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# Quality Effects on Beef from Cattle Fed High-Protein Corn Distillers Grains or Other Ethanol By-Products

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QUALITY EFFECTS ON BEEF FROM CATTLE FED HIGH-PROTEIN  
CORN DISTILLERS GRAINS OR OTHER ETHANOL BY-PRODUCTS

by

Kellen B. Hart

A THESIS

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QUALITY EFFECTS ON BEEF FROM CATTLE FED HIGH-PROTEIN  
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University of Nebraska, 2018

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The objective of this study was to evaluate the effects of feeding high protein corn distillers grains on fresh beef quality. Steers ( $n=300$ ) were fed one of five diets (6 pens with 10 head/pen): a corn control, 40% high protein dry distillers grains plus solubles (HP-DDGS), 40% dry distillers grains plus solubles (DDGS), 40% wet distillers grains plus solubles (WDGS), or 40% bran plus solubles (Bran). Eighteen Choice carcasses (3 head/pen) were randomly selected within each treatment ( $n=88$ ). Strip loins were aged for 2, 9, or 23 d, after which steaks were placed under retail display conditions for 0, 4, or 7 d. Dietary treatment had no effect on tenderness within each aging period ( $P > 0.05$ ). After 7 d of retail display, following all aging periods, steaks from cattle fed HP-DDGS had the greatest discoloration except for DDGS and Bran after 23 d ( $P < 0.05$ ). The steaks from cattle fed HP-DDGS also had lower ( $P < 0.05$ ) redness ( $a^*$ ) values than all other treatments following 7 d of retail display. After 7 d of retail display, steaks from cattle fed HP-DDGS had significantly greater lipid oxidation than all other treatments except WDGS ( $P < 0.01$ ). There was a trend ( $P = 0.07$ ) that steaks from cattle fed HP-DDGS had higher levels of polyunsaturated fatty acids and C18:2. These results suggest that feeding high protein

distillers grains has no detriment on tenderness, but may alter the lipid profile of the muscle resulting in decreased color stability, increased lipid oxidation and decreased shelf life.

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## INTRODUCTION

As the ethanol industry in America continues to grow, so does the amount of co-products created. When corn is converted to ethanol, only about 1/3 of the total input is transformed into ethanol (Saunders and Rosentrater, 2009). The other 2/3 are equally divided between carbon dioxide and distillers grains (Saunders and Rosentrater, 2009). Corn distillers grains are the unfermented grain residues that remain following the fermentation and distillation of ethanol from corn. In 2017, the ethanol industry in the United States generated 15.84 billion gallons of ethanol and a record 41.4 million metric tons of distillers grains, gluten feed and gluten meal (Renewable Fuels Association, 2018). Currently, Nebraska is the second largest ethanol producer with 26 operating ethanol plants in the state. These plants can produce a total of 2.23 billion gallons; 14 percent of the country's total production (Renewable Fuels Association, 2018).

Distillers grains have been increasingly used as a feedstuff in the production of cattle due to availability and the increasing prices of grains. However, distillers grains have been used as livestock feed since the 19<sup>th</sup> century (Klopfenstein et al., 2008). Dry distillers grains plus solubles (DDGS) have greater amounts of protein and fat compared to corn, which can lead to better cattle performance in the finishing stages prior to harvest (Klopfenstein et al., 2008). Distillers grains can be an affordable protein source for cattle producers, as well as profitable for the ethanol plants by increasing the value of the co-products. However, increased levels of DDGS in the diet can lead to negative attributes in meat quality (Mello et al., 2012).

As the ethanol industry evolves, the co-products can be altered. The advent of front-end fractionation allows diversification of the co-products created while increasing the

efficiency of the production of ethanol. Generally, the crude fat content of DDGS has been reported at 8-9% (Ganesan et al., 2009). Using the new technique of fractionation can decrease these fat levels to around 4% (Cereal Process Technologies, 2016). These lower fat distillers grains are often referred to as high protein distillers grains.

Therefore, the objective of this study was to better understand the implications of this new feedstuff (high protein dry distillers grains) in terms of meat quality. This information is vital to the Nebraska beef cattle industry in order to continue the production of high quality beef. More specifically, this study evaluated the effects of dietary treatments on 1) tenderness, 2) fatty acid profile of the muscle, and 3) impacts on retail display as it relates to discoloration and lipid oxidation.

## **REVIEW OF LITERATURE**

### **Use of corn ethanol by-products in beef production systems**

Since 1980, when there were 175 million gallons of ethanol produced, until today, there has been a steady increase in the total ethanol production across the U.S. Today there are 198 ethanol plants with a total capacity of producing 15.84 billion gallons (Renewable Fuels Association, 2018). Corn and sorghum are the two primary grains used when producing ethanol. Generally, the ethanol plants determine which type of grain they will use based on their geographical location (Depenbush et al., 2009a). Most ethanol plants are located in the Midwest; thus, they primarily utilize corn due to the large quantity of corn produced in this region (Raush and Belyea, 2006). According to the Renewable Fuels Association (2018), one bushel of corn weighing 56 pounds will yield 2.87 gallons of denatured fuel ethanol, 16.5 pounds of carbon dioxide, and 16.4 pounds of dried distillers grains at 10% moisture. It was estimated in 2007 that over 90% of cattle on feed in Nebraska were fed ethanol co-products as a portion of their diets (Waterbury et al., 2009). As the corn is fermented during the process of ethanol production the concentrations of protein, fat, and fiber increase by three times the original nutritional content of corn (Klopfenstein et al., 2008). The protein level in DDGS compared to corn increases from about 10 to 30%; thus, DDGS are usually used as a protein source in animal diets (Klopfenstein et al., 1978, 2008). Because distillers grains are cheaper than corn and have higher nutrient levels, they are a very efficient feedstuff for cattle producers (Klopfenstein et al., 2008). Distillers grains typically can be found in three different variations based on moisture level. Distance from the ethanol plant to the cattle producer plays a large role in the type of distillers grains that are fed. Dried distillers (DDGS; 10% moisture) and

modified distillers (MDGS; 50-55% moisture) are dried from wet distillers (WDGS; 65-70% moisture) which can increase the price to producers when purchasing these types of co-products. Advantages to DDGS, however, include increased storage life and easier transportation (Firkins et al. 1985), but drying can result in indigestible complexes with proteins and carbohydrates which can reduce energy levels (Depenbush et al., 2009a). Although MDGS have a higher feeding value than corn (111-125%), it appears that WDGS have a feed value of 121-178% that of corn, making it a more efficient feedstuff than corn (Watson et al., 2014). Paired with the lowest cost due to the absence of drying, WDGS may be the most cost effective distillers grains option (Klopfenstein et al., 2008) when feedlots are located near ethanol plants. Inclusion level of distillers grains can also play an important role in whether or not to utilize distillers grains. As the level of inclusion increases, so does the potential for cost savings compared to corn (Tonsor, 2006). Distillers grains at any moisture level included in the diet at 40% DM resulted in fewer days on feed and positive returns of up to \$40/head compared to a corn control diet (Buckner et al., 2011).

### **Types of corn milling and distillers grains produced**

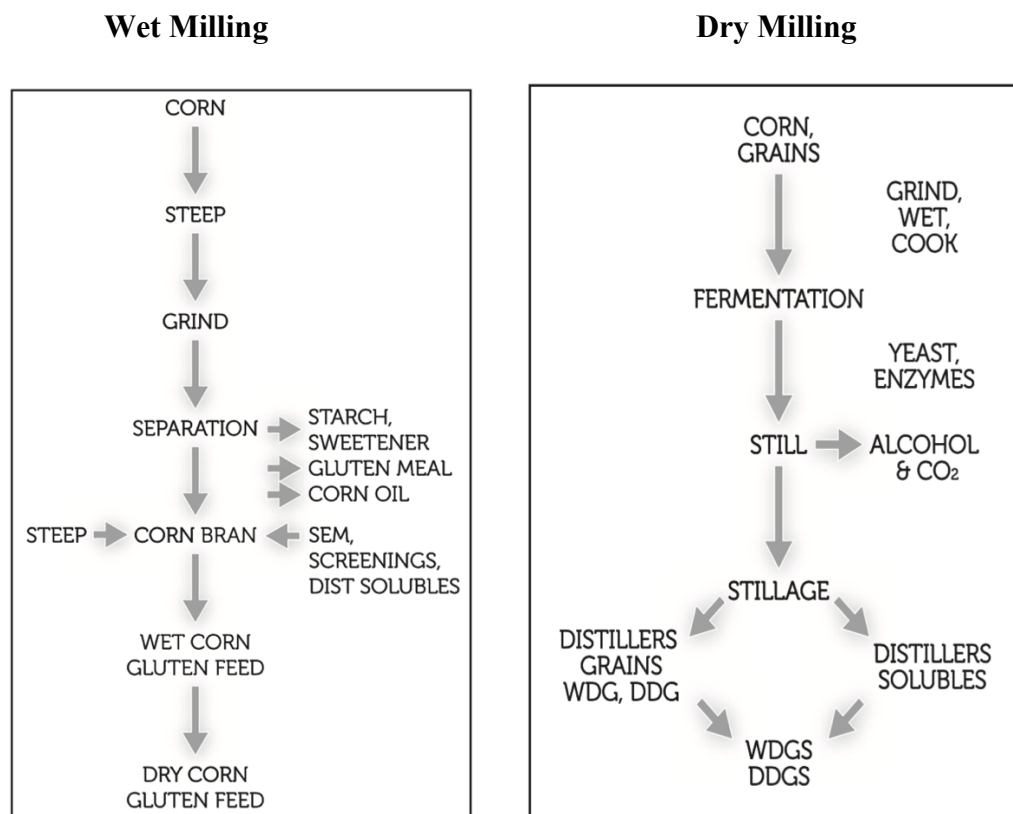
The type of milling that an ethanol plant employs will affect the co-product produced. The two primary pathways of corn ethanol production are wet milling and dry milling. However, ethanol producers are constantly looking for ways to improve yield and decrease cost by creating new co-products available for animal feed and human consumption (Singh et al., 2001; Winker- Moser and Breyer, 2011).

**Wet milling.** Only about 10% of all ethanol produced in the U.S. is created using the wet milling process (Renewable Fuels Association, 2018). The primary purpose of wet

milling is to isolate and recover starch to be fermented into a number of products such as germ meal, gluten meal and condensed fermented extractives (Rausch and Belyea, 2006). Number 1 or 2 corn is often used because many byproducts can be created that are suitable for human consumption (Stock et al., 2000). The first step in this process is the fractionation of the germ, fiber, and starch by steeping the kernels to solubilize the components around the germ. Using the natural differences in density and particle size the germ and fiber are then removed from the steepwater via screens and hydrocyclones. The steepwater can be partially evaporated to increase the protein concentration creating what is known as heavy steepwater, which contains 35-40% solids (Rausch and Belyea, 2006). The fiber can then be added back to the heavy steepwater and dried to form corn gluten feed (CGF). According to Rausch and Belyea (2006), 22-24% of all initial corn that enters the wet mill is converted into CGF. Approximately 70-75% of CGF produced in the U.S. is converted into pellets and shipped to Europe (Stock et al., 2000). The germ that is removed will subsequently be dried and pressed in order to produce corn oil. The only two fractions of corn in the slurry remaining will be protein and starch. The slurry will then be centrifuged and due to the lesser weight of protein than starch, the protein will be removed. This protein will be concentrated using a gluten thickener and dried, resulting in corn gluten meal (CGM) (Rausch and Belyea, 2006). Corn gluten meal accounts for about 5% of total corn input. Once the steepwater is fermented to form ethanol, the distillers solubles can be added to the previously removed bran resulting in wet corn gluten feed (WCGF). Wet corn gluten feed can be further dried, forming dry corn gluten feed (Erickson et al., 2010).

**Dry milling.** Today about 90% of grain ethanol produced in the U.S. comes from the dry milling process (Renewable Fuels Association, 2018). In the dry milling process,

the corn is initially ground to a diameter of about 1 mm (Kim et al., 2008). Water is added to the newly ground corn to form a slurry and heated to approximately 104°C. Alpha-amylase is added in order to break down the starch components (complex sugars) into dextrins in order to form mash. The mash temperature is held at 85°C and after a short period of time brought down to 32°C. Once cooled, glucoamylase is added to break down the dextrins into glucose and maltose, which allows for better fermentation of monosaccharides by the yeast that is added concurrently. The yeast ferments the starches and creates alcohol. The alcohol will be distilled off and what remains are the non-fermentable components of the grain, known as whole stillage. The remaining stillage will further be centrifuged to separate coarser grain particles, called wet grains, and the water and soluble solids fraction known as thin stillage. (Berger and Singh, 2010). Thin stillage can be evaporated into a syrup-like substance called condensed distillers solubles (CDS) which can be added back into the distillers grains to create distillers grains plus solubles (Stock et al., 2000).

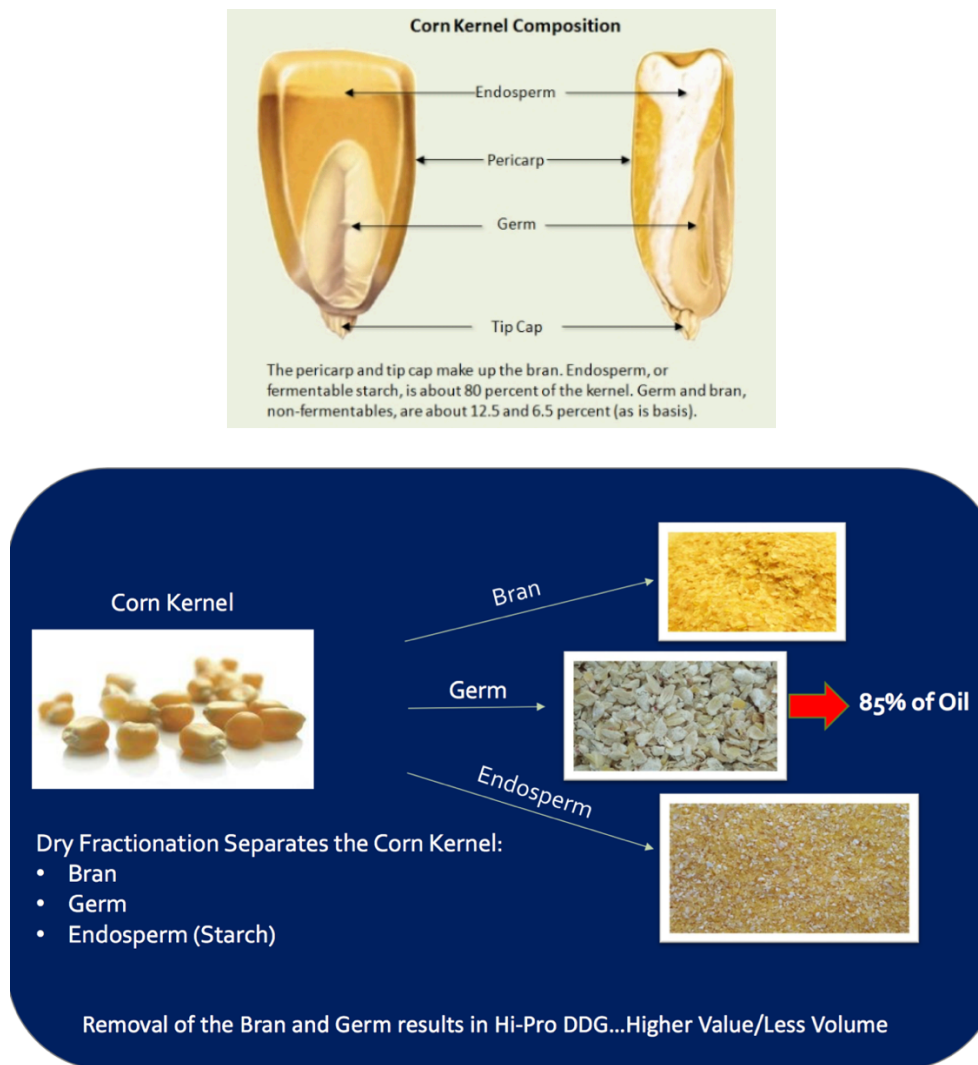


**Figure 1.** Schematic of the wet and dry milling process (Erickson et al., 2010)

**Dry fractionation.** Dry or front-end fractionation is the process of physically separating corn into its three fractions (endosperm, bran, and germ) prior to starting the ethanol production process. Germ, which makes up 12% of the kernel, is non-fermentable and contains 85% of the oil within corn. Removing the germ from the fermentation process allows ethanol producers to utilize this corn oil for other markets and creates more capacity for fermentation of available starches. Another 6% of the kernel is comprised of the bran, which is also a non-fermentable. Once the bran is removed, it can be combusted for energy due to its high levels of cellulose, hemicellulose, and lignin, fermented into cellulosic ethanol, or sold as a livestock feedstuff (Cereal Process Technologies, 2018). The removal of these two fractions increases the amount of starch available during the ethanol production



process (Ponnampalam et al., 2004). At this point, the endosperm is added into the same dry milling fermentation procedure as discussed in the previous section. Due to the removal of the non-fermentable bran and germ, there are lower costs associated with distillers grains production from dry fractionation as a result of less energy needed for drying and greater production of co-products (Rendleman and Shapouri, 2007).



**Figure 2.** Representation of corn kernel composition and dry fractionation separation (Cereal Process Technologies, 2016)

**Wet distillers grains plus solubles (WDGS).** Distillers grains can vary greatly depending on the amount of drying carried out by the ethanol processors. If the remaining

wet grains and solubles are combined with no drying steps the product is known as WDGS. Wet distillers grains plus solubles normally contain 65-70% moisture. Inclusion of WDGS in the diets of cattle has been widely shown to improve cattle performance at various inclusion rates. Watson et al. (2014) reported that WDGS inclusion in finishing diets can have a feeding value as high as 178% greater than corn. However, WDGS have been shown to have a relatively short shelf life of only 1-2 weeks, often resulting in utilization by feedlots that are within a 50 mile radius of the ethanol plant due to handling and storage concerns (Bothast and Schlicher, 2005).

