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Feeding Low-fat Wet Distillers Grains and Beef Quality

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Feeding low-fat wet distillers grains and beef quality

by

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Feeding low-fat wet distillers grains and beef quality

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Feeding distillers grains to cattle decreases shelf life stability of retail displayed steaks due to the increased polyunsaturated fatty acid (PUFAs) content. High levels of PUFAs cause rapid oxidation of the fats in meat. To improve shelf life, a low-fat distillers grain ration, without solubles (LFWDG; 4.72% fat), was compared to a traditional diet containing wet distillers grains plus solubles (TWDGS; 6.91% fat) and a corn-based control diet (Corn; 3.64% fat). Strip loins (*M. longissimus lumborum*) from 45 USDA Choice steers (15 per dietary treatment) were selected to determine the effects of diet on fatty acid profile, fat composition, oxidation, color changes during retail display, flavor and tenderness. After 4 days of simulated retail display, samples from cattle fed LFWDG had significantly ($P < 0.05$) more oxidation than TWDGS or controls as measured by objective and subjective color scores. By day five of simulated retail display, steaks from cattle fed LFWDG were less red in color (lower a^*) and had more visual discoloration than the other steaks ($P < 0.01$). The L^* and b^* values were not significantly different. Steaks from LFWDG were less tender ($P \leq 0.0006$) and had a more distinct off-flavor ($P \leq 0.02$) after display than TWDGS. Meat from LFWDG had about 10% more PUFAs

than TWDGS (4.86 vs. 4.46, respectively; $P = 0.08$). These data suggest fatty acids contained within the distillers grains are not biohydrogenated during digestion, while fatty acids in the soluble fraction are more readily hydrogenated in the rumen.

Consequently, diets formulated with LFWDG tend to compromise meat quality after extended retail display.

Key words: Beef, Distillers Grains, PUFA, Tenderness, Flavor, Oxidation

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INTRODUCTION

The recent growth of the ethanol industry has provided a low cost feedstuff to producers. However, in some instances, beef from cattle finished on wet distillers grains has reduced shelf stability and eating quality. The focus of this research study was to determine the effects of a low-fat wet distillers grains diet on beef quality. It was hypothesized that a diet containing Low-Fat Wet Distillers Grains (LFWDG) would result in beef with less polyunsaturated fatty acids (PUFAs) than a Traditional Wet Distillers Grains plus Solubles (TWDGS) diet, thereby minimizing problems associated with oxidation, discoloration and off-flavor development.

Increased levels of distillers grains in cattle diets have been shown to elevate the PUFA content in the muscles of cattle (de Mello Jr. et al., 2008*b* and Gill et al., 2008). Polyunsaturated fatty acids pose a threat to the shelf life and quality of beef because PUFAs contain multiple double bonds that are likely to become oxidized. Oxidation of these fatty acids can result in off flavors such as ketones and aldehydes which have an effect on the perceptions of the shelf life, taste, flavor and overall quality of beef (de Mello Jr., et al., 2008*b* and Roeber et al., 2005). Oxidation of fatty acids also causes brown color of the raw product in retail display and consumers will often reject beef that is brown in color. Consumers use color to indicate freshness and quality. Therefore, it was hypothesized that if the level of fat in the distillers grain fed to cattle was decreased, then the fatty acid profile of beef would be more supportive of sustained shelf life and uncompromised flavor.

The effects of feeding a low-fat wet distillers grain diet to cattle was compared to two different diets. One of these was a traditional wet distillers grain diet with solubles and the other was a corn-based diet. Based on projected differences in fatty acid profile and fat composition in the diet, differences in meat characteristics were hypothesized for oxidation rate, color changes during retail display, and flavor and tenderness scores during taste panel sessions. The goals for this study were: 1) determine the effects of a low-fat wet distillers grain diet on fatty acid profile and muscle composition 2) evaluate the effects of a low-fat wet distillers grain diet on the oxidation and color changes during retail display and 3) assess flavor and tenderness changes as a consequence of feeding low-fat distillers grains to cattle.

REVIEW OF LITERATURE

I. Distillers Grains

Ethanol has become a very important commodity to the Midwest and the by-product, distillers grains, have become an inexpensive yet nutritional feed source for cattle. Therefore, it is no large surprise that distillers grains have boomed in popularity as an animal feed. These grains play an important role in the finishing diet of cattle. Cattle diets with distillers grains, with or without solubles, are a good source of protein (Larson et al., 1993, Ham et al., 1994 and Schoonmaker et al., 2010) and can be used to replace other protein sources such as urea and soybean meal at a less expensive cost to the producer. The feeding value of wet distillers grains with solubles compared to corn is up to 130% or even more of the value of corn and the performance and carcass characteristics (for example ADG, higher yield grades and increased fat thickness at the

12th rib) of cattle on these finishing diets can be improved 30-40% in most instances (Larson et al., 1993, Erickson et al., 2005 and Kelzer, et al., 2010, Koger et al., 2010). Wet distillers grains are a product of the dry milling industry and their co-product - distillers solubles, more commonly known as “syrup”- are taken from the liquid portion of the mash. Bremer et al. (2009) developed a new procedure to measure the lipid content of the condensed corn distillers solubles showed that they contained 1.3 times the fat than WDGS. Estimates of wet distillers grains plus solubles (DM basis) are about 65% distillers grains and about 35% distillers solubles (Erickson et al., 2005). The distillers solubles portion can be sold directly or added to back to the wet distillers grains to form wet distillers plus solubles (Tjardes et al., 2002). Although nutrient concentrations may vary and solubles vary from each ethanol plant across the Midwest, solubles tend to be about 20-35% CP, about 25% fat and <5% fiber (Erickson et al., 2005, Lardy, 2007, Bremer et al., 2009, Vanness et al., 2009 and Corrigan et al., 2009).

While distillers grains reduce the costs of livestock production, it may be detrimental to the quality of the meat product. Previous research has demonstrated that high levels (40-50% DM) of distillers grains in cattle finishing diets may have a negative effect on the color stability of strip loins during retail display. For example, there was a greater ($P < 0.05$) percentage of steaks from steers feed at the upper inclusion level (40%, DM basis) of distillers grains in the diet that were reported moderately unacceptable as compared to those steaks from steers that were fed lower inclusion levels (10-20%; Roeber et al, 2005). de Mello, Jr. (2008c) fed 0-30% wet distillers grains plus solubles (WDGS) on a DM basis and found that including increasing levels of WDGS in a finishing diet can compromise the color and increase oxidation capacity of beef steaks.

The a* (redness) values steaks were significantly lower at day 3 and then even lower at day 6 of retail display when comparing the 0% and 15% or 30% WDGS inclusion levels. Koger et al. (2010) also showed that increasing percentages of WDGS (20-40%) in the finishing diet cause a subsequent increase in PUFAs, which led to decreased shelf life and increased rancidity of the product. Gill et al. (2008) suggested that feeding wet distillers grains at 15% of the diet DM, consumers noticed no effect on sensory attributes of retail displayed day 1 steaks.

Erickson et al. (2005) stated that approximately 1/3 of the DM of corn remains as the feed product following fermentation during the dry milling process. Furthermore, the nutrients that are in the DM portion of the remainder are concentrated 3-fold because more grains contain about 2/3 starch. The researchers give an example of oil in corn being 4%, the wet distillers grains portion will contain up to about 12% oil. This explains why WDG are of such high energy value.

II. Fat as a Component in Finishing Diets

Fat can be added to a finishing diet for cattle to improve efficiency and increase the daily gain of cattle. Cattle finishing diets contain high levels of concentrates. They also contain small amounts of forage to reduce the risk of acidosis and other physiological issues that ruminants have when consuming high concentrate diets (Huffman et al, 1992). However, relatively small amounts of fat can be added to the rest of the diet. Some diet constituents, like WDGS, already contain fat. Fat supplements can be, but are not limited to, corn oil, tallow and condensed corn distillers solubles. The addition of fat in finishing diets usually does not influence marbling score, marbling

texture or marbling distribution and has seldom been noted to have an impact on carcass traits, positively or negatively (Andrae et al., 2001 and de Mello, Jr., et al., 2008a).

However, there have been reports that indicate the addition of fat to the finishing diet tends to increase the subcutaneous and internal fat deposition within the animal, such as kidney, heart and pelvic fat when compared to the diets without additional fat added (Brandt and Anderson, 1990).

It is interesting to note that the feeding increased levels of various fats in the diet may have other effects on meat and meat quality other than the deposition of fat. The larger the percentage of WDGS in the diet, the more the PUFAs (which facilitates an increase in oxidation), decreases color stability and also reduces flavor desirability of the meat due to the production of off flavors (de Mello, Jr., et al., 2008). Bremer et al. (2010), compared five diets which were dry rolled corn (with no added fat), dry rolled corn with either tallow or corn oil, dry rolled corn with condensed corn distillers solubles and dry rolled corn with WDGS. They observed that although the condensed corn distillers solubles and the corn oil diets were similar in fat content (8.5% of diet DM); the two feeds appeared to be digested completely differently. The condensed corn distillers solubles supplemented diet had the greatest total tract DM digestibility and WDGS had the lowest total tract digestibility. The steers fed the condensed corn distillers solubles had a lower average ruminal pH and greater DM digestibility than those steers fed the other dietary treatments. This is interesting because the fat percentages were all very similar in the beginning of the study. Moreover, steers that were fed the condensed corn distillers solubles in the diet had greater fat and fatty acid digestibility than the corn or corn oil fed steers. These animals also digested the NDF better than the corn oil or tallow

fed steers as well. However, in all cases, the efficiency of fat absorption was not decreased with the steers that were fed high concentrations of fat.

III. Digestion

The incorporation of fat into finishing diets increases the energy content of the feed (Azain, 2004). Cattle fed an increased amount of fat in the diet tend to have increased daily gains, feed efficiencies, carcass weights, dressing percentages, higher marbling scores, quality grades and enhanced intramuscular fat deposition (Brandt et al., 1990 and Andrae et al., 2001). Wether lambs fed glycerin (which is a reduced-cost feed energy source from the biodiesel industry) at 0-20% of dietary DM had increasing ADG with increasing concentrations of glycerin up to 15% (Gunn et al., 2010). Conversely, Huffman et al. (1992), Andrae et al. (2000) and Depenbusch et al. (2008) have found differing effects of fat, oil and DGS in finishing diets. The addition of numerous types of fat or energy, such as bleachable fancy tallow, animal-vegetable oil blends and high oil corn for example, have been reported to have no effect on gain to feed ratios, feed intake or ADG. Palmquist (1994) noted that the composition of the fatty acids when they enter the small intestine could have an impact on their digestion; as the length of saturated fatty acids increase, digestion decreases, but as unsaturation increases, so does the digestion of fatty acids. Additionally it has been shown by Palmquist (1994) that digestibility will decrease with an increasing amount of fat intake.

