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Impact of Finishing Diets with De-Oiled Distillers Grains or Antioxidant Containing Supplement on Beef Shelf Life

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by

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In its entirety this dissertation intended to address the impact of feeding several forms of de-oiled corn distillers grains plus solubles and the effect of supplementing OmniGen-AF as a potential antioxidant source for extending beef shelf life. In study one we learned that despite the de-oiling process, greater inclusion levels (65%, DM basis) of de-oiled wet distillers grains plus solubles (WDGS) causes an increase in polyunsaturated fatty acids (PUFA) similar to the levels obtained with full-fat WDGS. Lower inclusion levels (35 and 50%, DM basis) of de-oiled WDGS have intermediate PUFA content in comparison to 65% de-oiled WDGS, full-fat WDGS and a corn control diet ($P < 0.01$). In study two cattle finished with 50% de-oiled dry distillers grain plus solubles (DDGS) also resulted in an increased PUFA content in muscle in relation to a corn control group ($P < 0.0001$). In this instance treatment by retail display interactions indicated that steaks from cattle on the 50% de-oiled DDGS diet had lower color and lipid stability at prolonged retail display times than did the steaks from cattle on the corn control diet ($P < 0.0001$). In general, the first two studies indicate that even after the de-oiling process and regardless of the moisture content of the distillers grains, feeding corn distillers grains plus solubles increases PUFA content, which in turn negatively impacts beef shelf life.

Therefore, it is important to consider this when utilizing these by-products for finishing rations, the addition of antioxidants may be beneficial to off-set any potential detrimental effects of distillesrs grain on beef shelf life. In study three, OmniGen-AF, a potential antioxidant supplement was evaluated. OmniGen-AF supplementation all through the finishing period caused an increase in PUFA content relative to cattle supplemented only through the receiving phase ($P = 0.01$). Feeding OmniGen-AF all through the finishing phase however did not alter color, lipid stability, or superoxide dismutase activity ($P > 0.05$). Therefore, in order to consider OmniGen-AF as an effective antioxidant source it may need to be fed at a greater concentration (greater than 4g/45.36kg BW/hd/d) or perhaps more potent antioxidants merit evaluation, particularly in feedlot rations utilizing corn distiller by-products.

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INTRODUCTION

As the demand for ethanol fuels increase there is a proportional increase of by-products that further maximize the use of starch-rich crops such as corn. Corn ethanol production generates 1/3 ethanol, 1/3 distillers grains and 1/3 carbon dioxide (Saunders and Rosentrater, 2009b). In the earlier days of ethanol production by-products from this industry where considered as lower-value products. However, currently, distillers grains represent an invaluable feed source for cattle and in 2007 an economic overview of ethanol co-products in Nebraska indicated that 91.2% of cattle on feed in Nebraska utilized ethanol by-products (Waterbury *et al.*, 2009).

Trends seeking to further diversify these by-products have now presented opportunities for superior economic benefits to the livestock industry as well as potential products for human consumption (Mathews and McConnell, 2009; Saunders and Rosentrater, 2009b). One of the newer innovations in ethanol plants has been to incorporate de-oiling steps prior to or after fermentation. Generally, fat content of dry distillers grains has been reported to range anywhere from 3 - 13%, but in most cases the fat content is closer to 8 - 9% fat (Ganesan *et al.*, 2009; Saunders and Rosentrater, 2009a; Winkler-Moser and Breyer, 2011). With new de-oiling techniques the fat content of de-oiled distillers grains has been reduced to as low as 2.1% fat on a dry matter basis (Ganesan *et al.*, 2009). The recovered oil is utilized for a wide range of products; including products for human consumption, biofuels, and commercial feed production (Winkler-Moser and Breyer, 2011). At the same time, the new de-oiled distillers grains are a more attractive feed source for ruminants due to an increase in protein and the extension of ethanol by-product shelf life (Watkins, 2007).

Given the availability and added performance advantages the trend of incorporating various forms of corn distillers grains in Nebraska beef operations continues to increase. Hence, understanding the implications in terms of meat quality associated to feeding corn distillers grains is essential for the continued production of high quality beef in Nebraska. Previous research conducted at the University of Nebraska has noted that there is a linear increase in polyunsaturated fatty acid content as dietary inclusion levels of full-fat wet distillers grains plus solubles increases ($P < 0.01$; Mello *et al.*, 2012a). This ultimately results in increased beef lipid oxidation and decreased shelf life, not favoring the use of greater inclusion levels of full-fat corn distillers grains. However, it has yet to be determined if the de-oiling process would aid in reducing beef polyunsaturated fatty acids and result in decreased lipid oxidation and extension of beef shelf life.

