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EFFECTS OF FEEDING HIGH LEVELS OF WET DISTILLERS GRAINS AND STRAW ON BEEF QUALITY

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by

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EFFECTS OF FEEDING HIGH LEVELS OF WET DISTILLERS GRAINS AND STRAW ON BEEF QUALITY

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This research was conducted to investigate the effects of feeding high levels of wet distillers grains plus solubles (WDGS) with straw on fatty acids profiles, lipid stability, retail color, and proximate composition (fats and moisture) of aged beef. Cattle (n = 336; 270 ± 9 kg) were fed corn/5% straw; 40 Distillers grain (WDGS)/5% straw; 70 WDGS/8% straw; 70 WDGS/25% straw; 77.5 WDGS/9% straw; 77.5 WDGS/17% straw, and 85 WDGS/10% straw on dry matter (DM) basis. There were significant increases in the proportions of polyunsaturated fatty acids (PUFA), omega 6, omega 3, and trans fatty acids when high levels of WDGS was compared to corn ($P \leq 0.05$). Similarly, there were significant increases in meat oxidation and discoloration during retail display when compared to corn ($P \leq 0.05$). Lipid oxidation showed significant treatment and day effects ($P < 0.05$). Steaks from cattle fed high levels of distillers grains and straw had significantly more ($P < 0.05$) amount of lipid oxidation (TBARS) by the end of retail display (d 7) when compared to steaks from cattle fed corn/5% straw. Higher levels of WDGS increased the amount of oxidation, a reflection of more PUFA in the steaks. Meat from cattle fed high levels of WDGS discolored more rapidly than meat from cattle fed lower levels of WDGS. There were significant ($P \leq 0.05$) treatment-by-day interactions for discoloration and instrumental color. There was a trend in decreasing redness from 0 day to 7 day of retail display

where beef from corn-fed cattle had the highest redness values at the end of retail display. Fat content was significantly ($P \leq 0.05$) greater in the strips of cattle fed high levels of WDGS compared to corn-fed steers. There were significantly lower ($P \leq 0.05$) moisture levels in strip steaks from cattle fed high levels of WDGS in comparison to steaks from cattle fed low levels of WDGS or corn. These data indicate that precautions against oxidation and discoloration are needed when cattle are fed high levels of WDGS, the meat is aged, and subjected to retail display in oxygen-permeable packaging.

Key words: beef, discoloration, lipid oxidation, wet distillers grain

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INTRODUCTION

In the U.S. the ethanol industry is growing rapidly. Distillers grains, co-products of ethanol production, are fed to livestock. The U.S. is focused on increasing biofuels production from 7 billion to 17 billion by the year 2017 or 20% replacement of transportation fuels in the next ten years (NGVAMERICA, 2010). Production of biofuels correlates directly with the production of distillers grains and as a consequence these byproducts will become plentiful feed resources for feedlot cattle.

Distillers grains may be less than 40% dry matter (DM) and are routinely fed as a supplemental protein source and high energy diet source for cattle. Few studies have been done with very high levels of distillers grains in the cattle diet. de Mello Jr. et al. (2009) fed up to 50% wet distillers grains plus solubles (WDGS) to steers. Feeding high levels of distillers grains to the cattle reduces dry matter intake (DMI) and has a quadratic effect on average daily gain (ADG) (Klopfenstein et al., 2008).

Distillers grains are an excellent source of energy and protein for feedlot cattle. Studies have shown that feeding distillers grains and corn to feedlot cattle has similar effects on quality and yield grades of carcass (Firkins et al., 1985). However, de Mello et al. (2008) showed linear increases in polyunsaturated fatty acids (PUFA) and omega 6 fatty acids in ribeye slices (M. *Longissimus thoracis*) of steers fed 0, 15, and 30% of WDG. Likewise, Schoonmaker et al. (2010) reported an increase in PUFA content of the loin when steers were fed a high forage diet (50% bromegrass on DM basis) compared to 0 and 20% wet distillers grains diet. Vander pol et al. (2009) demonstrated that the concentration of unsaturated fatty acids reaching the small intestine of cattle fed distillers grain was greater when compared with cattle fed corn

oil. This suggests that increasing levels of distillers grains in feedlot cattle diets may increase levels of PUFA in beef. High levels of PUFA are directly associated with meat oxidation and reduction in color stability thereby decreasing the shelf life of beef.

The University of Nebraska developed a research project where up to 85 % (DM basis) of wet distillers grains plus solubles (WDGS) were fed to feedlot cattle with addition of wheat straw. Straw was fed to maintain rumen pH and manage any sulfur related challenges. According to the NRC (1996), the recommended level of dietary sulfur in feedlot cattle is less than 0.3%, however the maximum tolerated total intake dose is 0.4% on a DM basis. Greater sulfur intake is liable to cause polioencephalomalacia.

Therefore the objectives of this research were to investigate the effects of feeding high levels of WDGS with straw on fatty acid profiles, proximate composition (fats and moisture), and retail color and lipid stability of aged beef. The effects on production traits and polioencephalomalacia have been reported (Rich et al., 2011).

REVIEW OF LITERATURE

I. Distillers grains

In the U.S., cereal grains have been fermented for ethanol since the beginning of twentieth century. At the same time, people started feeding distillers grains to cattle. Ethanol plants are mostly located in the Mid-western section of the U.S. because of the availability of raw materials (Raush and Belyea, 2006).

Ethanol in the U.S. is produced by wet milling, dry grinding, and dry milling processes. In the case of wet milling, the production capacity is large and a variety of co-products are produced; however it is more capital intensive in comparison to the dry grinding process where the production capacity and variety of co-products are less (McAloon et al., 2000; Wu, 2007). As a result, the numbers of dry grinders are growing in the U.S. at a rapid pace. According to the USDA (2006), a bushel of corn produces 2.81 gallons of denatured ethanol from dry grinding or 2.74 gallons of denatured ethanol from wet milling (Wu, 2007). As ethanol production increases there is an increase in distillers grains which directly adds up to the feed for cattle (Jenschke et al., 2007; Robinson et al., 2008).

The co-products produced from wet milling are wet or dried corn gluten feed, corn gluten meal and corn germ meal. The dry grinding process produces wet and dried distillers grains, wet and dried distillers grains with solubles, modified wet cake, and condensed distillers solubles (University of Minnesota, 2007).

In the wet milling process, the corn kernel is fractioned into germ, fiber, starch, and protein (Raush and Belyea, 2006; Richardson et al., 2002). In this process, starch is isolated and recovered in a highly purified stream by following five basic

steps: steeping, germ recovery, fiber recovery, protein recovery, and starch washing. In the very first step, corn is steeped in a weak solution of sulfuric acid which softens the kernel and leaches out solubles from the germ. Steeping is followed by separation of fiber particles and germ on the basis of their particle size and differences in their density; solid particles are separated by centrifugation into a starch and protein fractions (Corn Refiners Association, 2002).

In the dry grinding process, the entire corn kernel is ground using hammer mills or roller mills and then fermented. Grinding, cooking, liquefaction, simultaneous saccharification and fermentation, distillation of ethanol and removal of water are some of the basic steps for dry grinding (Raush and Belyea, 2006; Berger et al., 2010). Starch obtained from the wet milling and dry grinding process can be converted into glucose and fermented into ethanol by applying yeast species (Bothast et al., 2005; Davis, 2001).

Dry milling is often confused with dry grinding of corn. The basic difference is the increase in kernel moisture by 15 to 20% before dry milling; this step does not occur in the dry grinding process. Adding moisture during dry milling helps in significant germ swelling thereby increasing malleability of the germ (Raush and Belyea, 2006). The basic steps that follow are degermination, separation, and aspiration where density differences are used to separate whole germ and germ pieces (Berger and Singh, 2010). Products from this process - brewer's grits, meal, and flour are widely used for human consumption (Stock et al., 2000).

II. Types of Distillers grains

Bothast and Schlicher (2005) reported that dry grinding of grains (67%) is increasing tremendously in comparison to wet milling (33%) of grains on a production basis. Co-products for the dry distillation process are wet and dried distillers grains (WDG/DDG), wet and dried distillers grains with solubles (WDGS/DDGS), and condensed distillers solubles (CDS) (Minnesota Nutrition Conference, 2001; University of Minnesota, 2007; Berger and Singh, 2010; Bothast and Schlicher, 2005).

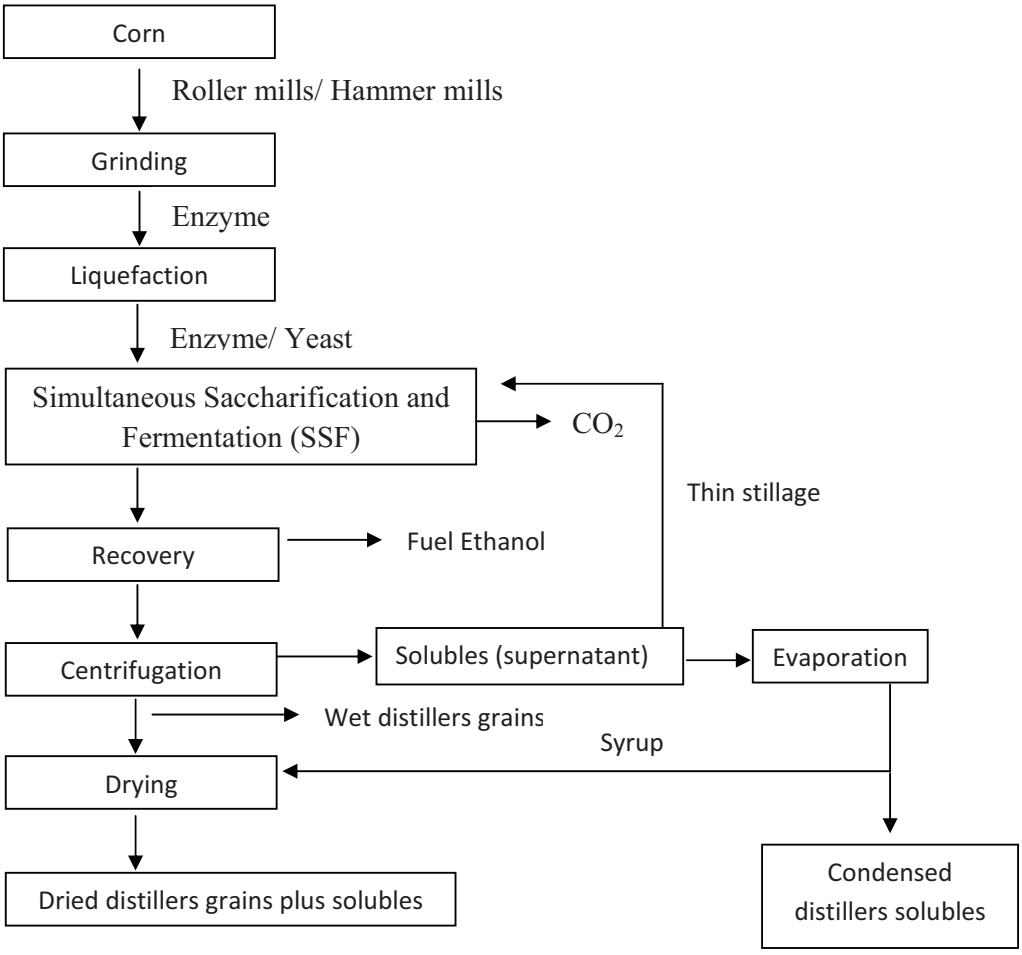

Fig 1. Schemetic diagram for distillers grains byproducts formation in a plant (SunGrant BioWeb, 2008; Bothast and Schlicher, 2005).

Varieties of coproducts are produced from the ethanol industry. Those coproducts differ each other in their nutrient content. Below is the table summarized by Lardy (2007) showing composition for various types of distillers grains expressed on dry matter basis.

Nutrient	Dried Distillers Grains	Dried Distillers Grains plus Solubles	Modified Wet Distillers Grains plus Solubles	Wet Distillers Grains plus Solubles	Condensed Distillers Solubles
DM, %	88 to 90	88 to 90	50	25 to 35	23 to 45
	DM Basis				
TDN, %	77 to 88	85 to 90	70 to 110	70 to 110	75 to 120
NEm, Mcal/cwt	89 to 100	98 to 100	90 to 110	90 to 110	100 to 115
NEg, Mcal/cwt	67 to 70	68 to 70	70 to 80	70 to 80	80 to 93
CP, %	25 to 35	25 to 32	30 to 35	30 to 35	20 to 30
DIP, % CP	40 to 50	43 to 53	45 to 53	45 to 53	80.0
UIP, % CP	50 to 60	47 to 57	47 to 57	47 to 57	20.0
Fat, %	8 to 12	8 to 12	12 to 15	10 to 18	9 to 15
Calcium, %	0.11 to 0.20	0.10 to 0.20	0.02 to 0.03	0.02 to 0.03	0.03 to 0.17
Phosphorus, %	0.40 to 1.15	0.40 to 0.80	0.50 to 1.42	0.50 to 0.80	1.30 to 1.45
Potassium, %	0.49 to 1.08	0.87 to 1.33	0.70 to 1.00	0.50 to 1.00	1.75 to 2.25
Sulfur, %	0.46 to 0.65	0.37 to 1.12	0.38 to 1.20	0.40 to 1.20	0.37 to 0.95

a. Wet distillers grains (WDG)

Starch from corn is fermented to ethanol and carbon dioxide (CO_2) where ethanol is distilled and the remaining liquid is centrifuged. The residue present after centrifugation is called WDG. This grain contains of 30 to 35% dry matter (DM) and 65 to 70% moisture. In addition, WDG contains fiber (44%), fat (15%), protein (30%), and mineral (3%) (Weiss et al., 2007; Ham et al., 1994) and has been

effectively used as a protein and energy source for ruminants (Firkins et al., 1985, Ham et al., 1994). The WDG contains high moisture levels so there is a very high risk of molding. In practice, wet distillers grains are produced by smaller ethanol plant close to the feedlots. As a result, there is little need to store WDG for a long time and hence molding is minimized.

Larson et al. (1993) fed dry-rolled corn and three different levels of wet distillers byproducts (5.2, 12.6, and 40%, DM basis) to yearlings and steers. In the experiment, low levels of wet distillers byproducts replaced the same amount of crude protein as supplied by soybean meal. Yearlings were 5, 10, and 20% more efficient (linear, $P < .01$) while calves were 2, 6, and 14% more efficient (linear, $P < .01$) when WDG were used as a source of energy and protein (Larson et al., 1993, Ham et al., 1994). The increase in energy value might be due to the combination of factors like reduced acidosis, increased energy utilization, and yeast end-products (Farran et al., 2006). Another study conducted by Vander Pol et al. (2009) concluded that propionate production was increased, greater fat digestibility occurred and more unsaturated fatty acids reached the duodenum when WDGS was fed in comparison to corn.

b. Wet distillers grains with solubles (WDGS)

Thin stillage which comes after the centrifugation (5 to 10% DM) step and is evaporated to produce condensed distillers solubles (CDS) are then added to WDG to produce wet distillers grains with solubles (WDGS, 30 to 35% DM) (Stock et al., 2000). Ham et al. (1994) documented the average nutrient composition of WDGS - starch (36.7%), crude protein (16.4%), fiber (27.3%), fat (8.2%) and mineral (5.3%).