**Dry distillers grains plus solubles (DDGS).** Production of DDGS follows the same process as WDGS, however, an additional drying step is included. Traditional DDGS are dried to about 10-12 % moisture, much less than the 65-70% found in WDGS. This additional drying has been shown to increase uniformity of the product as well as increase the shelf-life, allowing for further transportation to more distant cattle producers (Bothast and Schlicher, 2005). Although DDGS have a decreased level of F:G (ratio of feed eaten to weight gain) than that of WDGS, it is still a highly efficient feedstuff, with a feeding value of 112% greater than corn (Bremer et al., 2011). Due to the extensive drying that DDGS undergoes, additional costs are incurred by the plant which can become a cost deterrent for some cattle feeders. A recent report from the University of Nebraska, in which Nebraska feedlot owners were surveyed, indicated that no feedlots utilized DDGS, while use of WDGS and modified wet distillers grains levels doubled from 2010 to 2015 (Birch and Brooks, 2015).

**Bran plus solubles.** As the ethanol industry looks to improve their efficiency to increase ethanol yield and reduce cost, new methodology creates more diversity in the

market for animal feed co-products (Singh et al., 2001; Winker-Moser and Breyer, 2011). With the advent of dry fractionation and removal of the three corn fractions prior to fermentation, the new fiber stream can be utilized to feed livestock. The bran removed from the kernel is combined with condensed distillers solubles, which are removed after the stillage is centrifuged from the wet grains, resulting in a product known as bran plus solubles (Stock et al., 2000).

Bran plus solubles are similar in moisture level to that of WDGS at around 60%. Fat levels have been reported at around 10%, similar to DDGS and WDGS, while CP and NDF levels are also similar (Garland et al., 2019a). According to Garland et al. (2019a), cattle fed bran plus solubles increased ADG when compared to those fed WDGS and a corn control diet, but there was no difference when compared to DDGS. Similarly, cattle that were supplemented with bran plus solubles had improved F:G compared to those supplemented WDGS and an all-corn diet; there was a trend that bran plus solubles had a greater feeding value than DDGS. Bran plus solubles-fed cattle and DDGS-fed cattle had similar HCW and exhibited the greatest HCW values compared to all other treatments. Overall, bran plus solubles were reported to have a feeding value of 126% that of corn, making it a very efficient option when feeding to cattle in finishing diets, and could be a viable replacement for traditional distillers grains when the market is favorable.

**High protein dried distillers grains plus solubles.** After dry fractionation and the resulting endosperm is fermented and dried, what remains is referred to as high protein dried distillers grains. Due to the removal of the non-fermentable bran and germ, it is believed that the resulting higher crude protein levels are due to more efficient fermentation (Robinson et al., 2008). On a nutritional basis, it has been shown that distillers grains from

fractionated systems have higher levels of crude protein and are lower in fat content than traditional fermentation systems (U.S. Grains Council, 2012). Fat levels in traditional DDGS have been shown to average around 8-9 % on a dry matter basis, but using front-end fractionation will drop fat levels to around 4% (Ganesan et al., 2009; U.S. Grains Council, 2012). This can be attributed to the removal of the corn germ which contains up to 85% of the corn oil in a kernel (Cereal Process Technologies, 2016). However, Garland et al. (2019a) reported increased levels of dietary fat at 9.4% on a dry matter basis in high protein distillers grains.

When it comes to cattle performance, cattle fed high protein distillers grains were shown to have a greater ADG when compared to those fed WDGS and a corn control diet as well as having an increased HCW. Cattle fed high protein distillers grains were also reported as having a greater F:G than cattle fed corn or WDGS with no detriment to LMA, marbling scores, or fat depth. The overall feeding value of high protein distillers grains in this experiment was found to be 121% of corn making it a practical substitute for DDGS (Garland et al., 2019a). However, due to the large amount of capital investment that is needed to either build a new plant or refurbish an existing plant, almost all front-end fractionation in the United States has been discontinued (U.S. Grains Council, 2012).

Nutrient <sup>1</sup>	DDGS	HP-DDGS	WDGS	MDGS	Bran
DM, %	91.4	91.8	32.8	67.3	91.4
Fat, %	6.2	9.4	11.6	7.1	6.2
CP, %	32.5	36.0	30.1	16.4	32.5
NDF, %	31.6	32.0	30.2	22.0	31.6

<sup>1</sup> Nutrients expressed on a dry-matter basis

**Table 1.** Diet composition (DM basis) fed to finishing steers receiving either dried distillers grains plus solubles (DDGS), high protein dried distillers grains plus solubles (HP-DDGS), wet distillers grains plus solubles (WDGS) or corn bran plus solubles (Bran) (Garland et al., 2019a; Burhoop et al., 2018)

**Ethanol by-product inclusion limits.** Much of the distillers grains fed to finishing cattle are wet due to their higher feeding value compared to DDGS, 178% and 112%, respectively (Watson et al., 2014; Bremer et al., 2011). However, regardless of which distillers grains are used, there are a variety of factors that limit the amount that can be included in cattle diets. Primarily, since distillers grains can contain upwards of 1% of sulfur, polioencephalomalacia can occur. It is important to manage this by increasing the percentage of forage in the diet (Drewnoski et al. 2014).

Multiple studies have shown that increasing inclusion levels of WDGS from 0 – 50% in finishing cattle diets quadratically increases final BW (Larson et al., 1993; Ham et al., 1994; Leubbe et al., 2012; Watson et al., 2014). Watson et al. (2014) found that cattle performance improved in terms of ADG and F:G as inclusions of WDGS increased up to 40%, but slightly decreased at 50% inclusion rates. These results are consistent with

Trenkle et al. (2008) which described that a supplementation rate of 60% in diets for both steers and heifers decreased cattle performance.

Dried distillers grains plus solubles were reported to provide a quadratic increase in ADG as inclusion levels increased in finishing diets of cattle, as well as a cubic response in G:F (Klopfenstein et al., 2008). Ham et al. (1994) compared the use of DDGS at 40% inclusion compared to a corn control diet and observed that feeding DDGS resulted in a 24% greater feeding value than that of corn. Feeding value has also been proven to decrease as inclusion levels increase from 20% to 40% (Klopfenstein et al., 2008).

Although there is little literature surrounding the effects of high protein distillers grains at different inclusion levels on performance, Garland et al. (2019b) reported that inclusion of high protein distillers grains at 40% decreased dry matter digestibility and organic matter digestibility compared to those found at 20% inclusion levels. However, it was noted that energy intake was higher for cattle supplemented with 40% high protein distillers grains, which leads to increased cattle performance. In conclusion, data suggest distillers grains, regardless of the form, are optimal in finishing diets at levels between 20 - 50%.

### **Effect of feeding corn distillers grains on meat quality**

While the performance and economic benefits of distillers grains are the ultimate driving forces for their utilization as an alternative feed source to corn for cattle producers, it is important to understand the effects that this ethanol co-product has on aspects of meat quality. Detrimental effects on meat quality could cause decreased acceptability and potentially create consumer distrust in the beef industry. It is imperative to understand the

effects that certain feedstuffs can have on qualities such as color, tenderness, and fatty acid composition in order to better evaluate the efficacy of a new feed ingredient.

**Color.** The inclusion of distillers grains in cattle diets has been widely reported to have detrimental effects on meat color and stability (Roeber et al., 2005; Depenbusch et al., 2009b; Mello et al., 2012; Chao 2015; Ribeiro et al., 2018). A study conducted by Roeber et al. (2005) indicated that formulating cattle diets with high levels of distillers grains (40 – 50%) could have negative effects on meat color, but levels lower than 25% showed no detriment. However, Mello et al. (2012) indicated that strip loins from cattle supplemented with only 15% WDGS showed an increase in discoloration after 7 days of retail display compared to cattle fed a corn control diet.

Depenbusch et al. (2009b) reported that increasing levels of DDGS in heifer diets from 0 – 75% resulted in a linear decrease in lightness (decreased  $L^*$  values) at day 0 of retail display after 16 d of aging. Redness ( $a^*$ ) values after 7 days of retail display showed a linear decrease as increasing levels of DDGS were fed (Depenbusch et al., 2009b). These results are similar to the findings of a feeding trial that utilized 0% or 35% WDGS supplementation paired with steam-flaked corn or dry-rolled corn, where supplementation of WDGS increased darkness (lower  $L^*$  values) regardless of corn processing method after 2 d of aging and 1 d of retail display and were also less red after 7 d of retail display (Buttrey et al. 2013). Segers et al. (2011) evaluated the utilization of DDGS compared to soybean meal and corn gluten feed (CGF) and observed a decrease in redness (lower  $a^*$  values) in steaks from cattle fed DDGS versus those fed soybean meal after 7 d of aging and 9 d of retail display.

Although there are many reports documenting the detrimental effects on color caused by feeding distillers grains, there are a few studies contradicting these claims. Gill et al. (2008) concluded that there was no difference in discoloration from steaks from cattle supplemented either 15% DDGS or 15% WDGS compared to steaks from cattle fed steam-flaked corn after 11 d of aging and 7 d of retail display. A study conducted at the University of Nebraska-Lincoln compared WDGS fed at either 0%, 35%, 50% or 65%, either full-fat or de-oiled, and found that even after 7 days of retail display there were no differences among treatment in regards to discoloration of strip loins after 7 and 21 d of aging. (Domenech-Perez et. al., 2017). Although Gill et al. (2008) found no differences in discoloration across treatments, it was reported that steaks from cattle fed either corn or sorghum distillers grains had decreased  $a^*$  (redness) values than steaks from cattle fed steam flaked corn. Although there is undoubtedly variation among the scientific literature, it seems that the inclusion of corn distillers grains in cattle feed overall does have detrimental effects on meat color.

**Tenderness.** The effects of distillers grains on tenderness have been widely studied in the scientific literature. Numerous studies conclude that inclusion of distillers grains has no effect on meat tenderness (Roeber et al., 2005; Gill et al., 2008; Koger et al., 2010; Segers et al., 2011; Mello et al., 2012). Alternatively, Depenbusch et al. (2009b) utilized a sensory panel to conclude that as distillers grains inclusion increased from 0% to 75%, there was a linear increase in tenderness. This was hypothesized to have a potential connection with a linear decrease in the amount of connective tissue present as the percentage of included distillers grain increased (Depenbusch et al, 2009b). These results are very similar to those found in a study conducted by Gordon et al. (2002) where a



sensory panel found a linear increase in myofibrillar tenderness and overall tenderness on rib steaks as DDGS increased from 0% to 75% inclusion.

Recent work at the University of Nebraska-Lincoln has also identified an improvement in tenderness when DDGS or WDGS are supplemented (Senaratne, 2012; Chao, 2015; Kunze et al., 2016). Senaratne (2012) initially indicated that meat from cattle fed WDGS, without supplement of vitamin E, had increased tenderness as well as greater troponin-T degradation than steaks from cattle fed a corn control diet. These results led to the hypothesis that muscle membrane integrity could be altered and compromised as a result of feeding WDGS which could result in earlier postmortem calcium release and cause improved tenderness due to earlier activation of calpains. A follow up study to this was conducted and results indicated an increase in PUFA's in the sarcoplasmic reticulum which could potentially explain the increased tenderness (Chao, 2015). Kunze et al. (2016) further looked into this phenomenon and were able to isolate mitochondria in order to model calcium flux and its role in improved tenderness. This study showed that mitochondria from strip loins from cattle fed distillers grains had higher C18:2 levels, as well as total PUFA. Mitochondria from cattle fed corn tended ( $P = 0.08$ ) to retain more calcium than mitochondria from cattle that were finished on distillers grains, thus, indicating that greater PUFA content in the organelles may alter calcium flux due to increased susceptibility to oxidation resulting in increased tenderness (Kunze et al., 2016).

**Sensory evaluation.** Sensory analysis has shown feeding distillers grains has minimal effects on tenderness, juiciness, overall acceptability, and off flavors (Roeber et al., 2005; Gill et al., 2008; Buttrey et al., 2013; Mello et al., 2012). Some research has alternatively shown that distillers grains could potentially improve tenderness and juiciness

(Gordon et al., 2002; Leupp et al., 2009). Depenbusch et al. (2009b) suggested that these potential improvements in tenderness could be related to a trending linear decrease in amount of connective tissue present as amount of distillers grain inclusion increased. It was also noted that steaks from heifers supplemented with 45% and 60% DDGS had significantly more beef flavor intensity than steaks from cattle not fed distillers grains (Depenbusch et al., 2009b). Similar results were found by Leupp et al. (2009) in which steaks from cattle with 30% inclusions DDGS in their finishing stage tended to be more flavorful than steaks from corn control fed steers. Overall, the utilization of distillers grains as a supplement within cattle finishing diets seems to have minimal to no detriment on sensory attributes and consumer acceptability.

**Nutritional composition.** Few have studied the effects of distillers grains on nutritional composition. There are a few reports that distillers grains in several forms do not alter proximate composition (Mello et al., 2012; Domenech-Perez et al., 2017; Ribeiro et al., 2018). Segers et al. (2011) found that steaks from steers fed DDGS tended to have higher moisture content than steaks from steers fed CGF with no change in protein and fat content across the different diets. Although this study showed differences in moisture content, the general consensus appears to be that the use of distillers grains most likely does not change the nutritional composition of meat.

**Fatty acid profile.** The fatty acid composition of meat from ruminant animals is largely controlled by the amount of fat present in the diet (Jenkins, 1993; Houben et al., 2000; Buttrey et al., 2013). Feeding WDGS has been shown to alter ruminal pH compared to corn fed cattle which can change the acetate:propionate ratio, allowing for greater fat digestibility, and increasing the amount of unsaturated fatty acids that reach the duodenum

(Vander Pol et al., 2009). Zinn et al. (2000) emphasized the formation of micelles in the rumen due to the presence of unsaturated fatty acids. As unsaturated fatty acids are increasingly more available, bile salt micelles will cover more surface area, causing a decrease in ruminal biohydrogenation, and allow for more efficient digestibility and absorption of fats (Zinn et al., 2000). Some PUFA's have been shown to bypass the rumen and travel directly into the small intestine and subsequently into the bloodstream to be deposited into the muscle (Enser et al., 1996; Scollan et al., 2006). Vander Pol et al. (2009) found that diets containing WDGS had the least amount of C18:0 that reached the duodenum, as well as the greatest proportion of C18:1 *trans*, C18:1, and C18:2, compared to cattle fed either corn bran and corn gluten meal, with or without added corn oil, and dry rolled corn, with or without a corn oil supplementation. These results suggest that fatty acids in WDGS are not hydrogenated in the rumen to the same extent as other feedstuffs. A similar conclusion was found by Atkinson et al. (2006) who concluded that feeding diets higher in polyunsaturated fats can decrease ruminal biohydrogenation leading to more unsaturated fatty acids present in tissue. Buttrey et al. (2013) found that steers fed a diet containing 35% WDGS had reduced levels of C18:1 *cis*-9 and C16:1 *cis*-9, while there was a tendency that total fat content of the meat increased. Mello et al. (2012) found that steaks from cattle where 30% WDGS was used in a finishing diet had a lower proportion of C18:1(n-7) than steaks from cattle fed 0% or 15% WDGS. Polyunsaturated fatty acids, *trans* C18:2(n-6), and total n-6 fatty acids all increased linearly as inclusion of WDGS increased from 0% to 30% (Mello et al., 2012). Domenech-Perez et al. (2017) echoed these findings by reporting that meat from cattle fed either full-fat WDGS or 65% de-oiled WDGS had the highest levels of PUFA when compared to a corn control diet. Another

study conducted at the University of Nebraska-Lincoln indicated that the same response was seen for cattle fed MDGS where greater levels of linoleic acid (C18:2) and PUFA were found compared to steaks from cattle fed corn only diets (Ribeiro et al., 2018).

**Lipid oxidation.** Oxidation is a major non-microbial factor responsible for the deterioration of muscle quality (Descalzo et al., 2005). Lipid oxidation generally follows three steps: initiation, propagation, and termination (Kanner, 1994; Morrissey et al., 1998). The initiation step occurs with the removal of a hydrogen from a methyl carbon within a fatty acid to form a carbon-centered alkyl radical in the presence of an initiator, likely superoxide anion radicals ( $O_2^-$ ), perhydroxyl radicals ( $HO_2$ ), hydrogen peroxides ( $H_2O_2$ ), or hydroxyl radicals ( $HO$ ) (Kanner, 1994; Morrissey et al., 1998; Wong, 2018). As the number of double bonds within a fatty acid chain increase, the easier it is for them to be oxidized into lipid peroxides (Halliwell and Chirico, 1993). Animals have a built in mechanism, however, to counteract lipid oxidation by converting the free radicals to water by utilizing natural enzymes like superoxide dismutase, catalase, and glutathione peroxidase (Morrissey et al., 1998). However, immediately after death, biochemical changes in the animal alter the balance between prooxidative factors and the antioxidative capacity allowing for more rapid oxidation (Morrissey et al., 1998). The alkyl radical will then rapidly react with oxygen and create a peroxy radical (Wong, 2018). Propagation occurs when a hydrogen atom is transferred from a lipid to the chain carrying peroxy radical, which results in the formation of a hydroperoxide and another free radical (Wong, 2018). The final step, termination, takes place with the combination of peroxy radicals or alkyl radicals to form non-reactive products (Wong, 2018). The following figure displays the three phases of lipid oxidation (Fernandez et al., 1997).