Another element to explore in order to off-set the detrimental effects of lipid oxidation is the use of antioxidants and a deeper understanding of innate antioxidant defense systems in cells. OmniGen-AF (Phibro Animal Health, Quincy, IL), a feed supplement containing a proprietary blend of vitamin and minerals, is designed to augment the innate immune function in cattle by up-regulating specific gene activity. The potential exists that the supplementation with OmniGen-AF could provide much needed clarity on whether supplemental antioxidants could up-regulate cellular antioxidant enzymes to combat oxidation. A great deal of research has focused on secondary antioxidants that can be fed or applied *post-mortem* to retard lipid oxidation with some degree of success. Collectively, meat scientists have primarily focused on measuring secondary oxidative products like malonaldehyde which are derived from

primary oxygen radicals like superoxide and hydrogen peroxide. Although these measures are strongly correlated to the development of off-flavors due to lipid oxidation, limited research documents the role of antioxidant enzymes in muscle foods such as superoxide dismutase, catalase and glutathione peroxidase on protecting lipids against highly reactive forms of oxygen. A deeper understanding of the activity of these antioxidant mechanisms can provide a more profound understanding of how to target lipid oxidation at the primary radical level that would in turn not allow or greatly diminish the formation of secondary radicles associated with poor meat quality.

Therefore, the objectives of these studies are to address the following questions:

1. Does feeding de-oiled wet distillers grains plus solubles at increasing inclusion levels alter fatty acid profiles and beef shelf life in comparison to full-fat wet distillers grains plus solubles or a corn-control diet?
2. Does feeding cattle 50% de-oiled dry distillers grains alter the fatty acid composition and shelf life of beef in comparison to a corn-control diet?
3. Does the supplementation of OmniGen-AF have any impacts on fresh meat quality, particularly lipid oxidation and shelf life?
4. Does the supplementation of OmniGen-AF have any effects on superoxide dismutase activity?

The combination of these studies will address current issues associated with feeding various forms of corn ethanol by-products which play a major role in Nebraska's beef industry. Also, this work looks at beef lipid oxidation from a different perspective that would hopefully light the way to exploring new alternatives of ameliorating meat quality issues relating to oxidative stress with the potential of extending beef shelf life.

LITERATURE REVIEW

Use of corn ethanol by-products in beef production systems

In 2014, ethanol production in the United States set a new high record producing 14.3 billion gallons of ethanol across 29 states. Nebraska currently has 26 operating ethanol plants and is the second largest ethanol producing state after Iowa (Renewable Fuels Association, 2015). As the demand for ethanol fuels increases there is a proportional increase of by-products that further maximize the use of starch-rich crops such as corn. Corn ethanol production generates 1/3 ethanol, 1/3 distillers grains and 1/3 carbon dioxide (Saunders and Rosentrater, 2009b). The distillers grains by-product of the ethanol industry represents an invaluable feed source for cattle and in 2007 an economic overview of ethanol co-products in Nebraska indicated that 91.2% of cattle on feed in Nebraska utilized ethanol co-products (Waterbury *et al.*, 2009).

After the fermentation of corn for ethanol production, which contains 2/3 starch, there is a concentration of nutritional constituents such as protein, fat and fiber that is threefold the original amount of corn (Klopfenstein *et al.*, 2008). Klopfenstein *et al.* (2008) have indicated that protein is increased from 10 to 30%, fat can increase from 4 to 12%, fiber increases from 12 to 36%, and some micronutrients such as phosphorous can also increase from about 0.3 to 0.9% on a dry matter basis comparing corn to distillers grains, respectively. Interestingly, even after the removal of starch (energy source), full-fat distillers grains have more energy per kilogram on a dry matter basis than regular corn and thus are considered a valuable energy source for cattle diets (Klopfenstein *et al.*, 2008; Saunders and Rosentrater, 2009b).