Brandt et al. (1990) and Andrae et al. (2001) also noted an increase in gains, dressing percentages and quality grades with increasing fat in the diet. They also noted that added dietary fat altered fatty acid proportions and increased unsaturation of fatty

acids particularly in the longissimus muscle. Supplemental fats, most explicitly oils with high degrees of unsaturation, disturb ruminal fermentation and decreases fiber digestibility in ruminants (Coppock and Wilks, 1991). However, these same authors mentioned this is not the case for oilseeds such as cottonseed, likely due to the slow release of the oil into the rumen. Decreased digestibility is an issue when feeding supplemental fat most likely due to the toxic effect of long-chain fatty acids on the ruminal bacteria (Bock et al., 1991), however, palatability issues can also arise.

Feeding distillers grains alter the fatty acid profile enough to increase PUFA values in the muscle of cattle (de Mello, Jr. et al., 2008*b*). The digestion of the distillers grains in turn seem to affect the quality of the meat. The idea of rumen biohydrogenation was a relatively new concept to Reiser and Ramakrishna Reddy (1956). These scientists found that the rumen microorganisms may be responsible for the hydrogenation of the fat that is consumed from the diet. This fat is then digested, absorbed, randomly resynthesized and deposited into the tissue. The rumen is a fermentation vessel in which microorganisms make chemical changes to dietary components. Biohydrogenation is an important process in the rumen and is performed by the microorganisms present in the gut of the ruminant. These microorganisms transform PUFAs to saturated fatty acids (SFAs) through biohydrogenation and lipolysis (Azain, 2004 and Jenkins et al., 2008). Jenkins et al. (2008) mentioned that when lipids first enter the rumen they are lipolyzed by microbial lipases and this eventually results in the release of fatty acids. Once lipolysis is finished, the unsaturated fatty acids undergo biohydrogenation, in which PUFAs are converted into SFAs by the process of isomerization, followed by the hydrogenation of the double bonds (Jenkins et al., 2008).

Many factors can affect the rate of passage for ruminal contents and biohydrogenation, including amount and type of forage in the diet and even rumenal pH. For example, Duckett et al. (2002) found feeding high-oil corn or adding corn oil to corn rations increased the steers' intake, biohydrogenation and duodenal flow of unsaturated long chain fatty acids. Kucuk et al. (2001) found that the highest concentrate diet fed (81.6%) in their study supported the greatest duodenal flows of dietary unsaturated fatty acids and also had the highest flow of 18:1^{trans-11}.

Carbohydrates are the main source of energy for ruminants (Nozière et al., 2010). Carbohydrates get broken down into volatile fatty acids (VFA) which consist of propionate, acetate and butyrate. These VFA's can be absorbed through the wall of the rumen. Volatile fatty acid levels are lower in the rumen when high amounts of fat supplements are added and cellulose digestion is decreased (Brooks et al., 1954). Schoonmaker et al. (2010) found that with the increase of WDG in the diet, VFA production was decreased. Furthermore, Schoonmaker et al. (2010) indicated that some carcass characteristics such as lower marbling scores and decreased quality grades may be a result of the altered VFA production. Apparently, it seems that propionate, the primary gluconeogenic precursor in ruminants, is considered to be a large contributing substrate for marbling in the ruminant animal (Schoonmaker et al., 2010).

IV. Deposition/Depletion of Fat

Growth is commonly referred to as the increasing size of muscle, bone and fat depicted in a sigmoid curve. As an animal grows, fat is one of the last tissues to form in the body. Owens et al. (1993 and 1995) stated adipose tissue grows via hyperplasia and

regularly occurs throughout the adult life of an animal. However, fat partitioning is dependent upon the sex, age, stage of growth, hormonal regulation, intake of required nutrients, mature body size, and compensatory growth of the animal (Byers, 1982 and Owens et al., 1993). The addition of fat to the system ultimately comes from the fat sources in the diet, but has to come from metabolic processes either from plasma triacylglycerols via lipoprotein lipase or by de novo synthesis of fatty acids (Vernon, 1982). Pethick et al. (2004) disputed that intramuscular and other fat depots develop at similar rates and suggested that intramuscular fat is not a late maturing fat depot. It has been shown that increasing the net energy in the diet, increasing cereal grain content, feeding processed grain and increasing the lipid content of the diet can increase the intramuscular fat in beef animals during finishing (Pethick, et al., 2004). Byers et al. (1982) stated that the primary way in which nutritional factors regulate or direct the deposition of fat and protein is through the total amount of energy that is absorbed from the system.

Fat deposition not only can be influenced by diet of the animal, but is also due to breed differences and hormonal control. Dinh et al. (2010) concluded genetic variation in fatty acid synthesis and deposition between Angus, Brahman and Romosinuano breeds of cattle. There are also differences between age of cattle and the amount of fat deposition; Wood and Butler-Hogg (1982) stated that specific metabolic factors for fat depots play a role during the growth of an animal. According to Azain (2004), derivatives of fatty acids can have hormone-like effects and have previously shown the ability to regulate gene expression of preadipocytes that can affect the production of fat cells numbers and type. Trenkle et al. (1983) stated that cell hypertrophy seems to contribute more to fat

accretion than hyperplasia in growing animals, which is contradictory to that research by Owens et al. (1993 and 1995). Okumura et al. (2007) demonstrated intramuscular fat continues to increase with the age of a steer, although the rate at which intramuscular fat is deposited depends upon the type of muscle.

Koger et al. (2010) reported that fat thickness at the 12th rib was thicker in steers fed DGS than cattle fed a conventional corn diet. When fed crude glycerin (which can be converted to propionate—a gluconeogenic precursor—in the rumen), 12th rib fat also increased as dietary crude glycerin percentage increased (Gunn et al., 2010). Vander Pol et al. (2009) and Schoonmaker et al. (2010) both alluded to WDG with or without solubles being partially protected from biohydrogenation in the rumen which can allow an increased flow of unsaturated fatty acids to the small intestine where it would then get absorbed and deposited in adipose tissue. Westerling and Hedrick (1979) and Andrae et al. (2001) found differences in the types of fatty acids that are found in fat depend on the type of diet that is fed. For example, in fat from steers that were fed predominantly grass contained more saturated fatty acids and less unsaturated fatty acids than those found in cattle fed a predominantly grain-based diet. It was also found that consumers tend to prefer meat that has more total unsaturated fatty acids (Andrae et al., 2001).

V. Polyunsaturated Fatty Acids

Polyunsaturated fatty acids are fatty acids that contain more than one double bond. It has been noted that large amounts of PUFAs in meat, although not harmful to the human diet, can be detrimental to the shelf-life stability of the meat product, can create off-flavors and off-aromas (such as aldehydes and ketones), can cause the product to

oxidize more quickly, and also affects the oxidative stability of the product (Greene, 1969, Calkins and Hodgen, 2007, de Mello et al., 2008b, Wood et al., 2008, Warren et al., 2008 and Koger et al., 2010). Typical percentages of fatty acids in beef are about 47% SFA, 42% monounsaturated fatty acids and about 4% PUFA (Scollan, 2003). Scollan (2003) noted in ruminants fatty acids are not incorporated into triglycerides as much as they are incorporated into the phospholipids (which are present predominantly in the muscle as intramuscular fat). Diet of the animal has an impact on the fatty acids content in the meat, but it varies because linoleic acid (18:2n-6), for example, is the most abundant fatty acid in adipose tissue and muscle of cattle fed concentrate diets, however, only about 10% of the dietary linoleic acid is available to be deposited into tissue (Wood et al., 2008).

Jiang et al. (2009) found that conjugated linoleic acids (CLAs) were doubled in number in outer subcutaneous fat than muscle of ruminants, however PUFAs were most extensively found in the lean tissue of the animal. Fat is composed of neutral and polar portions in meat. Polyunsaturated fatty acids are found predominantly in the polar portion of the fats (Noci et al., 2005 and Jiang et al., 2009). However, diets high in PUFAs may contribute to off-flavors in the meat of beef animals, due to the production of off-flavors such as aldehydes and ketones (Calkins and Hodgen, 2007).

Elmore et al. (1999) conducted research to determine the impact of various fatty acid compositions had on aroma profiles and the eating quality of meat. It was postulated that even the slightest change in concentration of PUFAs (whether that be a result of degradation products such as ketones and aldehydes or even the thermal degradation of

lipids) might alter the aroma volatiles when cooking and thereby affecting the flavor and eating quality of the meat. The idea that the phospholipid fraction seems to contribute to the flavor of cooked meat more than the triglyceride fraction was further investigated in a study by Mottram and Edwards (1983). Oxidative changes and the interaction between lipid and lean may also give rise to flavors or even aromas in cooked meat (Mottram and Edwards, 1983). The Maillard reaction, the cooking effect on amino acids and sugars also referred to as non-enzymatic browning, is also thought to carry some influential flavor characteristics during the cooking of the product (Calkins and Hodgen, 2007 and Mottram and Edwards, 1983). In the study conducted by Elmore et al. (1999), conclusions were made that cooked beef samples that contained increased levels of PUFAs elicited higher concentrations of lipid oxidation products. Moreover, compounds formed from the Maillard reaction did not show any significant differences among the various diets that were fed cattle, instead it was the volatiles from lipids that showed the largest response and variation in volatiles after cooking. This could be in part due to the higher levels of aldehydes derived from increased levels of PUFAs in the meat samples (Elmore et al., 1999).

Polyunsaturated fatty acids do not only affect the flavor of fresh meat. Due to their multiple double bond nature, they are healthier for the human to consume (beef, fish and eggs are the main sources that are high in *n*-3 C₂₀ PUFA), although beef has been criticized in the past due to being too high in SFA. However, PUFAs are the main culprit for suppressed color stability due to their nature of being easily oxidized (Scollan et al., 2001, Karolyi et al., 2009, Calkins and Hodgen, 2007, de Mello, Jr., et al., 2008 and Koger et al., 2010).