1. Initiation:
  - a.  $RH + O_2 \rightarrow R^\bullet + \bullet OOH$
2. Propagation:
  - b.  $R^\bullet + O_2 \rightarrow ROO^\bullet$
  - c.  $RH + ROO^\bullet \rightarrow ROOH + R^\bullet$
  - d.  $ROOH \rightarrow RO^\bullet + \bullet OH$
3. Termination:
  - e.  $R^\bullet + R^\bullet \rightarrow R-R$
  - f.  $R^\bullet + ROO^\bullet \rightarrow ROOR$
  - g.  $ROO^\bullet + ROO^\bullet \rightarrow ROOR + O_2$

**Figure 3.** Steps and reactions involved in lipid oxidation (Adapted from Fernández et al., 1997)

**Lipid oxidation's impact on meat color.** The bright red cherry color of fresh meat is one of the largest indicators of freshness to consumers and can drive consumer purchases (Hood and Riordan, 1973; Gatellier et al., 2001). Reports indicate that 20% discoloration of meat leads to a 50% reduction of sales in a retail display case (Hood and Riordan, 1973). Hood and Riordan (1973) stated that the color of fresh meat depends on the relative amounts of three states of myoglobin at the surface: reduced myoglobin, oxymyoglobin, and metmyoglobin. The reduction of oxymyoglobin to metmyoglobin has been strongly linked to lipid oxidation (Faustman et al., 2010). As myoglobin is oxidized it generates a superoxide anion which rapidly dismutates to hydrogen peroxide, increasing further oxidation of myoglobin and unsaturated fatty acids (Gotoh and Shikama, 1976; Faustman et al., 2010). Zakrys et al. (2008) concluded that changes in oxymyoglobin and  $a^*$  values appeared to be instigated by lipid oxidation with a strong correlation to TBARS results. Meat from animals fed higher proportions of PUFA's has been shown to be more

susceptible to lipid oxidation and subsequently increased levels of discoloration (Nute et al., 2007). This can be linked to the interaction of highly reactive primary and secondary products formed by lipid oxidation, such as alkyls and peroxide radicals, and myoglobin to enhance myoglobin oxidation (Faustman et al., 2010). Grimsrud et al. (2008) indicated the incubation of specific products of lipid oxidation resulted in an increased metmyoglobin formation. Multiple studies from the University of Nebraska-Lincoln have demonstrated that the supplementation of distillers grains during cattle finishing diets, regardless of the form, can have a negative effect on lipid oxidation as well as decreased color stability during retail display (Mello et al., 2012; Ribeiro et al. 2018).

## **Conclusion**

Corn distillers grains are being increasingly utilized as a high energy feedstuff in cattle finishing diets. As the ethanol industry continues to grow and look for new ways to maximize production and economic benefits, they are continually producing co-products such as high protein distillers grains. It has been well documented that traditional DDGS or WDGS have a significantly higher feed efficiency than that of corn control diets, but this increased performance comes at a cost of greater levels of PUFA and decreased shelf life as a result of greater lipid oxidation. However, it is unclear if feeding high protein distillers grains will result in the same alterations of fatty acid profile and shelf life. Therefore, understanding the impacts on beef from feeding high protein distillers grains is invaluable for the production of high quality beef from Nebraska. More specifically, this study aims to evaluate the effects of feeding high protein distillers grains on 1) tenderness, 2) fatty acid profile of the muscle, and 3) impacts of retail display as it relates to discoloration and lipid oxidation on beef.

## **MATERIALS AND METHODS**

### **Quality Effects on Beef from Cattle Fed High-Protein Corn Distillers Grains and Other Ethanol By-Products**

#### **Cattle and dietary treatments**

A total of 300 cross-bred calf-fed steers (initial BW =  $282 \pm 10$  kg) were fed (University of Nebraska feedlot at Mead, NE) for 190 days on either a corn control diet (Control), or a diet containing 40% high protein dried distillers grains plus solubles (HP-DDGS), 40% dried distillers grains plus solubles (DDGS), 40% wet distillers grains plus solubles (WDGS), or 40% bran plus solubles (Bran). Inclusion rate of distillers grains were calculated on a dry matter basis. Steers were fed 50% Sweet Bran (Cargill, Blair, NE) and 50% alfalfa hay at two percent of BW for 5 days to identify initial BW for the trial. Steers were blocked and stratified by BW prior to being randomly assigned to a pen. Pens were randomly assigned to one of 5 treatments with 10 steers/pen and 6 pens/treatment resulting in a total of 30 pens. High protein dried distillers grains plus solubles and Bran were sourced from the same ethanol plant (Corn Plus, Winnebago, MN). Traditional WDGS and DDGS were sourced from E Energy (Adams, NE) and KAAPA Ethanol (Ravenna, NE), respectively. All diets contained 15% corn silage and 5% supplement containing 30g/ton Rumensin® (Elanco Animal Health, Greenfield, IN) and 8.8 g/ton Tylan® (Elanco Animal Health, Greenfield, IN). On d 1, steers were implanted with Revalor XS® (Merck Animal Health, DeSoto, KS). All dietary treatments are presented in Appendix I.

### **Sample collection**

All steers were transported June 27, 2017 to Greater Omaha Packing (Omaha, NE) and held 12 h prior to slaughter. Prior to grading, carcasses were chilled 48 h and tagged with a university number that corresponded to the pen in which cattle were fed. Once carcasses were USDA graded, three low Choice carcasses per pen were selected for a total of eighteen low Choice carcasses within each treatment ( $n = 90$ ). During carcass selection, two carcasses were incorrectly removed resulting in a final count of 88 carcasses. The two carcasses lost included one from the Bran treatment and one WDGS treatment animal fed in different pens. From the selected carcasses, strip loins (*Longissimus lumborum*; IMPS # 180, NAMP, 2007) from the right side of the carcass were marked using food grade carcass crayons (Industrial markers Dixon No. 1530R Joseph Dixon Crucible Co., New Jersey, N. J.) and a plastic carcass push pin (Beef brads, KMC-KK1075-064, Butchers and Packers supplies, Edmonton, AB) with a UNL meat lab number ranging from 1 to 88. Identified loins were vacuum packaged with corresponding laminated ID tags and transported to the Loeffel Meat Laboratory at the University of Nebraska-Lincoln.

### **Fabrication**

Immediately after arrival at the Loeffel Meat Lab 2 d postmortem, strip loins from the right side were divided into three equal sections and randomly assigned to one of the three aging periods (2, 9, or 23 d). Three 2.54 cm steaks were fabricated at each aging period (one steak used to measure Warner-Bratzler shear force for 0 d of retail display, one steak used to measure Warner-Bratzler shear force, visual discoloration, and objective color for 7 d of RD, and one steak was cut in half and trimmed of all subcutaneous fat for



lipid oxidation for 0 d of RD and all other laboratory analysis). One 1.27 cm steak was cut in half, trimmed of all subcutaneous fat, and utilized to measure lipid oxidation after 4 and 7 d of retail display. At d 2, steaks for laboratory analysis, lipid oxidation steaks for 0 d of RD, and all remaining sections of loins were vacuum packaged (MULTIVAC 500, Multivac, Inc., Kansas City, MO) in Prime Source Vacuum pouches (3 mil STD barrier, Prime Sources, St. Louis, MO). Steaks for laboratory analysis and lipid oxidation were frozen for further analysis ( $-80^{\circ}\text{C}$ ). The remaining portions of loins were aged ( $2^{\circ}\text{C}$ ) under dark storage. All steaks were separated from the loin from the anterior end of the loin. The same fabrication map (Appendix II) was used for all aging periods. At all aging periods, samples for tenderness, color, and lipid oxidation analysis were placed on foam trays (21.6 x 15.9 x 2.1 cm, Styro-Tech, Denver, CO) and overwrapped with an oxygen permeable film (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO). Trays were placed under simulated RD conditions for 4 and 7 d ( $3^{\circ}\text{C}$  under white fluorescence lighting at 1000 to 1800 lux) and randomly rotated daily. All frozen steaks utilized for laboratory analysis and lipid oxidation were tempered enough to finely dice, frozen in liquid nitrogen and then powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT) on August 24/25 and September 12/14, 2017 and held for 3 d and 14 d, respectively, at  $-80^{\circ}\text{C}$  until further analysis.

### **Proximate composition**

Moisture, fat, and ash (%) of raw meat samples were determined. Moisture and ash (%) were quantified with a LECO Thermogravimetric Analyzer in duplicate (Model 604-100-400, LECO Corporation, St. Joseph, MI). Total fat (%) was determined in triplicate by

ether extraction according to the Soxhlet procedure (AOAC, 1990)(See Appendix IV). Protein was determined by difference.

### **Tenderness determination**

Steaks (2.54 cm) were measured for tenderness via Warner-Bratzler Shear Force (WBSF). Internal temperature and weight was measured prior to cooking using a quick disconnect T-type thermocouple (TMQSS-062U-6, OMEGA Engineering, Inc., Stamford, CT) and a handheld thermometer (OMEGA 450-ATT, OMEGA Engineering, Inc., Stamford, CT) in the geometric center of the steaks. All steaks were cooked to an internal temperature of 35°C and turned over until they reached a target temperature of 70°C on an electric indoor grill (Hamilton Beach-31605A, Hamilton Beach Brands, Glen Allen, VA). After cooking final weights were recorded. The steak was then bagged (PB-90-C, .85 mil., 6x3x15in.) and stored overnight at 2°C. The following d, 6 (1.27 cm diameter) cores were removed with a drill press parallel to the muscle fibers and sheared using a Food Texture Analyzer (TMS- Pro, Food Technology Corp., Sterling, VA.) with a triangular Warner-Bratzler blade. The mean of the 6 cores was calculated for statistical analysis.

### **Objective and subjective color**

Objective color measurements were taken once daily for 7 days during simulated retail display at all aging time points. Steaks (2.54 cm) were placed on Styrofoam trays (21.6 x 15.9 x 2.1 cm, Styro-Tech, Denver, CO), overwrapped with oxygen permeable film (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO), and placed under retail display conditions (3°C under white fluorescence lighting at 1000

to 1800 lux). Commission internationale de l'éclairage (CIE)  $L^*$ ,  $a^*$ , and  $b^*$  values were obtained using a Minolta CR-400 colorimeter (Minolta, Osaka, Japan) set with a D65 illuminant, 2° observer, with an 8 mm diameter measurement area. Six measurements were made per steak and the mean was calculated for statistical analysis. The colorimeter was calibrated daily with a white ceramic tile (Calibration Plate, Serial No. 14933058, Konica Minolta, Japan). Lightness ( $L^*$ ) is measured with a range from 0 (black) to 100 (white),  $a^*$  measures redness with the range between red (positive) and green (negative), and  $b^*$  is a measure of yellowness from yellow (positive) to blue (negative). Color readings were recorded at the same time each day.

Visual discoloration was assessed daily during the 7 d of RD utilizing 5 trained panelists comprised of graduate students from the University of Nebraska-Lincoln. Panelists were trained using a standardized discoloration guide (Appendix VI). Discoloration % was approximated from 0% to 100% with 0% meaning no discoloration present and 100% being a fully discolored steak. Steaks were randomly rotated daily to minimize location effects.

### **Lipid oxidation (TBARS)**

Lipid oxidation was determined using thiobarbituric acid reactive substance values (TBARS) for all aging periods at 0, 4, and 7 d RD described by Ahn et al. (1998; see appendix VII). Five g of powdered meat were placed into a 50 mL conical tube to which 14 mL of distilled deionized water were added and 1 mL of butylated hydroxyanisole (BHA) solution (10% BHA: 90% ethanol). Samples were homogenized using a Polytron (POLYTRON® Kinimatica CH-6010, Switzerland) for 15 s at medium to high speed. The

samples were centrifuged (2,000 x g for 5 min at 10°C) and one mL of supernatant was transferred into a 15 mL conical tube with 2 mL of 2,4,6-tribromoanisole (TBA) 2,4,6-trichloroanisole (TCA) solution (15% TCA and 20 mM TBA in deionized distilled water). Tubes were then placed in a 70°C water bath for 30 min. After 30 min, tubes were cooled for at least 10 min in a water bath (22° C) and centrifuged (2,000 x g for 15 min at 10°C). Two hundred µL of supernatant was transferred to a 96-well plate in duplicate (Microtest III sterile 96 well flat bottomed microplate; Becton Dickinson & Company, Lincoln Park, NJ). Absorbance values were then read at 540 nm using a microplate spectrophotometer and compared to known standards (Model Epoch, Biotek, Winooski, VT). Results were expressed in mg of malonaldehyde per kg of tissue.

### **Sarcomere length**

Sarcomere length was determined using the helium-neon laser diffraction method described by Cross et al. (1981) and Dolazza and Lorenzen (2014). A few flecks of powdered meat sample were placed on a clear glass microscope slide. A single drop of 0.25 M sucrose solution was added to the slide and topped with a glass coverslip. The distance to the top of the slide from the base of the laser was 100 mm. A sheet of paper was placed below the stand in order to mark the two diffraction bands. Six sarcomeres per sample were determined and sarcomere length (µm) was determined by the equation provided by Cross et al. (1981):

Sarcomere length (µm) =

$$\frac{0.6328 \times D \sqrt{\left(\frac{T}{D}\right)^2 + 1}}{T}$$

Where:

$0.6328 = 632.8$  (the wavelength of the laser)  $\times 10^{-3}$

D = distance from specimen to diffraction pattern screen (100 mm)

T = spacing between diffraction bands (mm)

### **Fatty acid analysis**

Total lipids were extracted using the chloroform-methanol procedure by Folch et al. (1957). After extraction, lipids were converted to fatty acid methyl esters according to Morrison and Smith (1964) and Metcalfe et al. (1966). One g of powdered sample was homogenized with 5 mL of 2:1 chloroform:methanol and allowed to sit at room temperature (23°C) for 1 h. After, samples were filtered through Whatman #2 paper, brought up to a final volume of 10 mL with 2:1 chloroform:methanol, and vortexed for 5 s with 2 mL of 0.74% KCl. Samples were centrifuged (1,000 x g for 5 min at 5°C) and the top layer was aspirated off. After centrifugation, samples were dried on a heating block at 60°C under nitrogen purge. Once dry, 1 mL of 0.5 M NaOH in methanol was added, vortexed (5 s), and heated at 100°C for 10 min. One mL of 14% Boron Trifluoride in methanol was added, vortexed (5 s), and again heated at 100°C, this time for 5 min. Two mL of a saturated salt solution and 2 mL of hexane was added and vortexed (5 s). Samples were then centrifuged (1000 x g for 5 min at 5°C) and the hexane layer removed and analyzed using gas chromatography (TRACE 1310 Gas Chromatograph; ThermoFisher Scientific, Waltham, MA). Fatty acids were separated using a Chrompack CP-Sil 88 capillary column (0.25 mm by 100 m; Inlet temp: 260°C, Oven: 140°C hold for 5 min,

increase at 4°C/min to 240°C and hold for 15 min. FID temp: 280°C. Injected at 30:1 ratio) and identified based on their retention times compared to known commercial standards (NU-Check Prep, Inc., Elysian, MN; # GLC-68D, GLC-79, GLC-87, GLC-455, and GLC-458, see appendix VIII). The percentage of fatty acids were determined by the peak areas in the chromatograph and values were converted to mg/100 g tissue:

Fatty acid mg/100 g tissue = (% of fatty acid peak area \* fat content of samples) \* 1000

### **Free calcium concentration**

Free calcium was quantified according to the procedure described by Parrish et al. (1981) with modifications. Three grams of powdered sample were centrifuged at 196,000 x g (Beckman Optima XPN-90 Ultracentrifuge, Type 50.2 Ti rotor, Beckman Coulter, Brea, CA) at 4°C for 30 minutes. Seven hundred µL of the supernatant were collected and treated with 0.1 mL of 27.5% trichloroacetic acid (TCA). Samples were centrifuged at 6,000 x g (accuSpin Micro 17R, ThermoFisher Scientific, Waltham, MA) for 10 min at 4°C. Four hundred µL of supernatant were transferred to a syringe, and the volume was brought to 4 mL with deionized, distilled water. The diluted sample was filtered through a 13 mm diameter Millex-LG 0.20 µm syringe filter (Millipore, Bedford, MA). Calcium concentration was quantified at Ward Laboratories (Kearney, NE) using an inductively-coupled plasma emission spectrometer (iCAP 6500 Radial; Thermo Electron, Cambridge, UK) with appropriate calcium concentration standards.

**pH analysis**

Powdered sample from steaks from all aging periods with 0 d RD were weighed out in 10 g duplicates into 250 mL plastic beakers and placed on a stir plate. Ninety mL of distilled deionized water and a magnetic stir bar were added to ensure constant mixing during the measurement process. The pH was measured using a pH meter (Orion 410Aplus: ThermoFisher Scientific; Waltham, MA) that was calibrated using 4.0, 7.0, and 10.0 standards. The mean measurement of the duplicates was utilized for all analysis.

**Statistical analysis**

Statistical analysis was conducted with SAS (version 9.4, Cary, NC). Objective and subjective color data were analyzed as a split-plot repeated measures design with dietary treatment as the whole-plot, aging period as the split-plot and retail display time as the repeated measures. Tenderness and lipid oxidation data were analyzed as a split-split-plot design with treatment as the whole-plot, aging period as the split-plot and retail display time as the split-split-plot. Proximate composition and sarcomere were analyzed as a completely randomized design. Free calcium and pH were analyzed as 5 x 3 factorial arrangement of treatments. Data were analyzed using the PROC GLIMMIX procedure of SAS and pen was the experimental unit. All means were separated with the LS MEANS statement and the TUKEY adjustment with an alpha level of 0.05 and tendencies were considered at an alpha level of 0.1.

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## **Quality Effects on Beef from Cattle Fed High-Protein Corn Distillers Grains and Other Ethanol By-Products**

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## ABSTRACT

The objective of this study was to evaluate the effects of feeding high protein corn distillers grains on fresh beef quality. Steers ( $n=300$ ) were fed one of five diets for 190 d (6 pens with 10 head/pen): a corn control (Control), 40% high protein dry distillers grains plus solubles (HP-DDGS), 40% dry distillers grains plus solubles (DDGS), 40% wet distillers grains plus solubles (WDGS), or 40% bran plus solubles (Bran). Eighteen Choice carcasses (3 head/pen) were randomly selected within each treatment ( $n=88$ ). Strip loins were collected, divided into 3 equal portions, and aged for 2, 9, or 23 d. Steaks were fabricated at each aging period and placed under retail display (RD) conditions for 0, 4, or 7 d. Pen was the experimental unit. Hot carcass weight at harvest was 391 kg (SD 31.6 kg). Dietary treatment had no effect on tenderness (WBSF) within each aging period ( $P > 0.05$ ). After 7 d of retail display, following all aging periods, steaks from cattle fed HP-DDGS had the greatest discoloration except for DDGS and Bran after 23 d ( $P < 0.05$ ). The steaks from cattle fed HP-DDGS had lower ( $P < 0.05$ ) redness ( $a^*$ ) values than all other treatments following 7 d of retail display. Lipid oxidation increased ( $P < 0.001$ ) during retail display at all aging periods. A day of retail display-by-dietary treatment effect ( $P < 0.001$ ) was observed for lipid oxidation. After 7 d of retail display, steaks from cattle fed HP-DDGS had significantly higher TBARS than all other treatments except WDGS ( $P < 0.01$ ). A trend was found for sarcomere length ( $P = 0.07$ ), with steaks from cattle fed Bran having the longest sarcomere length compared to all other treatments. An aging effect was found for free calcium content ( $P < 0.001$ ) where steaks aged 9 and 23 d had significantly higher levels of free calcium than those aged 2 d. There was a tendency ( $P = 0.07$ ) that steaks from cattle fed WDGS contained more free calcium than steaks from cattle fed DDGS or

Bran. Although there were no statistical differences, there was a trend ( $P = 0.07$ ) that steaks from cattle fed HP-DDGS had higher levels of polyunsaturated fatty acids and C18:2. These results suggest that feeding high protein distillers grains has no detriment on tenderness, but may alter the lipid profile of the muscle resulting in decreased color stability, increased lipid oxidation and decreased shelf life.