In order to accurately evaluate the economic benefit of feeding distillers grains over un-processed corn, factors involving cattle performance (dry matter intake, average daily gain, feed to gain ratios or days on feed) as well as current corn prices (price of bushel of corn, price of different types of distillers grains, transportation costs, offer and demand or global commodity prices) must be considered (Erickson *et al.*, 2010). With several economic scenarios including distance from the ethanol plants to the feedlot, cattle performance and current co-product and corn prices, Buckner *et al.* (2011) found that there were positive returns of up to $40/head when feedlot cattle were fed WDGS, MDGS and DDGS in relation to a corn control diet given the improved cattle performance parameters, resulting in fewer days on feed with inclusions at 40% (DM). Under these scenarios cattle receiving WDGS outperformed cattle on MWDS and DDGS; however, after adjusting all feed co-products cost on a dry matter basis, the feeding value of WDGS was comparable to that of MDGS. Due to greater drying cost of DDGS and decreased cattle performance, feeding DDGS provided a lower feeding value than distillers grains with greater moisture contents (Buckner *et al.* 2011). Similarly, Tonsor (2006) evaluated different economic scenarios including either WDGS or DDGS at 20% or 40% DM with a range of corn prices and found that as the price of corn increased (particularly over $3.50/bushel) the value of feeding distillers grains over corn also increased. The economic benefits also became more apparent with greater inclusion levels of distillers grains, in some instances representing increases of up to $50/head with inclusions of 40% vs. 20% (Tonsor, 2006).

One economic benefit that favors the use of distillers grains is the fact that due to their nutrient concentration after milling, feedlot rations can sometimes eliminate or

reduce costs associated with some micro-nutrients or supplements given the greater nutrient density of distillers grains in comparison to un-processed corn (Mathews and McConnell, 2009). Conversely, sulfur content is also increased from 0.1 - 0.15% to 0.7% in the milling process which, combined with residues from sulfuric acid used to control pH and for cleaning purposes in ethanol plants, can cause polioencephalamalcia (polio) resulting in damage to the central nervous system that could result in death or poor performance thereafter even if treated on time (Erickson *et al.*, 2010). Vannessa *et al.* (2009) indicated in a study of 4,143 feedlot steers the incidence of polio was low (0.14%) with diets containing 0.46% sulfur, but there was a rise in polio (6.06%) incidence with diets containing 0.58% sulfur. In their study the use of phosphoric acid was evaluated as a potential substitute for sulfuric acid and although this replacement was successful for the fermentation process the cost did not justify the potential benefits (Vannessa *et al.*, 2009). Hence, inclusion levels of distillers grains in feed rations should consider a sulfur limit closer to 0.50% - a limit that can be obtained with inclusions of up to 50% WDGS (0.47% sulfur) and a roughage inclusion close to 15% (Erickson *et al.*, 2010; U.S. Grains Council, 2012).

Types of corn distillers grains used as cattle feed and how they are obtained

Starch rich crops, such as corn, are widely utilized for ethanol production as the starches are extracted to undergo several manufacturing processes to obtain ethanol as an energy source. Corn in particular, due to the great carbohydrate content (70-72% weight on a dry matter basis is starch), is a highly sought out crop where starch is converted to glucose and then converted to ethanol via fermentation (Bothast and Schlicher, 2005).

The milling process that corn undergoes can be classified as either wet or dry milling. In the wet milling process corn is subjected to an intensive steeping process by soaking corn kernels in water that soften the kernels in order to separate different components to obtain bran, starch, protein, germ and soluble components. Through additional processing steps these individual components are then converted to ethanol, syrups, some plastics, oils and several individual components like bran and fiber are mixed to generate gluten feed (Erickson *et al.*, 2010).

In the dry milling process corn is ground, cooked and fermented to produce ethanol and carbon dioxide. The remaining fractions after alcohol is generated and extracted are considered as stillage and these then become distillers grains (Berger and Singh, 2010; Bothast and Schlicher, 2005; Mathews and McConnell, 2009). More details on the production and the products obtained from both the wet and dry milling process of corn are explained below.

Wet milling process. It has been estimated that 33% of ethanol plants in the U.S. are wet milling operations (Bothast and Schlicher, 2005). Through the wet milling process corn is partitioned to generate products that are intended for human use as well as animal consumption. Typically, one bushel of corn, equivalent to 56 pounds, will yield 1.6 pounds of corn oil (extracted from the germ), 11 to 13 pounds of gluten feed (germ after oil extraction plus fiber and hulls), 2.6 pounds of gluten meal (gluten is separated to generate high-protein animal feed) and 2.5 gallons of ethanol (Bothast and Schlicher, 2005; Mathews and McConnell, 2009). In general, water is added to corn in order to undergo a steeping process, followed by grinding which subsequently goes through different separations that fraction the corn kernels to obtain corn bran, generating starch,

gluten meal, and corn oil as by-products. The corn bran is subjected to a second steeping step where distillers solubles generated after fermentation are added back to the bran portion to yield wet corn gluten feed. If the wet corn gluten feed undergoes a subsequent drying process then dry corn gluten feed is generated (Bothast and Schlicher, 2005; Erickson *et al.*, 2010).