VI. Meat Color and Flavor

Meat color is dynamic, even during retail display. Because of the Metmyoglobin Reducing Activity cycle (MRA cycle), scientists have been able to understand the way in which meat changes from purple (deoxymyoglobin) to red (oxymyoglobin) to brown (metmyoglobin). This scheme shows that when reducing equivalents are high, meat can turn from purple to red and back again, but as soon as the meat loses reducing equivalents, the meat is forced to the metmyoglobin state, which is unacceptable to consumers. In fact, Siegel (2010) found that consumers prefer meat that contains less than 40% metmyoglobin. Renner (1990) noted that once the surface of a piece of meat in a retail display reaches a low 20% metmyoglobin, consumers will reject it. Mancini and Hunt (2005) further reiterate meat purchases are influenced by color. This is because consumers would rather purchase a steak that has more eye appeal than one that does not. Thus, it is very important to have a good understanding and knowledge about the MRA cycle and how certain packaging systems extend the shelf life of a product (for example high oxygen modified atmosphere packaging).

Oxidation of meat products causes off-colors. Oxidation of proteins and lipids also influences the color and the flavor of the product. Lipid oxidation is dependent on the composition of phospholipids, PUFA, and concentrations of other elements, such as oxygen and metal ions (Calkins and Hodgen, 2007), which all seemingly increase when WDGS are fed (de Mello, Jr., et al., 2008c and Koger et al., 2010). This poses a problem to retailers as many cattle in the US are finished on concentrate diets, most containing WDGS in some percentage fed with a roughage source.

Protein oxidation, inherent when meat products are irradiated for food safety issues (Rowe et al., 2004), can facilitate oxidation via the production of free radicals. Protein oxidation is also apparent in modified atmosphere packaging (MAP). The reason for MAP packaged meat is to stabilize the bright red color of meat, the indicator of freshness by consumers (Renerre, 1990, Killinger et al., 2004 and Mancini and Hunt, 2005). Typically, oxygen comprises 70-80% and carbon dioxide fills the remaining 20-30% of the atmosphere in the package containing the meat (Lund et al., 2007 and Lund et al., 2007). Protein oxidation's main effect on meat quality is that of decreasing tenderness which can happen in different ways. Lund et al. (2007) noted in pork that the effect of protein oxidation is a result of bond formations and interactions between proteins. Interactions such as oxidation of cysteine thiol groups forming disulfide bonds, formation dityrosine, reactions with between carbonyls and lysine residues, intermolecular cross-links in myosin heavy chains and also the decreased ability for enzymes to function properly (which would work normally under non-oxidative conditions, tenderizing meat) are all possibilities. Lund et al. (2007), found in meat that is packaged in either skin packages or packages that contain no oxygen will not result in cross-linkages between myosin heavy chains.

Lipid oxidation differs from protein oxidation because lipids can be prevented or at least sustained from oxidation by implementing antioxidant, however protein oxidation cannot be prevented in this way (Lund et al., 2007). Lipid oxidation plays an important role in meat quality and flavor (Campo et al., 2006). Greene (1969) and Greene and Cumuze (1981) stated ferric heme pigments are able to catalyze the oxidation of lipids found in meat, due to the free radical intermediates that decompose the hemes. This

results in a conversion of a bright red color to a dull brown color, and often causing off-flavors. Rancid odor tends to stay with the product after cooking. The 2-Thiobarbituric Acid (TBA) assay is a common chemical test that is often used in laboratory settings to determine the amount of lipid oxidation in red meats. The TBA values correspond to the amount of oxidation in a piece of meat that can be recognized by an experienced taste panelist. It has been found that a threshold between 0.5 and 1.0 of TBA values can be detected by an experienced panelist (Greene and Cumuze, 1981).

From a pigment standpoint, there is a multitude of factors that influence the color of meat. The main factors which influence meat color are the muscle structure, amount of pigment present and the chemical state of the pigment. Muscle structure becomes an important factor when looking at pH of the meat in relation to the isoelectric point (pI) of major proteins (myosin), which is at pH of 5.4 (Huff-Lonergan and Lonergan, 2005). The pI of muscle is obtained as the net charge of the proteins is zero (the number of positive charges equals the number of negative charges). It is well known that the color of meat is associated with the pH of meat. For example, a piece of meat that has a low pH (5.2), will have a light color. This is because at a lower pH, the proteins' charges are more attracted to each other and therefore, will reflect light; this will make the surface of the meat appear light. In the opposite situation, when the pH is higher (7.0) the protein charges will begin to repel each other and because of this, the surface of the meat will be more open and will absorb light and appear dark in color.

Color of meat is dependent upon the concentration of meat pigments. Meat pigments vary among species, beef having more than lamb, lamb having more than pork

or poultry (Lawrie, 1998). Pigment concentration can also depend upon the age and sex of the animal as well; veal appearing less red than market beef animals. Meat pigments are impacted by packaging systems and postmortem conditions such as chilling, type of lighting in retail display, storage, distribution and surface microbial growth (Renerre, 1990 and Mancini and Hunt, 2005).

Finally, the chemical state of the pigment is other important concept when talking of meat color. The structure of myoglobin is such that it contains a heme ring with an iron molecule in the center which is attached to a histadine of globin and on the other end of the iron molecule a free binding site. When various compounds attach to the free binding site, various colors will be reflected. For instance, when oxygen is not present, and no ligand attaches to the free binding site, a purple color is emitted (deoxymyoglobin, ferrous state). When oxygen is present, the meat will be red in color (stable, oxymyoglobin, ferrous state). The increased amount of oxygen and other factors such as temperature and even pH can allow oxygen to penetrate deeper and deeper into the muscle's surface. When a hydroxyl group is attached, the meat will appear brown (metmyoglobin, ferric state) in color. Because meat is dynamic, changes will occur within the pigments with available reducing equivalents (Fox, Jr., 1966 and Mancini and Hunt, 2005).

Although it is pivotal to know that meat color diminishes when lipid or protein oxidation occurs in beef, it is equally important to be aware that issues in palatability arise, even after the product has been cooked (Greene and Cumuze, 1981 and Campo et al., 2006). Flavor is an important part of consumers coming back to buy products that

they know taste good (Carmack et al., 1995 and Calkins and Hodgins, 2007). Flavor consistency among beef products is a continuing research endeavor. Some aspects of flavor are already known, including knowledge about different muscles and their flavor and shelf life, knowing what happens to beef in retail display, packaging methods and cooking methods. Campo et al. (2006) demonstrated that as display time in a retail case increased, beef flavor decreased and there was an increase in off flavors. Campo et al. (2006) and Calkins and Hodgen (2007) found that diet of the animal has an effect on the meat product as well, as the cattle fed concentrate diets (as well as diets high in PUFAs) seemed to have the highest values of abnormal flavor.

There can be variations among muscles in beef-flavor-intensity, juiciness and tenderness due to factors such as diet fed to the animal, age, sex, breed, retail display time, dry aging versus wet aging and the perception of these differences vary from consumer to consumer. However, there is a concept known as the “halo effect” that can influence results that may not tell the whole story of the perception of the meat that is being tested. One common problem about sensory evaluation is when the taste panelist is asked a question, the answer may impact the response of following questions (Clark and Lawless, 1994). For example, if a sample of meat has some connective tissue or some off-flavor aspects within it, a taste panelist may discount the sample in its entirety even if it has a robust flavor and intense juiciness, rather than for docking the sample for the issue at hand being either the connective tissue or off-flavor.

VII. Conclusion

Wet distillers grains plus solubles has recently contributed to a large proportion of finishing diets in Nebraska feedlots. However, WDGS have been noted to alter the fatty acid composition of beef muscles. This alteration has ultimately led to compromising factors in the fresh product. Microorganisms transform PUFAs to SFAs by biohydrogenation in the rumen, however, it appears that WDGS affect the way in which fatty acids are deposited in muscle of beef animals. Moreover, Kucuk et al. (2001) showed that when ruminants were fed diets high in increasing increments of concentrate and lower levels of forage, duodenal content flows increased. This supports the idea that if PUFAs escaped the rumen before they could be biohydrogenated, they could then become absorbed by the small intestine, further being deposited into muscle tissue as PUFAs and not as SFAs.

Particle size, the digestibility of fiber, as well as pH in the rumen have been noted to influence rate of feedstuff passage in the rumen (Allen and Mertens 1988, Kucuk et al., 2001, Yang et al., 2001 and Seo et al., 2009). Larger particle size feedstuffs or forages will encourage rumination (remastication or cud-chewing) and the large particles will become smaller once they are re-chewed and then swallowed (Seo et al., 2009). Kucuk et al. (2001) noted that the flow of SFAs increased as dietary forage level increased. Increasing the amount of concentrate in the diet can lower the pH in the rumen and reduce the rate of biohydrogenation (Kucuk et al., 2001), which in turn could mean PUFAs will not be converted to SFAs as readily.

It is not completely clear what the role of fat in distillers grains is in the finishing diet for ruminants. What is known, however, is that wet distillers grains plus solubles do have an impact on the color, shelf-life stability, oxidation potential, and the fatty acid profile in the meat. Because of this, it was hypothesized that cattle fed a “low-fat” wet distillers grain diet without the “syrup” or solubles fraction would have better meat quality than meat from cattle fed WDGS.

Materials and Methods

MATERIALS AND METHODS

Animals, diets and sample collection

This study began in the fall of 2009 when ninety-six cross-bred, yearling steers from the University of Nebraska-Lincoln feedlot were separated into three different groups and were then fed three different finishing diets. These steers were fed for a total of 131 days. One diet consisted of low-fat wet distillers grains with no solubles (LFWDG), another diet consisted of traditional wet distillers grains with solubles (TWDGS) and the final diet was a corn-based, control diet. On February 24, 2010 the steers were harvested at Greater Omaha Packing Co., Inc. (Omaha, NE). Two days later, a group of graduate students from the ruminant nutrition discipline area collected data on the carcasses and a group from the meat science department randomly chose forty-five USDA Choice carcasses, 15 from each treatment, and removed their respective strip loins (IMPS # 180, NAMP, 2007). The respected strip loins were labeled by numbered pins and also by a carcass ink pen to be sure to keep identity then they were vacuum packaged and shipped to the Loeffel Meat Laboratory at the University of Nebraska-Lincoln. The

product was then aged for 14 days at $0.5\text{ }^{\circ}\text{C} \pm 2$ in the carcass cooler, and then the strip loins were fabricated. Seven steaks were cut from each strip loin, the breakdown is as follows; beginning at the most cranial end of the strip loin, two 2.5 cm steaks were cut for taste panels and two 2.5 cm steaks were also cut for Warner-Bratzler Shear Force (WBSF) testing. The remaining loin sections were cut into 1.3 cm thick steaks for the measurement of oxidation by using the thiobarbituric acid method (TBARS). TBARS steaks for day 7 and 4 were one steak that was divided into two portions, TBARS steak day 0 was its own steak but it also was a steak that proximate analysis was performed. Steaks were finally vacuum packaged using a MULTIVAC 500 (Multivac, Inc., Kansas City, MO) in Prime Source Vacuum Pouches (20.3 x 25.4 cm 3 mil STD Barrier). Steaks for retail display and day 7 taste panels were wrapped in oxygen-permeable polyvinyl chloride film for simulated retail display. Day zero steaks were vacuum packaged and immediately frozen in a $-38\text{ }^{\circ}\text{C} \pm 2$ freezer.