Nutrient composition and net energy gain from WDGS was higher than dry-rolled corn (DRC) and high moisture corn (HMC) (Ham et al., 1994; Lodge et al., 1997). In one of the initial research studies conducted by Farlin (1981), the resultant byproduct obtained from corn-based ethanol production had more energy per kg of DM than the starch contained within corn.

Vander Pol et al. (2009) fed 60 crossbred yearling heifers corn oil (0%, 2.5%, and 5%) or WDGS (0%, 20%, and 40%). For initial body weight (BW), final BW, hot carcass weight (HCW), or dry matter intake (DMI) there were no significant differences ($P > 0.01$) among treatments; however a linear decrease of DMI was observed as fat provided from WDGS or corn oil increased. There were no differences for average daily gain (ADG) and gain to feed (G:F) ratio. In another study, Loza et al. (2010) demonstrated feeding 30% WDGS resulted in increased G:F compared to steers fed with corn and wet corn gluten feed (WCGF). Feeding 30% WDGS also increased DMI and ADG in feedlot cattle. Following the same trend, Vander Pol et al. (2007) showed that feed efficiency at all levels of WDGS (0 to 50%) was greater than a corn-based diet.

c. Dry distillers grains (DDG)

In an ethanol production plant, dried residue remaining after the fermentation of starch with selected yeasts and enzymes results in a product called DDG. Research has shown that DDG can serve as a very good protein source for feedlot cattle (Tjardes et al., 2002). Commonly, the protein found in distillers grains contains about 50% undegraded intake protein (UIP) or bypass protein, and the remaining is degraded intake protein (DIP). DDG contain nearly 65% UIP and about 35% of

moisture (Stalker et al., 2005). Distillers grain has been considered as a protein supplement when fed at < 15% of the diet DM. Otherwise DDG are considered an energy source (Stalker et al., 2005). Stalker et al. (2005) indicated that DDG are more suitable for forage-based production systems. In addition, drying of distillers grains cost more due to the investment in fuel, equipment and transportation (Klopfenstein et al., 2008).

Aines et al. (1986) reported that DDG contain approximately 29.5% protein, 12.8% fiber, and 8% fat. In the research conducted by Stalker et al. (2005), heifers were feed 3 lb (DM)/head either three or six times a week with DDG. Heifers fed distillers grains six times per week had more weight than heifers fed three times a week. They showed that feeding increasing levels of DDG for heifers increases ADG and G: F and decreases the total forage intake. Decreases in ADG and G: F ratio in cattle may occur because high fat content of the diet depresses fiber digestion via negative effects on rumen micro flora.

d. Dry distillers grains with solubles (DDGS)

The DDG from ethanol plants, are mixed with solubles and the resulting distillers grain is called DDGS. This type of distillers co-product contains at least three times more nutrients (protein, energy) than processed grain (Minnesota Nutrition Conference, 2001). Proteins found in DDGS are mainly by-pass protein which passes the rumen and is digested from the duodenum (McDonald, 1954; Little et al., 1968; and Klopfenstein et al., 1978). Protein in DDGS has 2.6 times the mean escape values in comparison to soybean meal whereas DDG has only 2.3 times the mean escape values of soybean meal (Klopfenstein et al., 2008). The nutritional

composition of DDGS has been reported to be 27% protein, 11% fat, and 9% fiber (Minnesota Nutrition Conference, 2001).

Ham et al. (1994) studied inclusion of WDGS and DDGS in feedlot diets, replacing 40% of the DM of corn. The results showed cattle fed WDGS were more efficient than those fed DDGS and WDGS had 47% more feeding value than corn and DDGS had 24% more feeding value than corn. In another trial, Wilken et al. (2009) fed 192 steers calves with DDGS (90% DM) or modified wet distillers grains (MDGS, 30 to 50% DM) in combination with wet or dry forages. There were no significant differences ($P > 0.05$) between DDGS and MDGS for G:F and ADG of steers. However, ADG and G:F was higher for wet forage than dry forage (Wilken et al., 2009). Feeding WDGS decreases the acetate: propionate ratio in the rumen which might be due to the inclusion of solubles, resulting in greater fat digestibility and movement of more unsaturated fatty acids to the duodenum (Vander Pol et al., 2009; Leupp et al., 2009a).

Currently, studies concerning the use of DDGS are increasing as they may be a better fit for small operations and their feeding combinations with forage situation. However, the price for DDGS is higher because of fuel, equipment and transportation costs.

e. Condensed distillers solubles (CDS)

Thin stillage or ‘sweet water’ is the liquid that comes after enzymatic digestion of distillers grains. The liquid is distilled or evaporated to approximately 70% moisture to extract residual ethanol and the remaining portion is sold as CDS (Iowa Beef Center, 2007; Tjardes et al., 2002). This co product is highly palatable and

provides additional protein and energy to the whole diet, although excessive amounts of fat and mineral may exist (Rausch and Belyea, 2006; Kalscheur et al., 2008). In addition, CDS can be used to boost the consumption of other feed ingredients. The DM content of CDS ranges from 25 to 50%. Beside this, CDS contains about 29% protein, 9% fat, and 4% fiber (Minnesota Nutrition Conference, 2001). These CDS are generally used with low quality forage where laboratory analysis is recommended before of its use because of the potential for high sulfur content (Lardy, 2007).

Tjardes et al. (2002) documented that an increase in ADG and efficiency of gain when CDS was added up to 10% of the diet dry matter. Cao et al. (2009) found that rumen-degraded DM was significantly greater ($P < 0.001$) for WDG in comparison to DDG but degradation increases as percentage of CDS increases. However, intestinal digestibility was not affected by addition of CDS in the diet (Cao et al. 2009). Similarly, as the level of CDS (0.0, 5.4, 14.5, 19.1, and 22.1% of DDG DM) increases in the DDG supplementation level (0.25, 0.50, 0.75 or 1.0% body weight), there is a corresponding increase in protein and decreases in NDF and CP (Corrigan et al., 2009). Storage of CDS is also a major challenge because of its high moisture content. So, the material tends to gel and freeze in cold temperatures (Tjardes et al., 2002).

III. Advantages/ Disadvantages of feeding distillers grains

As a whole, distillers grains co-products can be divided into dry and wet forms. In general, these co-products vary from plant to plant and from day to day with respect to their composition. Thus, the question is not about whether we can feed co-

products, but rather, how much co- product can be given to the cattle with the given composition of fat, sulfur, moisture and other minerals (VanOverbeke, 2007).

a. Advantages

- Dry forms of distillers grains contain high amount of protein, fat, phosphorus, and other minerals. So, phosphorus and protein supplementation is reduced (Honeyman et al., 2007).
- Co-products provide beef producers an opportunity to potentially decrease the unit cost of production by maintaining similar levels of performance.
- The high energy content and medium protein levels of co-products are suitable to be mixed with other feed ingredients for different types of cattle and different production situations.
- In a meta-analysis of 9 different experiments, Klopfenstein et al. (2008) showed that feeding different levels of WDGS to cattle resulted in higher ADG and G:F compared to cattle fed corn-based diets. Feeding DDGS showed similar results but with less apparent feeding value in comparison with WDGS.

b. Disadvantages

- There is higher transportation and storage cost per unit of DM for WDGS and WDG (Tjardes et al., 2002).
- Wet forms of DG are not so stable because of high moisture content, so surface spoilage can be very high and occurs quickly (Tjardes et al., 2002).

- A browning reaction may occur when DG is exposed to heat which renders carbohydrate and protein unavailable to the animal (Tjardes et al., 2002).
- In a research conducted by Depenbusch et al. (2009), DDGS were fed in increasing levels to 75%. A linear decrease in fat thickness at the 12th rib and a linear increase of carcasses graded USDA Select was observed. In the same study, the best marbling scores were detected when steers were fed 45% DDGS.
- Research by de Mello et al., (2010a, 2010b) reported a linear increase of polyunsaturated fatty acids (PUFA) in meat which is responsible for oxidation of beef and ultimately degrades color and shelf life.
- Sulfur above 0.4% of dietary DM may lead to a disease called polioencephalomalacia in cattle which can be fatal to cattle. High levels of sulfur also interfere with copper absorption and metabolism (Tjardes et al., 2002).

IV. Distillers grains and beef production

Bremer et al. (2010) conducted a research where finishing steers were fed different levels of WDGS (up to 50% on DM basis) which resulted in superior performance compared to steers fed a corn diet. Increasing levels of WDGS improves cattle performance quadratically for DMI, ADG, 12th rib fat, and marbling score. Similar results were shown by Larson et al. (1993) and Vander Pol et al. (2006) where cattle were fed 40% and 50% of WDGS, respectively. In another study, feeding steers up to 30% of WDGS showed the best results for ADG and DMI while G:F was higher at 30 to 50% of the diet (Klopfenstein et al., 2008). Following these results, cattle

replacing corn DM with 20 to 40% WDGS gives the best results on performance and carcass characteristics.

Distillers grains are a major contributor of PUFA to beef. Increasing levels of distillers grains linearly increase the level of PUFA in beef (de Mello et al., 2009). These fatty acids are responsible for oxidation where the heme ring present in myoglobin oxidizes to the ferric state and is unable to combine with oxygen, giving brown color to the meat. Several studies have related feeding distillers grains and to beef color (Roeber et. al., 2005; Gill et al., 2008; Leupp et al., 2009b). Roeber et al. (2005) conducted a trial where steers were fed corn soybean meal diet containing 12.5 – 50% dried and wet distillers grains on DM basis. After 138 h of simulated retail display, steaks from steers fed high levels of WDG (50%) were more discolored than steaks from steers fed low levels of distillers grains (25%). Similarly, Gill et al. (2008) found that steaks from steers fed steam-flaked corn (SFC) had greater a* values than those from steers fed DG. In addition, the L* (measure for lightness) values increased for steaks from steers fed DG. Likewise, steaks from steers fed sorghum DG had lower L* values but greater values for a* in comparison to steaks from steers fed corn DG. To conclude, feeding higher levels of distillers grains co-products increased PUFA, lipid oxidation and decreased color stability and shelf life of beef.

V. Meat color and fatty acids

Beef color makes the first impression to the customer and is of utmost importance. The characteristic beef color is contributed by different meat pigments.

Those pigments includes hemoglobin, myoglobin and cytochrome c. Hemoglobin is present in the blood as an oxygen carrier from the lungs to the muscle cell or fiber and myoglobin (80 to 90 % of the total pigment of meat) is the pigment present in muscle fiber which takes oxygen from the carrier hemoglobin. Cytochrome c is associated with mitochondria and works in an electron transport chain (Aberle et al., 2001; Claus, 2007). All of these pigments can contribute to meat color.

Fatty acids are organic compounds derived from neutral lipids having a hydrocarbon chain and terminal carboxyl group. The hydrocarbon chain of fatty acid or naturally occurring fatty acids have even carbon number ranges-from four to twenty-eight. Fatty acids in ruminants are located in the adipose tissue and occur primarily as triglycerides; most of them are C16 and C18 carbon groups (Whetsell et al., 2003; Gunstone, 1996; Aberle et al., 2001). Triglycerides are formed by combining glycerol with three fatty acid molecules where each of the hydroxyl groups of glycerol combine with the carboxyl group from fatty acid and form an ester bond.

In general, fatty acids can be classified on the basis of length and the number of double bonds present (saturated, mono- or polyunsaturated) or essentiality in the diet (essential or non-essential). Saturated fatty acids are those where each carbon atom present is fully saturated with hydrogen atoms. In humans, saturated fatty acids are known to increase low density lipoprotein (LDL) - cholesterol in the blood. Similarly, monounsaturated fatty acids are lacking a pair of hydrogen bonds in a molecule however, these fatty acids are known to decrease LDL – cholesterol in human blood. Likewise, polyunsaturated fatty acids have more than one pair of hydrogen bonds lacking within a molecule. In the case of humans, PUFA are known to decrease both high density lipoprotein (HDL) also known as good cholesterol and

LDL. On the other hand, saturated fatty acids are known to increase the oxidative stability, however PUFA are not stable to oxidation and they become rancid (Whetsell et al., 2003). For instance, palmitic (C16:0), stearic (18:0), lauric (C12:0), and myristic (C14:0) are saturated fatty acids found in beef, whereas oleic acid (C18:1), elaidic (C18:1), palmitoleic (C16:1), transvaccenic (C18:1 trans -11), and vaccenic acid (C18:1 cis-11) are monounsaturated fatty acids (Whetsell et al., 2003). Whetsell et al. (2003) also documented the important PUFA in beef are linoleic (C18:2), alpha-linolenic (C18:3), arachidonic (C20:4), eicosapentaenoic (C20:5), docosapentaenoic (C22:5), and docosahexaenoic (C22:6) acids.

Omega-3 (ω-3; alpha-linolenic acid) and omega-6 (; linolenic acid) fatty acids are categorized under PUFA and are known as essential fatty acids. The ω-6 to ω-3 ratio in beef from grain fed cattle is about 4:1, whereas the ratio in beef from grass-fed cattle is about 2:1. The ω-3 fatty acids are best known for their health benefits in humans like cancer prevention, protection against cardiovascular disease, immune function, and anti-inflammatory functions. In contrary to this, non-essential fatty acids are hard fats from beef and pork and can provide excess calories.

a. Color and Lipid chemistry

Myoglobin, the primary pigment in muscle, contains a heme ring with iron as the central part of its structure. The state of the iron determines the interaction of myoglobin with other molecules (Aberle et al., 2001; Liu et al., 1995). Liu et al. (1995) documented that myoglobin having an oxidized state of Fe (Fe^{+++}) is unable to combine with oxygen, whereas the reduced state (Fe^{++}) is able to combine with water and O_2. In the case of intact muscle Fe in myoglobin combines with water and forms

deoxymyoglobin (deep purplish-red color) which is the natural color of uncut meat. After cutting the meat and exposure to air, the color changes to bright cherry red through oxygenation, a process called blooming (Aberle et al., 2001; Claus, 2007). The change in color is due to the binding of oxygen to the heme ring of myoglobin, forming oxymyoglobin. The Fe present in oxymyoglobin and deoxymyoglobin is in the reduced form, but in case of metmyoglobin (brown color of meat) the Fe changes to the Fe^{+++} state and the pigment cannot bind oxygen (Grobbel et al., 2006; Claus, 2007). The brown color of meat is the most undesirable color at the retail display.

Frei et al. (1989), Erickson et al. (2002), and Scislowski et al. (2005) reported that lipid oxidation occurs in three different stages: initiation, propagation, and termination. All these stages are associated with free radical chain reaction. In the initiation process, an endothermic reaction occurs where the hydrogen atom is spontaneously abstracted from the fatty acid pool by molecular oxygen. As a result, lipid free radicals are formed with the presence of oxidation initiators like metal catalyst, UV light or heat (Kanner and Rosenthal, 1992; Shahidi and Wanasundara, 1997). Once the free radicals or peroxyradicals are formed, these again hydrogen atoms from the hydrocarbon pool and form new free radicals and the process is easily propagated. Besides this, decomposition of instable hydroperoxides also occurs at the same time producing more peroxyradicals. This cycle is continuous until there is formation of more peroxyradicals or free radicals. The final stage of lipid oxidation occurs when the decomposition of lipid hydroperoxides (homolysis) creates non-radicals species: aldehydes, ketones, alcohols, hydrocarbons, esters, furans, and lactones (Kanner and Rosenthal, 1992) resoponsible for quality deterioration of meat (Erickson et al., 2002; Campo et al., 2006).