Dry milling process. It was estimated in 2007 that 82% of ethanol plants in the U.S. are dry milling operations (Renewable Fuels Association, 2007). In the dry milling process one bushel of corn will yield 2.8 gallons of ethanol (Bothast and Schlicher, 2005) and the remaining portion after the dry milling process yields approximately 17.5 pounds of dry distillers grains plus solubles (Mathews and McConnell, 2009). Briefly, the dry milling process consists of grinding corn with a hammer mill and then incorporating water to form a slurry which is then cooked (104°C) with enzymes (alpha-amylase) to create a mash. The enzymes convert complex sugars (starch) to simpler forms of sugars (dextrins). At this point, yeasts (*Saccharomyces Cerevisiae*) and more enzymes (glucoamylase) are added to promote the fermentation process that further simplifies sugars to glucose molecules and their fermentation generates fuel-grade ethanol. Particularly cellulose (glucan) and hemicellulose (arabinan and xylan) components making up corn fiber and are the fermentable sugars that augment ethanol yields (Kim *et al.*, 2008). To separate the alcohol, a distillation column is used to separate the ethanol from all other non-fermented fractions known as whole stillage. A subsequent centrifugation of the whole stillage then yields thin stillage (water with soluble solids) and the wet grains (solids). Thin stillage can also be evaporated to create the syrup which is then re-combined with the wet grains to generate distillers grains plus solubles, used

extensively in the cattle industry. Carbon dioxide is also generated with this process and is captured and sold mainly to beverage companies (Berger and Singh, 2010; Bothast and Schlicher, 2005).

Through the dry milling process according to the final moisture content, a vast array of distillers grains products are available. According to the 2002 USDA's ethanol cost of production survey (Shapouri and Gallagher, 2005), 70% of distillers grains are sold as dry distillers grains plus solubles (DDGS; 10% moisture), 21% are sold as wet distillers grains plus solubles (WDGS; 65–70% moisture) and the remaining 9% are sold as modified wet distillers grains plus solubles (MDGS; 50-55% moisture; Bothast and Schlicher, 2005; Shapouri and Gallagher, 2005). The type and amount of distillers grains inclusion in cattle diets is also greatly affected by the distance of the feedlot to the ethanol plant as studies have shown that these decrease as distance increases, particularly if feedlots are over 100 miles away from ethanol plants (Waterbury *et al.*, 2009).

Wet distillers grains plus solubles (WDGS). When the wet grains and solubles are combined with no additional evaporation steps the result is known as WDGS which have a final moisture content of 65-70%. From a nutritional standpoint, the incorporation of WDGS tend to improve cattle performance and several studies have quantified the added feeding value of WDGS in comparison to corn based diets and have found that the feeding value of WDGS can range from 35-47% greater than corn (Ham *et al.* 1994; Larson *et al.* 1993). The greatest limiting factors for the use of WDGS in feedlot rations is the added cost of transportation and the shortened shelf life (1 to 2 week shelf life) due to greater moisture content (Bothast and Schlicher, 2005). Thus, the WDGS market

primarily consists of operations closer to ethanol plants that can guarantee a steady supply of WDGS.

Modified wet distillers grains plus solubles (MWDG). A partial dehydration of the wet grains and the addition of the solubles is a practice that some ethanol plants also perform to generate MWGS with a final moisture content of about 50-55%. The slight reduction in moisture benefits feedlots that are slightly farther from ethanol plants and can also extend shelf life with minimal sacrifice to cattle performance.

Dry distillers grains plus solubles (DDGS). When wet grains are dramatically dehydrated and solubles are combined, the resulting cattle feed source is known as DDGS which have a final moisture content of 10-12%. The greatest advantage of using DDGS as cattle feed is that DDGS are more suitable for inter-state and international distribution given their ability to be pelletized (Mathews and McConnell, 2009) as well as ease of transporting less water weight and diminishing feed spoilage associated with greater moisture content of feed (Bothast and Schlicher, 2005). Although cattle performance is inferior to that of cattle fed WDGS, and the fact that there are added costs of drying incurred at the plant level, the benefits of transporting and maximizing the shelf life of feed outweigh the potential disadvantages of feeding DDGS vs. WDGS or MDGS. Also, even when cattle performance is not as great compared to WDGS, feeding DDGS still represents an added feeding value of up to 24% relative to corn (Ham *et al.*, 1994).