**Keywords:** beef, distillers grains, high protein, fatty acid composition

## INTRODUCTION

Distillers grains, a byproduct of corn ethanol production, have been used as a livestock feedstuff for over 100 years (Klopfenstein et al., 2008). As the ethanol industry continues to evolve to maximize profits, new co-products are being produced. One of the newest methods used in the ethanol industry is a process known as dry or front-end fractionation. Dry fractionation is the process of physically separating corn into its three fractions (endosperm, bran, and germ) prior to the fermentation process. Removing the non-fermentable germ, which contains 85% of the corn oil, allows ethanol producers to remove the oil for other markets and creates more capacity for fermentation of starches. Additionally, the removal of this corn oil can decrease the fat content of the resulting distillers grains (U.S. Grains Council, 2012). The bran will also not be introduced into the ethanol production process due to its non-fermentable nature and can be combusted for energy or sold as a livestock feed (Cereal Process Technologies, 2018). The removal of these two fractions increases the total amount of fermentable starch available during ethanol production (Ponnampalam et al., 2004). With only the endosperm being fermented, there are lower costs associated with the production of the distillers grains as a result of lower energy costs of drying and a greater stream of co-products (Rendleman and Shapouri, 2007). The resulting distillers grains from this method have been referred to as high protein. It is believed that the higher level of crude protein present is due to more efficient fermentation (Robinson et al., 2008). Crude fat content of traditional dried distillers grains plus solubles (DDGS) has been reported at 8-9%, while high protein dried distillers grains (HP-DDGS) can have a fat level of closer to 4%, on a dry matter basis (Ganesan et al., 2009; Cereal Process Technologies, 2016). Previous research conducted at the University

of Nebraska-Lincoln has shown that feeding cattle with diets containing distillers grains can alter the fatty acid profile resulting in increased lipid oxidation and decreased color stability (Mello et al., 2012; Chao 2015; Domenech-Perez et al., 2017; Ribeiro et al., 2018). However, it is unclear if feeding high protein distillers grains to cattle will cause the same detriments as its predecessors. Therefore, a full comprehension of how the inclusion of high protein distillers grains in cattle diets affects meat quality is imperative. More specifically, the objectives of this study were to evaluate how high protein distillers grains affect tenderness, fatty acid profile of the muscle, and impacts on retail display as related to discoloration and lipid oxidation on beef.

## **MATERIALS AND METHODS**

All procedures related to live animals for this study were approved by the Institutional Animal Care and Use Committee of the University of Nebraska-Lincoln. (IACUC #1282)

### *Cattle and dietary treatments*

A total of 300 cross-bred calf-fed steers (initial BW =  $282 \pm 10$  kg) were fed (Eastern Nebraska Research and Extension Center feedlot in Mead, NE) for 190 d on either a corn control diet (Control), or a diet containing 40% high protein dried distillers grains plus solubles (HP-DDGS), 40% dried distillers grains plus solubles (DDGS), 40% wet distillers grains plus solubles (WDGS), or 40% bran plus solubles (Bran). Inclusion rate of distillers grains were calculated on a dry matter basis. Steers were fed 50% Sweet Bran (Cargill, Blair, NE) and 50% alfalfa hay at two 2% of BW for 5 days to identify initial BW for the trial. Steers were blocked and stratified by BW prior to being randomly assigned to

a pen. Pens were randomly assigned to one of 5 treatments with 10 steers/pen and 6 pens/treatment resulting in a total of 30 pens. High protein dried distillers grains plus solubles and Bran were sourced from the same ethanol plant (Corn Plus, Winnebago, MN). Traditional WDGS and DDGS were sourced from E Energy (Adams, NE) and KAAPA Ethanol (Ravenna, NE), respectively. All diets contained 15% corn silage and 5% supplement containing 30 g/ton Rumensin® (Elanco Animal Health, Greenfield, IN) and 8.8 g/ton Tylan® (Elanco Animal Health, Greenfield, IN). On d 1, steers were implanted with Revalor XS® (Merck Animal Health, DeSoto, KS).

#### *Sample collection and fabrication*

All cattle were harvested at Greater Omaha Packing Co. in Omaha, NE. On day 2 postmortem, three low Choice carcasses per pen were identified for a total of eighteen low Choice carcasses within each treatment (n = 90). During carcass selection, two carcasses were lost resulting in a final count of 88 carcasses. The two carcasses lost included one from the Bran treatment and one from the WDGS treatment. Strip loins (Longissimus lumborum, IMPS # 180, NAMP, 2011) from the right side of the carcass were collected. Identified loins were vacuum packaged and transported to the Loeffel Meat Laboratory at the University of Nebraska-Lincoln. Strip loins were divided into three equal sections and each section was randomly assigned to one of the three aging periods (2, 9, or 23 d) immediately after arrival at the Loeffel Meat Laboratory 2 d postmortem. Three 2.54 cm steaks were fabricated at each aging period (one steak to measure Warner-Bratzler shear force at 0 d of retail display (RD), one steak to measure Warner-Bratzler shear force, visual discoloration, and objective color at 7 d of RD, and one steak was cut in half and trimmed of all subcutaneous fat and analyzed for lipid oxidation for 0 d of RD and all other



laboratory analysis). One 1.27 cm steak was cut in half, trimmed of all subcutaneous fat, and analyzed for lipid oxidation after 4 and 7 d of RD. At d 2, steaks for laboratory analysis, lipid oxidation steaks for 0 d of RD, and all remaining sections of loins were vacuum packaged in Prime Source Vacuum pouches (3 mil STD barrier, Prime Sources, St. Louis, MO). Steaks for laboratory analysis and lipid oxidation were frozen for further analysis (-80° C). The remaining portions of loins were aged (2°C) under dark storage. All steaks were separated from the loin from the anterior to posterior end of the loin. The same fabrication scheme was used for all aging periods. At all aging periods, samples for tenderness, color, and lipid oxidation analysis were placed on foam trays (21.6 x 15.9 x 2.1 cm, Styro-Tech, Denver, CO) and overwrapped with an oxygen permeable film (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO). Trays were placed under simulated RD conditions for 4 and 7 d (3° C under white fluorescence lighting at 1000 to 1800 lux) and randomly rotated daily. All frozen steaks utilized for laboratory analysis and lipid oxidation were tempered enough to finely dice, frozen in liquid nitrogen and then homogenized in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT) and held at -80° C until further analysis.

#### *Proximate composition*

Moisture, fat, and ash (%) of raw meat samples were determined. Moisture and ash (%) were quantified with a LECO Thermogravimetric Analyzer in duplicate (Model 604-100-400, LECO Corporation, St. Joseph, MI). Total fat (%) was determined in triplicate by ether extraction according to the Soxhlet procedure (AOAC, 1990)(See Appendix IV). Protein was determined by difference.

### *Tenderness determination*

Steaks (2.54 cm) were measured for tenderness via Warner-Bratzler Shear Force (WBSF). Internal temperature and weight was measured prior to cooking using a quick disconnect T-type thermocouple (TMQSS-062U-6, OMEGA Engineering, Inc., Stamford, CT) and a handheld thermometer (OMEGA 450-ATT, Engineering, Inc., Stamford, CT) in the geometric center of the steaks. All steaks were cooked to an internal temperature of 35° C and turned over until they reached a target temperature of 70° C on an indoor electric grill (Hamilton Beach-31605A, Hamilton Beach Brands, Glen Allen, VA). After cooking final weights were recorded. The steaks were then bagged individually (PB-90-C, .85 mil., 6x3x15in.) and stored overnight at 2° C and the following d, 6 (1.27 cm diameter) cores were removed with a drill press parallel to the muscle fibers and sheared using a Food Texture Analyzer (250 mm/min blade speed, TMS-Pro, Food Technology Corp., Sterling, VA.) with a triangular Warner-Bratzler blade (250 mm/min blade speed). The mean shear force of the 6 cores was calculated for statistical analysis.

### *Objective color*

Objective color measurements were made once daily during each day of retail display at all aging time points. Commission internationale de l'éclairage (CIE) L\*, a\*, and b\* values were obtained using a Minolta CR-400 colorimeter (Minolta, Osaka, Japan) set with a D65 illuminant, 2° observer, with an 8 mm diameter measurement area. Six measurements were made per steak and the mean was calculated for statistical analysis. The colorimeter was calibrated daily with a white ceramic tile (Calibration Plate, Serial No. 14933058, Konica Minolta, Japan). Color readings were recorded at the same time each day (1600 h).

### *Subjective discoloration*

Visual discoloration was assessed daily during the 7 d of RD utilizing 5 trained panelists comprised of graduate students from the University of Nebraska-Lincoln. Discoloration % was approximated from 0% to 100% with 0% meaning no discoloration present and 100% being a fully discolored steak. Steaks were randomly rotated daily to minimize location effects. Panelists were trained using a standardized discoloration guide (Appendix VI).

### *Lipid oxidation (TBARS)*

Lipid oxidation was determined using thiobarbituric acid reactive substance method (TBARS) for samples at 0, 4, and 7 d of RD at all aging periods, according to Ahn et al. (1998). Five g of powdered meat were placed into a 50 mL conical tube to which 14 mL of distilled deionized water and 1 mL of butylated hydroxyanisole (BHA) solution (10% BHA: 90% ethanol) were added. Samples were homogenized using a Polytron (POLYTRON® Kinimatica CH-6010, Switzerland) for 15 s at medium to high speed. The samples were centrifuged (2,000 x g for 5 min at 10°C) and 1 mL of supernatant was transferred into a 15 mL conical tube with 2 mL of 2,4,6-tribromoanisole (TBA) 2,4,6-trichloroanisole (TCA) solution (15% TCA and 20 mM TBA in deionized distilled water). Tubes were then placed in a 70°C water bath for 30 min. Tubes were cooled for at least 10 min in a water bath (22° C) and centrifuged (2,000 x g for 15 min at 10°C). Two hundred µL of supernatant was transferred to a 96-well plate in duplicate. Absorbance values were then read at 540 nm using a microplate spectrophotometer (Model Epoch, Biotek, Winooski, VT). Results were expressed in mg of malonaldehyde per kg of tissue.

### *Sarcomere length*

Sarcomere length was determined using the helium-neon laser diffraction method described by Cross et al. (1981) and Dolazza and Lorenzen (2014). A few flecks of powdered meat sample were placed on a clear glass microscope slide. A single drop of 0.25 M sucrose solution was added to the slide and topped with a glass coverslip. The distance to the top of the slide from the base of the laser was 100 mm. A paper sheet was placed below the stand in order to mark the two diffraction bands. Six sarcomeres per sample were determined and sarcomere length ( $\mu\text{m}$ ) was determined by the equation provided by Cross et al. (1981).

### *Fatty acid analysis*

Total lipids were extracted using the chloroform-methanol procedure by Folch et al. (1957). After extraction, lipids were converted to fatty acid methyl esters according to Morrison and Smith (1964) and Metcalfe et al. (1966). One g of powdered sample was homogenized with 5 mL of 2:1 chloroform:methanol and allowed to sit at room temperature (23°C) for 1 h. After, samples were filtered through Whatman #2 paper, brought up to a final volume of 10 mL with 2:1 chloroform:methanol, and vortexed for 5 s with 2 mL of 0.74% KCl. Samples were centrifuged (1,000 x g for 5 min) and the top layer was aspirated off. After centrifugation, samples were dried on a heating block at 60° C under nitrogen purge. Once dry, 1 mL of 0.5 M NaOH in methanol was added, vortexed (5 s), and heated at 100° C for 10 min. One mL of 14% Boron Trifluoride in methanol was added, vortexed (5 s), and again heated at 100° C, this time for 5 min. Two mL of a saturated salt solution and 2 mL of hexane was added and vortexed (5 s). Samples were then centrifuged (1000 x g for 5 min) and the hexane layer removed and analyzed using gas

chromatography (TRACE 1310 Gas Chromatograph; ThermoFisher Scientific, Waltham, MA). Fatty acids were separated using a Chrompack CP-Sil 88 capillary column (0.25 mm by 100 m; Inlet temp: 260° C, Oven: 140° C hold for 5 min, increase at 4° C/min to 240° C and hold for 15 min. FID temp: 280° C. Injected at 30:1 ratio) and identified based on their retention times compared to known commercial standards (NU-Check Prep, Inc., Elysian, MN; # GLC-68D, GLC-79, GLC-87, GLC-455 and GLC-458, see appendix VIII). The percentage of fatty acids were determined by the peak areas in the chromatograph and values were converted to mg/100 g tissue:

Fatty acid mg/100 g tissue = (% of fatty acid peak area \* fat content of samples) \* 1000

#### *Free calcium concentration*

Free calcium was quantified according to the procedure described by Parrish et al. (1981) with modifications as described. Three grams of powdered sample were centrifuged (196,000 x g) at 4°C for 30 minutes. Seven hundred µL of the supernatant were collected and treated with 0.1 mL of 27.5% trichloroacetic acid (TCA). Samples were centrifuged (6,000 x g) for 10 min. Four hundred µL of supernatant were transferred to a syringe, and the volume was brought to 4 mL with deionized, distilled water. The diluted sample was filtered through a 13 mm diameter Millex-LG 0.20 µm syringe filter (Millipore, Bedford, MA). Calcium concentration was quantified at Ward Laboratories (Kearney, NE) using an inductively-coupled plasma emission spectrometer (iCAP 6500 Radial; Thermo Electron, Cambridge, UK) with appropriate calcium concentration standards.

#### *pH analysis*

Powdered sample from steaks from all aging periods with 0 d RD were weighed out in 10 g duplicates into 250 mL plastic beakers and placed on a stir plate. Ninety mL of

distilled deionized water and a magnetic stir bar were added to ensure constant mixing during the measurement process. The pH was measured using a pH meter (Orion 410Aplus; ThermoFisher Scientific; Waltham, MA) that was calibrated using 4.0, 7.0, and 10.0 standards. The mean measurement of the duplicates was utilized for all analysis.

#### *Statistical analysis*

Statistical analyses were conducted with SAS (version 9.4, Cary, NC). Objective and subjective color data were analyzed as a split-plot repeated measures design with dietary treatment as the whole-plot, aging period as the split-plot and retail display time as the repeated measures. Tenderness and lipid oxidation data were analyzed as a split-split-plot design with treatment as the whole-plot, aging period as the split-plot and retail display time as the split-split-plot. Proximate composition and sarcomere length were analyzed as a completely randomized design. Free calcium and pH were analyzed as 5 x 3 factorial arrangement of dietary treatments by aging period. Data were analyzed using the PROC GLIMMIX procedure of SAS and pen was the experimental unit. All means were separated with the LS MEANS statement and the TUKEY adjustment with an alpha level of 0.05.

## **RESULTS AND DISCUSSION**

#### *Proximate composition*

In this study, dietary treatment had no effect ( $P > 0.05$ ) on proximate composition of steaks or marbling scores (Table 1). The grade distribution of all carcasses were as follows: 1.4% low Prime, 4.4% high Choice, 23.3% average Choice, 45.6% low Choice, and 25.3% Select. The mean values for the proximate composition were: 71.31% moisture, 19.99% protein, 6.89% fat, and 1.81% ash. Other studies have also shown that the

supplementation of distillers grains in multiple forms and inclusion levels does not alter the proximate composition of beef (Mello et al., 2012; Domenech-Perez et al., 2017; Ribeiro et al., 2018). However, Segers et al. (2011) concluded that steaks from steers fed DDGS tended to have higher moisture levels than those fed corn gluten feed and Buttrey et al. (2013) found that steers fed a 35% inclusion of WDGS had steaks that tended ( $P < 0.10$ ) to have greater fat content compared to steaks from cattle not fed WDGS.

#### *Tenderness determination*

Steaks aged 2 d showed differences ( $P = 0.08$ ; Figure 1) in tenderness across dietary treatments, and a retail display effect ( $P < 0.001$ ) was identified. Steaks from cattle fed HP-DDGS had greater WBSF ( $P = 0.08$ ) than steaks from cattle fed WDGS, but was similar to all other dietary treatments. Steaks aged for 9 d exhibited no differences across dietary treatments ( $P = 0.26$ ). After 23 d of aging, steaks from cattle fed Bran tended ( $P = 0.07$ ) to have lower WBSF than steaks from cattle fed HP-DDGS and Control. A significant aging time-by-dietary treatment effect ( $P = 0.01$ ) was seen. As steaks from HP-DDGS, DDGS, and Bran increased in aging time, an increase of tenderness was observed. There were no differences ( $P > 0.05$ ) in tenderness between HP-DDGS and Control across aging periods, however, steaks from cattle fed HP-DDGS had numerically greater shear force values than all other treatments at 2 d of aging. As expected, placing steaks in retail display for 7 d increased tenderness for steaks aged 2, 9, and 23 d ( $P < 0.001$ ). These results are in agreement with Popowski (2011) who found that steaks from steers fed 35% HP-DDGS did not differ ( $P = 0.25$ ) from steaks from steers fed either a corn control diet or 35% DDGS after 2 d of aging. Steaks from cattle fed WDGS had decreased (numerically lower) shear force values compared to steaks from cattle fed all other treatments after 2 d of aging

( $P = 0.08$ ). This is consistent with Chao (2015), who reported that at 2 d of aging, steaks from cattle finished with WDGS were more tender than steaks fed a corn control diet, but there was no difference as aging time increased. In the present study, after 23 d of aging steaks from cattle fed Bran tended to have the lowest WBSF values compared to steaks from all other treatments. Steaks from cattle fed Bran also exhibited the greatest decrease in shear force values from 2 d of aging to 23d (4.4 kg and 3.2 kg, respectively).