Reactive oxygen species (ROS) are chemically reactive molecule containing oxygen. These molecules are formed by several different mechanisms such as interaction of ionizing radiation or from byproducts of cellular respiration can cause protein oxidation. Protein oxidations are very similar to lipid oxidations however, ROS or free radicals cleave the amino acid chain and peptide backbone resulting in the formation of certain structural alterations (Kanner and Rosenthal, 1992). These structural alterations in beef relate to the formation of carbonyl content and decreased sulfhydryl content which has positive correlations with fresh meat color and tenderness (Rowe et al., 2004). Living cells can normally defend ROS through the use of enzymes such as superoxide dismutases, glutathione peroxidases, and peroxiredoxins, and α- tocopherol in fresh meat; however these enzymes get inactivated after the normal cell death (Erickson et al., 2002).

b. Factors influencing meat color

Typical beef color may be influenced by a number of factors. One of the important factors is diet. Feeding vitamin E (α- tocopherol) to cattle provides protection against oxidation (Aberle et al., 2001; Senaratne et al., 2009a). Senaratne et al. (2009a) showed that vitamin E mitigates the surface discoloration problem of aged beef fed with 40% WDG. Beef contains high concentrations of myoglobin pigment and iron, which catalyze oxidation and diminish color, flavor, texture, and nutritive value (Soyer et al., 2010).

Processing practice is another area where oxidation of fresh beef occurs. During the processing of fresh meat (cooking, mincing, deboning, chopping, and emulsification), crushing of muscle fiber helps in minimizing particle size but results

in exposure of lipids to air and activation of pro-oxidants or oxidative catalysts giving meat a brown color (Erickson, 2002).

Likewise, stress is another factor that determines the color of displayed meat. High temperatures, rapid glycolysis (pH drop), and early onset of postmortem rigor mortis are some of the uniqueness of stressed animals. Muscles of such animals become pale, soft, and exudative after normal chilling period (Aberle et al., 2001).

Age, sex and exercise are additional contributing factors that influence the color of beef. As age of animal progresses, the myoglobin content of the muscle fiber increases. Males have more myoglobin pigment than females (Aberle et al., 2001). As a result the muscle looks dark in color in retail display (USDA, 2008; Aberle et al., 2001).

VI. Fatty acids deposition and depletion

The amount of fat present in the beef can be determined by the type of feedstuffs given to the cattle. In one of the studies conducted by Larson et al. (1993), fat percentage of corn, WDGS, and thin stillage were identified as 3.8, 13.7 and 8, respectively. Similarly, in another study conducted by Ham et al. (1994) fat percentage of WDGS and thin stillage were 8.2 and 5.4, respectively. So, it is clear diets containing WDGS provide more fat inside the rumen. Rumen microorganisms produce microbial lypolytic enzymes which hydrolyzes the ester linkage of dietary fatty acid glycerol and form glycerol and free fatty acids. Glycerol produced after hydrolysis changes into volatile fatty acids (VFA) in the rumen (Jenkins et al., 1994; Arpigny et al., 1999). Unsaturated fatty acids, those are having 18 carbons, produced after hydrolysis are transformed into a variety of trienoic, dienoic, and monoenoic

isomers as they are biohydrogenated (Bauman et al., 2000). Biohydrogenation of unsaturated fatty acids leads to the production of saturated fatty acids (SFA) through isomerization. The isomerization inside the rumen produces *trans* fatty acids intermediates together with conjugated linoleic acids (CLAs) (Harfoot and Hazlewood, 1988). However, Jenkins et al. (2002) have written that accumulation of linoleic (18:2 ω-6) fatty acid (the predominant fatty acid from grain diets) or long chain fatty acids in the rumen can stop complete biohydrogenation. Wood et al. (2008) showed that only 10% of dietary linoleic acid is transferred to tissues where in cattle these acids are found more in muscle than in adipose tissue. Zinn et al. (2000) reported that absorption of fatty acids depends on the extent of micelle growth in the small intestine. Extensive growth of micelles in the duodenum helps in absorption of more unsaturated fatty acids.

Nielsen et al. (2000) has found that diets rich in PUFA are more prone to plasma lipid peroxidation composed with diets containing saturated and monounsaturated fatty acids. Scislowski et al. (2005) found the similar results when steers were fed with an oil rich diet which increased the PUFA: SFA ratio and peroxidation index. According to the research, steers fed the oil rich diet had an increase in α-linolenic acid (18:3 ω-3) content in plasma lipids which is a more peroxidizable substrate. However, steers having ruminal manipulation of fatty acids showed no difference between plasma lipids and their fatty acids composition and no peroxidation as well (Scislowski et al., 2005). In contrast, fatty acids absorbed through duodenal manipulation showed changes in plasma lipids and thereby promote lipid peroxidation. According to Nielsen et al. (2000), peroxidation capacity of

18:3 ω-3 is greater than 18:2 ω-6 in plasma lipids and might be due to the presence of more bonds in the fatty acid chain.

VII. Lipid oxidation

Lipid depots in meat can be classified as intermuscular fat and as intramuscular tissue lipids (Love and Pearson, 1971). These lipids bring qualitative attributes to the fresh meat; however some qualitative attributes like color stability, drip loss, and oxidative rancidity can be influenced (Enser, 2001). Based on the molecular structure, lipids (organic compound containing carbon, hydrogen, and oxygen) are divided into 1) simple lipids, 2) compound lipids, and 3) derived lipids. Simple lipids are esters of fatty acids with various types of alcohol whereas compound lipids contain an inorganic or organic group in addition to fatty acid and glycerol (Vance and Vance, 2006). Derived lipids are obtained on hydrolysis of simple and compound lipids. Compound lipids and derived lipids are much more susceptible to oxidation because of the presence of PUFA's (Deligeorgis and Simitzis, 2010).

a. Effect of fatty acids and minerals on oxidation

Lipids (triglycerides) are considered as a concentrated caloric source and have two main functions: providing essential fatty acids to the body and acting as an absorption vehicle for fat-soluble vitamins and other nutrients (German, 1999). Lipids, as a food component, are also well known for their instability and may undergo radical chain reaction for oxidation. German (1999) further wrote that lipids or fatty acids are accountable for the production of oxidative fragments. In German's

(1999) definition, oxidative fragments are volatile compounds and perceived as the off flavor of rancidity in food components. Lipids or fatty acids are also responsible for the degradation of proteins, vitamins, and pigments which bring the qualitative deterioration in meat. In addition, lipids also help in formation of cross link between lipids and other macromolecules resulting in the formation of non-nutritive polymers (German, 1999). The oxidation mechanism of lipid is better understood as oxygen-dependent deterioration of polyunsaturated fatty acids by autocatalytic action of free radicals. For instance, in the case of unsaturated fats, the susceptibility to oxidation is dependent on the availability of allelic hydrogens for reactions with peroxy radicals (Frankel, 1980). Therefore, the degree of oxidation of fresh meat mainly depends on the saturation of fatty acids present in the muscle. However, antioxidants and prooxidants (free radicals) also have an impact on lipid oxidation (Enser, 2001). Thus, fatty acids are more responsible for the oxidative deterioration of meat.

Transitional metals like copper, iron, cobalt, and manganese are essential in minute quantities for proper growth, development, and physiology of animals. On the other hand, transitional metals ions act like pro-oxidants which create oxidative stress through the production of reactive oxygen species (ROS) (Lauridsen et al., 1999). The oxidation-reduction active transitional metals react with O_2 molecules to generate O_2^- radicals by monovalent reduction of O_2 molecules (Fellenberg et al., 2006). Generated O_2^- radicals undergoes fast dismutation and produce H_2O_2, which is an important generator of hydroxyl species ($HO^\bullet$), a most reactive radical species (Fellenberg et al., 2006; Keher, 1993). Free radical species are responsible for the oxidative damage of lipid, especially phospholipids present in the cell membrane, being positively correlated with the unsaturation of its fatty acids.

b. Relationship between fatty acids and color

Bright cherry red color of beef is an indicator of freshness and the first perception about meat quality for consumers. The cherry red color of meat is due to the presence of oxymyoglobin content of the meat, whereas formation of unattractive brown color or metmyoglobin is due to the oxidation of iron to the ferric state (Yin and Faustman, 1993). Deviation of meat color from the normal or acceptable to unacceptable or brown color is dependent on several factors. Some of the related factors are pH of the meat, pigment content, physical characteristics of muscle, and the diets fed to the feedlot cattle before slaughter. Also, types of diet that have been fed to the cattle before slaughter play an important role on color contribution (Lanari et al., 2002; Jenschke et al., 2008; Depenbusch et al., 2009).

In one of the studies conducted by de Mello Jr. et al. (2008), feeding cattle with 30% WDGS elevated the levels of PUFA, conjugated linoleic acid (CLA), linoleic isomer 18:2 and total fatty acids in beef. Similar results were shown by de Mello Jr. et al. (2009), and Senaratne et al. (2009b) where cattle feed different levels of WDGS linearly increase the levels of PUFA and other fatty acids in beef. Kanner and Harel (1985) investigated the interrelationship between the oxidation of PUFA and heme Fe of oxymyoglobin. Metmyoglobin formation after oxidation of PUFA has an interrelationship with heme ring of oxymyoglobin where Fe is oxidized to the ferric state promoting cellular oxidation in meat.

Similarly, Hur et al. (2003) showed that oxidized lipids initiate the oxidation of myoglobin forming metmyoglobin, an unattractive brown color of meat. The relationship between lipid oxidation and metmyoglobin formation was further clarified through a report documented by Schaefer et al. (1995) where the scientific

crew proposed that the products from lipid oxidation are more polar so they can easily enter the cytoplasmic barrier and hasten the oxidation of muscle pigment. Lipid oxidation is the primary cause for color and flavor deterioration of meat, however feeding diets and packaging systems can also affect the qualitative aspect of meat as well. The oxidation of meat degrades color and reduces the shelf life, affecting consumer satisfaction and economic gain consequently. The oxidation of lipid or rancidity generally involves the oxidation of polyunsaturated fatty acids (PUFA).

VIII. Rumen function

The rumen is a fermentation vat which provides constant temperature, pH, and good mixing region for consumed ration in an anaerobic environment (Bowen, 2009). To maintain the normal rumen pH and for lubrication, saliva is produced inside the body in large quantities. Saliva consists of bicarbonate and is responsible for buffering large quantities of acid produced in the rumen after starch digestion (Bowen, 2009). The rumen microbial ecosystem is very diverse and the fermentation of starch and its products depends on the types and activities of microorganisms in the rumen (Sikkema et al., 1990; Russell et al., 1992; Lee et al., 2000). Microorganisms present in the rumen are responsible to ferment the ingested feedstuffs into volatile compounds (Russell et al., 1992; McAllister et al., 1994). Russell et al. (1992) further elaborate that the volatile fatty acids like acetate and butyrate are the primary energy source for the animal, whereas propionate is used through gluconeogenesis only when there is shortage of other forms of energy in the animal body. In abnormal cases, rapid fermentation of feedstuffs inside the rumen leads to an accumulation of lactic acid. As a result, problems like acidosis, a decline in fiber digestion, decreased feed intake and

in severe case even death is possible (Russell et al., 1992). On the other hand, fermentation of starch is dependent on different factors and some of them are different feed treatments, storage methods, and the type of cereal grains that were fed to the cattle. Non-fermented feedstuffs escape ruminal degradation and are determined by fermentation and passage rate of the feed (Waldo et al., 1972).

IX. Sulfur in Distillers Grains

Sulfur is an essential mineral for a ruminant's diet. Nix (2003) has mentioned that a high amount of sulfur is found in cutaneous part of the body, however each and every cell contains a certain percentage of sulfur. Sulfur is associated with other useful compounds within the body including amino acids (methionine, cystine, cysteine and taurine) and B vitamins (thiamine and biotin) (Nix, 2003; Hoog, 2007).

Despite the fact that sulfur is an essential mineral for ruminants, high inclusion of sulfur in the animals' diet proved to be fatal. Diet containing high levels of distillers grains contain elevated levels of sulfur. Moreover, the percentage of sulfur in the diet depends upon the ethanol plants specific procedure (Hoog, 2007). The National Research Council (NRC, 1996) recommends a sulfur concentration of 0.15%, which is needed for formation of amino acids and the B vitamins thiamine and biotin, as well as for use in some detoxification reactions to maintain normal function of body cells. The maximum tolerated total intake dose is 0.4% on DM basis and more than this dose is liable to cause polioencephalomalacia (PEM) reported as fatal to ruminants (Larson, 2010; Okine, 2010). The PEM is caused by the production of hydrogen sulfide gas due to rumen fermentation. The gas is rebreathed into the lungs and carried to the brain causing necrosis to the cerebrocortical region (Hoog, 2007).

Straw is beneficial in a ruminant diet because high or long fiber diet prevents the cattle from polioencephalomalacia by producing massive amount of saliva that buffers the pH and decreasing the sulfur concentration inside the rumen.

X. Role of fiber

Livestock species have an ability to digest fiber via microbial digestion and extract essential nutrients and energy. Straw or fiber content is entirely made up of cell walls which contain highly lignified structural carbohydrates, small amounts of protein, and minerals (Antongiovanni et al., 1991; Kung, 2000). As discussed earlier, bacteria and protozoa are responsible for rumen fermentation. Kung (2000) further describes that there are two different types of bacteria based on their metabolic functions: amylolytic bacteria responsible for starch digestion and fibrolytic bacteria responsible for fiber digestion or fermentation. Therefore, the type of bacteria present in the rumen depends on the type of feed given to the cattle. Fiber contained in the diet is fermented to acetic acid whereas starch contained in the diet is fermented to propionic acid. Both acids are the precursor of energy to the cattle (Kung, 2000; Russell et al., 1992).

The pH of the rumen has an effect on fiber digestion. The pH of the rumen is affected by different factors and some of them are described by Kung (2000) and Bowen (2009). Some of them are difference in fiber length, insufficient fiber, types of diet, and chewing time. As long as animal gets high amounts of fiber there will be a large production of saliva which serves as a buffering agent and checks the ruminal pH. On the other hand, high concentrate rations (high in readily fermentable carbohydrates) increase rumen sulfide concentration and induce a disease in cattle

known as polioencephalomalacia (Larson, 2010; Okine, 2010). High or readily fermentable carbohydrate produce high amount of acetic acid (Russell et al., 1992) which increases the concentration of rumen sulfide. In this case, high or long fiber diet prevents the cattle from polioencephalomalacia by producing massive amount of saliva that buffers the pH and decreasing the sulfur concentration inside the rumen. According to NRC (1996), the recommended level of dietary sulfur is less than 0.3%, however the maximum tolerated total intake dose is of 0.4% on DM basis which is liable to cause polioencephalomalacia. In a research conducted by Rich et al. (2011), cattle were fed 40% WDGS and 5% straw had the greatest ($P < 0.01$) value for ADG, G:F when compared to cattle fed as much as higher as 85% WDGS and 10% straw, but no cases were reported as polioencephalomalacia.

Conclusion

Distillers grains co-products from ethanol industry are being feed to the beef cattle. Diet mixing ratio, formulation, and number of days fed in a feedlot may affect the beef quality, composition, and shelf life, however feeding high levels of distillers grains aggravate the problems of beef. Researchers have shown that increasing levels of distillers grains increase PUFA's linearly in aged beef. To minimize the problem, antioxidant (Vitamin E) was tried and positive results were found. The underlying chemistry behind oxidation of beef is due to incomplete biohydrogenation of fatty acids inside the rumen. To increase biohydrogenation through rumen micro flora, a good buffering system should be active which can be achieved through the feeding of a high fiber diet given to feedlot cattle. For example, addition of straw to the feedlot cattle can serve this function.