Retail Display

Day 4 and day 7 steaks were placed into labeled foam trays (Stryo-Tech Foam Manufacturing, Denver CO, white foam tray 21.3 x 14.8 x 1.6 cm 36K02SW). Labels were randomly assigned to the treatments so that there would be no bias as to the treatment of each steak during retail display. These steaks were overwrapped with the same oxygen-permeable polyvinyl chloride film and were randomly placed into two retail display cases (Tyler Refrigeration Corporation, model LNSC5, MI, USA) with a temperature controlled at $2\text{ }^{\circ}\text{C} \pm 2$, to simulate retail display conditions. In this simulation, the steaks were continually exposed to 3 sets of two continuous 1000-1800 lux warm white fluorescent lighting (Phillips Inc., F32T8/TL741 ALTO 700 Series, 32

WATT B7, Royal Philips Electronics, Amsterdam, Netherlands). The steaks were taken out of the retail display case at day 4 or day 7, vacuum packaged and frozen in a $-28\text{ }^{\circ}\text{C} \pm 2$ freezer.

Objective and Subjective Color

Objective color measurements were made each day for seven days at about 10:00 am each day. The measurements were made with a Minolta Chromameter CR-400 (Minolta Camera Company, Osaka, Japan) with a 8 mm diameter measurement area and a 11 mm diameter illumination area, illuminant D65 and a 2° standard observer, and the recorded measurements included L^* (psychometric lightness; black = 0, white = 100), a^* (red = positive values, green = negative values) and b^* (yellow = positive values, blue = negative values). The chromameter was calibrated every day by normal standards with a white calibration plate that came with the machine from the manufacturer. Six different readings were obtained randomly on each steak. Percent discoloration, or subjective color, was estimated every day at about the same time as objective color scoring for seven days by seven graduate students who had previous experience with tabulating subjective color scores. Zero percent discoloration was given to a steak that had no discoloration, and as the percentages increased, so did the discoloration amount of the steak.

Oxidation

Day 0, day 4 and day 7 steaks were removed from the freezer and were allowed to partially thaw (until crusted), then they were immediately cut into small cubes, flash frozen in liquid nitrogen and then powdered in a blender (Warning Commercial Blender, Torrington, CT Model 51BL32, 120 volts, AC, 60 Hz, 30 Amps). The thiobarbituric acid

assay (TBARS) analysis was performed by using the standard protocol by Buege and Aust (1978), modified by Ahn et al. (1998).

Warner-Bratzler Shear Force

Steaks were removed from the freezer and placed into the cooler to be thawed for 24 hours at 5 °C. The steaks were then grilled to 71°C (medium degree of doneness). Steaks were grilled on a Hamilton Indoor/Outdoor grill (Hamilton Beach/Proctor Silex, Inc., Catalog No. HB9, Model 31605A Series Type G16 Grill, 120 v~ 60 Hz, 1200 W) and a thermocouple (Type T, Model L-0044T Fine Wire Thermocouples Omega Engineering, Inc., Stamford, CT) used with a thermometer (Omega digital Thermometer, S/N 01120275, Model 450-ATT, T-Thermocouple, Stamford, CT) was placed in the geometric center of the steak using a pointed syringe to get the wire in the correct location. Initial and final weights were recorded to calculate drip loss of each steak, and also the initial and final temperatures were recorded. Steaks were cooked on one side until the center temperature reached 35 °C and then turned over. The steaks were then left on the grill until the steak reached 71 °C. Once the steaks' temperature and weight were recorded, they were placed on a tray and covered with oxygen-permeable polyvinyl chloride film and placed in a walk-in cooler overnight. The next morning, each cooked steak had six 1.3 cm cores removed (using a Delta 20.3 cm Drill Press, Mfg. Date/Ser. No. W9609, Model 11-950, Delta International Machinery Corp., Pittsburgh, PA) and were sheared using a tabletop WBSF machine (Salter Brecknell, Model 235 6X: Motor for Shearer—Bodine Electric Company, Small Motor S/N 0291KUIL 0009 Chicago, IL) and results were recorded for each of the six cores that were sheared.

Taste Panel

Steaks from 0 and 7 day of retail display were boxed and shipped to the University of Florida for consumer evaluation. The steaks were shipped to Florida due to the project deadline soon approaching and unavailability of the consumer taste panel due to the ongoing construction in the Animal Science building. Results and protocols were obtained from Dr. D. Dwain Johnson, professor of Meat Science, for the steaks. Prior to cooking, steaks were thawed for 18 h at 4 °C. Steaks were cooked on grated, non-stick electric grills (Hamilton Indoor/Outdoor grill, Hamilton Beach/Proctor Silex, Inc., Catalog No. HB9, Model 31605A Series Type G16 Grill, 120 v~ 60 Hz, 1200 W) that were preheated for 20 minutes. Steaks were turned once when the internal temperature reached 35 °C and then were allowed to finish cooking until they reached an internal temperature of 71 °C (AMSA, 1995). Internal temperatures were monitored by copper-constantan thermocouples (Omega Engineering Inc., Stamford, CT) placed in the geometric center of each steak and recorded using a 1100 Labtech Notebook for Windows 1998 (Computer Boards Inc., Middleboro, MA). Upon reaching 71 °C, steaks were served to panelists while still warm. Panelists evaluated 6 samples, 2 sample cubes that were 1.27 cm³ per sample, served in warmed covered containers. Sensory sessions were conducted once or twice daily in a positive pressure ventilated room with lighting and cubicles designed for objective meat sensory analysis. A 7–11 member sensory panel trained according to AMSA sensory evaluation guidelines (AMSA, 1995) evaluated each sample for juiciness (8=extremely juicy; 1=extremely dry), flavor (8=extremely intense beef flavor; 1=extremely bland beef flavor), tenderness (8=extremely tender; 1=extremely tough), connective tissue (8=none detected; 1=abundant amount), and off-

flavor (1=extreme off-flavor, 6=no off-flavor detected). Along with objectively scoring off-flavor, if an off-flavor was noticed the panelists were asked to describe or characterize the off-flavor to the best of their ability.

Proximate Analysis, Fatty Acid Profile and Mineral Analysis

Fatty acid profiles were obtained by using gas chromatography (GC) analysis in Dr. Tim Carr's lab, professor of Nutrition and Health Sciences. Samples were prepared by using the fatty acid analysis technique by Folch et al. (1957), Morrison and Smith (1964) and Metcalfe et al. (1966). The column that was used was the Chrompack CP-Sil 88 (0.25 mm x 100 m). Injector temperature was set at 270°C and the Detector temperature was set at 300°C. Head pressure was 40 psi and the flow rate was at 1.0 mL/min.

Moisture and total fat of pulverized samples were measured. Two grams of the pulverized muscle sample were measured in duplicate and were measured using the LECO thermo-gravimetric analyzer (LECO Corporation, model 604-100-400, St. Joseph, MI). The total fat was measured as outlined by AOAC (1990), the Soxhlet extraction method.

Frozen, powdered meat samples were sent to Ward Laboratories, Inc., (Kearney, NE) for mineral analysis of the following, Ca, P, K, Mg, Zn, Fe, Mn, Cu, S, and Na. The mineral composition was measured using the standard procedure of Ward and Gray (1994). Ca, P, K, Mg, S, and Na was expressed on a percent on dry matter basis, while Zn, Fe, Mn and Cu were reported in ppm on a dry matter basis.

Statistical Analysis

Data from this experiment were analyzed using the 2009 9.2 SAS® Institute, Inc., USA. Data were analyzed as a split-plot design where dietary treatment was the whole plot and the day in retail display was the split-plot. Data were analyzed using the GLIMMIX procedure and when significance ($P \leq 0.05$) was indicated by ANOVA, mean separations were performed using the LSMEANS and DIFF functions of SAS. The study was completed in its entirety by mid-May 2010.

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MANUSCRIPT

Running Head: Low-fat wet distillers grains and beef quality

Feeding Low-fat Wet Distillers Grains and Beef Quality^{1,2}

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Abstract

The incorporation of distillers grains into cattle diets decreases shelf life due mostly to increased PUFA, which allow rapid oxidation. To combat this, a low-fat distillers grain ration, without solubles (LFWDG; 4.72% fat), was compared to a traditional diet containing wet distillers grains plus soluble (TWDGS; 6.91% fat) and a corn-based control diet (Corn; 3.64%). Strip loins (*M. longissimus lumborum*) from 45 USDA Choice steers (15 per dietary treatment) were used to test the effects of diet on fatty acid profile, fat composition, oxidation, color changes during retail display, flavor and tenderness of beef steaks. After 4 days of simulated retail display, samples from cattle fed LFWDG had significantly more oxidation than samples from TWDGS or Corn. By day five of simulated retail display, meat from LFWDG were less red in color (lower a^*) and had more visual discoloration than other treatments ($P \leq 0.0001$). L^* and b^* were not significantly different. Samples from LFWDG were less tender ($P \leq 0.0006$) and had more off-flavor ($P \leq 0.02$) after display than TWDGS. Meat from LFWDG had about 10% more PUFA than TWDGS (4.86 vs 4.46, respectively; $P = 0.08$). These data suggest fatty acids contained within the distillers grains not biohydrogenated during digestion, while fatty acids in the soluble fraction are more readily hydrogenated in the rumen. Consequently, diets formulated with LFWDG tend to compromise meat quality after extended retail display.

Index terms: Beef, Distillers Grains, PUFA, Tenderness, Flavor, Oxidation

Introduction

By-products from the ethanol industry, distillers grains from corn in particular, are being used in increasing amounts as a feedstuff in the cattle industry due to the nature of their availability, nutrient value, and low cost (Leupp et al., 2009). Distillers grains (DG) are an inexpensive, yet nutritive feedstuff. Wet distillers grains are a product of the dry milling industry of ethanol production and depending upon the plant and its capabilities; the relative amounts of distillers grains and distillers soluble mixed together is variable (Erickson et al., 2005).

de Mello Jr. et al. (2008) previously reported a correlation between the amount of distillers grains in the diet and increased oxidation in the meat, resulting in the meat turning brown faster. The discoloration of the myoglobin occurs as a result of increased PUFA's in beef according to de Mello Jr. et al. (2008) and Gill et al. (2008). Polyunsaturated fatty acids are susceptible to oxidative rancidity and therefore decrease shelf life. It is hypothesized that a low-fat diet of WDG may reduce the concentration of PUFA leading to an increased shelf life. The focus of this research was to compare the effects of a low-fat WDG diet without the addition of solubles (LFWDG) to a traditional wet distillers grains diets with solubles (TWDGS) and a corn-based control diet concerning their effect on fatty acid profile, fat composition, oxidation, color changes during retail display, flavor and tenderness of beef strip loins.

