 degrees C) for about four hours or overnight before weighing back.

Calculation: $(\text{Original weight including filter paper and paper clip} - \text{Fat extracted sample weight}) / \text{Sample Wt} * 100 - \% \text{ Moisture} = \% \text{ Fat}$

APPENDIX XIV: Phenol-sulfuric acid method for total carbohydrates

1. To 1 ml of a solution containing <math><0.1\text{ mg/ml}</math> of carbohydrate, add 50 μl of 80% (w/w) phenol
2. Add 2.5 ml of concentrated sulfuric acid and vortex *immediately*
3. Let stand 10 min
4. Cool in water bath for 10-25 min
5. Read absorbance at 490 nm
6. Estimate sugar concentration from a standard curve prepared with glucose, arabinose+xylose, or fructose

Standard curve: Make a stock solution of $\sim 0.1\text{ mg/ml}$ of sugar standard

Standard	Stock solution (ml)	Water (ml)	Approx. concentration (mg/ml)*
0	0	1.0	0
1	0.2	0.8	0.02
2	0.4	0.6	0.04
3	0.6	0.4	0.06
4	0.8	0.2	0.08
5	1.0	0	0.1

*Use actual concentration of stock solution to calculate concentration in standards

Reference: Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 1956; 28: 350-356

APPENDIX XV: Determination of non-heme iron

Rhee, K.S. and Ziprin, Y.A. 1987. Modification of the Schricker nonheme iron method to minimize pigment effects for red meats. *J. Food Sci.* 52:1174-1176.

1. Weigh $5 \text{ g} \pm 0.05 \text{ g}$ of powdered sample into 50 mL (2.5 x 1.5 cm) screw-cap tubes and add 0.2 mL NaNO_2 solution (156 ppm NaNO_2 based on meat weight). Mix well with spatula and leave in tube to mix again.
2. Add 15 mL acid solution to tubes, caps, and shake vigorously.
3. Heat samples in water shaker bath for 20 hours at 65°C with vigorous agitation. Use pump to trickle water into bath overnight so it does not run dry.
4. Remove samples from water bath and cool to room temperature. Do not agitate less than 1 hour before pipetting.

5. SAMPLE

Transfer 1 mL of the middle liquid brown phase (acidic liquid above the meat phase) to small (15 x 75) centrifuge tube and mix with 5 mL of color reagent. Vortex. *Carefully pipette to avoid particulate. Read against the Reagent Blank (use as reference).*

6. LIQUID PHASE WITHOUT COLOR REAGENT BLANK

Transfer 1 mL of the middle liquid brown phase to another set of centrifuge tubes and mix with 5 mL 21:20 sodium acetate solution. *Read against the Liquid Phase Blank (use as reference).*

7. REAGENT BLANK

Mix duplicate reagent blanks in the same size tube using 1 mL HCL-TCA acid mixture + 5 mL color reagent.

8. LIQUID PHASE BLANK

Mix duplicate liquid phase blanks in the same size tube using 1 mL HCL-TCA acid solution + 5 mL 21:20 sodium acetate solution.

9. Prepare standards according to table below.

10. Transfer 1 mL of each standard to a 15 x 75 mm centrifuge tube and mix 5 mL color reagent. The gradient is pale pink color.

11. Centrifuge all samples, blanks, and standards at 3800 x g (6,000 rpm with Sorvall SE-12 rotor and Sorvall RC 5B centrifuge, DuPont Co., Wilmington, DE) for 10 min.
12. Set up a standard curve at 540 nm on the spectrophotometer.
13. Measure absorbance of all samples and blanks at 540 nm.

CONCENTRATION ($\mu\text{g/mL}$)	mL 50 mg/100 mL Fe STANDARD*	mL Acid Mixture
0.5	0.025	24.975
1.0	0.050	24.950
1.5	0.075	24.925
2.0	0.100	24.900
2.5	0.125	24.875
3.0	0.150	24.850
3.5	0.175	24.825
4.0	0.200	24.800
4.5	0.225	24.775
5.0	0.250	24.750

*Standard obtained from Sigma

REAGENTS

NaNO₂ Solution: *Daily*

Prepare NaNO₂, 0.39% (w/v), with fresh distilled deionized water each day. (0.39 g in 100 mL)

40% TCA

Make from 100% TCA by mixing 200 mL brought to 500 mL with H₂O. (100% TCA made from new bottle of 500g with 500 mL added)

Acid Solution

Mix 6 N HCL and 40% TCA in equal volumes 1:1.

Color Reagent: *Daily*

Mix water:saturated sodium acetate solution:bathophenathroline disulfonate reagent (20:20:1) fresh each day. (Daily should need 100:100:5 mL or 250:250:12.5 mL or 300:300:15 mL)

Sodium Acetate Solution

Stir 400 g sodium acetate (trihydrate) into 500 mL distilled deionized water. When the solution (initially very cold) warms to room temperature, add more sodium acetate until crystals remain undissolved.

Bathophenanthroline Disulfonate Solution

Dissolve 0.162 g bathophenanthroline disulfonic acid in 100 mL water. Add 2 mL thioglycolic acid (in freezer). Store in amber bottle.

21:20 Sodium Acetate Solution (for blanks)

Mix 105 mL H₂O and 100 mL of sodium acetate solution.