MATERIALS AND METHODS

Experimental design

Crossbreed steers (n = 336; 270 ± 9 kg) were randomly allotted to one of seven finishing diets containing corn wet distillers grains plus solubles (WDGS) and wheat straw. Diets contained corn/5% straw (n=15); 40% distillers grain (WDGS)/5% straw (n=15); 70% WDGS/8% straw (n=15); 70% WDGS/25% straw; 77.5% WDGS/9% straw (n=9); 77.5% WDGS/17% straw (n=9), and 85% WDGS/10% straw (n=12) were fed on DM basis. The diet containing corn/5% straw, 40% WDGS/5% straw, 70% WDGS/8% straw, 77.5% WDGS/9% straw were fed for 183 d and the diet containing 70% WDGS/25% straw, 77.5% WDGS/17% straw, and 85% WDGS/10% straw were fed 225 d so as to attain similar final body weights (BW). Steers were slaughtered at Greater Omaha Packing Co. (Omaha, Nebraska). Carcasses were chilled for 48 h before grading. After grading, strip loins (IMPS # 180 PSO2; NAMP, 2007) 9-15 per treatment (except for 70% WDGS/25% straw no samples were collected because of low USDA grades), all USDA Choice, were collected from n=75 animals. The loins were vacuum packaged, transported under refrigeration to Loeffel meat laboratory at the University of Nebraska-Lincoln and aged for 20 days at 2^{o} C.

Fabrication and assignment of steaks

The m. *Longissimus lumborum* from Strip loins (n = 75) were cut into 3 steaks (2.54 cm- thick) after 20 days of postmortem aging. The first steaks were vacuum packaged using nylon- polyethylene vacuum pouches (3 mil STD barrier, Prime Sources, St. Louis, OM) on a Multivac Packaging machine (MULTIVAC C500, Multivac Inc., Kansas City, MO) for laboratory analysis to analyze fat, day 0 TBARS,

and proximate analysis and were kept in a freezer at -20^{o} C. The second steaks were packaged on Styrofoam trays (measurement, Styro-Tech, Denver, CO) and overwrapped with oxygen permeable polyvinyl chloride film (prime source meat film, 5000 FT, Bunzl distribution, St. Louis, MO) and placed on a table in a cooler maintained at 2^{o} C for a simulated retail display for 7 days starting from day 0. Steaks from each muscle for seven days were for color and TBA analysis. The third steaks were first cut into half (to optimize sample size) and the halves were placed in Styrofoam trays (measurement, Styro-Tech, Denver, CO) and overwrapped with oxygen permeable polyvinyl chloride film (prime source meat film, 5000 FT, Bunzl distribution, St. Louis, MO) and placed on a same type of cooler and temperature for 4 days of retail display to provide the required retail display conditions. Steaks from each muscle for four days were for TBA analysis.

Retail display

Strip loins (M. *longissimus lumborum*) steaks, that were overwrapped for 7 and 4 days of aging were placed on a table in a cooler maintained at 2^{o} C. The steaks were exposed to continuous 1000-1800 lux warm white fluorescence lighting (PHILIPS F32T8/TL741 ALTO 700 Series, Royal Philips Electronics, Amsterdam, Netherlands). Overwrapped 7 days steaks were monitored every day starting from day 0 for color evaluation. Day 4 and day 7 steaks were vacuum packaged on their respective retail display days and kept in the freezer (- 20^{o} C) for TBARS testing.

Subjective color evaluation

Subjective color evaluation was done by three-four trained panelists from the Department of Animal Science at University of Nebraska Lincoln. Color evaluation

was an estimate of the discoloration percentage that ranges from 0 to 100% discoloration. Panelists were trained through open discussion and also by looking at a percentage discoloration chart (Appendix 4).

Objective color evaluation

Objective color was read with a Hunter Lab® Mini Scan XE Plus (Model 45/0-L, Hunter Laboratory Associates, Inc., Reston, VA) a portable colorimeter equipped with a 2.54 cm aperture and using illuminant D65 at 10° standard observer. Color was read on the strip loin steaks that were kept for 7 day of retail display, at 24 hr intervals. The average of three L* (measure of darkness to lightness), a* (measure of redness), and b* (measure of yellowness) readings were taken. Before taking the readings every day, the colorimeter was standardized using a black tile and a white tile (X= 78.5, Y= 83.2, and Z= 88.7).

Lipid oxidation analysis

Steaks in the simulated retail display condition for 0, 4, and 7 d were used to measure the oxidation status. At the end of the retail display period, samples were vacuum packaged and kept at -80° C. The thiobarbituric acid assay (TBA) was used to measure the amount of malondialdehyde present in the sample. The TBA assay was described by Buege and Aust (1978) and modified by Ahn et al. (1998).

Samples for oxidation tests were diced and powdered after dipping in liquid nitrogen using a Waring commercial blender (Model 51BL32, Waring Commercial, Torrington, CT). Powdered samples were stored at -80°C until they were used for assigned testing within 3 months. For the procedure of TBA analysis, fourteen mL of deionized, distilled water, 1 mL of butylated hydroxyanisole (10% BHA in 90%

ethanol) were added to 5 g of pulverized sample. After homogenizing for 15 sec using a polytron (POLYTRON® Kinimatica CH-6010, Switzerland), the homogenate was centrifuged for 2000 × g for 5 min. One mL of homogenate was mixed with 2 mL of 2-thiobarbituric acid and trichloroacetic acid mixture (15% TCA (w/v) and 20 mM TBA in double distilled water (ddH_2O)) and vortexed for 5 sec. The sample mixture was incubated at 70°C in a water bath for 30 min to develop color. After samples were cooled in a cold-water bath for 10 min, the sample mixture was centrifuged at 2000 × g for 15 min. Finally, duplicate 200 µL aliquots of each sample were transferred into wells on a 96-well plate and the absorbance was read at 540 nm (Dynatech microplate reader - Dynex Technologies, model MR 5000, VA, USA) to calculate the mg of malonaldehyde per kg of tissue using 1,1,3,3-tetraethoxypropane as the standard solution.

Fatty acid analysis

Fatty acid profiles were determined by gas chromatography (GC) as described by Folch et al. (1957). For fatty acid profile analysis, meat from 0 d steaks was diced and powdered after dipping in liquid nitrogen using a Waring commercial blender (Model 51BL32, Waring Commercial, Torrington, CT). One gram of pulverized meat was dissolved in 5 mL of 2:1 chloroform:methanol (v/v) to extract the lipid fraction after vortexing for 5 sec and letting them stand for 1 h at room temperature. The homogenate was filtered through Watman #2 filter paper into a 13 × 150 mm screw-cap tube and the final volume was brought up to 10 mL with 2:1 chloroform:methanol mix. Then, 2 mL of 0.74% KCl solution was added to the lipid extract and vortexed for 5 sec to separate extracted proteins. Samples were centrifuged at 1000 × g for 5 min. Following centrifugation, the aqueous layer was aspirated and evaporated to

dryness under nitrogen at 60°C (Folch et al., 1957). Then, 0.5 mL of 0.5 M NaOH in methanol was added, vortexed again for 5 sec and heated for 5 min at 100°C under nitrogen for saponification. To methylate the fatty acids, 0.5 mL of boron trifluoride in 14% methanol was added and heated for 5 min at 100°C to methylate the fatty acids (Metcalfe et al., 1966). One milliliter of saturated salt solution and 1 mL of hexane were added and vortexed for 5 sec. Following centrifugation at 1000 × g for 5 min, the hexane layer was removed and placed in a GC vial. The GC vial was purged with nitrogen, capped and stored at -80°C for a week until the sample was read on a Hewlett-Packard Gas Chromatograph (Agilent Technologies, model 5890A series, Santa Clara, CA) attached to a Hewlett-Packard Autosampler (Agilent Technologies, model 6890A series, Santa Clara, CA). Total fatty acids converted to methyl esters were separated on a fused silica column (Chrompack CP-Sil 88; 0.25 mm × 100 m, Santa Clara, CA), which was placed in an oven programmed from 140°C for 10 min to 220°C at a rate of 2°C/min for 20 min. Total run time was 70 min. The injector and detector were programmed to work at 270°C and 300°C, respectively. Each lipid extract was separated into fatty acids by using helium as the carrier gas at a flow rate of 1 mL/min. Individual fatty acids of each sample were determined by comparison of retention times with known standards. Each fatty acid was expressed as a percentage of total fatty acid methyl esters extracted.

Proximate analysis

Total fat content and moisture content were determined. The Soxhlet extraction procedure with anhydrous ether was used to determine total fat content as described by AOAC (1990). Total moisture levels were quantified by pulverizing two grams of day 0 muscles samples in duplicates after immersing in liquid N_2 and using

LECO Thermo-gravimetric analyzer (LECO Corporation, model 604-100-400, St. Joseph, MI). Before pulverizing, subcutaneous fat of the day 0 steaks were properly trimmed.

Statistical analysis

Oxidation data (TBA values) were analyzed as a split plot design where diet considered as a whole plot and days of retail display as the split plot. Effects of diet and day were considered main effects. Animal (whole muscle) within diet was considered the whole plot error and day by diet the split plot error terms, respectively. Color data were analyzed as a split plot repeated measures design. Subjective color evaluation for trained panelist is analysed through randomized complete block design. Mineral, fatty acid profile and fat content data were analysed as a complete randomized design.

Data were analyzed using the GLIMMIX procedure of SAS (Version 9.2, SAS Institute inc. 2009). Where significance ($P \leq 0.05$) was indicated by ANOVA, mean separations were performed using the LSMEANS and DIFF functions of SAS.

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Running Head: Wet distillers grains and straw affect beef quality.

Effects of feeding high levels of wet distillers grains and straw on lipid oxidation and color stability of beef muscle[1,2]

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Written in the style of Journal of Animal Science

Abstract

The objective of the research was to measure the effect of feeding high levels of wet distillers grains plus solubles and straw on retail shelf life of beef. Cattle (n = 336; 270 ± 9 kg) were fed one of 7 diets - (corn/5% straw (n=15); 40 Distillers grain (WDGS)/5% straw (n=15); 70 WDGS/8% straw (n=15); 70 WDGS/25% straw; 77.5 WDGS/9% straw (n=9); 77.5 WDGS/17% straw (n=9), and 85 WDGS/10% straw (n=12)) on DM basis. Strip loins from USDA Choice, 9-15 per treatment (except for 70 WDGS/25% straw, no samples were collected because of low USDA grades), were sampled after 20 days of postmortem aging. Lipid oxidation showed significant treatment and day effect ($P < 0.05$). Steaks from cattle fed high levels of distillers grains and straw had significantly higher ($P < 0.05$) amount of lipid oxidation (TBARS) by the end of retail display (d 7) when compared to steaks from cattle fed corn/5% straw. By day d 4, steaks from cattle fed 77.5 WDGS/17% straw and 85 WDGS/10% straw were discolored (>20%). Steaks from corn/5% straw fed cattle always had the lowest discoloration. The amount of polyunsaturated fatty acids, (ω-3), and (ω-6) fatty acids were significantly greater ($P < 0.05$) in the strips from cattle fed high levels of WDGS and straw when compared to strips from cattle fed corn. Likewise, steaks from cattle fed corn diet had more monounsaturated fatty acids (14:1, 16:1, 17:1, and 18:1) when compared to steaks from cattle fed high levels of WDGS and straw. The amounts of *trans*-fatty acids where higher ($P < 0.05$) in the strips from cattle fed high level of WDGS and straw when compared to the strips from cattle fed corn. These data indicate that precautions against oxidation and discoloration are needed when cattle are fed high levels of WDGS, the meat is aged, and subjected to retail display in oxygen-permeable packaging.

Key words: beef, discoloration, oxidation, shelf life, wet distillers grains

INTRODUCTION

The production of the distillers grains as a byproduct of ethanol is tremendously increasing. The annual production of distillers grains increased from about 1 million to 10 million tons from 1998 to 2006. This is estimated to be 16 million tons in 2016 (Weiss et al., 2009).

Distillers grains may be less than 40% dry matter (DM) and are routinely fed as a supplemental protein source and high energy diet source for cattle. Few studies have been done with very high levels of distillers grains in the cattle diet. de Mello Jr. et al. (2009) fed up to 50% wet distillers grains plus solubles (WDGS) to steers. Feeding high levels of distillers grains to the cattle reduces dry matter intake (DMI) and has a quadratic effect on average daily gain (ADG) (Klopfenstein et al., 2008).

Detrimental effects to meat quality were observed when cattle were fed WDGS (de Mello Jr. et al., 2008; 2009; Senaratne et al., 2009). Almost all the research has shown that polyunsaturated fatty acid (PUFA) composition increases in beef irrespective of the age group of cattle and muscle types. The PUFA are easily oxidized and are therefore responsible for degrading meat quality - notable shelf life, taste and flavor – during retail display.

The objectives of this study were to determine the relationships between high levels of WDGS and straw on PUFA, proximate composition, and color stability of M. *Longissimus lumborum.*

MATERIALS AND METHODS

Experimental design

Crossbreed cattle (n = 336; 270 ± 9 kg) were randomly allotted to one of seven finishing diets containing corn wet distillers grains plus solubles (WDGS) and wheat straw. Diets contained corn/5% straw (n=15); 40% distillers grain (WDGS)/5% straw (n=15); 70% WDGS/8% straw (n=15); 70% WDGS/25% straw; 77.5% WDGS/9% straw (n=9); 77.5% WDGS/17% straw (n=9), and 85% WDGS/10% straw (n=12) were fed on DM basis. The diets containing corn/5% straw, 40% WDGS/5% straw, 70% WDGS/8% straw, 77.5% WDGS/9% straw were fed for 183 d and the diet containing 70% WDGS/25% straw, 77.5% WDGS/17% straw, and 85% WDGS/10% straw were fed 225 d so as to attain similar final body weights (BW). Cattle were slaughtered at Greater Omaha Packing Co. (Omaha, Nebraska). Carcasses were chilled for 48 h before grading. After grading, strip loins (IMPS # 180 PSO2; NAMP, 2007) 9-15 per treatment (except for 70% WDGS/25% straw no samples were collected because of low USDA grades), all USDA Choice, were collected from n=75 animals. The loins were vacuum packaged, transported under refrigeration to Loeffel Meat Laboratory at the University of Nebraska-Lincoln and aged for 20 days at 2^{o} C.

Fabrication and assignment of steaks

The m. *Longissimus lumborum* from strip loins (n = 75) were cut into 3 steaks (2.54 cm- thick). The first steaks were vacuum packaged using nylon-polyethylene vacuum pouches (3 mil STD barrier, Prime Sources, St. Louis, MO) on a Multivac Packaging machine (MULTIVAC C500, Multivac Inc., Kansas City, MO) for laboratory analysis of fatty acids, day 0 oxidation (Thiobarbituric Acid Reactive Substances = TBARS), and proximate analysis and were kept in a freezer at -20^{o} C.