De-oiled distillers grains plus solubles. A greater demand for ethanol production results in the generation of a diversity of by-products that were considered as additional but less valued products. However, the maximization of these by-products has shifted the way in which these by-products are viewed and they are now considered

valuable resources not only for the livestock industry but also as potential products for human consumption (Mathews and McConnell, 2009; Saunders and Rosentrater, 2009b). As explained by Berger and Singh (2010) and Bothast and Schlicher (2005), research has now focused on using hybrid corn with greater starch content, the conversion of corn fiber to ethanol and the development of novel and higher valued co-products. As part of these new trends to further diversify the use of by-products and develop new products, de-oiling steps have now been added to many ethanol plants that can now recover oil from the dry milling process to be utilized either for human consumption, biofuels or commercial feed production (Winkler-Moser and Breyer, 2011).

Fat content of DDGS has been reported to range anywhere from 3 to 13%, but in most cases the fat content is closer to 8-9% fat (Ganesan *et al.*, 2009; Saunders and Rosentrater, 2009a; Winkler-Moser and Breyer, 2011). With new de-oiling techniques the fat content of de-oiled distillers grains has been reduced to as low as 2.1% fat on a dry matter basis (Ganesan *et al.*, 2009). Recent trends to remove corn oil have looked at the removal at the front or back end of production in the dry milling process. In both instances, dry mill plants can add equipment to their operations to remove corn oil prior to or after the fermentation of corn, generating oil useful for the production of biodiesel while adding value to the remaining by-products (Watkins, 2007).

Although front end extraction methods of corn oil are more expensive (three times as costly), the oil extraction at this point is cleaner and has greater yields in comparison to back end extraction (Watkins, 2007). However, if the plant has a market for the more refined oil the investment may be worth the added inputs for front end extraction (Watkins, 2007). On the contrary, the back end extraction of oil takes place after the

ethanol has been distilled and uses centrifugation in the whole or thin stillage portions to fraction off a portion of the soluble fats (Watkins, 2007; Winkler-Moser and Breyer, 2011). Separating the oil at this point would mean that out of every bushel of corn 2 pounds of oil would be extracted leaving about 16 pounds of de-oiled DDGS. Given that the oil extracted via centrifugation on the back end of the dry milling process is not as pure as oil from the front end extraction, this oil is then better suited for biodiesel or addition to poultry and swine diets. Also, the new de-oiled distillers grains are a more attractive feed source for ruminants due to an increase in protein and the extension of ethanol by-product shelf life (Watkins, 2007).

Ethanol by-product inclusion limits

In terms of nutritional value, the incorporation of distillers grains, particularly those with higher moisture content, are advantageous. However, due to self-limiting factors such as sulfur content and fiber requirements for proper ruminal digestion there are inclusion limits that should be followed. One of the other limiting factors on the amount of distillers grains to incorporate is also the market price of these co-products. For instance, typically co-products are priced at about 80-95% the price of corn; however, in some cases where corn prices have been very high (~$5/bushel) the co-products have not followed the same trend and have been available for 50% the price of corn (Erickson *et al.*, 2010).

Work conducted at the University of Nebraska has demonstrated that feeding WDGS at 40% inclusion in the diet has a 14% increase in feed efficiency which points to a 35% greater feeding value in relation to corn (Klopfenstein *et al*., 2008; Larson *et al*.,

1993). Studies aiming to decipher appropriate inclusion levels of WDGS have found that there is a quadratic relationship in ADG and gain to feed ratios, a cubic effect of feeding value and an overall increase in feed efficiency as incorporation of WGDS increases from 0 – 50% in ten percent increments (Vander Pol *et al*., 2006). In conclusion, data suggest that inclusion levels of WDGS between 30 – 50% are more advantageous in terms of ADG and gain to feed ratios.

In a study conducted by Ham *et al.* (1994) evaluating the feeding values of DDGS and WDGS in feedlot rations it was found that DDGS at 40% inclusion resulted in a 24% increase in feeding value. Further studies echoed the findings of Vander Pol *et al.* (2006) where a quadratic response in ADG and a cubic response in gain to feed ratio was observed with inclusion of DDGS ranging from 0 – 40% inclusion levels (Buckner *et al*., 2007; Klopfenstein *et al*., 2008). In the case of DDGS it seems as though optimal inclusion levels range from 20 – 30% in terms of cattle performance.

Effect of feeding corn distillers grains on meat quality

Conversion and performance parameters are of maximum importance in the evaluation of alternate feed sources for cattle rations as these greatly drive economic profitability. On the other hand, meat quality is just as important when determining the implications of a new feed ingredient as this can largely benefit or have detrimental effects in terms of meat quality parameters and can potentially alter consumer perception or acceptability of meat products. Hence, having a better understanding of parameters such as color, tenderness, nutritional composition, and fatty acid composition are important in the evaluation of alternate feed ingredients.