#### *Fatty acid profile*

The fatty acid profiles of all the dietary treatments are reported in mg/100 g of tissue basis (Table 2). Differences ( $P < 0.05$ ) were found in the amount of margaric acid (C17:0) present in the muscle across dietary treatments. Amount of C17:0 was greatest in beef from cattle fed WDGS, intermediate in beef from cattle fed Control, and lowest in beef from cattle fed HP-DDGS, Bran, and DDGS. Mello et al. (2012) also noted that steaks from cattle supplemented with 15% WDGS had greater levels of C17:0 compared to steaks from cattle fed Control. Popowski (2011) reported that back fat from HP-DDGS contained a greater concentration of C17:0 than fat from DDGS, but was not different than fat from cattle fed a corn control. There were no differences ( $P > 0.05$ ) found in amount of mono-unsaturated fatty acids (MUFA), stearic acid (C18:0), linoleic acid (C18:2),  $\alpha$ -linolenic acid (C18:3), or polyunsaturated fatty acids (PUFA).

Differences were identified at  $P = 0.08$  for elaidic acid (C18:1T), where steaks from cattle fed HP-DDGS had greater values for C18:1T than steaks from cattle fed Control (389.10 mg/100g, and 179.98 mg/100g, respectively). Similar results were found by Mello et al. (2012) who determined that as increasing levels of WDGS were fed to cattle, C18:1T also increased. Domenech-Perez et al. (2017) also reported that supplementing cattle with



65% WDGS resulted in greater levels of C18:1T in steaks than the amounts that were found in steaks from cattle fed corn finishing diets.

Dietary treatments were shown to be different at  $P = 0.07$  for linoleic acid (C18:2). Following the same pattern as C18:1T, steaks from cattle fed HP-DDGS had the greatest levels of C18:2, followed by WDGS, Bran and DDGS, with steaks from cattle fed Control containing the least amount of C18:2. Linoleic acid totals were also greater for steaks from cattle fed distillers grains compared to corn in multiple other studies conducted at the University of Nebraska (Mello et al., 2012; Domenech-Perez et al., 2017; Ribeiro et al., 2018).

Polyunsaturated fatty acid content exhibited no difference ( $P = 0.31$ ). Popowski (2011) noted a difference ( $P < 0.001$ ) in the levels of PUFA with meat from cattle fed HP-DDGS having a greater level of PUFA than meat from cattle fed a corn control.

It has been stated that fatty acid composition of meat from ruminant animals is largely controlled by the feedstuff used (Jenkins, 1993; Houben et al., 2000; Buttrey et al., 2013; Ribeiro et al., 2018). When greater amounts of grain are included in ruminant diets, the pH of the rumen will become more acidic which reduces the biohydrogenation of fatty acids (Plascencia et al., 1999; Atkinson et al., 2006). As a result, more unsaturated fatty acids reach the duodenum where they interact with bile salt micelles and are more efficiently absorbed into the bloodstream (Zinn et al., 2000). Vander Pol et al. (2009) found that by feeding WDGS, a greater amount of PUFA was reaching the duodenum. These findings were supported by Duckett et al. (2002) when they found that feeding higher lipid diets increased duodenal flow of palmitic, stearic, oleic, linoleic, and arachidonic acid by 30%. Once the fatty acids have reached the bloodstream, they can then be deposited into

the muscle (Enser et al., 1996; Scollan et al., 2006). Due to higher levels of PUFA in WDGS compared to corn, it can be inferred that the increased C18:2 deposition in the muscle tissue in this experiment could be from this dietary supply (Ham et al., 1994).

### *Objective color*

Muscle  $L^*$  and  $a^*$  exhibited dietary treatment by retail display interactions ( $P < 0.001$ ). The  $L^*$  values for steaks from cattle fed WDGS were greater (lighter;  $P < 0.05$ ) than all treatments at 0 d of retail display, other than steaks from cattle fed HP-DDGS which were similar. There were no differences ( $P > 0.05$ ) in lightness among the other dietary treatments at 0 d of RD. After 7 d of RD, steaks from cattle fed HP-DDGS had greater  $L^*$  values than steaks from the Control cattle ( $P = 0.004$ ). Gill et al. (2008) found that meat from cattle fed 15% distillers grains had lighter meat than cattle fed steam-flaked corn ( $P < 0.05$ ). These results contrast findings by Depenbusch et al. (2009) who reported, as the proportion of DDGS fed to heifers increased linearly from 0 – 75%, there was a linear decrease in lightness (lower  $L^*$ ) at RD 0 ( $P = 0.04$ ).

Redness ( $a^*$ ) was greater ( $P < 0.05$ ) for steaks from cattle fed DDGS than steaks from the Bran treatment, WDGS treatment, and Control on 0 d of RD. The HP-DDGS steaks had lower ( $P < 0.05$ ) redness values than all other treatments following 7 d of retail display, while Control steaks had the greatest  $a^*$  value ( $P < 0.05$ ; Figure 2). Popowski (2011) determined that after 4 d of RD, steaks from steers fed HP-DDGS and DDGS had lower ( $P < 0.01$ )  $a^*$  values than steaks from steers fed a corn control. This is consistent with the findings of multiple studies that indicated a decrease in redness after the utilization of distillers grains in cattle diets (Depenbusch et al., 2009; Leupp et al., 2009; Mello et al., 2012).

A 3-way interaction of dietary treatment, aging time, and days of retail display was identified for  $b^*$  values ( $P = 0.006$ ). At 2 and 23 d aging with 0 d retail display there were no differences among dietary treatments ( $P > 0.05$ ). However, during 9 d of aging with 0 d of RD, steaks from cattle supplemented with DDGS was significantly more yellow than steaks from Control fed cattle ( $P = 0.03$ ). For steaks with 2 d of aging and 7d of RD, Control, WDGS, and DDGS were all significantly more yellow than the HP-DDGS treatment. At 9 d of aging with 7 d of RD, steaks from Bran and DDGS had greater yellowness values ( $P < 0.05$ ) compared to HP-DDGS. There were no differences ( $P > 0.05$ ) between dietary treatments at 23 d of aging and 7 d of RD.

#### *Subjective discoloration*

Discoloration results for all aging periods, days in RD, and dietary treatments are reported in Table 3. After 7 d of RD following 2 and 9 d of aging, steaks from cattle fed HP-DDGS had more discoloration ( $P < 0.05$ ) than all other treatments, which were similar. Following 23 d of aging, steaks from cattle fed HP-DDGS had the most discoloration and all treatments except WDGS were more discolored than Control ( $P < 0.05$ ) after 6 and 7 d of retail display. These results are consistent with Popowski (2011) who found an increase in surface discoloration for steaks from steers fed HP-DDGS compared to steaks from steers fed either DDGS or a corn control ( $P < 0.01$ ).

Meat purchasing decisions have been noted to be largely affected when fresh meat color contains 20% surface discoloration, resulting in a decrease in sales by up to 50% (Hood and Riordan, 1973). In this study, only after 9 d of aging and 7 d of RD, steaks from cattle fed HP-DDGS were already reaching discoloration values of 19.9%. After 23 d of

aging steaks from all dietary treatments except Control and WDGS surpassed the 20% discoloration mark after 6 d of RD.

Since lipid oxidation has been tightly linked to myoglobin oxidation, it was expected that a lower fat feedstuff would result in more stable meat color (Greene, 1969). However, as noted above, the trend ( $P = 0.07$ ) of an increase of C18:2 in meat samples from cattle fed HP-DDGS could explain this discoloration pattern. It has been well documented that an increase in PUFA can result in increased lipid oxidation (Morrissey et al., 1998; Faustman et al., 2010). Lipid oxidation can be a major contributor to myoglobin oxidation due to the creation of free radicals that interact with myoglobin, oxidizing it from the oxymyoglobin form into metmyoglobin (Faustman et al., 2010). Nute et al. (2007) reported that meat from animals fed diets with greater levels of PUFA present is more susceptible to being oxidized resulting in greater discoloration. Although there were no significant differences among dietary treatments for total PUFA, the increased levels of C18:2 could be the cause of the increased surface discoloration.

#### *Lipid oxidation (TBARS)*

A retail by treatment interaction ( $P < 0.001$ ) was observed for lipid oxidation (Table 4). After 7 d of retail display, steaks from cattle fed HP-DDGS had greater TBARS values ( $P < 0.05$ ) than all other treatments except steaks from WDGS fed cattle. There were no differences in lipid oxidation between steaks from cattle fed WDGS, Bran, and DDGS. The least lipid oxidation occurred in steaks from cattle fed Control, which were different ( $P < 0.05$ ) from steaks from all other dietary treatments except those from the DDGS treatment. These results are consistent with results from Popowski (2011) where ground beef from

cattle fed HP-DDGS had greater levels of TBARS than ground beef from steers fed corn or DDGS ( $P < 0.001$ ).

An aging time-by-days of retail display interaction was also noted ( $P < 0.001$ ) where steaks aged for 23 d and 7 d of RD had the greatest TBARS values compared to all other periods. Within each aging period, steaks from d 7 of RD had the highest TBARS values followed by steaks from 4 d of RD having greater values than steaks from 0 RD.

Lipid oxidation by-products can affect color, aroma, flavor, and overall shelf life (Morrissey et al., 1994; Gray et al., 1996; Ladeira et al., 2014) Campo et al. (2006) surmised that TBARS values of 2.28 mg/kg were considered the limiting threshold of oxidized beef acceptability. In this study, steaks from cattle fed HP-DDGS surpassed this limit (2.40 mg malonaldehyde/kg) after 4 d of RD while no other treatments reached this level until 7 d of RD. After 7 d of RD, all treatments had reached the threshold of acceptability. Lipid oxidation in meat has been strongly linked to myoglobin oxidation, meaning the oxidation of one can accelerate oxidation of the other (Faustman et al., 2010). These data, along with our discoloration results as described in the previous section, follow a similar pattern where beef from cattle fed distillers grain and other ethanol co-products have the greatest levels of lipid oxidation and discoloration, with HP-DDGS having the most detrimental effects for both.

#### *Free calcium concentration*

There were no statistical differences ( $P = 0.07$ ) in free calcium content among dietary treatments, but there was a tendency where steaks from WDGS had the greatest free calcium levels and Bran steaks the lowest (68.15  $\mu\text{M}$ , and 62.05  $\mu\text{M}$ , respectively). As aging increased, there was a significant increase of free calcium levels in the meat ( $P <$

0.001) (2 d: 60.06  $\mu\text{M}$ ; 9 d: 66.27  $\mu\text{M}$ ; 23 d: 67.82  $\mu\text{M}$ ). These results are congruent with the tenderness results discussed previously where there were no differences in tenderness across treatments.

Calcium has a major role in meat tenderization postmortem. Calcium is sequestered in the sarcoplasmic reticulum which regulates the amount of calcium ions in the sarcoplasm of the muscle fiber. It has been shown that after harvest, the calcium concentration in the sarcoplasm increases due to the inability of the sarcoplasmic reticulum to accumulate the ions (Greaser et al., 1969). Over time, calcium concentration in the sarcoplasm has been shown to increase (Parrish et al., 1981; Ji and Takahashi, 2006; Senaratne, 2012). Calcium release activates the proteolytic enzyme calpains, which has been largely attributed to early postmortem tenderness (Koohmarie, 1994; Taylor and Koohmarie, 1998). It has been shown that feeding distillers grain to cattle can also alter the fatty acid profile of organelles within the cell such as the sarcoplasmic reticulum and mitochondria, resulting in earlier postmortem calcium release and improved tenderness (Chao, 2015; Kunze et al., 2016). The lack of differences in amount of PUFA could be an indication of why there were no differences across dietary treatments for free calcium concentration nor tenderness.

#### *Sarcomere length*

Sarcomere lengths differed with a p-value of 0.07, where sarcomeres from steaks from cattle fed Bran were longer than all other treatments. The mean sarcomere length of strip loins from cattle fed Control, WDGS, Bran, DDGS, and HP-DDS were 1.67, 1.69, 1.74, 1.69, and 1.69  $\mu\text{m}$ , respectively (Figure 3). Wheeler and Koohmarie (1999) report that sarcomere length from non-shortened longissimus dorsi was 1.69  $\mu\text{m}$ , compared to those that were cold shortened, at 1.36  $\mu\text{m}$ . Other studies focusing on the feeding of

distillers grains have also reported no differences in sarcomere length (Chao, 2015; Ribeiro, 2017).

### *pH*

A day of aging effect was found for pH ( $P < 0.001$ ). However, the difference in values were likely of little practical significance (5.48 2 d, 5.44 9 d, 5.49 23 d, respectively). The pH of meat generally decreases from 7.4 in living muscle tissue to an ultimate pH in beef of around 5.3-5.7 within 24 h of slaughter. This drop in pH is due to the accumulation of  $H^+$  ions through the process of glycolysis and hydrolyzing ATP to ADP (Aberle et al., 2012).

## **CONCLUSION**

Feeding cattle HP-DDGS at 40% inclusion increased lipid oxidation and discoloration in steaks compared to those fed a corn control finishing diet. Although there were no statistical differences in the fatty acid profile across treatments, there was a trend that meat from cattle fed HP-DDGS had greater amounts of C18:2 than steaks from cattle fed Control. These findings could be the cause of the decreased display life of steaks from cattle fed HP-DDGS at longer aging times and retail display times. While there were no detriments in terms of tenderness, the increase in lipid oxidation and color instability could result in significant economic ramifications due to shorter shelf life.

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**Table 1. Proximate composition<sup>1</sup> of strip loin steaks (*L. lumbarum*) aged for 2 d from cattle fed dried distillers grains plus solubles (DDGS), high protein dried distillers grains plus solubles (HP-DDGS), wet distillers grains plus solubles (WDGS), corn bran plus solubles (Bran), or a corn control diet. (n=30)**

Treatment	Moisture	Fat	Ash	Protein <sup>2</sup>	Marbling Score <sup>3</sup>
Control	71.16	7.03	1.74	20.06	463
WDGS	71.02	7.38	1.81	19.79	453
Bran	71.76	6.47	1.85	19.93	454
DDGS	71.25	6.49	1.82	20.44	480
HP-DDGS	71.36	7.07	1.85	19.72	461
<i>SEM</i>	0.90	1.05	0.14	0.53	14.5

<sup>1</sup>There were no differences among treatments ( $P > 0.05$ )

<sup>2</sup>Protein was calculated by subtracting the sum of moisture, fat, and ash from 100

<sup>3</sup>400 = Small<sup>0</sup>, 500 = Modest<sup>0</sup>



**Table 2. Amount<sup>1</sup> of fatty acids from strip loin steaks (*L. lumbrorum*) aged for 2 d from cattle fed dried distillers grains plus solubles (DDGS), high protein dried distillers grains plus solubles (HP-DDGS), wet distillers grains plus solubles (WDGS), corn bran plus solubles (Bran), or a corn control diet. (n=30)**

Fatty Acid	Dietary Treatment					SEM	P-value
	Corn	WDGS	Bran	DDGS	HP-DDGS		
C14:0	225.41	226.26	195.39	185.20	239.57	34.94	0.52
C15:0	35.01	42.34	30.90	28.50	35.64	3.77	0.17
C15:1	70.80	65.27	71.25	67.19	50.76	15.08	0.87
C16:0	1808.83	1958.65	1684.94	1593.28	1898.69	185.38	0.64
C16:1	246.74	202.24	195.16	184.05	220.73	23.47	0.41
C17:0	91.42 <sup>ab</sup>	132.86 <sup>a</sup>	77.81 <sup>b</sup>	72.06 <sup>b</sup>	82.51 <sup>b</sup>	10.75	0.02
C17:1	79.63	95.76	63.40	68.26	72.10	8.83	0.16
C18:0	787.79	1109.47	918.64	788.79	88.87	89.60	0.15
C18:1T <sup>2</sup>	179.98	355.21	267.81	194.52	389.10	54.84	0.08
C18:1V <sup>2</sup>	107.08	113.75	95.37	83.76	106.82	14.45	0.63
C18:2	286.87	440.00	405.94	386.66	544.83	53.69	0.07
C18:3	10.04	11.80	7.60	5.54	17.52	3.72	0.27
C20:3	20.14	22.66	23.10	20.95	19.38	2.98	0.88
C22:4	10.17	7.61	11.46	10.03	5.46	2.01	0.30
C22:5	18.20	12.65	16.15	13.23	11.21	2.56	0.26
Total	6720.02	7932.05	6731.93	6189.55	7416.18	660.07	0.43
SFA <sup>2</sup>	2963.80	3492.94	2924.14	2683.05	3170.17	303.33	0.45
MUFA <sup>2</sup>	3294.90	3833.14	3221.83	2944.63	3532.87	301.18	0.36
PUFA <sup>2</sup>	461.32	605.97	585.97	561.87	713.13	76.86	0.31

<sup>1</sup>Amount (mg/100 g tissue) of fatty acid in powdered loin sample determined by gas chromatography

<sup>2</sup> C18:1T: Elaidic acid; C18:1V: Vaccenic acid; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids

<sup>a,b</sup> Means in the same row with different superscripts differ ( $P < 0.05$ )

**Table 3. Discoloration (%) of strip loin steaks (*L. lumbarum*) from steers fed either a corn control diet, 40% high protein dry distillers grains plus solubles (HP-DDGS), 40% dry distillers grains plus solubles (DDGS), 40% wet distillers grains plus solubles (WDGS), or 40% bran plus solubles (Bran) with 2, 9, and 23 d of aging at 5, 6, and 7 d of retail display. (n=30)**