The second steaks were packaged on Styrofoam trays (measurement, Styro-Tech, Denver, CO), overwrapped with oxygen-permeable polyvinyl chloride film (prime source meat film, 5000 FT, Bunzl distribution, St. Louis, MO) and subjected to a 7 d simulated retail display. The third steaks were first cut in half (to optimize sample size) and the halves were placed in Styrofoam trays (measurement, Styro-Tech, Denver, CO) and overwrapped with oxygen permeable polyvinyl chloride film and placed in the retail display to provide d 4 samples for oxidation (TBARS) analysis.

Retail display

Strip loins (M. *Longissimus lumborum*) steaks for retail display were placed on a table in a cooler maintained at 2^{o} C. The steaks were exposed to continuous 1000-1800 lux warm white fluorescence lighting (PHILIPS F32T8/TL741 ALTO 700 Series, Royal Philips Electronics, Amsterdam, Netherlands). Steaks intended for the entire 7 d display period were monitored every day starting from day 0 for color evaluation. Day 4 and day 7 steaks were vacuum packaged on their respective retail display days and kept in the freezer (- 20^{o} C) for TBARS testing.

Subjective color evaluation

Subjective color evaluation was done by three-four trained panelists from the Department of Animal Science at University of Nebraska, Lincoln. They were trained in the University by looking at a 0 to 100% discoloration chart. Color evaluation was an estimate of the discoloration percentage that ranges from 0 to 100% discoloration. Panelists were trained through open discussion and also by looking at a percentage discoloration chart (Appendix 4).

Objective color evaluation

Objective color was read with a Hunter Lab® Mini Scan XE Plus portable colorimeter (Model 45/0-L, Hunter Laboratory Associates, Inc., Reston, VA) equipped with a 2.54 cm aperture and using illuminant D65 at 10º standard observer. Color was read on the strip loin steaks that were kept for 7 d of retail display, at 24 hr intervals. The average of three L* (measure of darkness to lightness), a* (measure of redness), and b* (measure of yellowness) readings were taken. Before taking the readings every day, the colorimeter was standardized using a black tile and a white tile (X= 78.5, Y= 83.2, and Z= 88.7).

Lipid oxidation analysis

Steaks in the simulated retail display condition for 0, 4, and 7 d were used to measure the oxidation status. At the end of the retail display period, samples were vacuum packaged and kept at -80º C. The thiobarbituric acid reactive substance (TBARS) was used to measure the amount of malondialdehyde present in the sample. The TBARS assay was described by Buege and Aust (1978) and modified by Ahn et al. (1998).

Samples for oxidation tests were diced after taking out from the freezer and powdered right after dipping in liquid nitrogen using a Waring commercial blender (Model 51BL32, Waring Commercial, Torrington, CT). Powdered samples were stored at -80°C until they were used for assigned testing within 3 months. For the procedure of TBARS analysis (Buege and Aust, 1978; Ahn et al., 1998), 14 mL of deionized, distilled water, 1 mL of butylated hydroxyanisole (10% BHA in 90% ethanol) were added to 5 g of pulverized sample. After homogenizing for 15 sec using

a polytron (POLYTRON® Kinimatica CH-6010, Switzerland), the homogenate was centrifuged for 2000 × g for 5 min. One mL of homogenate was mixed with 2 mL of 2-thiobarbituric acid (TBA) and trichloroacetic acid mixture (15% TCA (w/v) and 20 mM TBA in double distilled water (ddH_2O)) and vortexed for 5 sec. The sample mixture was incubated at 70°C in a water bath for 30 min to develop color. After samples were cooled in a cold-water bath for 10 min, the sample mixture was centrifuged at 2000 × g for 15 min. Finally, duplicate 200 µL aliquots of each sample were transferred into wells on a 96-well plate and the absorbance was read at 540 nm (Dynatech microplate reader - Dynex Technologies, model MR 5000, VA, USA) to calculate the mg of malonaldehyde per kg of tissue using 1,1,3,3-tetraethoxypropane as the standard solution.

Fatty acid analysis

Fatty acid profiles were determined by gas chromatography (GC) as described by Folch et al. (1957). For fatty acid profile analysis, meat from 0 d steaks was diced, and powdered by dipping in liquid nitrogen and politroning, using a Waring commercial blender (Model 51BL32, Waring Commercial, Torrington, CT). One gram of pulverized meat was dissolved in 5 mL of 2:1 chloroform:methanol (v/v) to extract the lipid fraction after vortexing for 5 sec and letting them stand for 1 h at room temperature. The homogenate was filtered through Watman #2 filter paper into a 13 × 150 mm screw-cap tube and the final volume was brought up to 10 mL with 2:1 chloroform:methanol mix. Then, 2 mL of 0.74% KCl solution was added to the lipid extract and vortexed for 5 sec to separate extracted proteins. Samples were centrifuged at 1000 × g for 5 min. Following centrifugation, the aqueous layer was aspirated and evaporated to dryness under nitrogen at 60°C (Folch et al., 1957). Then,

0.5 mL of 0.5 M NaOH in methanol was added, vortexed again for 5 sec and heated for 5 min at 100°C under nitrogen for saponification. To methylate the fatty acids, 0.5 mL of boron trifluoride in 14% methanol was added and heated for 5 min at 100°C to methylate the fatty acids (Metcalfe et al., 1966). One milliliter of saturated salt solution and 1 mL of hexane were added and vortexed for 5 sec. Following centrifugation at 1000 × g for 5 min, the hexane layer was removed and placed in a GC vial. The GC vial was purged with nitrogen, capped and stored at -80°C for a week until the sample was read on a Hewlett-Packard Gas Chromatograph (Agilent Technologies, model 5890A series, Santa Clara, CA) attached to a Hewlett-Packard Autosampler (Agilent Technologies, model 6890A series, Santa Clara, CA). Total fatty acids converted to methyl esters were separated on a fused silica column (Chrompack CP-Sil 88; 0.25 mm × 100 m, Santa Clara, CA), which was placed in an oven programmed from 140°C for 10 min to 220°C at a rate of 2°C/min for 20 min. Total run time was 70 min. The injector and detector were programmed to work at 270°C and 300°C, respectively. Each lipid extract was separated into fatty acids by using helium as the carrier gas at a flow rate of 1 mL/min. Individual fatty acids of each sample were determined by comparison of retention times with known standards. Each fatty acid was expressed as a percentage of total fatty acid methyl esters extracted.

Proximate analysis

Total fat and moisture contents were determined. The Soxhlet extraction procedure with anhydrous ether was used to determine total fat content as described by AOAC (1990). The subcutaneous fat of the day 0 steaks were properly trimmed. Total moisture levels were quantified by pulverizing two grams of day 0 muscles

samples in duplicates after immersing in liquid N_2 and using LECO Thermo-gravimetric analyzer (LECO Corporation, model 604-100-400, St. Joseph, MI).

Statistical analysis

Oxidation data (TBARS) were analyzed as a split plot design where diet was considered as the whole plot and days of retail display as the split plot. Effects of diet and day were considered main effects. Animal (whole muscle) within diet was considered the whole plot error and day by diet the split plot error terms, respectively. Color data were analyzed as a split plot repeated measures design. Subjective color evaluation for trained panelist is analyzed through randomized complete block design. Mineral, fatty acid profile and fat content data were analyzed as a complete randomized design.

Data were analyzed using the GLIMMIX procedure of SAS (Version 9.2, SAS Institute inc. 2009). Where significance ($P \leq 0.05$) was indicated by ANOVA, mean separations were performed using the LSMEANS and DIFF functions of SAS.

RESULTS AND DISCUSSION

Visual discoloration

For the first 2 days of retail display (0 and 1 day) the discoloration percentage was zero for all treatments (Table 1). It was clear that strips from cattle fed very high levels of WDGS (77.5 DG/17% Straw, 80 DG/10% Straw) start discoloring at the 2nd day of retail display and became more prominent toward the end of retail display. Likewise, strips from cattle fed corn/5% straw showed less discoloration ($P < 0.05$) from d 4 to d 7 of retail display when compared to strips from cattle fed very high levels of WDGS. Strips packaged in PVC from cattle fed corn/5% straw discolor at a

slower rate in retail display than other treatments. Senaratne et al. (2009) demonstrated that discoloration in steaks largely depends upon the levels of WDGS (0%, 20%, and 40%- DM basis) where steaks from cattle fed higher levels of WDGS discolor faster regardless of the aging period. However, the redness of steak is due to the presence of oxymyoglobin which is formed by the reaction of oxygen and myoglobin. If the ambient environment of steak has lots of oxygen, oxymyoglobin becomes more stable, otherwise it is reduced to metmyoglobin. Metmyoglobin is accountable for the brownish color in steaks and is referred to as discoloration.

Instrumental color

Data for L*, a*, and b* are shown on Table 2, 3, and 4, respectively. The L* (lightness) values first decreased and then increased from d 0 to 7 of retail display (Table 2). The values for L* positively correlates with beef carcass lean maturity and negatively correlates with the pH of the beef (Wulf and Wise, 1999; Page et al., 2001). Behrends et al. (2003) concluded that a* for redness is a good indicator of color acceptability. Roeber et al. (2005) reported that diets containing DG (40 to 50% - DM basis) negatively affect the color stability (a*) of strip loin steaks but concluded that 10 to 25% of WDGS could be included in the diet with no adverse effects. The redness values decreased for strips from d 0 to 4 for all treatments (Table 3). However, strips from cattle fed corn were significantly more red (higher a* value) on d 5 and 6 of retail display than steaks from cattle fed high levels of WDGS. To conclude, steaks from corn-fed cattle, compared to steaks from cattle fed high levels of WDGS, retained redness to a greater extent during retail display. Roeber et al. (2005) and Roberson, (2004) reported that the redness is more likely due to the presence of xanthophylls and other oxygen derivatives of carotenes such as yellow to

orange carotenoid pigments present in the corn. For b*, a positive value indicates yellow and a negative value indicates blue color in beef. Yellowness values (b*) decreased for all treatments from d 0 of retail display to d 7 (Table 4).

Lipid oxidation

Oxymyoglobin becomes stable only when there is high partial pressure and concentration of oxygen. A high concentration of oxygen ultimately leads to higher lipid oxidation. In support of this statement, Jakobsen and Bertelsen (2000) found lipid oxidation in beef steaks under high O_2 packing. Strips from cattle fed 85 DG/10% straw had the significantly high levels of TBARS on d 7 when compared to strips from cattle fed corn/5% straw, 40 WDGS/5% straw, and 77.5 WDGS/9% straw (Table 5). However, it had the same TBARS levels as in the strips from cattle fed 70 WDGS/8% straw and 77.5 WDGS/17% straw. TBARS is an indication of polyunsaturated fatty acids in a steak, responsible for development of rancidity in meat in case of long term retail display. Senaratne et al. (2009) showed that the increase in aging also increases the TBARS value in a steak from cattle fed 0%, 20%, and 40% of WDGS with or without distillers soluble and vitamin E supplementation. However, beef from cattle fed vitamin E-supplemented diets has lower TBARS values in comparison with cattle fed non-supplemented diets. In this study, very high levels of WDGS with straw in finishing diets help to boost up lipid oxidation in strip steaks.

Fatty acids

As shown in Table 6, strips from cattle fed high levels of WDGS and straw had significantly low levels of monounsaturated fatty acids in comparison to strips from cattle fed corn or control diets. Levels of Myristoleic (14:1), Palmitoleic (16:1), Heptadecanoic (17:1), and Oleic acid (18:1) decreased significantly on strips from

cattle fed high levels of WDGS and straw. However, the levels of Linoleic (18:2), Linolenic (18:3), Stearic acid (18:0), Nonadecanoic (19:0), Eicosanoic acid (20:0) increased significantly in the strips of cattle fed high level of WDGS and straw diet when compared to strips from cattle fed corn diet.

Strips from corn/5% straw fed cattle had significantly lower amount of PUFA, (ω 3) and (ω 6) fatty acids when compared to the strips from cattle fed high levels of WDGS and straw (Table 6). However, there is no difference in ω-3 content on strips from cattle fed corn/5% straw and 40 WDGS/5% straw diets. *Trans*-fatty acids significantly increased in the strips of cattle fed high levels of WDGS and straw when compared to the strips of cattle fed corn diet. de Mello Jr. et al. (2009) reported that there were positive and linear relationships between levels of WDGS fed (0%, 15%, and 30%-on DM basis) and total *trans* fatty acids for ribeye slices (M. *Longissimus thoracis*, M. *Psoas major*, M. *Infraspinatus*). In other research, Senaratne et al. (2009) reported that WDGS (0%, 20%, and 40% with or without vitamin E supplementation and distillers soluble) diets significantly increased all the *trans* fats and PUFA on tenderloins and strip loins. However, there was minimal effect of vitamin E on fatty acids profile of strip loins and tenderloins. *Trans*-fatty acids like 18:1 Δ13*t*, 18:2 Δ9*t*, and CLA *c12, t10* increased significantly in strips from cattle fed high levels of WDGS and straw. In addition, 18:1 Δ14*t* significantly increased in strips from cattle fed 77.5 WDGS/17% straw and 85 WDGS/10% straw. Likewise, the amounts of CLA c9, *t*11 in all strips were similar except in strips from cattle fed 40 WDGS/5% straw.

Vander Pol et al. (2009) reported that WDGS, as a byproduct from distillers grain, have greater fat digestibility in comparison to whole grains. Cattle fed WDGS can accumulate high concentration of 18:1 *trans*, 18:1(ω 9), and 18:2(ω 6) in the

duodenum (Vander Pol et al., 2009). Unsaturated fats are biohydrogenated to saturated fatty acids (SFA) inside the rumen and isomerization of unsaturated *cis* to *trans* fatty acids occurs (Harfoot and Hazlewood, 1988). Zinn et al. (2000) reported that unsaturated fatty acids inside the duodenum promote the absorption of PUFA by increasing the surface area of micelles. As a result, greater absorption of PUFA, ω 3, and ω 6 fatty acids will occur in the intestinal lamellae which are then reflected in the muscles and finally to the meat.

de Mello Jr. et al. (2009) had also reported that the levels of modified wet distillers grains - 0% to 50% (MWDGS) had no effect on marbling score, marbling distribution and intramuscular fat but had significant linear effect on fatty acid profile and PUFA. Conjugated linoleic acid (CLA) is produced during ruminal biohyrogenation of linoleic acid and also synthesized from animal tissue *trans*-11 C18:1. (Harfoot, 1981; Bauman et al., 1999). T*rans*-11 C18:1 helps in synthesizing *cis*-9, *trans*-11 C18:2 endogenously by the action of the enzyme Δ^9-desaturase (Griinari et al. 1999). There were significant increases in *trans* fat isomers in the strips from cattle fed high levels of WDGS and straw which is due to the action of rumen microorganisms on unsaturated fats making them more *trans* in configuration (Mozaffarian et al., 2006). But, *trans* fat was not significantly higher on strips from cattle fed very high levels of distillers grains (85 WDGS/10% straw and 77.5 WDGS/17% straw). This might be due to high concentration of straw in the diet which protects fatty acids from entering into the duodenum. Increased levels of WDGS linearly increase PUFA level for top blades, tenderloins, and striploins (de Mello Jr. et al. 2009). Long chain fatty acids are most often found in the membrane phospholipids and their presence reflects the total lipids in the leaner meats (Bas and

Sauvant 2001; Raes et al. 2004). The PUFA having weak double bonds between carbon atoms are responsible for producing detrimental effects on color and other sensory attributes in steaks by more rapid oxidation.