Materials and Methods

Animals, diets, and sample collection

Cross-bred, yearling steers from the University of Nebraska-Lincoln feedlot (n = 96) were allocated to three different finishing diets and fed for 131 days. The diets

consisted of low-fat wet distillers grains with no solubles (LFWDG), traditional wet distillers grains with solubles (TWDGS) and a corn-based, control diet (Table 1). After slaughter forty-five USDA Choice carcasses, 15 from each treatment, were randomly selected and their respective strip loins (IMPS # 180, NAMP, 2007) were removed, vacuum packaged and shipped to the Loeffel Meat Laboratory at the University of Nebraska-Lincoln. After aging at $0.5^{\circ}\text{C} \pm 2$ (14 days post-mortem) the strip loins were fabricated into strip steaks. There were seven steaks cut from each strip loin. Four 2.5 cm steaks were cut for taste panels and Warner-Bratzler Shear Force (WBSF) testing. The remaining loin sections were cut into 1.3 cm thick steaks for measurement of oxidation using the thiobarbituric acid assay (TBARS). Steaks were vacuum packed using a MULTIVAC 500 (Multivac, Inc., Kansas City, MO) in Prime Source Vacuum Pouches (20.3 x 25.4 cm 3 mil STD Barrier) or wrapped in oxygen-permeable polyvinyl chloride film for simulated retail display. Day zero steaks were vacuum packaged and immediately frozen in a $-38 \pm 2^{\circ}\text{C}$ freezer.

Retail Display

Two steaks from each strip loin (day 4 and day 7) were placed into foam trays (Stryo-Tech Foam Manufacturing, Denver CO, white foam tray 21.3 x 14.8 x 1.6 cm 36K02SW) and were overwrapped with oxygen-permeable polyvinyl chloride film and were randomly placed into two retail display cases (Tyler Refrigeration Corporation, model LNSC5, MI, USA) with a temperature controlled at $2 \pm 2^{\circ}\text{C}$, to simulate retail display conditions. In this simulation, the steaks were continually exposed to 3 sets of two continuous 1000-1800 lux warm white fluorescent lighting (Phillips Inc., F32T8/TL741 ALTO 700 Series, 32 WATT B7, Royal Philips Electronics, Amsterdam,

Netherlands). The steaks were taken out of the retail display case at day 4 or day 7, vacuum packaged and frozen in a -28 ± 2 °C freezer.

Objective and Subjective Color

Color was evaluated for seven days. The measurements were made with a Minolta Chromameter CR-400 (Minolta Camera Company, Osaka, Japan) with a 8 mm diameter measurement area and a 11 mm diameter illumination area using illuminant D65 and a 2° standard observer, and the recorded measurements included L* (psychometric lightness; black = 0, white = 100), a* (red = positive values, green = negative values) and b* (yellow = positive values, blue = negative values). The chromameter was calibrated every day by manufacture recommendations. Six different readings were obtained randomly on each steak. Percent discoloration was estimated every day for seven days by panelists that had previous experience tabulating subjective color scores (n = 7).

Oxidation

Day 0, day 4 and day 7 steaks were cut into small pieces while still partially frozen, flash frozen in liquid nitrogen and then powdered using a blender (Warning Commercial Blender, Torrington, CT Model 51BL32, 120 volts, AC, 60 Hz, 30 Amps). The thiobarbituric acid assay (TBARS) analysis was performed by using the protocol by Buege and Aust (1978), modified by Ahn et al. (1998).

Warner-Bratzler Shear Force

Steaks were thawed for 24 hours at 5°C and were grilled to 71°C. Steaks for WBSF testing were grilled (Hamilton Indoor/Outdoor grill, Hamilton Beach/Proctor Silex, Inc., Catalog No. HB9, Model 31605A Series Type G16 Grill, 120 v~ 60 Hz, 1200 W). A thermocouple (Type T, Model L-0044T Fine Wire Thermocouples Omega

Engineering, Inc., Stamford, CT) used with an thermometer (Omega digital Thermometer, S/N 01120275, Model 450-ATT, T-Thermocouple, Stamford, CT) was placed in the geometric center of the steak. Steaks were cooked on one side until the center temperature reached 35°C and then turned over. They were cooked to 71°C, covered with oxygen-permeable polyvinyl chloride film and placed in a cooler overnight. Within a 24 hour time period each cooked steak had six 1.3 cm cores removed (using a Delta 20.3 cm Drill Press, Mfg. Date/Ser. No. W9609, Model 11-950, Delta International Machinery Corp., Pittsburgh, PA) and sheared using a tabletop WBSF machine (Salter Brecknell, Model 235 6X: Motor for Shearer—Bodine Electric Company, Small Motor S/N 0291KUIL 0009 Chicago, IL) .

Taste Panel

Steaks from 0 and 7 day of retail display were shipped to the University of Florida for consumer evaluation. Prior to cooking, steaks were thawed for 18 h at 4 °C. Steaks were cooked on grated, non-stick electric grills (Hamilton Indoor/Outdoor grill, Hamilton Beach/Proctor Silex, Inc., Catalog No. HB9, Model 31605A Series Type G16 Grill, 120 v~ 60 Hz, 1200 W) that were preheated for 20 minutes. Steaks were turned once when the internal temperature reached 35 °C and then were allowed to finish cooking until they reached an internal temperature of 71 °C (AMSA, 1995). Internal temperatures were monitored by copper-constantan thermocouples (Omega Engineering Inc., Stamford, CT) placed in the geometric center of each steak and recorded using a 1100 Labtech Notebook for Windows 1998 (Computer Boards Inc., Middleboro, MA). Upon reaching 71 °C, steaks were served to panelists while still warm. Panelists evaluated 6 samples, 2 sample cubes that were 1.27 cm³ per sample, served in warmed covered containers. Sensory

sessions were conducted once or twice daily in a positive pressure ventilated room with lighting and cubicles designed for objective meat sensory analysis. A 7–11 member sensory panel trained according to AMSA sensory evaluation guidelines (AMSA, 1995) evaluated each sample for juiciness (8=extremely juicy; 1=extremely dry), flavor (8=extremely intense beef flavor; 1=extremely bland beef flavor), tenderness (8=extremely tender; 1=extremely tough), connective tissue (8=none detected; 1=abundant amount), and off-flavor (1=extreme off-flavor, 6=no off-flavor detected). Along with objectively scoring off-flavor, if an off-flavor was noticed the panelists were asked to describe or characterize the off-flavor to the best of their ability.

Proximate Analysis, Fatty Acid Profile and Mineral Analysis

Fatty acid profiles were obtained by using gas chromatography (GC) analysis. Samples were prepared by using the fatty acid analysis technique by Folch et al. (1957), Morrison and Smith (1964) and Metcalfe et al. (1966). The column that was used was the Chrompack CP-Sil 88 (0.25 mm x 100 m). Injector temperature was set at 270°C and the Detector temperature was set at 300°C. Head pressure was 40 psi and the flow rate was at 1.0 mL/min (Table 3 and Figure 6).

Moisture and total fat of pulverized samples were measured. Two grams of the pulverized muscle sample were measured in duplicate and were measured using the LECO thermo-gravimetric analyzer (LECO Corporation, model 604-100-400, St. Joseph, MI). The total fat was measured as outlined by AOAC (1990), the Soxhlet extraction method (Table 4 and Figure 7).

Frozen, powdered meat samples were sent to a commercial laboratory (Ward Laboratories, Inc., Kearney, NE) for mineral analysis of the following, Ca, P, K, Mg, Zn,

Fe, Mn, Cu, S, and Na. The mineral composition was measured using the standard procedure of Ward and Gray (1994). Ca, P, K, Mg, S, and Na was expressed on a percent on dry matter basis, while Zn, Fe, Mn and Cu were reported in ppm on a dry matter basis (Table 5).

Statistical Analysis

Data from this experiment were analyzed using the 2009 9.2 SAS® Institute, Inc., USA. Data were analyzed as a split-plot design where dietary treatment was the whole plot and the day in retail display was the split-plot. Data were analyzed using the GLIMMIX procedure and when significance ($P \leq 0.05$) was indicated by ANOVA, mean separations were performed using the LSMEANS and DIFF functions of SAS.

Results and Discussion

There were no significant differences for b^* (blueness) or L^* (lightness) (Figures 2 and 3, $P = 0.776$ and $P = 0.9097$, respectively) values among treatments. However, for a^* (redness) values there was a significant treatment x day interaction $P < 0.0001$, respectively (Figure 1). The objective a^* values of steaks from cattle fed LFWDG declined at a significantly ($P < 0.05$) faster rate and to a greater degree (Figure 1) than other treatments. This decline started at day 4 of retail display. Estimated percent discoloration (Figure 4) closely paralleled the objective color data in that meat from cattle fed LFWDG had greater discoloration than the other treatments at day 5 of retail display. Lipid oxidation values were significantly larger at days 4 and 7 in the LFWDG diet when compared to TWGDS and the corn-based diets (Figure 5).

There was no interaction between day and dietary treatment when observed for any sensory attribute (Table 2). As days of aging increased (during retail display),

tenderness scores increased, ratings for amount of connective tissue improved (less connective tissue detected), and off-flavor was more apparent ($P < 0.01$). When differences in sensory ratings and WBSF existed, they favored the TWDGS diet which was equal or superior to the control diet (Table 2). There was no significant difference in shear force values between the treatments. Overall tenderness and off-flavor results were significant ($P \leq 0.0006$) and ($P \leq 0.02$), respectively, in that meat from cattle fed LFWDG were similar to corn-fed controls but has less desirable ratings than meat from cattle fed TWDGS.

Mineral composition of the steaks were generally similar, although the LFWDG diet resulted in lower levels of Fe and Na ($P \leq 0.05$) than the TWDGS diet (Table 5). Although Fe levels were lower in the LFWDG diet, steaks still exhibited higher levels of oxidation.

The control diet had much less PUFA than diets containing DG, and a difference in PUFA of 0.40 (about 10%, $P = 0.08$) was found between LFWDG (4.86) and TWDGS (4.46). This difference would support the increased amount of discoloration and lipid oxidation between the two diets containing DG, especially toward the end of the retail display period. An increase in total trans fatty acids was also significantly different ($P \leq 0.05$) in comparison to the corn-based diet. Fat percent and moisture content was not significantly different among the dietary treatments (Table 4).