Calculations

(Absorbance of incubated liquid phase with color reagent) – (Absorbance of incubated liquid phase without color reagent) = Final Absorbance

Use Final Absorbance in the following equation:

$$\text{Final Concentration} = (\text{Final Absorbance} - \text{intercept})/\text{slope}$$

Take the intercept and slope from the standard curve in step 12. (Slope tends to be ~ 0.06)

The final equation is as follows:

$$\text{Mg nonheme FE/g meat} = \text{Concentration } (\mu\text{g/mL}) \times \frac{(15+0.2+\text{moisture in 5g meat})}{5\text{g}} \times 1\text{mL}$$

APPENDIX XVI: Determination of heme iron concentration and total pigment

Modified procedure of:

Hornsey, H.C. 1956. The color of cooked cured pork. I. Estimation of nitric oxide-haem pigments. *J. Sci. Food Agric.* 7:534-540.

Lee, B.J., Hendricks, D. G. and Cornforth, D.P. 1998. Antioxidant effects of carnosine and phytic acid in a model beef system. *J. Food Sci.* 63:394-398.

1. Weigh 2 g+ 0.02 g of powdered sample into screw cap tubes.
2. Add needed de-ionized water to tube so the total volume of water is 0.72mL.
(.72mL-moisture %=amt. of water to add
ie: if meat has 70% moisture then 70% of .72=0.504ml, so
.72 ml - 0.504 = 0.216 ml of water to add)
3. Add 8.1 mls of acetone to tube.
4. Add 0.2 ml of hydrochloric acid to tube.
5. Polytron solution using a Polytron set at position 5 (10,800 rpm) for 15 sec.
6. Filter solution through #2 Whatman filter paper (90 mm in diameter).
7. Filtrate is then immediately read using the Cary 100 Varian UV/Visual spectrophotomer at 640 nm.

Calculations:

Total Pigment (ppm) = absorbance 640 x 680.

Heme-Iron (ppm) = Total pigment (ppm) x 8.82/100

Notes:

Acetone 90% 8.1ml

HCl 2% .18ml (@.2ml)

Water 8% .72ml

9 ml

Perform addition of acetone and HCl and polytroning under hood.

Keep away from direct light as much as possible.

Do 12-24 tubes at a time to keep readings as accurate as possible.

APPENDIX XVII: Fatty acid determination with neutral and phospholipid layer separation

1. Weigh out 4 g of pulverized muscle tissue into centrifuge tube.
2. Add 10 mL of 2:1 chloroform:methanol (v/v) for muscle tissue or 3 mL for subcutaneous fat.
3. Vortex for 5 s and let stand for 1 h at room temperature.
4. Filter homogenate through Whatman #2 filter paper into 13 x 150 mm screw cap tube bringing the final volume with chloroform:methanol to 15 mL for muscle lipid and 5 mL for subcutaneous fat extract. If stopping at this point, purge test tube with nitrogen, cap tube, and store at -80°C.
5. Add 2 mL of a 0.74% KCl solution for muscle lipid extract or 1 mL for subcutaneous fat tissue extract and vortex for 5 s. If stopping at this point, purge test tube with nitrogen, cap tube, and store at 0°C for no more than 24 h.
6. Centrifuge samples at 1000 x g for 5 min. Following centrifugation, aspirate off the aqueous phase (top layer). If stopping at this point, purge test tube with nitrogen, cap tube, and store at -80°C.
7. Evaporate to dryness under nitrogen at 60°C.
8. Plate dried samples onto aluminum thin layer chromatography (TLC) plates (Silica Gel 60 w/o indicator, Catalog No.: M5547-7, Thermo Fisher Scientific Inc.)

9. Place TLC plates in a tank with a 75:25:2 hexane:diethyl ether:acetic acid solution, and allow to run until the solution had travelled to the top of the plate, approximately 45 min. Upon completion, remove plate from the tank and allow the solvent to evaporate.
10. Stain dried plates with primulin dye (5mg of primulin in 100 mL of acetone\water (80\20)). The neutral and phospholipid regions are identified and marked (with pencil) under a blacklight using a known standard that is also ran on the same plate.
11. Regions of interest are cut out, folded up, and placed in separate glass tubes.
12. Submerge the neutral layer samples in 100% chloroform and the phospholipid samples in 100% methanol. Purge with nitrogen, cap, and place tubes in a 4°C cooler for 45 min to extract the fatty acids.
13. After extraction, remove the folded up plates from the tube and evaporate the samples to dryness under nitrogen at 60°C.
14. Add 0.5 mL of a 0.5 M NaOH in methanol. Vortex for 5 sec. Heat for 5 min at 100°C
15. Add 0.5 mL of boron trifluoride in 14% methanol. Vortex for 5 sec. Heat for 5 min at 100°C.
16. Add 1 mL of a saturated salt solution and 1 mL of hexane. Vortex for 5 sec.
17. Centrifuge samples at 1000 x g for 5 min. Following centrifugation, remove hexane layer (top layer) making sure not to disrupt the aqueous phase (lower layer) and place in

GC vial. For neutral lipid samples: Purge GC vial with nitrogen, cap and crimp cap, and store at -80°C until sample is ready to be read on the GC.

For phospholipid samples: Evaporate samples in GC vial to dryness under nitrogen at 60°C . Add $100\ \mu\text{L}$ of hexane to concentrate the sample. Samples are then re-pipetted into $100\ \mu\text{L}$ polyspring inserts (which are placed inside the GC vial). Purge GC vial with nitrogen, cap and crimp cap, and store at -80°C until sample is ready to be read on the GC.

GC Settings

Column- Chrompack CP-Sil 88 (0.25 mm x 100 m)

Injector Temp- 270°C

Detector Temp- 300°C

Head Pressure- 40 psi

Flow Rate- 1.0 mL/min

Temperature Program- Start at 140°C and hold for 10 min. Following 10 min, raise temperature $2^{\circ}\text{C}/\text{min}$ until temperature reaches 220°C . At 220°C , hold for 20 min.