Proximate analysis

The detail of the proximate analysis is given in Table 7. Steaks (d 0) from cattle fed 77.5 WDGS/17 % straw and 40 WDGS/5% straw showed significantly higher amount of intramuscular fat which coincides with marbling score of strips. However, there were no differences in the fat content between strips from cattle fed control, 40 WDGS/5% straw, and 70 WDGS/8% straw diets. Brackebusch et al. (1991) showed similar results on high marbling and fat content of longissimus composition and composition of major muscles. On the other hand, the amount of fat decreased in steaks from cattle fed high levels of WDGS diet (77.5 WDGS/9% straw and 85 WDGS/10% straw). Although strips from cattle fed very high levels of WDGS (85 WDGS/10% straw) contained significantly lower moisture levels when compared to the rest of the diets, There were no differences in moisture levels among strips from cattle fed control, 40 WDGS/5% straw, 70 WDGS/8% straw, or 77.5 WDGS/9% straw.

Conclusion

Ratio of concentrate to roughage, proportion of distillers grains in the diet, and number of days fed in a feedlot affect beef quality, composition, and shelf life. Feeding high levels of distillers grains (> 70% DM) creates problems with color, shelf-life, and oxidation. In this research, there was an increase in the level of PUFA, *trans* fatty acids, omega-3, and omega-6 fatty acids in the strips of cattle fed high levels of WDGS. Consequently, there was increased visual as well as instrumental

discoloration during retail display. Likewise, lipid oxidation was higher in strips from cattle fed high levels of WDGS. There were no benefits to beef quality when straw was added to diets containing high levels of WDGS.

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Table 1. Visual discoloration of strips steaks (M. *longissimus lumborum*) from cattle fed corn, high level WDGS, and straw.

	Days at display							
Treatments	0	1	2	3	4	5	6	7
Corn/5% St[1]	0^{E}	0^{E}	0^{E}	0.43^{DEc}	4.72^{Dc}	11.84^{Cd}	32.33^{Bb}	62.62^{Ab}
40 WDGS/5% St[2]	0^{E}	0^{E}	0^{E}	0.70^{Ec}	23.01^{Dab}	41.00^{Cabc}	66.11^{Ba}	83.78^{Aa}
70 WDGS/8% St[3]	0^{E}	0^{E}	0^{E}	0.27^{Ec}	11.38^{Dbc}	38.07^{Cbc}	65.22^{Ba}	89.22^{Aa}
77.5 WDGS/9% St[4]	0^{E}	0^{E}	0^{E}	0.03^{Ec}	12.19^{Dbc}	28.22^{Cdc}	58.70^{Ba}	80.85^{Aa}
77.5 WDGS/17% St[5]	0^{E}	0^{E}	0.19^{E}	5.74^{Eb}	34.26^{Da}	60.22^{Cab}	82.59^{Ba}	95.19^{Aa}
85 WDGS/10% St[6]	0^{F}	0^{F}	0.14^{F}	8.67^{Ea}	33.56^{Da}	63.61^{Ca}	81.81^{Ba}	93.75^{Aa}
S.E.M.[7]	3.21	3.21	3.21	3.21	3.21	3.74	3.74	3.74

[A, B, C, D, E, F] Means in the same row having different superscripts are significant at $P \leq 0.05$.

[a, b, c, d] Means in the same column having different superscripts are significant at $P \leq 0.05$.

[1]Corn/5% St: corn diet and 5% straw.

[2]40 WDGS/5% St: diet with 40% WDGS and 5% straw.

[3]70 WDGS/8% St: diet with 70% WDGS and 8% straw.

[4]77.5 WDGS/9% St: diet with 77.5% WDGS and 9% straw.

[5]77.5 WDGS/17% St: diet with 77.5% WDGS and 17% straw.

[6]85 WDGS/10% St: diet with 85% WDGS and 10% straw.

S.E.M.[7]: Standard Error of Mean.

Table 2. L* values of strips steaks from cattle (M. *longissimus lumborum*) fed corn, high level WDGS, and straw.

	Days at display							
Treatments	0	1	2	3	4	5	6	7
Corn/5% St[1]	40.19^{A}	37.4^{Bab}	35.92^{Ba}	36.94^{Bab}	37.32^{Bab}	37.02^{Ba}	36.97^{Bb}	39.01^{ABab}
40 WDGS/5% St[2]	40.94^{A}	39.1^{BCa}	36.68^{Ca}	37.68^{Ca}	37.87^{Ca}	37.92^{BCa}	38.88^{Ca}	40.61^{ABa}
70 WDGS/8% St[3]	39.87^{A}	37.9^{BCab}	37.41^{Ca}	37.03^{Ca}	37.04^{Cabc}	37.13^{Ca}	37.64^{Cab}	39.14^{ABab}
77.5 WDGS/9% St[4]	40.44^{A}	38.6^{BCab}	38.56^{BCa}	37.36^{Da}	38.44^{Ca}	37.77^{CDa}	38.15^{CDab}	40.06^{ABa}
77.5 WDGS/17% St[5]	39.69^{A}	36.99^{Bb}	36.41^{BCa}	34.89^{Db}	35.28^{CDc}	36.51^{Ba}	36.86^{Bb}	37.41^{Bb}
85 WDGS/10% St[6]	39.87^{A}	38.3^{ABab}	37.29^{BCa}	35.72^{Dab}	35.97^{CDbc}	37.51^{Ba}	38.43^{ABab}	38.95^{Aab}
S.E.M.[7]	0.69	0.86	2.11	0.90	0.75	0.75	0.72	0.69

$^{A, B, C. D, E}$ Means in the same row having different superscripts are significant at $P \leq 0.05$.

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

[1]Corn/5% St: corn diet and 5% straw.

[2]40 WDGS/5% St: diet with 40% WDGS and 5% straw.

[3]70 WDGS/8% St: diet with 70% WDGS and 8% straw.

[4]77.5 WDGS/9% St: diet with 77.5% WDGS and 9% straw.

[5]77.5 WDGS/17% St: diet with 77.5% WDGS and 17% straw.

[6]85 WDGS/10% St: diet with 85% WDGS and 10% straw.

S.E.M.[7]: Standard Error of Mean.

Table 3. a* values of strips steaks (M. *longissimus lumborum*) from cattle fed corn, high level WDGS, and straw .

	Days at display							
Treatments	0	1	2	3	4	5	6	7
Corn/5% St[1]	25.20^{Aab}	21.05^{Ba}	19.01^{Cab}	17.91^{Dab}	16.43^{Ea}	15.92^{Ea}	14.13^{Fa}	10.17^{Ga}
40 WDGS/5% St[2]	25.11^{Aab}	21.21^{Ba}	18.67^{Cb}	16.27^{Dc}	13.86^{Eb}	12.57^{Fb}	10.49^{Gb}	8.95^{Hab}
70 WDGS/8% St[3]	23.62^{Ac}	21.93^{Ba}	18.41^{Cb}	16.56^{Dbc}	15.50^{Eab}	12.82^{Eb}	10.05^{Fb}	8.15^{Gb}
77.5 WDGS/9% St[4]	24.43^{Abc}	21.29^{Ba}	18.96^{Cab}	17.31^{Dabc}	14.44^{Eab}	13.74^{Eab}	11.32^{Gb}	9.08^{Hab}
77.5 WDGS/17% St[5]	26.01^{Aa}	22.10^{Ba}	19.81^{Cab}	17.55^{Dabc}	14.76^{Eab}	12.09^{Eb}	10.02^{Gb}	7.91^{Hb}
85 WDGS/10% St[6]	25.09^{Aab}	21.91^{Ba}	20.08^{Ca}	18.12^{Da}	15.20^{Eab}	11.66^{Eb}	9.48^{Gb}	7.94^{Hb}
S.E.M.[7]	0.51	0.85	0.56	0.65	1.06	0.94	0.96	0.70

$^{A, B, C, D, E, F, G, H}$ Means in the same row having different superscripts are significant at $P \leq 0.05$.

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

[1]Corn/5% St: corn diet and 5% straw.

[2]40 WDGS/5% St: diet with 40% WDGS and 5% straw.

[3]70 WDGS/8% St: diet with 70% WDGS and 8% straw.

[4]77.5 WDGS/9% St: diet with 77.5% WDGS and 9% straw.

[5]77.5 WDGS/17% St: diet with 77.5% WDGS and 17% straw.

[6]85 WDGS/10% St: diet with 85% WDGS and 10% straw.

S.E.M.[7]: Standard Error of Mean.

Table 4. b* values of strips steaks (M. *longissimus lumborum*) from cattle fed corn, high level WDGS, and straw.

	Days at display							
Treatments	0	1	2	3	4	5	6	7
Corn/5% St[1]	21.30^{Aa}	18.11^{Ba}	16.35^{Cab}	16.23^{Ca}	15.61^{Da}	12.09^{Fb}	12.80^{Eab}	9.71^{Gb}
40 WDGS/5% St[2]	20.47^{Aab}	17.89^{Ba}	16.68^{Cab}	15.98^{Da}	14.70^{Eab}	11.66^{Gbc}	12.53^{Fbc}	10.44^{Hb}
70 WDGS/8% St[3]	18.61^{Ac}	17.46^{Ba}	16.12^{Cb}	15.24^{Da}	14.47^{Eab}	10.76^{Gc}	11.82^{Fc}	9.67^{Hb}
77.5 WDGS/9% St[4]	20.45^{Aab}	17.84^{Ba}	17.34^{Bab}	16.35^{Ca}	14.34^{Dab}	11.64^{Fbc}	12.38^{Ebc}	9.83^{Gb}
77.5 WDGS/17% St[5]	20.16^{Ab}	18.04^{Ba}	17.06^{Bab}	15.60^{Ba}	14.16^{Cb}	13.88^{Ca}	13.72^{Ca}	14.63^{BCa}
85 WDGS/10% St[6]	19.90^{Ab}	17.20^{Ba}	17.36^{Ba}	16.09^{Ca}	14.99^{Dab}	14.58^{Da}	13.52^{Ea}	13.24^{Ea}
S.E.M.[7]	0.41	0.53	0.52	0.51	0.53	0.47	0.39	0.75

$^{A, B, C, D, E, F, G, M}$ Means in the same row having different superscripts are significant at $P \leq 0.05$.

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

[1]Corn/5% St: corn diet and 5% straw.
[2]40 WDGS/5% St: diet with 40% WDGS and 5% straw.
[3]70 WDGS/8% St: diet with 70% WDGS and 8% straw.
[4]77.5 WDGS/9% St: diet with 77.5% WDGS and 9% straw.
[5]77.5 WDGS/17% St: diet with 77.5% WDGS and 17% straw.
[6]85 WDGS/10% St: diet with 85% WDGS and 10% straw.
S.E.M.[7]: Standard Error of Mean.

Table 5. Treatment versus day interaction of strip steaks (M. *longissimus lumborum*) containing thiobarbituric acid reactive substances (malondialdehyde mg/kg) from cattle fed high level WDGS with straw.

		Day		
		0	4	7
Treatment	Corn/5% St[1]	0^{C}	0.57^{Bd}	1.34^{Ac}
	40 WDGS/5% St[2]	0.12^{C}	1.89^{Bbc}	3.79^{Ab}
	70 WDGS/8% St[3]	0.25^{C}	2.51^{Bab}	4.77^{Aa}
	77.5 WDGS/9% St[4]	0.09^{C}	1.38^{Bcd}	3.73^{Ab}
	77.5 WDGS/17% St[5]	0.02^{C}	2.27^{Babc}	4.57^{Aab}
	85 WDGS/10% St[6]	0.20^{C}	2.65^{Ba}	4.70^{Aa}
	S.E.M.[7]	0.32	0.32	0.32

$^{A, B, C}$ Means in the same row having different superscripts are significant at $P \leq 0.05$.
$^{a, b, c, d}$ Means in the same column having different superscripts are significant at $P \leq 0.05$.
[1]Corn/5% St: corn diet and 5% straw.
[2]40 WDGS/5% St: diet with 40% WDGS and 5% straw.
[3]70 WDGS/8% St: diet with 70% WDGS and 8% straw.
[4]77.5 WDGS/9% St: diet with 77.5% WDGS and 9% straw.
[5]77.5 WDGS/17% St: diet with 77.5% WDGS and 17% straw.
[6]85 WDGS/10% St: diet with 85% WDGS and 10% straw.
S.E.M.[7]: Standard Error of Mean.

Table 6. Weight percentage[1] of fatty acids of strip (M. *longissimus lumborum*) steaks from cattle fed high level of weight distillers grains with straw.

Fatty acids	Dietary Treatments (% WDGS –DM basis)						*P*-Value	S.E.M.[7]
	Corn 5% St[1]	40 WDGS 5% St[2]	70 WDGS 8% St[3]	77.5 WDGS 9% St[4]	77.5 WDGS 17% St[5]	85 WDGS 10% St[6]		
12:0	0.07	0.06	0.07	0.08	0.07	0.07	0.84	0.008
14:0	3.12^{a}	2.91^{ab}	2.87^{ab}	2.63^{b}	2.69^{b}	2.68^{b}	0.08	0.15
14:1-(n-5)	0.75^{a}	0.55^{b}	0.43^{bc}	0.43^{bc}	0.46^{bc}	0.42^{c}	<0.01	0.05
15:0	0.55^{a}	0.53^{ab}	0.47^{bc}	0.47^{bc}	0.38^{d}	0.46^{c}	<0.01	0.02
ISO 16:0	0.43^{a}	0.26^{c}	0.44^{a}	0.39^{ab}	0.31^{bc}	0.39^{ab}	<0.01	0.05
16:0	26.23^{a}	24.44^{b}	23.56^{c}	22.68^{cd}	22.48^{d}	23.39^{cd}	<0.01	0.38
16:1- (n-7)	3.85^{a}	2.54^{b}	2.31^{bc}	2.08^{c}	2.47^{bc}	2.25^{bc}	<0.01	0.16
17:0	1.52^{a}	1.46^{a}	1.18^{b}	1.21^{b}	0.84^{c}	1.00^{c}	<0.01	0.06
ISO 18:0	0.29^{bc}	0.23^{c}	0.43^{a}	0.38^{ab}	0.36^{ab}	0.42^{a}	<0.01	0.04
17:1- (n-7)	1.30^{a}	0.86^{b}	0.68^{c}	0.68^{cd}	0.52^{d}	0.56^{cd}	<0.01	0.06
18:0	12.70^{d}	14.94^{c}	16.42^{b}	16.35^{bc}	17.86^{ab}	18.74^{a}	<0.01	0.57
18:1T	2.14^{c}	4.74^{b}	6.73^{a}	7.51^{a}	5.60^{b}	5.49^{b}	<0.01	0.45
18:1- (n-9)	38.38^{a}	35.90^{b}	31.74^{c}	32.21^{c}	34.75^{b}	32.25^{c}	<0.01	0.81
18:1-(n-7)	1.49^{a}	1.06^{b}	1.39^{a}	1.47^{a}	0.81^{b}	0.94^{b}	<0.01	0.15
18:1 Δ13	0.11^{d}	0.21^{b}	0.24^{ab}	0.27^{a}	0.17^{c}	0.22^{b}	<0.01	0.02
18:1 Δ14	0.04^{b}	0.07^{ab}	0.07^{ab}	0.07^{ab}	0.08^{a}	0.08^{a}	0.11	0.02
18:2T	0.08^{c}	0.19^{b}	0.2^{ab}	0.23^{ab}	0.23^{a}	0.20^{ab}	<0.01	0.02
19:0	0.07^{c}	0.24^{b}	0.26^{ab}	0.28^{ab}	0.30^{a}	0.28^{a}	<0.01	0.02
18:2- (n-6)	3.27^{d}	5.45^{c}	6.83^{a}	6.69^{a}	5.85^{a}	6.30^{ab}	<0.01	0.26
20:0	0.04^{b}	0.11^{a}	0.11^{a}	0.13^{a}	0.14^{a}	0.14^{a}	<0.01	0.02
18:3- (n-3)	0.12^{b}	0.21^{a}	0.21^{a}	0.25^{a}	0.22^{a}	0.25^{a}	<0.01	0.02
20:1- (n-9)	0.25^{c}	0.35^{bc}	0.29^{bc}	0.41^{ab}	0.52^{a}	0.31^{bc}	<0.01	0.06
CLA *c9, t11*	0^{b}	0.01^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0.1	0.004
CLA *c12, t10*	0.01^{c}	0.05^{b}	0.06^{ab}	0.10^{a}	0.09^{ab}	0.10^{a}	<0.01	0.02
20:2	0.07	0.08	0.05	0.03	0.04	0.10	0.33	0.03
20:3- (n-6)	0.26^{ab}	0.21^{b}	0.30^{a}	0.30^{a}	0.23^{b}	0.26^{ab}	0.02	0.03
20:4- (n-6)	0.83^{a}	0.57^{b}	0.91^{a}	0.82^{a}	0.60^{b}	0.80^{a}	<0.01	0.07
22:4	0.02	0.03	0.03	0.02	0.03	0.03	0.96	0.02
22:5- (n-3)	0.02	0	0.02	0.04	0.02	0.01	0.61	0.02
Trans	2.37^{d}	5.28^{c}	7.31^{ab}	8.20^{a}	6.18^{bc}	6.09^{c}	<0.01	0.48
PUFA	4.67^{d}	6.80^{c}	8.62^{a}	8.50^{a}	7.32^{bc}	8.06^{ab}	<0.01	0.33
OMEGA 3	0.15^{c}	0.21^{bc}	0.23^{ab}	0.29^{a}	0.25^{ab}	0.26^{ab}	<0.01	0.03
OMEGA 6	4.37^{d}	6.26^{c}	8.07^{a}	7.84^{a}	6.70^{bc}	7.40^{ab}	<0.01	0.32
OMEGA6:3	29.39	30.49	32.52	28.21	28.28	29.13	0.77	2.64