There were significant differences in sensory attributes for off-flavor and also overall tenderness. However, although they were significant the numerical difference between steaks from cattle fed TWDGS and steaks from cattle fed LFWDG is 0.17 on an eight point scale, meaning a this difference would be hard to differentiate; but the

statistics found that the taste panelists' preferences were consistent session after session. The statistics show that there is not only a significant difference between the treatments for color (a^* and percent discoloration), but more importantly, consumers will use visual appraisal when purchasing steaks at the retail display counter. Therefore, among these two significant findings, the most important is color.

Although the LFWDG diet contained less fat on a percentage basis than the TWDGS diet (4.72% vs 6.91%), more of that fat was found in the DG portion of the diet. Much of the fat in the TWDGS diet came from the distillers solubles. We hypothesize that fats in the distillers soluble are readily hydrogenated in the rumen while those contained within the DG fraction are more protected from biohydrogenation. This would explain why meat from cattle fed the LFWDG diet tended to have more PUFA which would help explain why there was a decreased shelf life and increased oxidative rancidity of the samples from the LFWDG diets toward the end of retail display. In summary, LFWDG decreases shelf life and increases oxidative rancidity in retail displayed strip loin steaks.

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Table 1. Diet composition of finishing diets fed to yearling steers, expressed as percentage of diet DM.

Constituent	Diet Composition		
	TWDGS	LFWDG	Corn
Dry Rolled Corn	25.0	25.0	42.5
High Moisture Corn	25.0	25.0	42.5
Wet Distillers Grains plus Solubles	35.0	-	-
Wet Distillers Grains	-	35.0	-
Sorghum Silage	10.0	10.0	10.0
Supplement	5.0	5.0	5.0
CP, %	17.8	17.9	13.6
Fat, %	6.91	4.72	3.64
Sulfur, %	0.41	0.37	0.12

Table 2. Sensory Attributes of strip loin (*M. longissimus lumborum*) steaks from steers traditional levels of WDGS, low-fat WDG and corn-based diets.

Attributes ²	Dietary treatments ¹			P-value
	Corn	TWDGS	LFWDG	
Juiciness	5.24	5.18	5.08	0.15
Beef Flavor Intensity	5.61	5.63	5.65	0.88
Overall Tenderness	5.71 ^b	5.89 ^a	5.67 ^b	0.0006
Connective Tissue	6.20	6.21	6.16	0.74
Off-flavor	5.46 ^b	5.59 ^a	5.42 ^b	0.02
WBSF (kg), 0 d	2.85	2.81	2.93	
WBSF (kg), 7 d	2.72	2.79	2.84	

¹Corn, TWDGS(traditional fat WDGS), LFWDG (low fat WDG).

² Juiciness (1 extremely dry – 8 extremely juicy); Beef flavor Intensity (1 extremely bland – 8 extremely intense); Overall Tenderness (1 extremely tough – 8 extremely tender); Connective Tissue (1 abundant amount – 8 none detected); Off-flavor (1 strong/extreme off-flavor – 8 none detected).

^{A, b} Means in the same row having different superscripts are different at $P \leq 0.05$.

Table 3. Weight percentage of fatty acids¹ and fat content of strip loin (*M. longissimus lumborum*) steaks from steers fed traditional WDGS, low-fat WDG and corn-based diets.

Fatty Acid	Dietary Treatments			P-value
	Corn	TWDGS	LFWDG	
10:0	0.03	0.05	0.03	0.41
12:0	0.05	0.04	0.04	0.83
14:0	2.82	2.59	2.62	0.27
14:1 (n-5)	0.65	0.61	0.63	0.68
15:0	0.53 ^a	0.41 ^b	0.46 ^b	0.01
iso 16:0	0.16	0.15	0.16	0.86
16:0	24.67 ^a	23.01 ^b	23.50 ^b	0.01
16:1 (n-7)	3.12 ^a	2.72 ^b	2.84 ^{ab}	0.03
17:0	1.65 ^a	1.43 ^b	1.37 ^b	0.0001
iso 18:0	0.08	0.11	0.10	0.20
17:1 (n-7)	1.50 ^a	1.22 ^b	1.22 ^b	<0.0001
18:0	13.02	13.98	13.12	0.12
18:1 <i>trans</i>	2.46 ^b	3.49 ^a	3.68 ^a	0.01
18:1 (n-9)	42.55	42.19	41.55	0.58
18:1 (n-7)	1.11	0.92	0.85	0.24
18:1 Δ13	0.15	0.16	0.18	0.81
18:1 Δ14	0.14 ^b	0.18 ^a	0.19 ^a	0.03
18:2 <i>trans</i>	0.04 ^b	0.08 ^a	0.07 ^a	0.0003
19:0	0.06	0.07	0.08	0.75
18:2 (n-6)	2.16 ^c	3.43 ^b	3.81 ^a	<0.0001
20:0	0.43 ^b	0.48 ^{ab}	0.52 ^a	0.05
18:3 (n-3)	0.07 ^b	0.10 ^a	0.11 ^a	0.02
20:1 (n-9)	0.07	0.06	0.04	0.21
18:2 <i>cis</i> 9 <i>trans</i> 11	0.03 ^b	0.06 ^{ab}	0.07 ^a	0.02
18:2 <i>cis</i> 10 <i>trans</i> 12	0.01	0.01	0.01	0.29
20:3 (n-6)	0.12 ^b	0.17 ^a	0.17 ^a	0.0013
20:4 (n-6)	0.39	0.44	0.45	0.44
20:5 (n-3)	0.03	0.03	0.03	0.66
22:4 (n-6)	0.05	0.06	0.05	0.06
22:5 (n-3)	0.09	0.09	0.10	0.70
PUFA ²	2.99 ^b	4.46 ^a	4.86 ^a	<0.0001
MUFA	51.76	51.54	51.17	0.79
Total Trans	2.50 ^b	3.57 ^a	3.75 ^a	0.01
SFA	43.51	42.33	42.00	0.19
Others	1.74 ^b	1.67 ^b	1.97 ^a	0.01
Omega 3	0.19 ^b	0.22 ^{ab}	0.23 ^a	0.10
Omega 6	2.72 ^b	4.09 ^a	4.48 ^a	<0.0001
Omega 6: Omega 3	16.99	19.29	20.15	0.50

¹Weight percentage values are relative proportions of all peaks observed by Gas Chromatography.

²*P*-value comparing LFWDG to TWDGS = 0.08.

^{a, b, c}Means in the same row having different superscripts are significant at $P \leq 0.05$.

Table 4. Percent fat and moisture content of strip loins (*M. longissimus lumborum*).

Attributes	Dietary treatments ¹			P-value
	Corn	TWDGS	LFWDG	
Fat, %	7.33	7.17	7.14	0.9707
Moisture Content, %	69.71	69.71	69.81	0.9839

¹Corn, TWDGS (traditional fat WDGS), LFWDG (low fat WDG).

Table 5. Least square means of mineral composition of strip loins (*M. longissimus lumborum*) from cattle fed different dietary regimes.

	Diet Composition ¹		
	Corn	TWDGS	LFWDG
Ca ²	0.02733 ^b	0.03933 ^a	0.03067 ^{a,b}
P ²	0.1840	0.1880	0.1873
K ²	0.3907	0.3960	0.3987
Mg ²	0.03133	0.03467	0.03267
Zn ³	34.5267	35.0267	33.9333
Fe ³	41.2667 ^{a,b}	50.200 ^a	35.9333 ^b
Mn ³	1.4667 ^a	1.000 ^{a,b}	0.4667 ^b
Cu ³	2.4400 ^a	1.8000 ^{a,b}	1.5400 ^b
S ²	0.1867	0.1887	0.1907
Na ²	0.05067 ^b	0.05467 ^a	0.05133 ^b

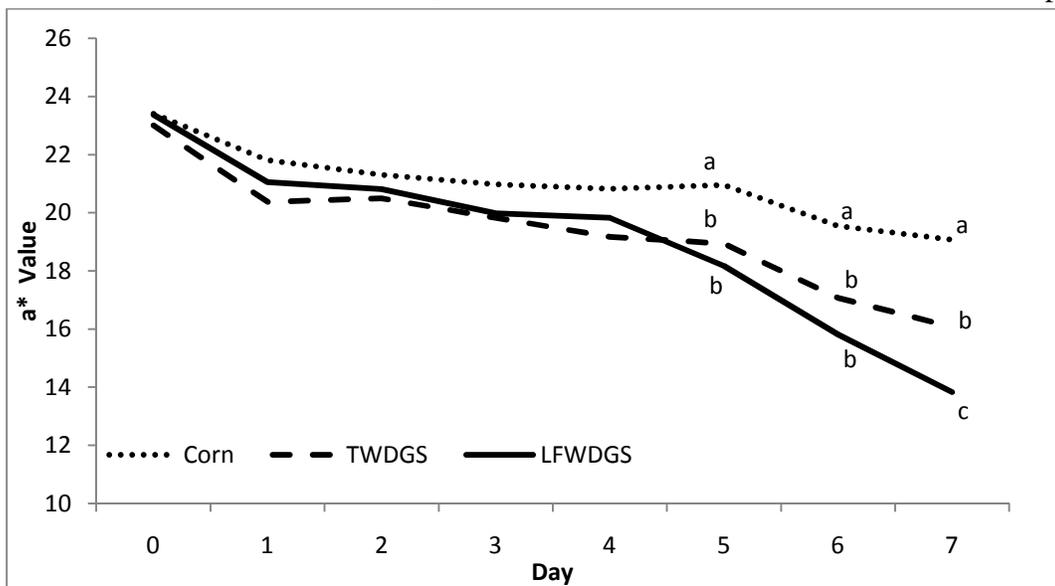
¹ Corn, TWDGS (traditional levels of fat wet distillers grains with solubles), LFWDG (low fat wet distillers grains).

² % on dry matter basis.

³ ppm on dry matter basis.

^{a,b} Means in same rows having different superscripts are different at $P \leq 0.05$.

Figure 1. a^* (redness) values for strip loin (*M. longissimus lumborum*) steaks from steers traditional levels of WDGS, low-fat WDGS and corn-based diets in retail display.