Dietary treatments	Aging period			2			9			23		
	Days in retail display			5	6	7	5	6	7	5	6	7
Control				0.29 <sup>a</sup>	0.72 <sup>a</sup>	1.59 <sup>b</sup>	0.13 <sup>a</sup>	0.68 <sup>b</sup>	1.68 <sup>b</sup>	3.13 <sup>a</sup>	15.75 <sup>c</sup>	38.85 <sup>c</sup>
WDGS				0.47 <sup>a</sup>	1.77 <sup>a</sup>	4.74 <sup>b</sup>	0.31 <sup>a</sup>	1.64 <sup>ab</sup>	5.67 <sup>b</sup>	6.02 <sup>a</sup>	34.45 <sup>bc</sup>	63.63 <sup>b</sup>
Bran				0.07 <sup>a</sup>	1.06 <sup>a</sup>	2.89 <sup>b</sup>	0.87 <sup>a</sup>	3.26 <sup>ab</sup>	6.79 <sup>b</sup>	18.98 <sup>a</sup>	46.24 <sup>ab</sup>	68.30 <sup>ab</sup>
DDGS				0.59 <sup>a</sup>	1.92 <sup>a</sup>	4.82 <sup>b</sup>	0.15 <sup>a</sup>	1.92 <sup>ab</sup>	4.83 <sup>b</sup>	13.88 <sup>a</sup>	45.05 <sup>ab</sup>	74.19 <sup>ab</sup>
HP-DDGS				0.44 <sup>a</sup>	4.86 <sup>a</sup>	15.32 <sup>a</sup>	1.74 <sup>a</sup>	7.67 <sup>a</sup>	19.88 <sup>a</sup>	16.30 <sup>a</sup>	64.31 <sup>a</sup>	85.79 <sup>a</sup>

<sup>a-d</sup> Means in the same column with different superscripts are different ( $P < 0.05$ ) SEM Day 2 = 0.78, SEM Day 9 = 1.25, SEM Day 23 = 3.75

**Table 4. Lipid oxidation value (TBARS; mg malonaldehyde/kg of meat) of strip loin steaks (*L. lumbarum*) from steers fed either a corn control diet, 40% high protein dry distillers grains plus solubles (HP-DDGS), 40% dry distillers grains plus solubles (DDGS), 40% wet distillers grains plus solubles (WDGS), or 40% bran plus solubles (Bran) with 0, 4, and 7 d retail display. (n=30)**

Dietary treatment	Days in retail display		
	0	4	7
Control	0.67 <sup>a</sup>	1.45 <sup>a</sup>	2.38 <sup>c</sup>
WDGS	0.77 <sup>a</sup>	2.04 <sup>a</sup>	4.27 <sup>ab</sup>
Bran	0.73 <sup>a</sup>	2.08 <sup>a</sup>	3.86 <sup>b</sup>
DDGS	0.85 <sup>a</sup>	1.64 <sup>a</sup>	3.19 <sup>bc</sup>
HP-DDGS	0.80 <sup>a</sup>	2.40 <sup>a</sup>	5.15 <sup>a</sup>

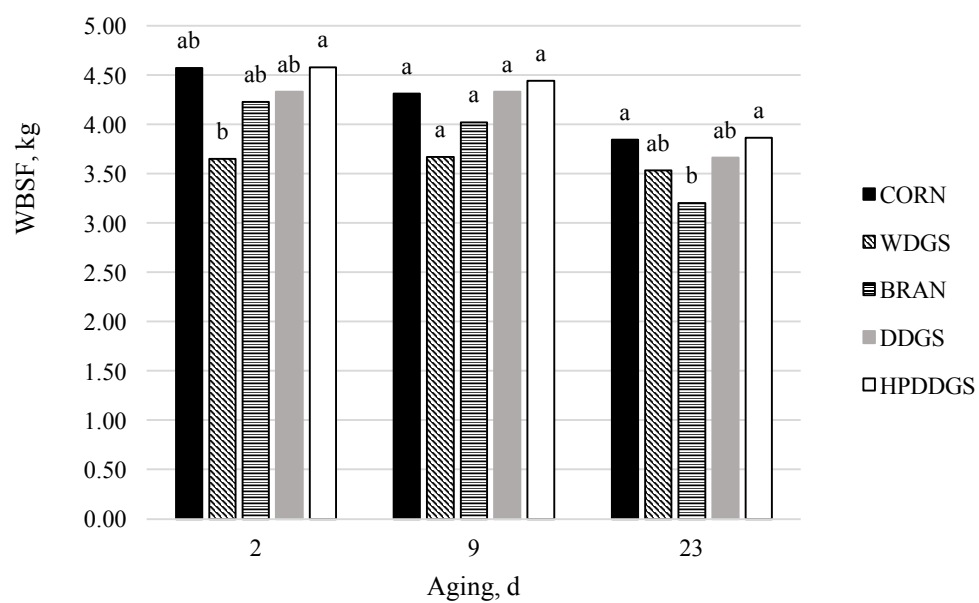
<sup>a-c</sup> Means in the same column with different superscripts are different ( $P < 0.05$ )  $SEM = 0.23$

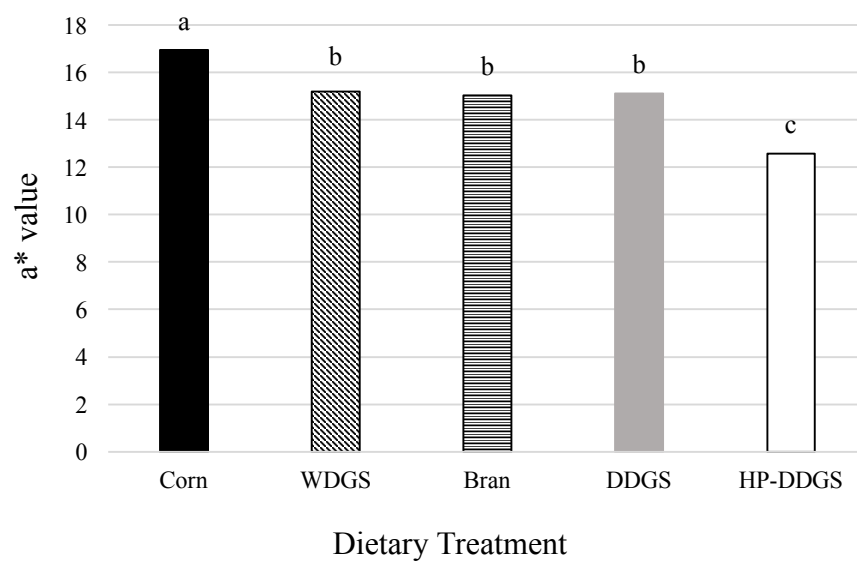
## Figure Legends

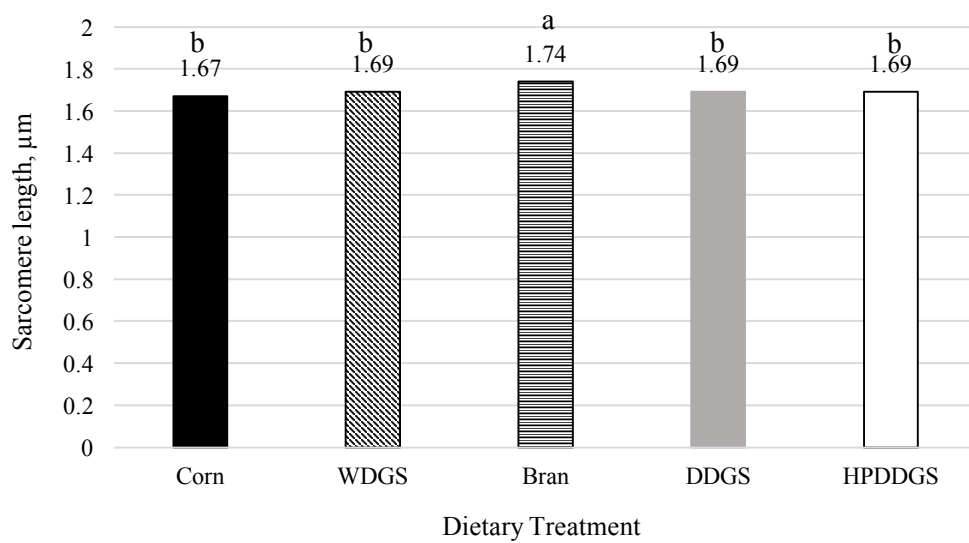
**Figure 1.** Warner-Bratzler shear force (WBSF) of strip loins steaks (*L. lumbarum*) from steers fed dried distillers grains plus solubles (DDGS), high protein dried distillers grains plus solubles (HP-DDGS), wet distillers grains plus solubles (WDGS), corn bran plus solubles (Bran), or a corn control diet with 0 d retail display. Superscripts within the same aging period are different ( $P > 0.05$ ;  $SEM$  Day 2 = 0.24,  $SEM$  Day 9 = 0.26,  $SEM$  Day 23 = 0.17)

**Figure 2.** Treatment by retail display interaction ( $P < 0.001$ ;  $SEM = 0.30$ ) for a\* value of strip loin steaks from all dietary treatments after 7 days of retail display.

**Figure 3.** Sarcomere length from strip loins (*L. lumbarum*) aged for 2 d from steers fed dried distillers grains plus solubles (DDGS), high protein dried distillers grains plus solubles (HP-DDGS), wet distillers grains plus solubles (WDGS), corn bran plus solubles (Bran), or a corn control diet with 0 d retail display. ( $P < 0.10$ ;  $SEM = 0.02$ )

**Figure 1.**

**Figure 2.**

**Figure 3.**

## RECOMMENDATIONS FOR FUTURE RESEARCH

These results indicate that supplementation of 40% high protein distillers grain to cattle will decrease color stability as well as increase lipid oxidation. I believe that more research is needed to understand how varying levels of inclusion could potentially affect these attributes. Interestingly though, a significant increase in PUFA content or other unsaturated fatty acids such as Linoleic (C18:2) or Linolenic (C18:3) acids that are often linked to these types of quality detriments was not found. Therefore, an additional study concentrating on the mechanism that resulted in the increase of lipid oxidation and decreased color stability should be conducted, due to the economic impact that could result. The consumer acceptability of beef from cattle fed high protein distillers grains is still largely unknown, so conducting a consumer taste panel to evaluate these characteristics is imperative to ensure that producers feeding high protein distillers grains to cattle will be able to sell their product.

Additionally, as aging increased, there was a sharp increase in tenderness in steaks from cattle fed Bran. This drastic tenderness difference could not be explained by any of our results, so a future study should attempt to explain potential sources of this change.

To summarize, based on our findings, some suggestions for future research should include:

1. Conduct a feed trial with varying levels of supplementation of high protein distillers grain.



2. Attempt to evaluate alternative mechanisms that result in an increase in lipid oxidation and decreased color stability, without altering the fatty acid profile of the muscle.
3. Conduct a taste panel in order to ensure that there are no negative perceptions by consumers.
4. Examine further the source of increased tenderness in steaks from cattle finished with Bran.

## APPENDIX I.

## Finishing diet composition (Study 1)

**Diet composition (DM basis) fed to finishing steers receiving either dried distillers grains plus solubles (DDGS), high protein dried distillers grains plus solubles (HP-DDGS), wet distillers grains plus solubles (WDGS), corn bran plus solubles (Bran), or a corn control diet.**

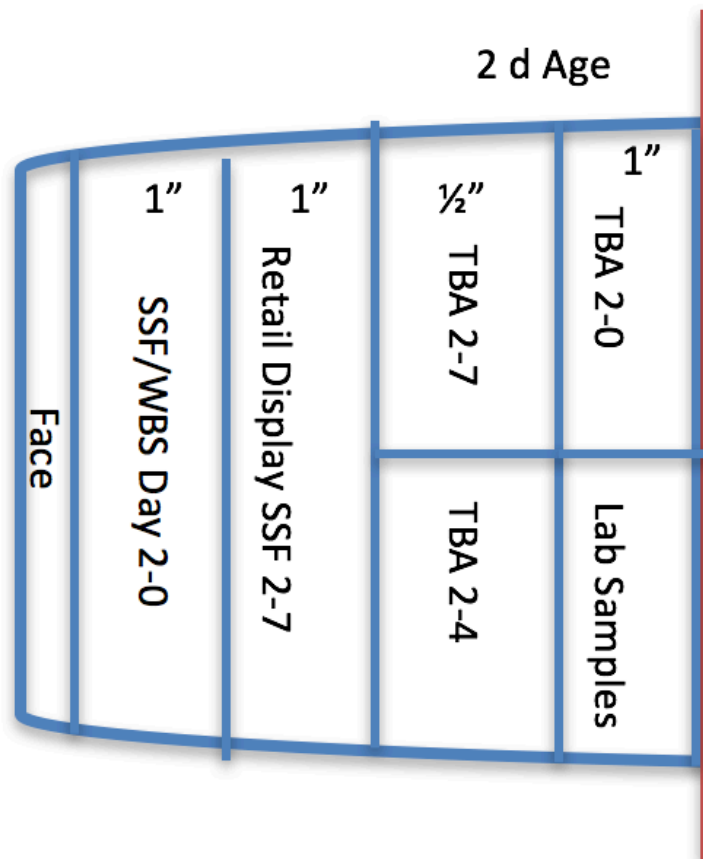
	Corn <sup>1</sup>	DDGS	HP-DDGS	WDGS	Bran
<b>Ingredient, % of DM</b>					
High-moisture Corn	39.25	20.50	20.50	20.50	20.50
Dry-rolled Corn	39.25	20.50	20.50	20.50	20.50
Corn Silage	15.00	15.00	15.00	15.00	15.00
HP-DDGS	-	-	40.00	-	-
DDGS	-	40.00	-	-	-
WDGS	-	-	-	40.00	-
Bran	-	-	-	-	40.00
Supplement <sup>2</sup>	6.5	4	4	4	4
<b>Analyzed Composition %</b>					
DM	-	91.4	91.8	32.8	91.4
Fat	-	6.2	9.4	11.6	6.2
CP	-	32.5	36.0	30.1	32.5
NDF	-	31.6	32.0	30.2	31.6

<sup>1</sup> Corn: Corn-based control diet with 50:50 blend of high-moisture and dry-rolled corn; DDGS: Dry distillers grains plus solubles; HP-DDGS: High Protein distillers grains; WDGS: Wet distillers grains plus solubles; Bran: Corn bran plus condensed distillers solubles

<sup>2</sup> Formulated to contain 30 g/ton Rumensin and 8.8 g/ton Tylan

## APPENDIX II.

Fabrication map (followed for all aging periods)



### APPENDIX III.

Discoloration (%) of strip loin steaks (*L. lumbarum*) from steers fed either a corn control diet, 40% high protein dry distillers grains plus solubles (HP-DDGS), 40% dry distillers grains plus solubles (DDGS), 40% wet distillers grains plus solubles (WDGS), or 40% bran plus solubles (Bran) with 2 d of aging at all d of retail display.

Days in retail display	0	1	2	3	4	5	6	7
Dietary treatments								
Control	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.11 <sup>b</sup>	0.29 <sup>e</sup>	0.72 <sup>b,c,d,e</sup>	1.59 <sup>b,c,d,e</sup>
WDGS	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.25 <sup>e</sup>	0.47 <sup>c,d,e</sup>	1.77 <sup>b,c,d,e</sup>	4.74 <sup>b,c,d</sup>
Bran	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.02 <sup>e</sup>	0.07 <sup>e</sup>	1.06 <sup>b,c,d,e</sup>	2.89 <sup>b,c,d,e</sup>
DDGS	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.15 <sup>e</sup>	0.59 <sup>b,c,d,e</sup>	1.92 <sup>b,c,d,e</sup>	4.82 <sup>b,c</sup>
HP-DDGS	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.00 <sup>e</sup>	0.03 <sup>e</sup>	0.44 <sup>ed</sup>	4.86 <sup>b</sup>	15.32 <sup>a</sup>

<sup>a-e</sup>Means with different superscripts differ ( $P < 0.05$ )  $SEM = 0.78$

**Discoloration (%) of strip loin steaks (*L. lumbrorum*) from steers fed either a corn control diet, 40% high protein dry distillers grains plus solubles (HP-DDGS), 40% dry distillers grains plus solubles (DDGS), 40% wet distillers grains plus solubles (WDGS), or 40% bran plus solubles (Bran) with 9 d of aging at all d of retail display.**

Days in retail display	0	1	2	3	4	5	6	7
Dietary treatments								
Control	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.09 <sup>c</sup>	0.13 <sup>c</sup>	0.68 <sup>c</sup>	1.68 <sup>b,c</sup>
WDGS	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.06 <sup>c</sup>	0.31 <sup>c</sup>	1.64 <sup>b,c</sup>	5.67 <sup>b,c</sup>
Bran	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.29 <sup>c</sup>	0.87 <sup>b,c</sup>	3.26 <sup>b,c</sup>	6.79 <sup>b,c</sup>
DDGS	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.03 <sup>c</sup>	0.15 <sup>c</sup>	1.92 <sup>b,c</sup>	4.83 <sup>b,c</sup>
HP-DDGS	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.18 <sup>c</sup>	1.74 <sup>b,c</sup>	7.67 <sup>b</sup>	19.89 <sup>a</sup>

<sup>a-c</sup>Means with different superscripts differ ( $P < 0.05$ )  $SEM = 1.25$

**Discoloration (%) of strip loin steaks (*L. lumbrorum*) from steers fed either a corn control diet, 40% high protein dry distillers grains plus solubles (HP-DDGS), 40% dry distillers grains plus solubles (DDGS), 40% wet distillers grains plus solubles (WDGS), or 40% bran plus solubles (Bran) with 23 d of aging at all d of retail display.**

Days in retail display	0	1	2	3	4	5	6	7
Dietary treatments								
Control	0.00 <sup>g</sup>	0.00 <sup>g</sup>	0.00 <sup>g</sup>	0.01 <sup>g</sup>	0.49 <sup>g</sup>	3.13 <sup>g</sup>	15.75 <sup>f,g</sup>	38.85 <sup>d,e</sup>
WDGS	0.00 <sup>g</sup>	0.00 <sup>g</sup>	0.07 <sup>g</sup>	0.08 <sup>g</sup>	1.40 <sup>g</sup>	6.02 <sup>g</sup>	34.45 <sup>d,e,f</sup>	63.63 <sup>b,c</sup>
Bran	0.00 <sup>g</sup>	0.00 <sup>g</sup>	0.00 <sup>g</sup>	0.04 <sup>g</sup>	2.81 <sup>g</sup>	18.98 <sup>e,f,g</sup>	46.24 <sup>c,d</sup>	68.30 <sup>a,b</sup>
DDGS	0.00 <sup>g</sup>	0.00 <sup>g</sup>	0.00 <sup>g</sup>	0.11 <sup>g</sup>	2.27 <sup>g</sup>	13.89 <sup>f,g</sup>	45.05 <sup>c,d</sup>	74.19 <sup>a,b</sup>
HP-DDGS	0.00 <sup>g</sup>	0.00 <sup>g</sup>	0.00 <sup>g</sup>	0.27 <sup>g</sup>	1.64 <sup>g</sup>	16.30 <sup>f,g</sup>	64.31 <sup>b,c</sup>	85.79 <sup>a</sup>

<sup>a-g</sup>Means with different superscripts differ ( $P < 0.05$ )  $SEM = 3.75$

## **APPENDIX IV.**

### **Fat extraction with Soxhlet method**

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

1. Check ground glass connections. They should be wiped clean with a dry paper towel and given a thin coating of stopcock grease.
2. Each boiling flask must contain boiling stones. This helps prevent violent boiling of the solvent which could be dangerous.
3. Load samples into soxhlet tubes, arrange them so that no samples are above the level of the top bend in the narrower tubing on the outside of the soxhlet. (The soxhlet will only fill with the solvent up to this point before cycling back down into the boiling flask.) In general, the large soxhlets will hold about 20 two-gram samples and the small soxhlets from 4-6.
4. Fill the large (500ml) boiling flasks with 400ml of solvent and the small (125ml) flasks with 100ml of solvent. Do this under the fume hood.
5. Fit the soxhlet onto the boiling flask. Very carefully, bring the assembly into the extraction room and fit it onto the condenser. Make sure all ground glass connections are snug and each boiling flask is resting on the heating element. The ceramic fiber sheet should be covering the bare metal surfaces on the burners completely.
6. Turn on the water supply to the condensers (usually a quarter turn). Check later to make sure condensers are cool enough – if not, increase water flow.