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

[a, b, c, d] Means in the same row having different superscripts are significant at $P \leq 0.05$.

[1]Corn/5% St: corn diet and 5% straw.

[2]40 WDGS/5% St: diet with 40% WDGS and 5% straw.

[3]70 WDGS/8% St: diet with 70% WDGS and 8% straw.

[4]77.5 WDGS/9% St: diet with 77.5% WDGS and 9% straw.

[5]77.5 WDGS/17% St: diet with 77.5% WDGS and 17% straw.

[6]85 WDGS/10% St: diet with 85% WDGS and 10% straw.

S.E.M.[7]= Standard Error of Mean.

Table 7. Percentage levels of fat and moisture of strips (M. *longissimus lumborum*) from cattle fed high level of WDGS with straw.

Attributes	Treatments						*P*-Value	S.E.M.[7]
	Corn 5% St[1]	40 WDGS 5% St[2]	70 WDGS 8% St[3]	77.5 WDGS 9% St[4]	77.5 WDGS 17% St[5]	85 WDGS 10% St[6]		
Marbling	Mt 42[abc]	Mt 64[a]	Mt 52[ab]	Mt 22[bc]	Mt 61[a]	Mt 21[c]	0.02	12.54
Fat content (%)	9.25[bc]	10.38[ab]	9.25[bc]	7.53[d]	10.79[a]	8.68[dc]	0.0003	0.54
Moisture content (%)	71.95[a]	71.62[a]	71.53[ab]	71.47[ab]	70.72[b]	69.87[c]	< 0.0001	0.34

LS-means with the different letters [a, b, c, d] are significantly different at $P < 0.05$.

[1]Corn/5% St: corn diet and 5% straw.

[2]40 WDGS/5% St: diet with 40% WDGS and 5% straw.

[3]70 WDGS/8% St: diet with 70% WDGS and 8% straw.

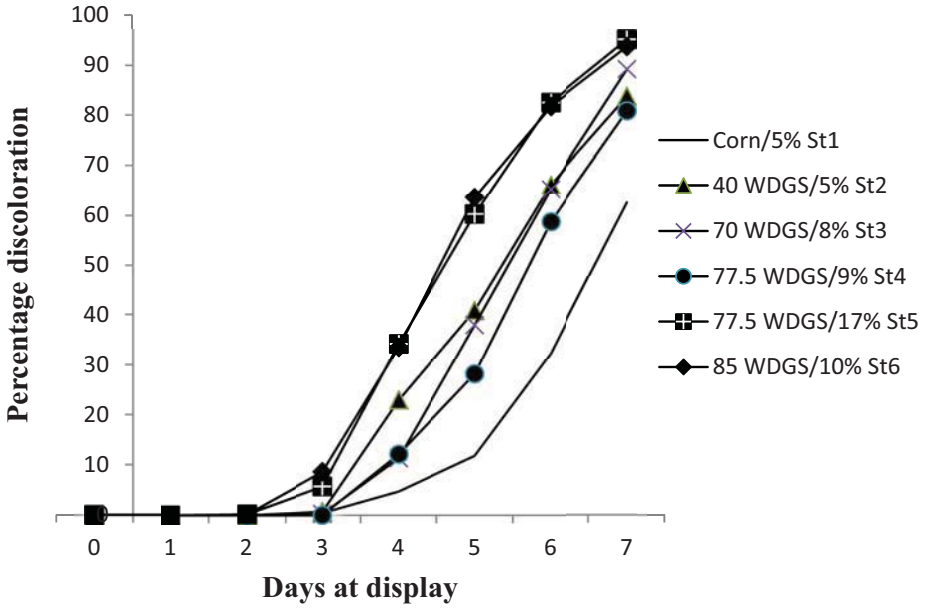
[4]77.5 WDGS/9% St: diet with 77.5% WDGS and 9% straw.

[5]77.5 WDGS/17% St: diet with 77.5% WDGS and 17% straw.

[6]85 WDGS/10% St: diet with 85% WDGS and 10% straw

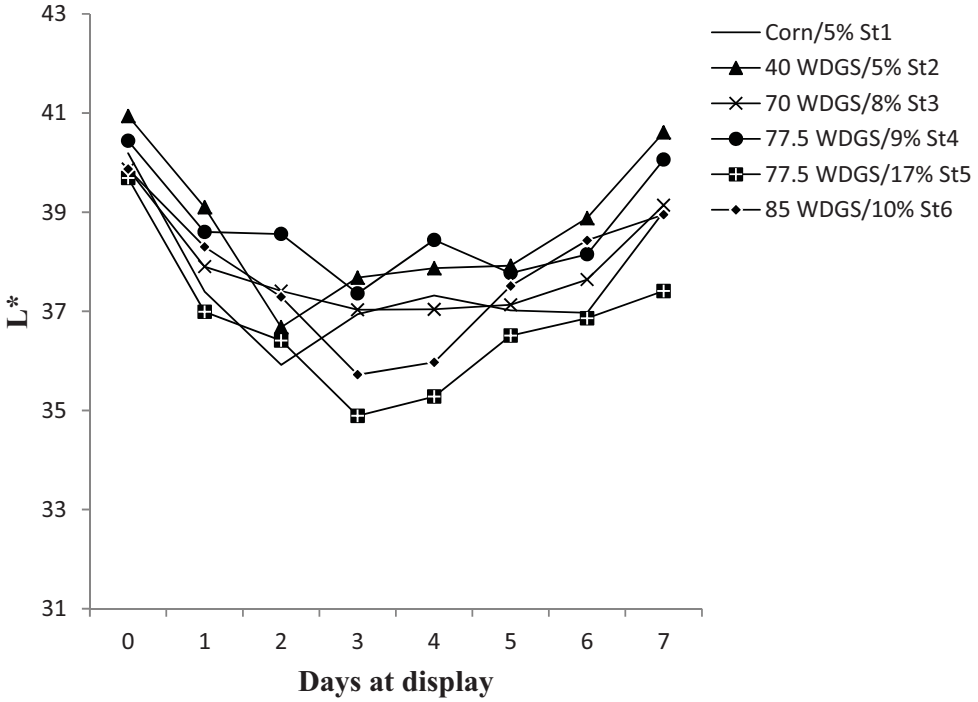
S.E.M.[7]= Standard Error of Mean.

Figure 1. Visual discoloration of strips steaks (M. *longissimus lumborum*) from cattle fed corn, high level WDGS, and straw.



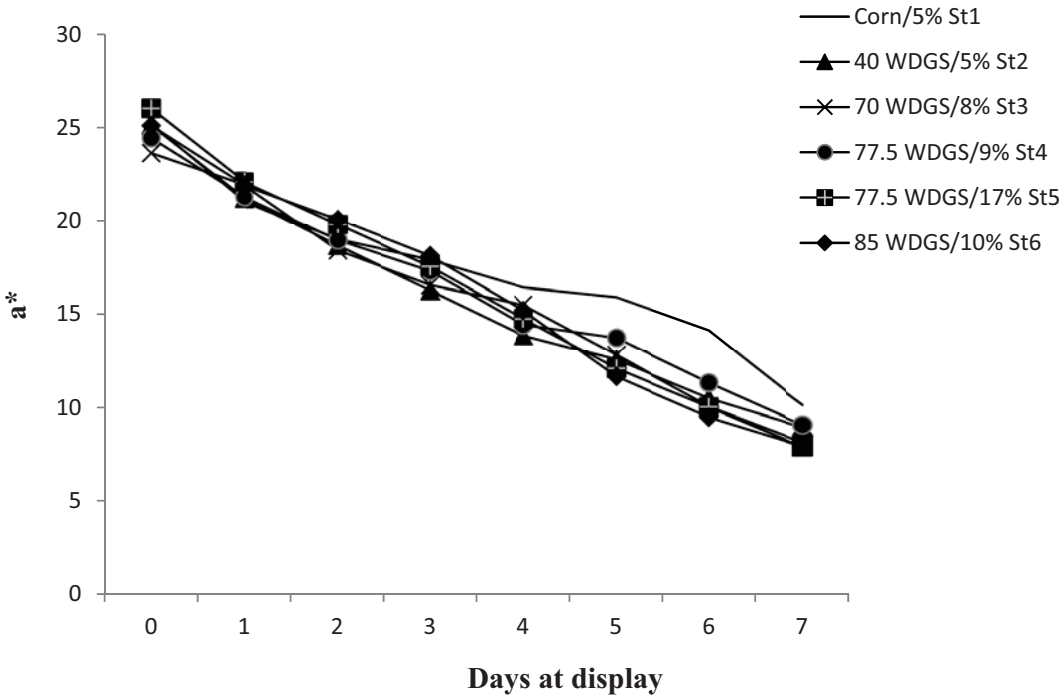
[1]Corn/5% St: corn diet and 5% straw.
[2]40 WDGS/5% St: diet with 40% WDGS and 5% straw.
[3]70 WDGS/8% St: diet with 70% WDGS and 8% straw.
[4]77.5 WDGS/9% St: diet with 77.5% WDGS and 9% straw.
[5]77.5 WDGS/17% St: diet with 77.5% WDGS and 17% straw.
[6]85 WDGS/10% St: diet with 85% WDGS and 10% straw.

Figure 2. L* values of strips steaks (M. *longissimus lumborum*) from cattle fed corn, high level WDGS, and straw.



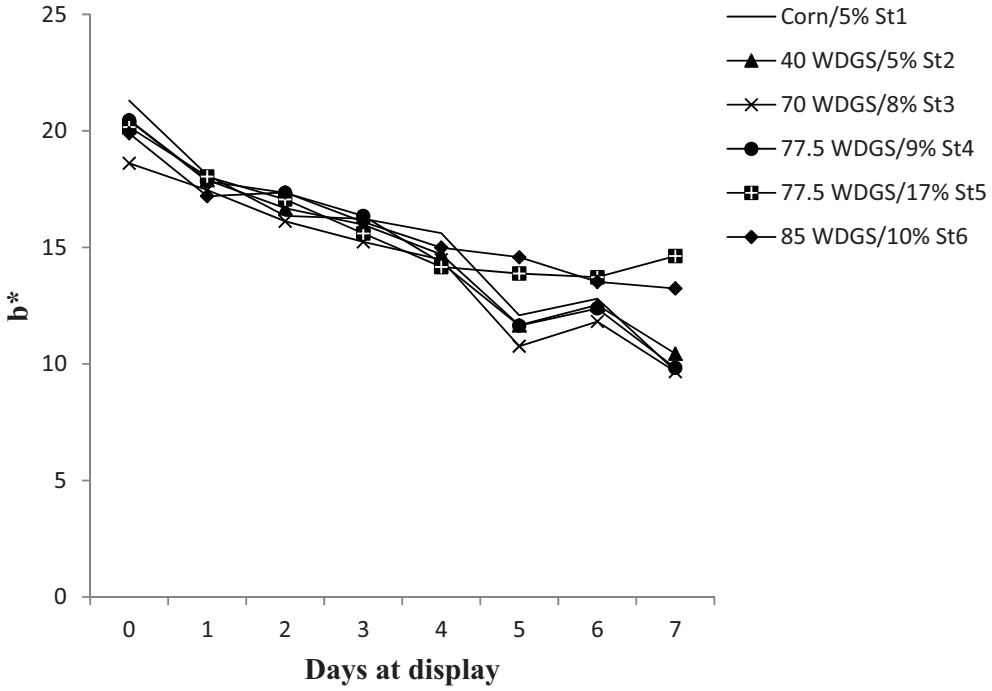
[1]Corn/5% St: corn diet and 5% straw.
[2]40 WDGS/5% St: diet with 40% WDGS and 5% straw.
[3]70 WDGS/8% St: diet with 70% WDGS and 8% straw.
[4]77.5 WDGS/9% St: diet with 77.5% WDGS and 9% straw.
[5]77.5 WDGS/17% St: diet with 77.5% WDGS and 17% straw.
[6]85 WDGS/10% St: diet with 85% WDGS and 10% straw.

Figure 3. a* values of strips steaks (M. *longissimus lumborum*) from cattle fed corn, high level WDGS, and straw.