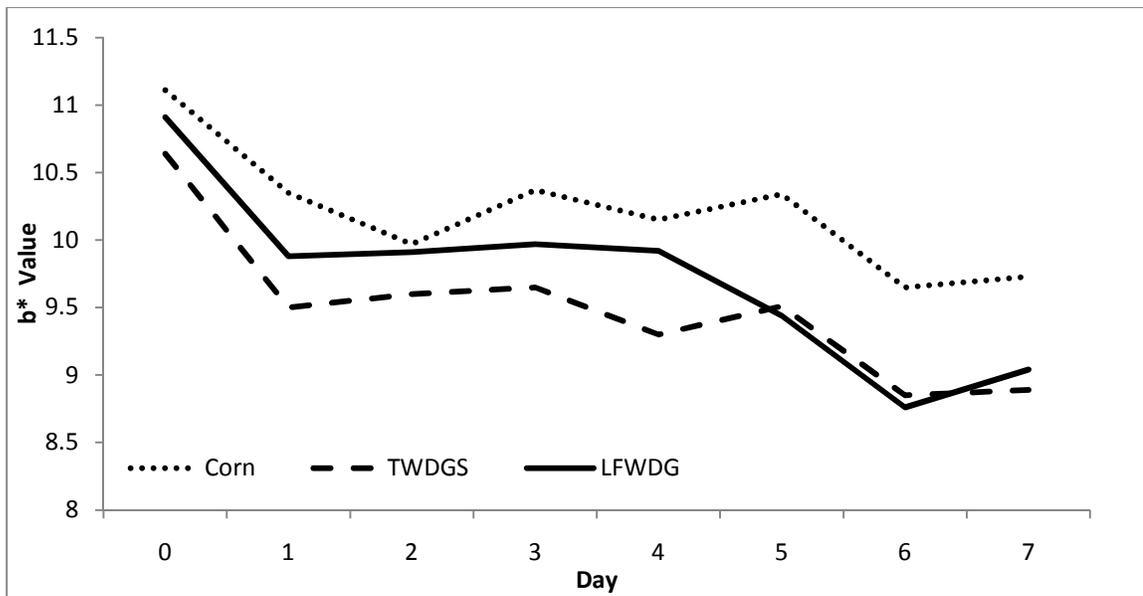
Corn, TWDGS (traditional levels of fat wet distillers grains with solubles),

LFWDGS (low fat wet distillers grains).

^{a, b, c} Means having different superscripts are different within days of display $P \leq 0.05$.

SE = 0.6003.

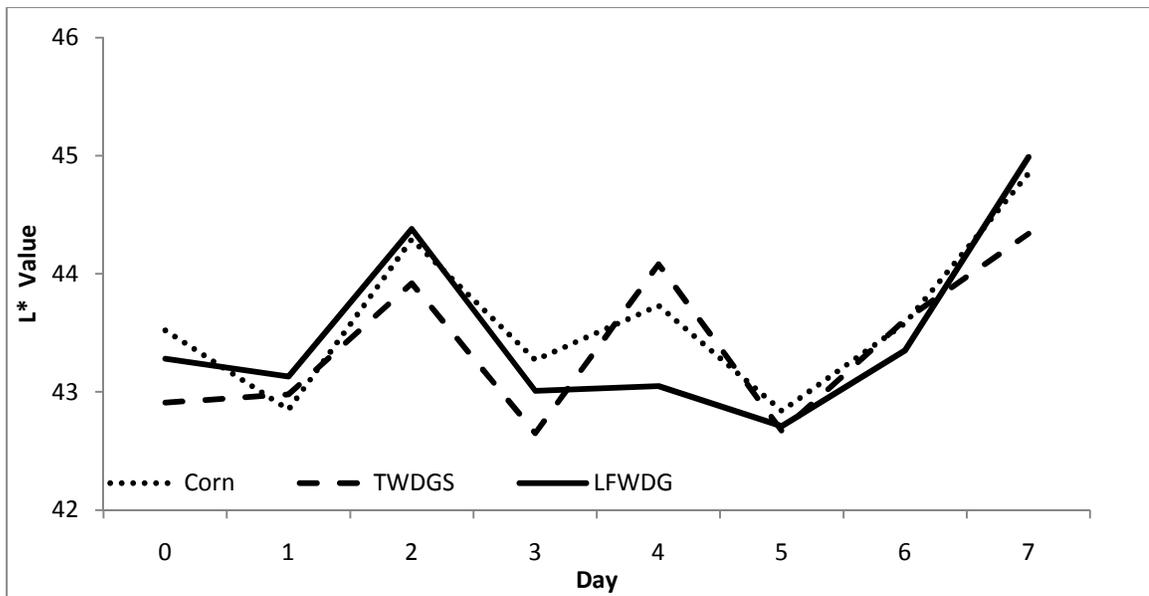
Figure 2. b^* (blueness) values for strip loin (*M. longissimus lumborum*) steaks from steers traditional levels of WDGS, low-fat WDG and corn-based diets in retail display.



Corn, TWDGS (traditional levels of fat wet distillers grains with solubles),
LFWDG (low fat wet distillers grains).

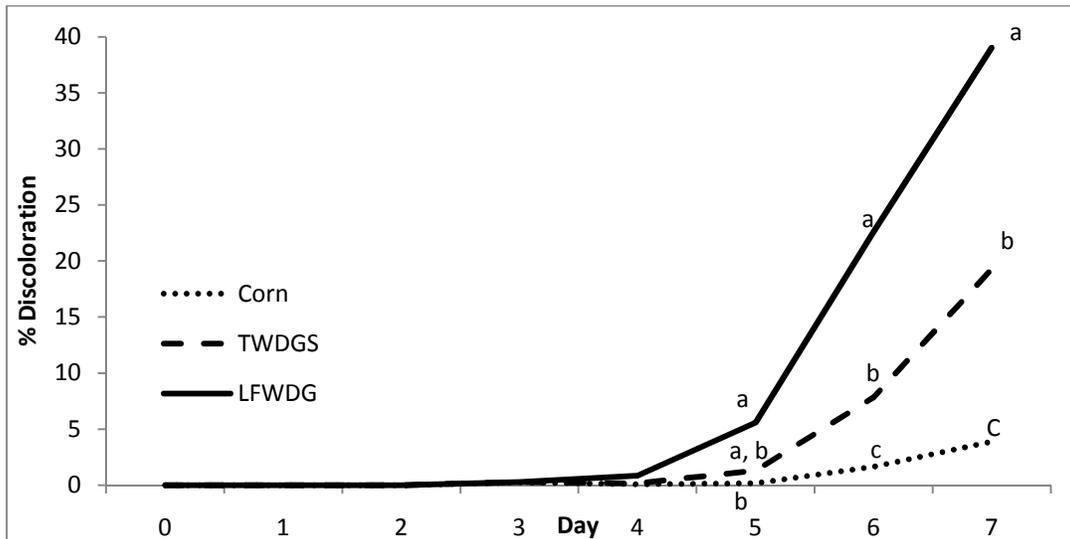
SE = 0.2709.

Figure 3. L* (lightness) values for strip loin (*M. longissimus lumborum*) steaks from steers traditional levels of WDGS, low-fat WDG and corn-based diets in retail display.



Corn, TWDGS (traditional levels of fat wet distillers grains with solubles),
LFWDG (low fat wet distillers grains).
SE = 0.4725.

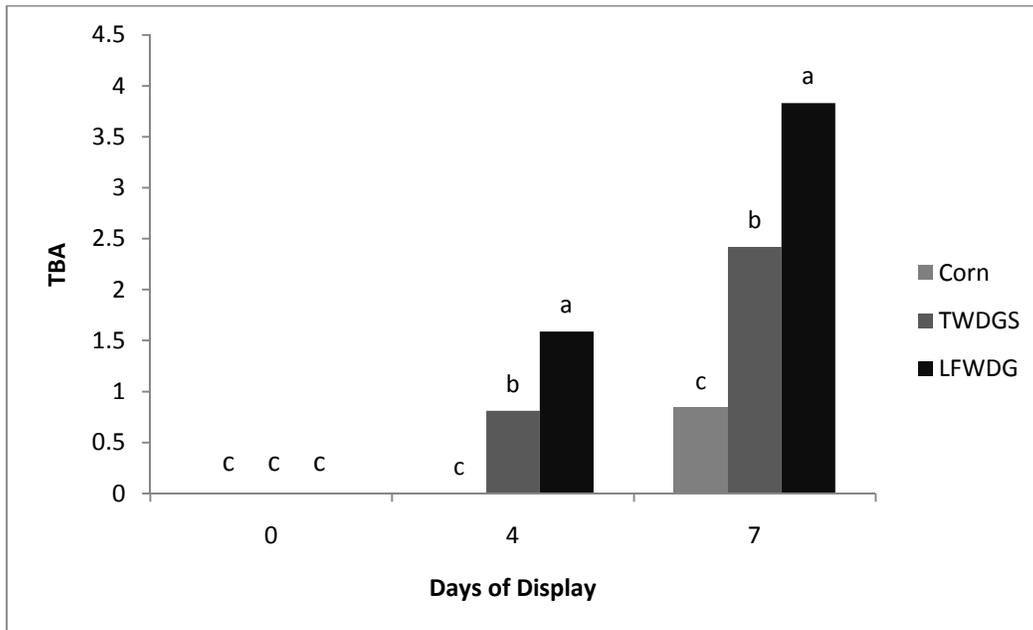
Figure 4. Percent discoloration of strip loin (*M. longissimus lumborum*) steaks from steers traditional levels of WDGS, low-fat WDG and corn-based diets in retail display.



Corn, TWDGS (traditional levels of fat wet distiller grains with solubles),
LFWDG (low fat wet distiller grains).

^{a, b, c} Means having different superscripts are different within days of display $P \leq 0.05$.

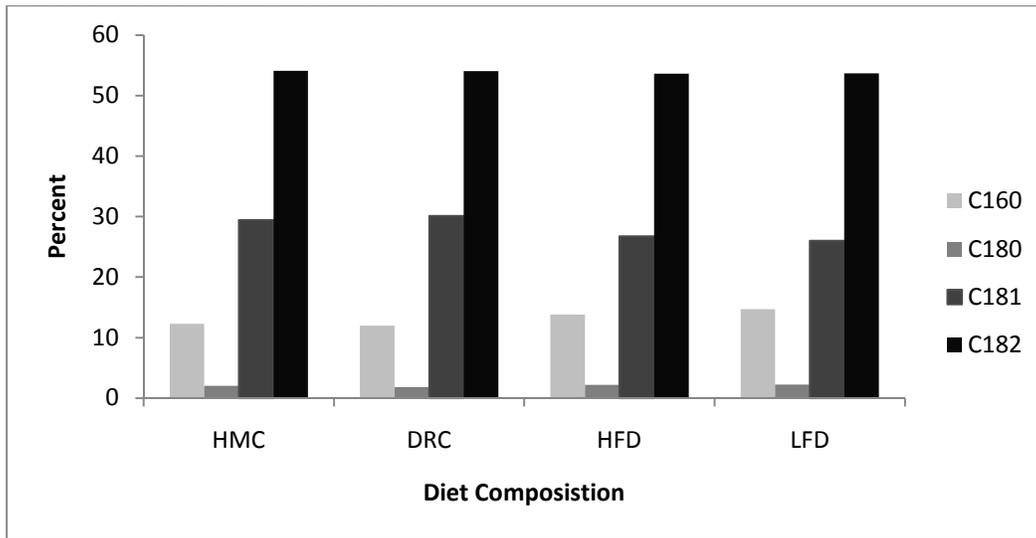
Figure 5. Lipid oxidation values for strip loin (*M. longissimus lumborum*) steaks from steers traditional levels of WDGS, low-fat WDG and corn-based diets.