Color. Inclusion of WDGS seems to have an effect on meat color (Mello *et al.*, 2012a; Roeber *et al.*, 2005); which appears to be a dose dependent response. Some studies indicate that inclusion of 15 or 30% WDGS are sufficient to decrease redness (lower a* values) of strip loin steaks under retail display conditions after 7 d of display ($P < 0.05$; Mello *et al.*, 2012a). Decreased redness has also been associated with 30% inclusion of WDGS on tenderloin and top blade steaks under retail display in comparison to meat from cattle not receiving WDGS (Mello *et al.*, 2012a). Similar observations were noted by Roeber *et al.* (2005) where they state that inclusion levels of over 40% distillers grains can result in lower color stability in a retail setting whereas inclusion of up to 25% distillers grains can be included with little to no effect on retail display color stability.

Likewise, in a feeding trial including 0% or 35% WDGS with either steam-flaked corn or dry-rolled corn, steaks from cattle receiving 35% WDGS, irrespective of corn processing method, had darker meat at day one of retail display ($P = 0.01$) and tended to be less red by seven days of retail display ($P = 0.06$) than steaks from cattle not being finished with WDGS (Buttrey *et al.*, 2013). In accordance, Depenbusch *et al.* (2009) found that loin steaks from heifers fed increasing levels of DDGS from 0 - 75% had a linear decrease in brightness (lower L*) with increasing concentrations of DDGS at day 0 of retail display ($P = 0.04$). However, as retail time increased, a quadratic increase in brightness was seen where samples from cattle fed between 30 - 45% DDGS reached maximum brightness and decreased thereafter ($P \leq 0.03$). Also, there was a linear decrease in redness after 7 day retail display resulting from increasing inclusion levels of DDGS ($P = 0.04$; Depenbusch et al., 2009). Similarly, Leupp *et al.* (2009) found that including 30% DDGS either at the growing or finishing phase of production reduced L*

($P = 0.04$), a* ($P < 0.001$) and b* ($P = 0.01$) values compared to a dry-rolled corn diet. Similar findings were established by Segers *et al.* (2011) that identified that steaks fed 25% DDGS had increased discoloration after 9 days of retail display and greater a* reduction on retail display ($P < 0.01$). This also agrees with Gordon *et al.* (2002) that noted a decrease in a* and b* values with inclusion of DDGS in heifer finishing diets ($P < 0.05$).

On a similar note, a study working with ground loin and round beef samples found that samples from cattle not receiving DDGS had greater L* values ($P = 0.02$) and tended to have greater a* values ($P = 0.10$) than samples from cattle on 25% or 50% DDGS (Gunn *et al*., 2009). In contrast to all the findings mentioned above, Gill *et al.* (2008) found that samples from cattle fed 15% corn distillers grains had lighter meat and lower a* values than cattle on steam flaked corn ($P < 0.05$). On the contrary, some research points to greater a* values ($P < 0.05$) with inclusion of 25% DDGS vs. 50% DDGS (Roeber *et al*., 2005). These findings were also observed by Aldai *et al.* (2010) that said that steaks from cattle fed 40% corn DDGS had greater oxymyoglobin content and where brighter than meat from cattle fed 20% corn DDGS ($P < 0.05$). Although there is not a complete consensus in the literature, for the most part it seems as though inclusion of WDGS or DDGS, especially at greater concentrations, does have detrimental effects on meat color on retail display.

Tenderness. Varied results have been found in terms of how feeding corn distillers grains affect meat tenderness. Numerous researchers have indicated that the inclusion of DDGS or WDGS at different concentrations and feeding phases does not alter meat tenderness measured via WBSF or sensory panel (Gill *et al*., 2008; Koger *et*

al., 2010; Leupp *et al*., 2009; Mello *et al*., 2012a; Roeber *et al*., 2005; Segers *et al*., 2011). Conversely, Depenbusch *et al.* (2009) found that through sensory evaluation panelist identified a linear increase in tenderness with increasing levels of DDGS ranging from 0 - 75%. Parallel to this, Gordon *et al.* (2002) used a trained sensory panel that detected a slight but significant ($P < 0.05$) linear increase in tenderness in steaks from heifers fed DDGS also ranging from 0 – 75%. Likewise, Aldai *et al.* (2010) indicated that meat from cattle fed 20% or 40% corn DDGS were found to be more tender ($P < 0.05$) than samples from cattle on a barley-based control diet. These findings were also evidenced by panelist's perception of lower connective tissue in steaks from cattle receiving DDGS.