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

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



## APPENDIX V.

### Objective color ( $L^*$ , $a^*$ , $b^*$ ) calibration instructions and helpful tips

#### Minolta Calibration Procedures

##### 1. Before Calibration:

Calibrate Minolta in the same temperature conditions as the measurements being taken.

- Place the Minolta in the environment where samples will be measured about 5 or 10 minutes before calibrating so it can become equilibrated with the temperature.

Calibrate with same materials as you will be taking measurements.

- If the measurements will not be taken directly on the meat surface, you must calibrate the Minolta with the same material it will be measuring through. For example, if you want to take readings from samples that are wrapped in overwrap, you must put some overwrap around the measuring head “eye” while calibrating using the white tile.

##### 2. Turn the power to the measuring head *ON*.

3. Turn the power to the data processor *ON* while holding down the [DELETE/UNDO] key at the same time.

- Release the [DELETE/UNDO] key when you hear a BEEP. (This deletes any previous data that might still be stored in the data processor)

4. When the screen turns on, the question “Initial set ok?” appears, press the [Measure Enter] key.

5. Once you get to the measurement screen, press the [Index Set] key.

- Use the *arrows and the [Measure Enter] key* to adjust all the following settings:
  - Printer: On
  - Color space: Off
  - Protect: On
  - Auto Average: However many readings wanted per sample (1-30)
  - Illuminant: D65
  - Back light: Off
  - Buzzer: On
  - Disp. Limit
- Press the [Esc] key to return to the measurement screen.

6. Press the [Calibrate] key while in the measurement screen.

7. Enter in the numbers listed on the calibrating white tile for the D65 setting using the following:

[< >] keys and the numeric pad  
 - (The [< >] keys move the cursor)  
 - D65 settings:  
 Y: 93.13 x: 0.3164 y: 0.3330

8. Set up the measuring head so that it is resting on the LCD screen and the “eye” is facing up.

- Place the white calibration tile on the measuring head, near the middle of the tile.

9. Press either the measurement button on the measuring head OR the [Measure Enter] key on the data processor after making sure the ready lamp is ON.

- Make sure the white tile is completely on the measuring head “eye”.
- The Calibration is complete after the lamp flashes 3 times and the screen returns to the measurement screen.
- Do not move the measuring head during calibration.

10. Press the [Color Space] key until the L\*, a\*, b\* screen shows up.

11. Calibration is finished and the Minolta is ready

- To save battery life, turn both the measurer and data collector off after calibration is finished until you need it for measuring. The calibration and settings will not be erased.
- When turning back on for measurements, ONLY turn on the power buttons. DO NOT hold down the [DELETE/UNDO] key at the same time. This will delete the calibration and settings and all of the steps will have to be repeated.

## Cleaning

- Wipe machine down with a soft, clean dry cloth. Never use solvents such as thinner or benzene.
- If the white calibration tile becomes dirty, wipe it gently with a soft, clean dry cloth. If dirt is difficult to remove, wipe it with lens cleaner and cloth, then dry.

### Minolta Helpful Tips

#### 1. Make a separate data sheet

- The Minolta prints out data with sample numbers 1 to 2000. In order to correlate it back to a sample, you must make a separate data sheet that has a place to record meat sample i.d. and its corresponding Minolta number.

#### 2. Batteries

- The measuring head requires 4 AAA batteries and the data processor requires 4 AA batteries

#### 3. The auto protect setting

- The Minolta can only record and store up to 2000 readings, once you go past 2000 readings it will start deleting older readings.
- When the auto protect is on it will automatically prevent the 2001<sup>st</sup> reading from being taken so you cannot accidentally overwrite other data.

#### 4. Auto Average Function

- During calibration, if you set the Auto Average function to a reading number above 1, for example 5, you only have to hit the measure button once and it will automatically take all 5 readings then print out the average.
- It only allows a second or two between readings so make sure you are paying attention and move the measuring head to where you want it before it automatically takes the next reading.

#### 5. Recalibrate regularly

- If using the Minolta all day, or for long periods of time, make sure to recalibrate it regularly.

#### 6. DELETE/UNDO KEY

- If you accidentally take a reading, hitting the [DELETE/UNDO] key will delete the last reading.
- If you accidentally delete a reading by hitting the [DELETE/UNDO] key, hitting the [DELETE/UNDO] key again will restore the previous reading.

## 7. Printer Paper

- The paper that the data is printed on is sensitive to heat and light. The printed data should be kept in a dark cool place, like a desk drawer. In order to prevent losing any data, you must make a photocopy of the printout in order to preserve it for long-term storage.

## 8. More than One Color Space on Print Out

If you want to print more than 1 color space (Example: L\*a\*b\* AND XYZ) on the print out slip:

1. Press the [Index Set] key. Use the arrows and the [Measure Enter] key to adjust all the following settings:
  - o Color space: On
  - o Disp. Limit: press the [Measure Enter] key to select this option
  - o Once inside the Disp. Limit option, go through the list and change all the color spaces that you DO NOT want to OFF.
2. Press the [Esc] key until you return to the measurement screen.

## 9. Change Measurements to a Different Color Space

If you get done measuring and realize that you meant to measure in a different color space (For example: measured everything using Yxy and meant to use L\*a\*b\*), you can correct it using these steps:

1. While in the measurement screen, press the [Color Space] key until your desired color space (in this example: L\*a\*b\*) appears.
2. Press the [Data List] key while in the measurement screen.
3. Select the desired page using the up and down arrows.
  - o If you only have one page it will show up as P00, select this one.
4. Once you have the desired page selected, press the [Measure Enter] key.
5. Press the [Print/Feed] key
6. Select “All Meas. Data” using the up and down arrows.
7. Press the [Measure Enter] key
  - o This will reprint all the stored data in your newly selected color space (L\*a\*b\* in this example).
8. Press the [Esc] key to return to the measurement screen.

**APPENDIX VI.****Visual guide for percent surface discoloration****0%****5%****10%****20%****30%****40%****50%****60%**



**70%**



**80%**



**90%**



**100%**

## APPENDIX VII.

### 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 $\mu$ L TEP (97%) bring volume to 100 mL ddH<sub>2</sub>O

Working Solution: Dilute stock solution to 1:3 (TEP Solution:ddH<sub>2</sub>O) ( $1 \times 10^{-3}$  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<sub>2</sub>O

Dissolve 2.88 g TBA in warm ddH<sub>2</sub>O first, then add TCA (150g) and ddH<sub>2</sub>O to 1L

#### BHA (Butylated HydroxyAnisole) Stock Solution:

Make 10% stock solution by dissolving in 90% ethanol

10g BHA dissolved in 90mL ethanol (90%) + 5mL ddH<sub>2</sub>O

#### Standards: In Duplicate

		<u>Moles of TEP</u>
Blank:	1 mL ddH <sub>2</sub> O	
Standard 5:	100 $\mu$ L working TEP + 1.90 mL ddH <sub>2</sub> O	( $5 \times 10^{-5}$ M)
Standard 4:	1 mL Std. 5 + 1 mL ddH <sub>2</sub> O	( $2.5 \times 10^{-5}$ M)
Standard 3:	1 mL Std. 4 + 1 mL ddH <sub>2</sub> O	( $1.25 \times 10^{-5}$ M)
Standard 2:	1 mL Std. 3 + 1 mL ddH <sub>2</sub> O	( $0.625 \times 10^{-5}$ M)
Standard 1:	1 mL Std. 2 + 1 mL ddH <sub>2</sub> O	( $0.3125 \times 10^{-5}$ M)

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

#### TBA Procedure:

1. Mix all reagents and standards before beginning.
2. Transfer 5g powdered sample into a 50mL conical tube; add 14 mL of ddH<sub>2</sub>O and 1.0 mL of BHA.
3. Homogenize for 15 sec with a polytron.
4. Centrifuge for 2000xg for 5 min.
5. Transfer 1mL of homogenate or standard to 15mL 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 cold water bath for 10 min.
9. Centrifuge tubes at 2000xg 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 x 10<sup>-8</sup> moles 1, 1, 3,3-tetraethoxypropane)/5ml

A= Absorbance of standard

MW = MW of malonaldehyde

E = sample equivalent

P = percent recovery

Final Calculation: .012 x concentration x 72.063 x 10<sup>-6</sup> = mg of Malonaldehyde/kg tissue

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

Ahn, D. U., D. G. Olsen, C. Jo, X. Chen, C. Wu, and J. I. Lee. 1998. Effect of muscle type, packaging, and irradiation on lipid oxidation, volatile production, and color in raw pork patties. *Meat Sci.* 49:27-39.

Buege, J. A. and Aust, S. D. 1978. Microsomal Lipid Peroxidation. *Methods in Enzymology.* 52:302-310.



## APPENDIX VIII.

### Fatty acid determination

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

1. Add 5 mL of 2:1 chloroform:methanol (v/v) for muscle tissue or 3 mL for subcutaneous fat.
2. Vortex for 5 s and let stand for 1 h at room temperature.
3. Filter homogenate through Whatman #2 filter paper into 13 x 150 mm screw cap tube bringing the final volume with chloroform:methanol to 10 mL for muscle lipid and 5 mL for subcutaneous fat extract. If stopping at this point, purge test tube with nitrogen, cap tube, and store at -80°C.
4. Add 2 mL of a 0.74% KCl solution for muscle lipid extract or 1 mL for subcutaneous fat tissue extract and vortex for 5 s. If stopping at this point, purge test tube with nitrogen, cap tube, and store at 0°C for no more than 24 h.
5. Centrifuge samples at 1000 x g for 5 min. Following centrifugation, aspirate off the aqueous phase (top layer). If stopping at this point, purge test tube with nitrogen, cap tube, and store at -80°C.
6. Evaporate to dryness under nitrogen at 60°C.
7. Add 1 mL of a 0.5 M NaOH in methanol. Vortex for 5 sec. Heat for 10 min at 100°C.
8. Add 1 mL of boron trifluoride in 14% methanol. Vortex for 5 sec. Heat for 5 min at 100°C.
9. Add 2 mL of a saturated salt solution and 2 mL of hexane. Vortex for 5 sec.
10. Centrifuge samples at 1000 x g for 5 min. Following centrifugation, remove hexane layer (top layer) **making sure not to disrupt the aqueous phase** (lower layer) and place in GC vial. Purge GC vial with nitrogen, cap and crimp cap, and store at -80°C until sample is ready to be read on the GC.

### GC Settings

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

**Injector Temp-** 270°C

**Detector Temp-** 300°C

**Head Pressure-** 40 psi

**Flow Rate-** 1.0 mL/min

**Temperature Program-** Start at 140°C and hold for 10 min. Following 10 min, raise temperature 2°C/min until temperature reaches 220°C. At 220°C, hold for 20 min.

## **APPENDIX IX.**

### **MATERIALS AND METHODS**

#### **Potential Errors in Determination of Longissimus Muscle Area in Carcasses from Heifers Fed with or without Zilpaterol Hydrochloride**

##### **Cattle and dietary treatments**

Twenty English-influenced crossbred heifers (initial BW =  $556 \pm 7$  kg BW) from the Eastern Nebraska Research and Extension Center feedlot were fed either a corn control diet (Control; n = 10), or a diet containing zilpaterol hydrochloride (ZH; n = 10) in December 2013. Heifers were fed in individual tie stalls and randomly assigned to treatment. Heifers were blocked by treatment (two heifers per block) so water was not shared across treatments. The ZH diet fed the same Control diet supplemented with ZH at 8.33 mg/kg BW on a DM basis. Heifers were acclimated for a 3-wk period prior to the study. During acclimation, heifers were haltered and acclimated to being restrained in a tie stall environment and to human contact. For delivery of ZH, five percent of the high-moisture corn in the diet was replaced with 4.9853% fine ground corn and ZH at 0.0147% calculated to supply ZH at 8.33 mg/kg on a DM basis. Five percent of the Control diet was replaced with fine ground corn to ensure similarity between the two diets. All supplements were individually mixed into individual heifer's daily allotment prior to feeding. Heifers were fed once daily for 20 d followed by a 3-d withdrawal period of ZH.

### **Sample collection**

On d 25, 26, and 27, heifers were harvested at the Loeffel Meat Laboratory located at the University of Nebraska – Lincoln. Heifers were assigned randomly to one of the three harvest days, and within days, harvest order was alternated based on treatment resulting in extended withdrawal times for heifers harvested on different days (4, 5, and 6 d post ZH supplementation). Rib-loin sections were cut caudal to the 13<sup>th</sup> rib and cranial to the 11<sup>th</sup> rib from 10 carcasses: 5 from heifers supplemented with ZH and 5 from heifers from the Control treatment. Consecutive slices (3-4 mm thick) from each rib-loin section were cut at 90 degrees to the long axis of the longissimus muscle on a band saw. To ensure structural integrity, the sections were frozen and tempered so that the muscles remained firm during cutting.

### **Image collection**

Each slice (3-4 mm) was placed on a stationary platform below a camera stand and images were captured using a digital Nikon D5100 camera (Lens: Nikon AF-S DX VR Zoom-Nikkor 55-200mm f/4-5.6G IF-ED). An image of a USDA beef ribeye grid was also obtained to ensure accurate calibration of longissimus muscle area (LMA). The LMA was traced using a tablet computer (ASUS), allowing for image magnification to ensure accurate tracings were made. The LMA was determined for each slice. Rib angles were measured on carcasses in the Loeffel Meat Lab at the University of Nebraska-Lincoln using a protractor (56854-15BK, Staedtler, Nuremburg). Ribeye images were printed and Levator costae area was calculated using a digital planimeter (Tamaya Planix 6, Sokkia

Corp., Overland Park, KS). A USDA beef ribeye grid was measured to calibrate muscle area.

### **Statistical analysis**

Statistical analysis was done with SAS (version 9.4, Cary, NC). Longissimus muscle area data were analyzed as a completely random design with animal being considered the experimental unit. All data were analyzed using the PROC GLIMMIX procedure of SAS. Means were separated with the LS MEANS and DIFF functions with  $\alpha = 0.05$ . Alpha levels between 0.05 and 0.10 were considered to be trends.

## **APPENDIX X.**

### **Potential Errors in Determination of Longissimus Muscle Area in Carcasses from Heifers Fed With or Without Zilpaterol Hydrochloride**

The author acknowledges the assistance of C.R. Calkins, J.J. O'Daniel, G. Konda Naganathan, K.I. Domenech-Perez, T.B. Schmidt, and J. Kammermann in the research discussed in the following chapter.

## ABSTRACT

This study was conducted to evaluate certain sources of potential variation in determination of longissimus muscle area (LMA) in strip loins of 10 carcasses from heifers fed with or without zilpaterol hydrochloride (ZH). Cross sections of the rib-loin were taken cranial to the 11<sup>th</sup> rib and caudal to the 13<sup>th</sup> rib, 3-4 millimeters thick at 90 degrees perpendicular to the long axis of the longissimus muscle. Natural variation of LMA from ribs 11 – 13 was 17.5%. Up to 9% LMA variation occurred between the 12<sup>th</sup> and 13<sup>th</sup> rib. Deviation in cutting perpendicular to the long axis of the muscle could contribute 6.9% error in LMA. There was a trend ( $P = 0.09$ ) that the loins from heifers supplemented with zilpaterol hydrochloride had larger mean longissimus muscle area than those not supplemented (102.1 sq. cm and 93.0 sq. cm, respectively). There were no differences ( $P = 0.53$ ) in the range of LMA among carcasses from heifers fed zilpaterol hydrochloride and controls. These data reinforce the written directions of the USDA to separate the longissimus muscle between the 12<sup>th</sup> and 13<sup>th</sup> ribs by a cut as close to 90 degrees as possible.