Corn, TWDGS (traditional levels of fat wet distillers grains with solubles),
LFWDG (low fat wet distillers grains).

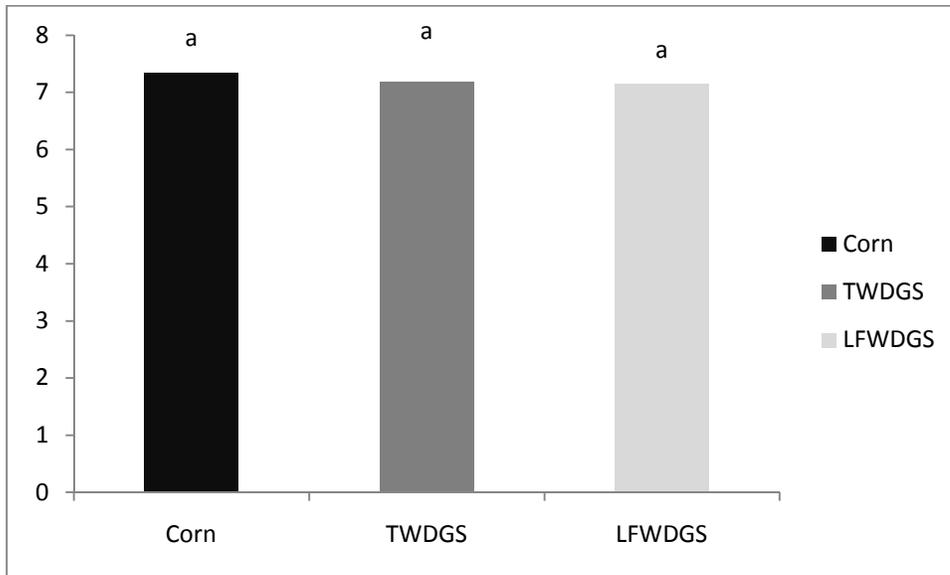
^{a, b, c} Means having different superscripts are different within days of display $P \leq 0.05$.

Figure 6. Percent of important fatty acids in traditional, low-fat and corn-based diets constituents fed to cattle.



C16:0 (Saturated) is Palmitic Acid, C18:0 (Saturated) is Stearic Acid,
C18:1 (Monounsaturated) is Oleic Acid, C18:2 (Polyunsaturated) is Linoleic Acid ($\omega 6$).

Figure 7. Fat percentage of strip loin (*M. longissimus lumborum*) steaks from steers fed traditional levels of WDGS, low-fat WDG and corn-based diets.



^aMeans mean value of the samples taken for each diet. Values are not significantly different.

RECOMMENDATIONS FOR FUTURE RESEARCH

This research unveiled significant findings and even findings that were not expected when this study began. The biggest and most significant finding that discovered was that the hypothesis of the research was that a diet that consisting of Low-Fat Wet Distillers Grains (LFWDG) would result in beef with less polyunsaturated fatty acids (PUFAs) than a Traditional Wet Distillers Grains plus Solubles (TWDGS) diet, thereby minimizing issues associated with oxidation, discoloration and off-flavor development. However, once the data began rolling in from the study, the results were not as expected; they were essentially opposite.

The LFWDG diet actually contained less fat on a percentage basis than the TWDGS diet (4.72% vs. 6.91%), however, this led the research team to conclude the study with another hypothesis, being that fats contained within the WDG portion have a greater impact on PUFAs content in the meat than the fats that are contained within the solubles fraction of the diet. It turns out that the cattle fed the LFWDG diet actually consumed a higher concentration of the WDG than the cattle that consumed the TWDGS diet (this diet had the DG “diluted” in a sense due to the addition of the solubles fraction). There is information in the literature that talks about protected fats. If there could be a study that could look into WDG and see if the fats are in a sense, “protected”, then there would be a valid information and supporting research that would hold the new hypothesis. This could be done using castrated steers to determine the extent biohydrogenation that happens in the rumen with the different diets. The study may also

benefit from looking into specific microbial populations and niches when these diets are fed.

It might be worthwhile to see if feeding a supplemental antioxidant to cattle consuming LFWDG to see how it would affect the fatty acid profile of the beef as well as the consumer acceptability and color of the product. If antioxidants are not fed, it would also be interesting to topically apply antioxidants to the surface of the freshly cut beef to see if there would be any shelf life sustainability from that intervention. It would also be interesting to further research the cost and effectiveness of feeding either of the diets, if antioxidants are fed, how much that would cost the producer. It might also be helpful to know if plants that are making LFWDG are finding another outlet for the solubles that are not being put back in to the product. Finally, a study on other usages of LFWDG in other livestock feeding applications could be preformed to see if there is any benefit to those systems.

APPENDICES

Appendix 1. Moisture and Ash Analysis

Non-Ruminant Nutrition Lab

Moisture

1. Weigh dried crucible and record weight.
2. Weigh 1 g of powdered meat sample into dry crucible and record the weight.

As the meat sample thaws and becomes a paste, spread the paste evenly along the sides of the crucible.

3. Place crucibles in oven at 100°C overnight.
4. Remove from oven and cool to room temperature in desiccator and weigh again.

Record weight.

Ash

4. Place crucible with dried sample in muffle furnace. Set furnace to ramp slowly to 600°C and ash for 6 hours at 600°C.
5. Allow furnace to cool to 200°C before opening it. Cool crucible plus ash in desiccator, then weigh back. Record weight.

Calculations:

Moisture = $\frac{\text{initial weight} - \text{final weight of dried sample}}{\text{sample weight}} \times 100$

Ash = $\frac{\text{dried sample weight} - \text{final weight of ashed sample}}{\text{sample weight}} \times 100$

Appendix 2. 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 $\frac{1}{2}$ 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 3. Thiobarbituric Acid Assay.

Buege and Aust (1978), Modified by Ahn et al. 1998

TEP Solution (1,1,3,3-Tetraethoxypropane) (Make new weekly)

Stock Solution: Dilute 99 μ l TEP (97%) bring volume to 100 mL ddH₂O

Working Solution: Dilute stock solution to 1:3 (TEP Solution:ddH₂O) (1×10^{-3} M)

TBA/TCA (2-Thiobarbituric Acid/Trichloroacetic Acid) Stock Solution: 1L

15% TCA (w/v) and 20 mM TBA (MW 144.5) reagent in ddH₂O.

Dissolve 2.88 g TBA in warm ddH₂O first, then add TCA (150g) and ddH₂O to 1L

BHA (ButylatedHydroxyAnisole) Stock Solution:

Make 10% stock solution by dissolving in 90% ethanol.

10g BHA dissolved in 90 mL ethanol (90%) + 5mL ddH₂O

Standards: In duplicate

Blank:	1 ml ddH ₂ O	<u>Moles of TEP</u>
Standard 5:	100 μ l working TEP + 1.90 mL ddH ₂ O	(5×10^{-5} M)
Standard 4:	1 mL Std. 5 + 1 mL ddH ₂ O	(2.5×10^{-5} M)
Standard 3:	1 mL Std. 4 + 1 mL ddH ₂ O	(1.25×10^{-5} M)
Standard 2:	1 mL Std. 3 + 1 mL ddH ₂ O	($.625 \times 10^{-5}$ M)
Standard 1:	1 mL Std. 2 + 1 ml ddH ₂ O	($.3125 \times 10^{-5}$ M)

Remove 1 mL of Standard 1 and discard it, leaving 1 mL behind.

Procedure

- Mix all reagents and standards before beginning.
- Transfer 5 g of powdered sample into a 50 ml conical tube, add 14 ml of ddH₂O and 1.0 mL of BHA (Butylated hydroxyanisole).
- Homogenize for 15 sec with a polytron
- Centrifuge for 2000xg for 5 minutes.
- Transfer 1 ml of homogenate or standard to 15 ml conical tube
- Add 2 ml of TBA/TCA solution, vortex.
- Incubate in a 70°C water bath for 30 min to develop color.
- Cool samples in a cold water bath for 10 min.
- Centrifuge tubes at 2000xg for 15 min.
- Transfer duplicate aliquots of 200 µl from each tube into wells on a 96 well plate.
- Read absorbance at 540nm.

Calculations: mgs of malonaldehyde/kg of tissue

$$K(\text{extraction}) = (S/A) \times MW \times (10^6/E) \times 100$$

Where S=Standard concentration (1×10^{-8} moles 1,1,3,3-tetraethoxypropane)/5ml.

A=Absorbance of standard MW=MW of malonaldehyde (72.063 g/mole)

E= sample equivalent (1) P=Percent recovery

Final calculation: .012 x concentration x 72.063x10⁶ = mgs Malonaldehyde/kg of tissue

Reagents (Sigma): TBA- T5500; TCA- T9159; TEP- T9889; BHA- B1253

Appendix 4. Fatty Acid Determination

Weigh out 1 g of pulverized muscle tissue. If extracting subcutaneous fat, weigh out 0.1 g of pulverized subcutaneous fat into centrifuge tube.

1. Add 5 mL of 2:1 chloroform:methanol (v/v) for muscle tissue or 3 mL for subcutaneous fat.
2. Vortex for 5 s and let stand for 1 h at room temperature.
3. Filter homogenate through Whatman #2 filter paper into 13 x 150 mm screw cap tube bringing the final volume with chloroform:methanol to 10 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.
4. 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.
5. 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.
6. Evaporate to dryness under nitrogen at 60°C.
7. Add 0.5 mL of a 0.5 M NaOH in methanol. Vortex for 5 sec. Heat for 5 min at 100°C
8. Add 0.5 mL of boron trifluoride in 14% methanol. Vortex for 5 sec. Heat for 5 min at 100°C.
9. Add 1 mL of a saturated salt solution and 1 mL of hexane. Vortex for 5 sec.
10. 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. 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°C/min until temperature reaches 220°C. At 220°C, hold for 20 min.