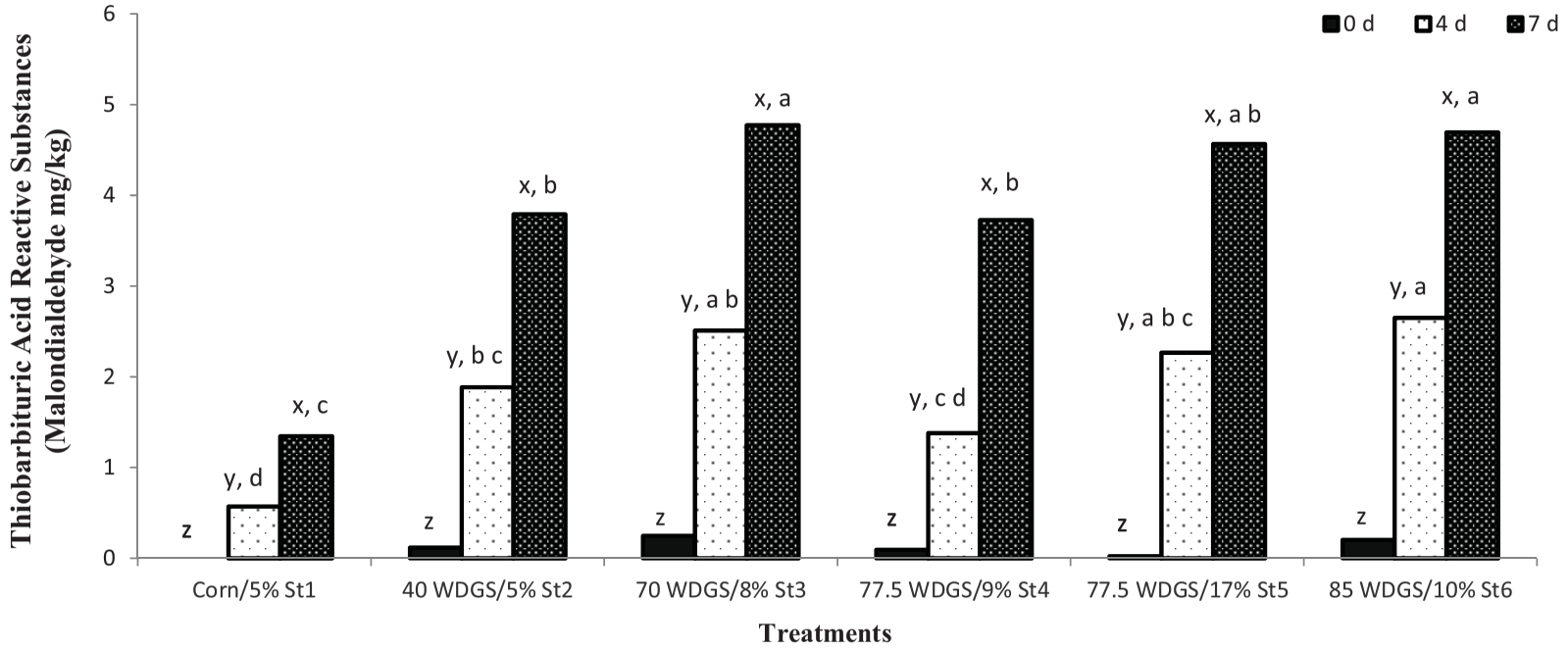
[1]Corn/5% St: corn diet and 5% straw.
[2]40 WDGS/5% St: diet with 40% WDGS and 5% straw.
[3]70 WDGS/8% St: diet with 70% WDGS and 8% straw.
[4]77.5 WDGS/9% St: diet with 77.5% WDGS and 9% straw.
[5]77.5 WDGS/17% St: diet with 77.5% WDGS and 17% straw.
[6]85 WDGS/10% St: diet with 85% WDGS and 10% straw.

Figure 4. b* values of strips steaks (M. *longissimus lumborum*) from cattle fed corn, high level WDGS, and straw.



[1]Corn/5% St: corn diet and 5% straw.
[2]40 WDGS/5% St: diet with 40% WDGS and 5% straw.
[3]70 WDGS/8% St: diet with 70% WDGS and 8% straw.
[4]77.5 WDGS/9% St: diet with 77.5% WDGS and 9% straw.
[5]77.5 WDGS/17% St: diet with 77.5% WDGS and 17% straw.
[6]85 WDGS/10% St: diet with 85% WDGS and 10% straw.

Figure 5. Thiobarbituric Acid Reactive Substances (malondialdehyde) present in the strip steaks (M. *longissimus lumborum*) from cattle fed high levels WDGS with straw.

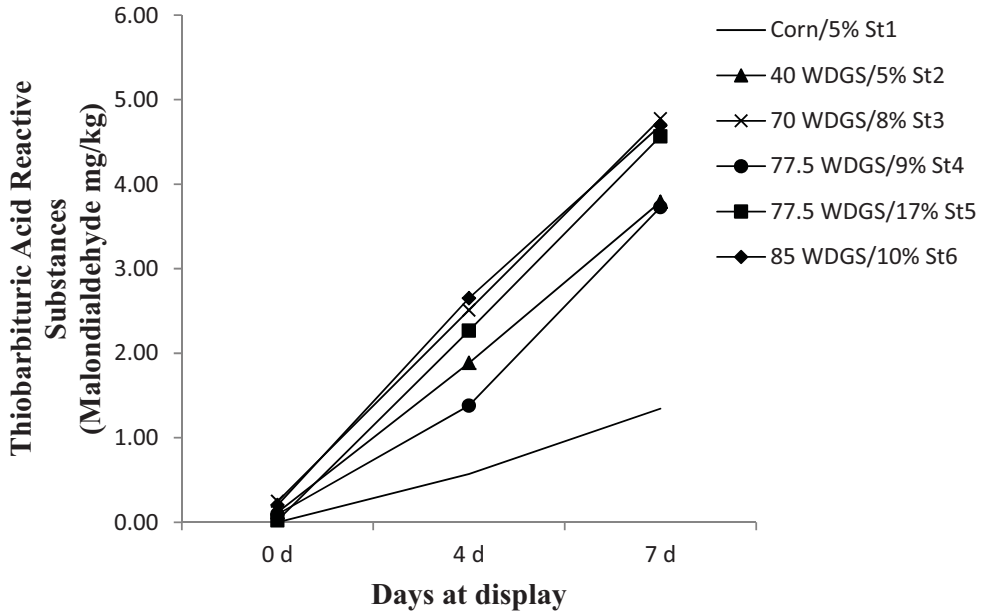


[a, b, c, d] Means within each retail display day with different letters are significantly different ($P \leq 0.05$).

[x, y, z] Means within each treatment having different letters significantly different ($P \leq 0.05$).

[1]Corn/5% St: corn diet and 5% straw.
[2]40 WDGS/5% St: diet with 40% WDGS and 5% straw.
[3]70 WDGS/8% St: diet with 70% WDGS and 8% straw.
[4]77.5 WDGS/9% St: diet with 77.5% WDGS and 9% straw.
[5]77.5 WDGS/17% St: diet with 77.5% WDGS and 17% straw.
[6]85 WDGS/10% St: diet with 85% WDGS and 10% straw.

Figure 6. Amount of malondialdehyde mg/kg from strips (M. *longissimus lumborum*) of cattle fed high level of WDGS with straw.



[1]Corn/5% St: corn diet and 5% straw.
[2]40 WDGS/5% St: diet with 40% WDGS and 5% straw.
[3]70 WDGS/8% St: diet with 70% WDGS and 8% straw.
[4]77.5 WDGS/9% St: diet with 77.5% WDGS and 9% straw.
[5]77.5 WDGS/17% St: diet with 77.5% WDGS and 17% straw.
[6]85 WDGS/10% St: diet with 85% WDGS and 10% straw.

Future recommendations

Distillers grains are an excellent source of energy and protein for feedlot cattle. In addition, distillers grains are cheaper than corn and have a higher energy value when compared to corn. Research has shown that distillers grains can enhance the feed efficiency where low quality roughage may be incorporated in the diet without altering responses in DMI, ADG, and G:F. Lower or equal to 40% DM is routinely fed as supplemental protein and high energy diet source to the cattle.

In this research, there is an increase in the level of PUFA, *trans* fatty acids, omega-3, omega-6, in the strips of cattle fed high levels of WDGS. Strips from cattle fed high levels of WDGS increases visual as well as instrumental discoloration in during retail display. Likewise, lipid oxidation was higher in strips from cattle fed high levels of WDGS. The increase in the amount of PUFA leads to rancidity and discoloration of strip steaks during retail display. There did not seem to be any changes to beef quality of added straw when high levels of WDGS were fed. Therefore, it would be interesting to investigate the effects of different levels of antioxidants applied on the steaks. It would be exciting to see whether different levels of applied antioxidants reduce the rancidity or lipid oxidation of steaks from cattle fed high levels of distillers grains in a retail display case. In addition, it would be interesting to investigate the sensory evaluation of steaks from cattle fed high levels of distillers grains that are applied with antioxidants. Trained panelists can be used to detect the flavor, tenderness, juiciness in the steaks. Likewise, Warner-Bratzler shear force can be done to observe the tenderness of steaks from cattle fed high levels of distillers grains. Finally, the research will help us to find out if there are any changes

occur in tenderness value or flavor profile of steaks from cattle fed high levels of distillers grain and treated with antioxidants during retail display.

Appendices

Appendix 1

Proximate Analysis

1. Place crucibles in drying over at 100°C for 4 h and then in the desiccator

2. Place 2 g of pulverized muscle tissue into a crucible

3. Moisture and ash are determined using the following program

Trait	Covers	Ramp Rate	Ramp Time	Ramp Temp	End Temp
Moisture	off	4°C/min	26 min	25°C	130°C
Ash	off	16°C/min	29 min	130°C	600°C

Trait	Atmosphere	Flow Rate	Hold Time	Constant Wt	Constant Time
Moisture	off	4°C/min	26 min	25°C	130°C
Ash	off	16°C/min	29 min	130°C	600°C

Equations:

Initial Wt W [Initial]

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

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

Fat Content

1. Weigh 2 g of pulverized muscle tissue on Whatman #2 filter paper

2. Fold and place in Soxhlet apparatus with ethyl ether drip for 48 h

3. % Fat = (Wet Weight-Dry Weight)/Wet Weight

Appendix 2

Fatty Acid Analysis

Folch et al. (1957). Morrison and Smith (1964) and Metcalfe et al. (1966)

1. Weigh out 1 g of pulverized muscle tissue. If extracting subcutaneous fat, weigh out 0.1 g of pulverized subcutaneous fat into centrifuge tube.
2. Add 5 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 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.
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. Add 0.5 mL of a 0.5 M NaOH in methanol. Vortex for 5 sec. Heat for 5 min at 100°C.
9. Add 0.5 mL of boron trifluoride in 14% methanol. Vortex for 5 sec. Heat for 5 min at 100°C.
10. Add 1 mL of a saturated salt solution and 1 mL of hexane. Vortex for 5 sec.

11. 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.

Appendix 3

Thiobarbituric Acid Reactive Substances 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 µl TEP (97%) bring volume to 100 mL ddH_2O.

Working Solution: Dilute stock solution to 1:3 (TEP Solution:ddH_2O) (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 ddH_2O.

Dissolve 2.88 g TBA in warm ddH_2O first, then add TCA (150 g) and ddH_2O to 1L.

BHA (Butylated Hydroxy Anisole) stock Solution:

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

10 g BHA dissolved in 90 mL ethanol (90%) + 5 mL ddH_2O.

Standards: In duplicate

		Moles of TEP
Blank:	1 mL ddH_2O	
Standard 5:	100 µl working TEP + 1.90 mL ddH_2O	(5×10^{-5} M)
Standard 4:	1 mL Std. 1 + 1 mL ddH_2O	(2.5×10^{-5} M)
Standard 3:	1 mL Std. 2 + 1 mL ddH_2O	(1.25×10^{-5} M)
Standard 2:	1 mL Std. 3 + 1 mL ddH_2O	(0.625×10^{-5} M)
Standard 1:	1 mL Std. 4 + 1 mL ddH_2O	(0.3125×10^{-5} M)
	Remove 1 mL of Standard 1 and discard it, leaving 1 mL behind.	

Procedure:

1. Mix all reagents and standards before beginning.

2. Transfer 5 g powdered sample into a 50 mL conical tube; add 14 mL of ddH_2O and 1.0 mL of BHA.

3. Homogenize for 15 sec with a polytron.

4. Centrifuge for 2000×g for 5 min.

5. Transfer 1 mL of homogenate or standard to 15 mL conical tube.

6. Add 2 mL of TBA/TCA solution, vortex.

7. Incubate in a 70°C water bath for 30 min to develop color.

8. Cool samples in a coldwater bath for 10 min.

9. Centrifuge tubes at 2000×g for 15 min.

10. Transfer duplicate aliquots of 200 µl from each tube into wells on a 96-well plate.

11. Read absorbance at 540 nm.

Calculations: mg 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)/5 mL

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

E = Sample equivalent (1) P = percentage recovery

Final calculation: $0.012 \times \text{concentration} \times (72.063 \times 10^6) = \text{mg of Malonaldehyde/kg of tissue}$

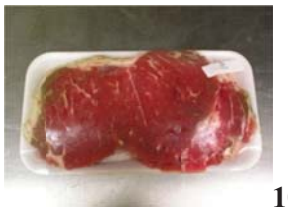
Reagents (Sigma): TBA- T5500; TCA – T9159; TEP – T9889; BHA – B1253

Appendix 4

Percentage discoloration chart


0%


5%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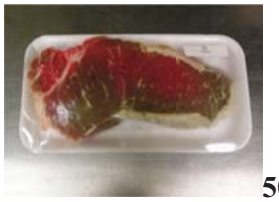
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Appendix 5

Diet nutrient analysis

	Dietary Treatments (% WDGS –DM basis)					
	Corn 5% Straw	**40 WDGS/5% Straw**	**70 WDGS/8% Straw**	**77.5 WDGS/9% Straw**	**77.5 WDGS/17% Straw**	**85 WDGS/10% Straw**
CP, %	13.30	18.24	24.74	26.36	26.01	27.98
Ca, %	0.70	0.70	0.84	0.90	0.88	0.95
P, %	0.28	0.53	0.70	0.75	0.73	0.79
K, %	0.65	0.65	0.91	0.97	0.95	1.04
EE, %	3.89	7.24	9.42	9.96	9.67	10.50
S, %	0.05	0.38	0.57	0.61	0.60	0.66
C:P	2.50	1.32	1.20	1.20	1.20	1.20

CP= Crude Protein; Ca= Calcium; P= Phosphorus; K= Potassium; EE= Ether Extract; S= Sulfur; C:P= Calcium to phosphorus ratio.

Appendix 6

Finishing diet composition

Ingredient	Corn/5% Straw	40 WDGS/5% Straw	70 WDGS/8% Straw	77.5 WDGS/9% Straw	77.5 WDGS/17% Straw	85 WDGS/10% Straw
WDGS	0.00	40.00	70.00	77.50	77.50	85.00
DRC	85.29	50.30	16.77	8.40	0.00	0.00
Straw	4.71	4.70	8.23	9.10	17.50	10.00
FGC	1.15	3.07	2.77	2.65	2.79	2.53
Limestone	1.71	1.69	1.99	2.11	1.97	2.23
Molasses	5.00	0.00	0.00	0.00	0.00	0.00
Urea	1.46	0.00	0.00	0.00	0.00	0.00
Salt	0.30	0.00	0.00	0.00	0.00	0.00
KCl	0.13	0.00	0.00	0.00	0.00	0.00
Tallow	0.13	0.13	0.13	0.13	0.13	0.13
Trace Mineral	0.05	0.05	0.05	0.05	0.05	0.05
Thiamin	0.02	0.02	0.02	0.02	0.02	0.02
Ionophore	0.02	0.02	0.02	0.02	0.02	0.02
Vitamin Premix	0.02	0.02	0.02	0.02	0.02	0.02
Anitbiotic	0.01	0.01	0.01	0.01	0.01	0.01
Total %	100.00	100.00	100.00	100.00	100.00	100.00

WDGS= Wet distillers grains with soluble; DRC= Dry rolled corn; Straw= Corn stalks; FGC= Fine ground corn; KCl= Potassium chloride; Ionophore= Rumensin; Antibiotic= Tylan.