Previous and current studies at the University of Nebraska-Lincoln have also identified that there is increased tenderness associated with the feeding of WDGS or DDGS (Chao, 2015; Kunze *et al*., 2016; Senaratne, 2012). Initially, it was noted that meat from cattle fed WDGS without vitamin E had greater tenderness, evident through greater troponin-T degradation, than cattle fed a control diet with no inclusion of WDGS (Senaratne, 2012). Senaratne's findings led to the hypothesis that the integrity of beef muscle membranes could potentially be altered by feeding WDGS which could ultimately result in greater calcium leakage and lead to improved tenderness. Closely following this train of thought Chao (2015) found that there is increased concentration of PUFA's in the sarcoplasmic reticulum (organelle of primary calcium storage) that could explain the increased tenderness at day 2 post-mortem of cattle feed WDGS vs. a corn-based control diet given greater membrane fluidity and oxidative potential that would allow for greater calcium leakage. More recently, Kunze *et al.* (2016), have been able to

isolate mitochondria (an organelle that also sequesters calcium) to further explore this calcium flux theory and use this organelle as a model that further explores the relationship of feeding distillers grains and its effects on improved tenderness. So far, Kunze *et al.* (2016) have been able to validate that there is an increase in PUFA content in organelle membranes associated with feeding DDGS. Also, meat samples from a corn control diet with no distillers grains tend (P = 0.08) to retain more calcium in the mitochondria than do samples from cattle fed 50% DDGS, primarily due to greater oxidative potential with increase PUFA concentrations in organelle membranes that result in greater calcium flux (Kunze *et al*., 2016).

Sensory evaluation. Some sensory analysis focusing on attributes such as tenderness, juiciness, overall acceptability and off-flavors have demonstrated that the inclusion of corn distillers grains has minimal to no effects on eating quality (Buttrey *et al*., 2013; Gill *et al*., 2008; Jenschke *et al*., 2007; Mello *et al*., 2012a; Roeber *et al*., 2005). However some research has been able to identify benefits associated with the use of corn WDGS or DDGS (Gordon *et al*., 2002; Leupp *et al*., 2009). In some instances improvement in overall tenderness were noted with inclusion of WDGS or DDGS (Gordon *et al*., 2002). Aldai *et al.* (2010) found that initial and overall sensory tenderness, as well as perception of connective tissue, was lower (more desirable) for samples from cattle fed corn DDGS in comparison to those from cattle on a barley-based diet (P < 0.05). The previous authors also noted that, in general, greater ratings for overall palatability were obtained in samples from steers fed DDGS and that particularly meat from cattle fed 20% DDGS were rated as having greater flavor intensity and

desirability than meat from the 40% DDGS group. In their study off-flavors and sustainable juiciness were unaffected by diet.

Trends (P = 0.10) for improvement in juiciness and flavor have also been observed by Leupp *et al.* (2009) when steers were finished with 30% DDGS. Just as well, Depenbusch *et al.* (2009) have also reported an improvement in beef flavor in steaks from heifers fed 45 and 60% DDGS in relation to heifers with no inclusion of DDGS. Therefore, it seems as though the inclusion of corn distillers grains has negligible to beneficial effects in sensory properties determined by both trained and untrained beef consumers.

Nutritional composition. Although proximate composition is generally not recorded in the bulk of research exploring the effects of feeding corn distillers grains on meat quality a handful of studies have reported slight to no changes in nutritional composition of meat. Segers *et al.* (2011) detected a trend (P = 0.07) where samples from cattle fed 25% DDGS had greater moisture values than cattle on corn gluten feed with no effects on protein or lipid concentration ($P \geq 0.13$). Aldai *et al.* (2010) compared the proximate composition from cattle fed 20% or 40% corn or wheat DDGS versus a control barley-based finishing diet and report a slight increase in protein concentration in the control group, intermediate values for the corn DDGS group, and lowest values for the wheat DDGS group (21.6%, 21.3%, 21.1%, respectively; $P < 0.05$). However, fat and moisture content in the previous study were not affected by dietary treatment. Other studies have determined that feeding several forms of corn distillers grains does not alter moisture, protein or fat composition of meat (Jenschke *et al*., 2008; Mello *et al*., 2012a; Mello *et al*., 2012b).