**Key words:** beef, longissimus, ribeye area, yield grade, zilpaterol hydrochloride

## INTRODUCTION

In order to determine yield grade in beef, sides are split between the 12<sup>th</sup> and 13<sup>th</sup> ribs to expose the longissimus muscle. The total longissimus muscle area (LMA) at that location is used in calculating a final yield grade. Yield grade impacts carcass value by influencing estimates of carcass cutability or as an estimate of the relative amount of lean meat (Tatum, 2007). Belk et al. (1998) surmised that when the current USDA yield grade system is utilized perfectly, it is reliable in assigning carcasses based on composition, however, they found that there is a large amount of variation that can be introduced during this process. Several studies have focused on the variability of LMA introduced by the individual determining the muscle area, however, exceedingly few examine the natural variation due to the shape of the muscle (Borggaard et al., 1996; Belk et al., 1998; Cannell et al., 1999; Steiner et al., 2003). Hedrick et al. (1965) and Steiner et al. (2003) indicated that a large amount of LMA variation can occur within the same carcass if the sides are not ribbed at the same angle. Additionally, the supplementation of zilpaterol hydrochloride (ZH) to cattle in the finishing stage has been shown to increase HCW as well as LMA (Plascencia et al., 1999; Vasconcelos et al., 2008; Hilscher et al., 2015) Montgomery et al. (2009) indicated that supplementing cattle with ZH for 20 d increased LMA by 7.2 sq. cm. Similar results were described by Hilton et al. (2009) when they determined that steers supplemented with ZH for 30 d resulted in an 8.8 sq. cm increase in LMA. Understanding the natural variation of the muscle shape, as well as the potential error introduced during ribbing, is vital to ensuring that the USDA yield grading program remains an accurate part of a value-based marketing system. It is also necessary to understand how growth promotants can alter muscle shape and size. This study was conducted to evaluate variation

that could arise when determining LMA, including supplementation of ZH, location of ribbing, and ribbing angle.

## **MATERIALS AND METHODS**

### ***Fabrication and LMA determination***

Rib-loin sections were cut caudal to the 13<sup>th</sup> rib and cranial to the 11<sup>th</sup> rib from 10 carcasses: 5 from heifers supplemented with zilpaterol hydrochloride (ZH) (8.33 mg/kg of dry matter) and 5 from heifers not supplemented with ZH (CON). Consecutive slices (3-4 mm thick) from each rib-loin section were cut at 90 degrees to the long axis of the longissimus muscle on a band saw. To ensure structural integrity, the sections were frozen and tempered so that the muscles remained firm during cutting. Each slice was placed on a stationary platform below a camera stand and images were captured using a digital Nikon D5100 camera (Lens: Nikon AF-S DX VR Zoom-Nikkor 55-200mm f/4-5.6G IF-ED). An image of a USDA beef ribeye grid was also obtained to ensure accurate calibration of LMA. The LMA was traced using a tablet computer (ASUS), allowing image magnification to ensure accurate tracings were made. Ribeye images were printed and Levator costae area was calculated using a digital planimeter (Tamaya Planix 6, Sokkia Corp., Overland Park, KS). A USDA beef ribeye grid was measured to calibrate muscle area. The LMAs were determined for all slices. Rib angles were measured on carcasses in the Loeffel Meat Lab at the University of Nebraska-Lincoln using a protractor (Staedtler 56854-15BK).



### *Statistical Analysis*

Data were analyzed as a completely random design with animal being considered the experimental unit. All data were analyzed using the PROC GLIMMIX procedure of SAS. Means were separated with the LS MEANS and DIFF functions with  $\alpha = 0.05$ . Alpha levels between 0.05 and 0.10 were considered to be trends.

## **RESULTS AND DISCUSSION**

Heifers supplemented with zilpaterol hydrochloride tended ( $P = 0.09$ ; Table 1) to have a larger LMA than control heifers (102.1 sq. cm and 92.9 sq. cm, respectively). These data are consistent with multiple research studies showing that supplementation of zilpaterol hydrochloride increases LMA in cattle (Casey et al., 1997; Plascencia et al., 1999; Montgomery et al., 2009; Arp et al., 2014). There were no differences ( $P = 0.53$ ) in the range of longissimus muscle area among carcasses from heifers fed ZH and controls.

The location of ribbing between the 12<sup>th</sup> and 13<sup>th</sup> rib can cause a large amount of variation in LMA. Between the 12<sup>th</sup> and 13<sup>th</sup> ribs, the mean LMA was 99.4 sq. cm and the mean range in LMA was 8.9 sq. cm (Table 2). These data also indicate that as the longissimus muscle approaches a rib, the LMA will decrease, and later increase once past the location of the rib (Figure 1). The mean LMA at the location of the 12<sup>th</sup> rib was 98.6 sq. cm. Similar results were reported by Stouffer (1961), who ribbed cattle in 5 different locations between the 12<sup>th</sup> and 13<sup>th</sup> ribs and indicated a significant difference in LMA between the separate locations. The midpoint between the 12<sup>th</sup> and 13<sup>th</sup> rib in their study showed the larger LMA (75.0 sq. cm) with decreasing LMA as the location moved nearer the ribs measuring 66.0 sq. cm at the 12<sup>th</sup> rib and 70.8 sq. cm at the 13<sup>th</sup> rib (Stouffer, 1961).

Our results indicate that the most consistent LMA was located in the posterior half from the midpoint between the 12<sup>th</sup> and 13<sup>th</sup> ribs, with a mean range LMA of only 0.75 sq. cm. In this study, depending upon the location of the cut between the 12th and 13th ribs, the LMA could vary by as much as 9.0%. This equates to approximately 0.4 yield grade units. That is, a carcass that should receive a yield grade of 3.2 could present a LMA supporting a grade of 2.8.

Part of the variation in LMA could additionally occur because of the presence of the Levator costae muscles. These muscles are largest in cross-section between the ribs and very small in cross-sections near the rib. The maximum mean muscle area of the Levator costae in between the 13<sup>th</sup> and 12<sup>th</sup> rib was 5.0 sq. cm, while the maximum mean area between the 11<sup>th</sup> and 12<sup>th</sup> ribs was 7.7 sq. cm. Inclusion of this muscle in calculation of LMA could potentially lead to incorrect reporting of LMA by up to 5%. The function of the Levator costae muscles is to aid in respiration by allowing movement of the lower ribcage (Goldman et al., 1985). They originate at the tip of the transverse process of the thoracic vertebrae and terminate at the anterior edge of the following rib, spanning the distance of one rib to another (Goldman et al., 1985). Images of the Levator costae muscles can be seen in Figures 2, 3, and 4.

Additional inaccuracy could occur by failing to cut “across the ribeye muscle perpendicular to the outside skin surface of the carcass at an angle toward the hindquarter which is slightly greater (more nearly horizontal) than the angle made by the 13th rib”, as stated in section 54.104 of the United States Standards for Grades of Carcass Beef by the USDA (USDA, 2016). An angle of 68 degrees (22 degrees from the desired 90 degree angle) can be created by closely following the curvature of the 13th rib, potentially

overestimating LMA by 7.9%. In this study, an incorrect cutting angle could overestimate LMA as much as 7.7 sq. cm, an additional 0.4 yield grade units. Results reported by Carpenter and Palmer (1961) also showed that ribbing the carcass as described by the USDA compared to following the contour of the 12<sup>th</sup> rib altered LMA by 4.4 sq. cm. Collectively, variation of muscle size and improper ribbing could alter LMA as much as 16.8 sq. cm (16.9%), the equivalent of 0.8 yield grade units.

## **CONCLUSIONS**

Feeding ZH to heifers tended to increase the LMA in this study. Variation of LMA can arise from the natural variation of muscle size up to 17.7%. Due to the combination of LMA variation and improper ribbing technique, LMA could vary by as much as 16.9% between the 12<sup>th</sup> and 13<sup>th</sup> ribs. These data reinforce the written directions of the USDA to separate the longissimus muscle between the 12<sup>th</sup> and 13<sup>th</sup> ribs by a cut as close to 90 degrees as possible.

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**Table 1. Mean longissimus muscle area measurements, in sq. cm., from heifers fed with or without zilpaterol hydrochloride across an entire rib-loin section. ( $P = 0.09$ ;  $n=10$ ).**

Treatment	Mean LMA	Mean LMA standard deviation	Mean maximum within a carcass	Mean minimum within a carcass	Mean LMA range within a carcass	Mean LMA range standard deviation
Control	92.9	3.2	100.6	84.5	16.1	4.5
Zilpaterol Hydrochloride	101.9	10.3	109.7	91.6	18.1	5.2
All Treatments	97.4	7.1	105.2	87.7	17.4	4.5
<i>SEM</i>	3.41		5.16	3.16	2.13	
<i>P-value</i>	0.09		0.12	0.15	0.53	

**Table 2. Mean longissimus muscle area measurements, in sq. cm., from heifers fed with or without zilpaterol hydrochloride between the 12<sup>th</sup> and 13<sup>th</sup> ribs ( $P > 0.10$ ; n=10)**

Treatment	Mean LMA between 12 <sup>th</sup> and 13 <sup>th</sup> ribs	Mean LMA standard deviation between 12 <sup>th</sup> and 13 <sup>th</sup> ribs	Mean maximum within a carcass	Mean minimum within a carcass	Mean LMA range within a carcass	Mean LMA range standard deviation
Control	94.8	5.2	98.7	90.3	7.7	2.6
Zilpaterol Hydrochloride	103.9	11.0	108.4	98.7	9.7	1.9
All Treatments	99.4	7.7	103.2	94.8	9.0	2.6
<i>SEM</i>	2.96		3.12	2.80	0.76	
<i>P-value</i>	0.12		0.11	0.15	0.22	



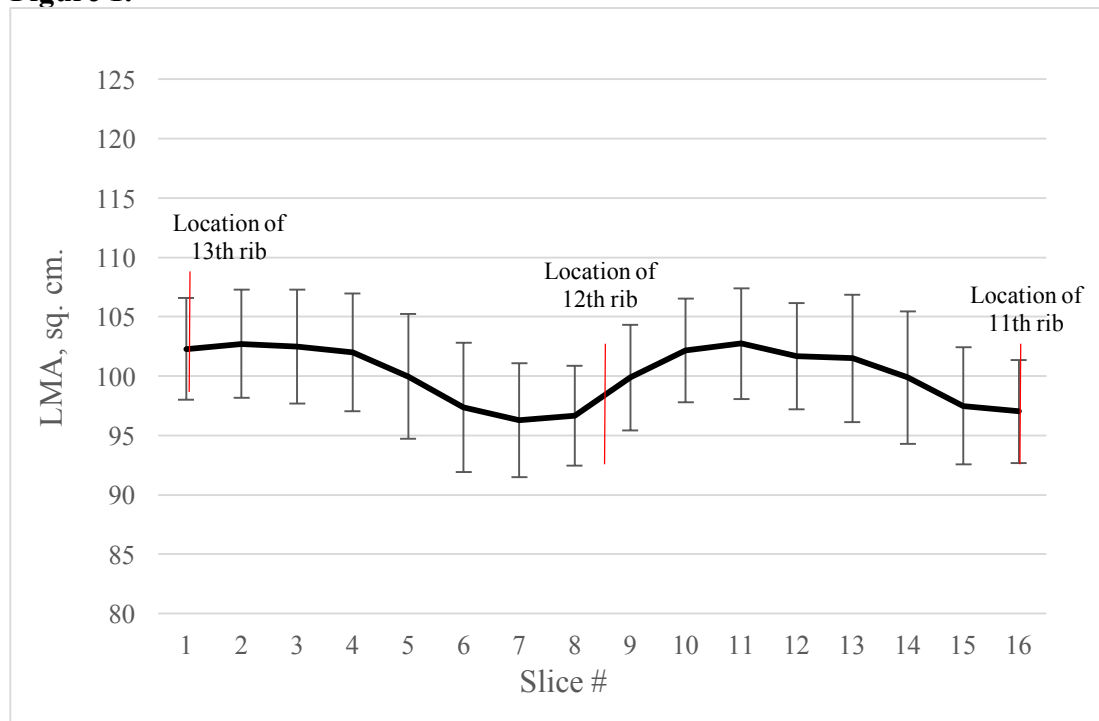
### Figure Legends

**Figure 1.** Mean LMA, sq. cm, from heifers fed with or without zilpaterol hydrochloride between 11<sup>th</sup> and 13<sup>th</sup> ribs.

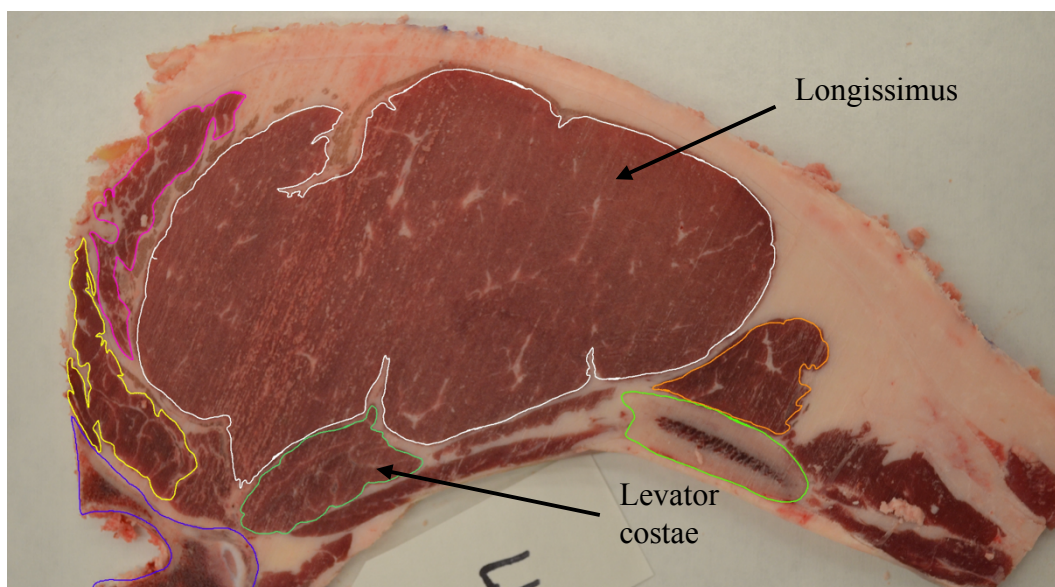
**Figure 2.** Cross section of the Longissimus and Levator Costae.

**Figure 3.** Levator costae muscles adjacent to the 10<sup>th</sup>, 11<sup>th</sup>, 12<sup>th</sup>, and 13<sup>th</sup> ribs. Iliocostalis and Longissimus thoracis removed.

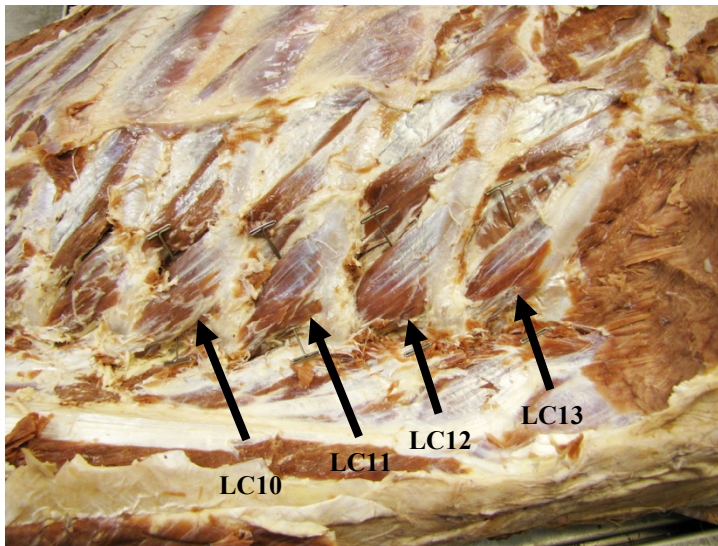
**Figure 4.** Levator costae muscle removed from the 12<sup>th</sup> rib.

**Figure 1.**

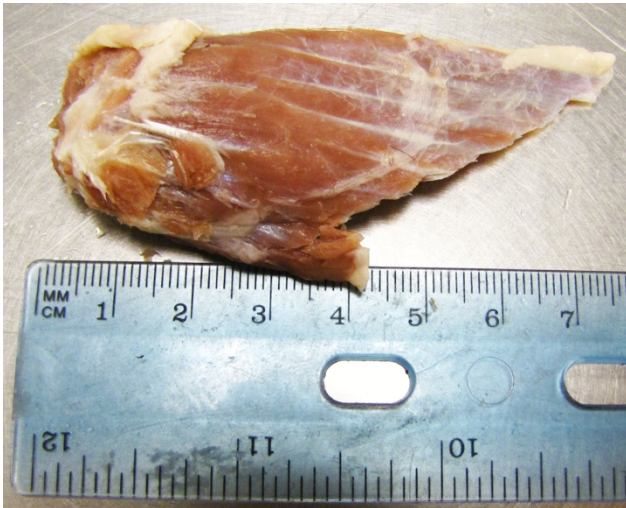
**Figure 2.**



**Figure 3.**



**Figure 4.**



## APPENDIX XI.

## Finishing diet composition (Study 2)

**Diet Composition on a DM basis fed to finishing heifers.**

	Treatment	
	Control	ZH <sup>1</sup>
Ingredient, % of DM		
High Moisture Corn	51.00	51.00
Sweet Bran®	40.00	40.00
Wheat Straw	5.00	5.00
Fine Ground Corn	1.8710	1.8710
Limestone	1.6400	1.6400
Salt	0.3000	0.3000
Tallow	0.1000	0.1000
Beef Trace Mineral	0.0500	0.0500
Rumensin-90	0.0150	0.0150
Vitamin A-D-E	0.0165	0.0165
Tylan-40	0.0075	0.0075
Supplement <sup>2</sup>		
Fine Ground Corn	5.00	4.9853
Zilpaterol Hydrochloride	-	0.0147

<sup>1</sup>Heifers receiving zilpaterol hydrochloride for 20 d with 3 d withdrawal

<sup>2</sup>The control supplement contained fine ground corn only. Zilpaterol hydrochloride (ZH) supplement contained (DM basis) 0.0147% Zilmax® (Merck Animal Health) Type A medicated article and 4.9853% fine ground corn and supplied zilpaterol hydrochloride (ZH) supplementation (8.33 mg / kg on a DM basis). Supplement was fed for 20 d.