Fatty acid profile. It would be expected that the generation of corn ethanol by-products such as WDGS and DDGS would have a lower energy feeding value in relation to corn given that starch is removed for the generation of ethanol. However, it has been established that WDGS are not only considered a valuable protein source they are also found to be a good energy source for cattle. This conundrum gave way to experiments that intended to understand this discrepancy and it has been established that cattle fed WDGS have lower ruminal pH compared to cattle fed corn control diets. The shift in ruminal pH thus alters the acetate to propionate ratios, where in cases of lower pH, propionate becomes prevalent leading to greater fat digestion as well as increased unsaturated fatty acids reaching the small intestine and being protected from the harsh environment of the rumen. Hence, the greater proportion of propionate coupled with the greater fat digestion and greater fatty acids reaching the duodenum could explain the greater energy feeding value of WDGS in relation to regular corn diets (Vander Pol *et al*., 2009).

As was explained by Zinn *et al.* (2000) fatty acids, particularly unsaturated fatty acids form micelles that are responsible for greater fatty acid absorption and are more efficiently utilized. Vander Pol *et al.* (2009) noted that perhaps with the addition of WDGS in cattle diets there is a protection phenomenon which protects unsaturated fatty acids from biohydrogenation thus making more fatty acids available as an energy source. These observations are in accordance to the observations of Atkinson *et al.* (2006) that indicate that greater proportions of grains in cattle diets lead to greater unsaturated fatty acid due to less biohydrogenation. Conversely, in cases of greater ruminal

biohydrogenation the intestinal digestibility of fat is greatly impaired (Plascencia *et al*., 2003).

As part of the experiments conducted by Vander Pol *et al.* (2009) it was observed that greater ($P < 0.10$) proportions of C16:0, C18:1 trans, C18:1, and C18:2 reach the duodenum in cattle fed 40% WDGS in relation to cattle fed a composite diet consisting of corn bran and corn gluten meal with and without corn oil and a dry rolled corn control diet with and without the addition of corn oil. These findings once again support the idea that fatty acids in WDGS appear to have an added protection to keep them from biohydrogenation in comparison to other fatty acid sources such as corn oil (Vander Pol *et al*., 2009). Buttrey *et al.* (2013) stated that beef from individually-fed crossbred steers including 35% WDGS in their finishing diets resulted in decreased C16:1*cis*-9, C18:1*cis*-9 and C18:1*cis*-11 fatty acids, and tended to increase total fat concentration in steaks. Similarly, Mello *et al.* (2012a) found that meat from cattle finished on 30% WDGS had a lower proportion of C18:1(n-7) than meat from cattle fed 0% or 15% WDGS ($P < 0.01$). It was also noted that there was a linear increase PUFA content as well as trans, C18:2(n-6), and total n-6 fatty acids as inclusion levels of WDGS increased from 0 - 30% ($P < 0.01$). However, finishing diet did not seem to alter total lipid content ($P = 0.19$) or marbling attributes such as texture and distribution (Mello *et al*., 2012a).

Lipid oxidation. Previous research has noted that inclusion of WDGS in feedlot finishing rations causes an increase in steak lipid oxidation, measured via thiobarbituric acid reactive substances (TBARS) values, under retail display conditions, thus limiting beef shelf life (Buttrey *et al.*, 2013; Koger *et al*., 2010). Similarly, Mello *et al.* (2012) determined that beef cattle fed greater concentrations of WDGS (30% DM basis) resulted

in an increase in TBARS values for top blade and strip loin steaks ($P < 0.01$) in comparison to 0% or 15% inclusion of WDGS; yet, no differences were found for tenderloin steaks ($P = 0.19$). On the contrary, other researchers have indicated that lipid oxidation of beef was unaffected in dietary treatments including DDGS (Depenbusch *et al*., 2009; Gunn *et al*., 2009). Mixed results were seen in the work of Gill *et al.* (2008) who found that when comparing corn DDGS and WDGS, treatments including DDGS had greater TBARS values than did the meat from cattle fed WDGS ($P < 0.05$).

Manipulation of fatty acid profiles through dietary treatments

Given the recent interest in increase consumption of poly-unsaturated fatty acids (PUFA's), particularly ω-3 fatty acids, many investigators have looked at dietary means to enhance their prevalence in meat. As explained by Wood *et al.* (2008), monogastric animals have the ability of depositing the PUFA's from the diet directly into muscle mass; whereas ruminants, due to bacterial biohydrogenation, can only deposit a limited amount of PUFA's in muscle. Ruminant diets, although containing little fat, are rich in PUFA's yet due to biohydrogenation, SFA are dominant in ruminant muscle in relation to PUFA's (Warren *et al.,* 2008).

Particularly for ruminants, the use of unprotected lipids has been explored as well as protected lipids. The incorporation of unprotected lipids such as plant and fish oils has been attempted with some degree of success (Scollan *et al.*, 2001). Although unprotected from the harsh environment of the rumen, some PUFA's can bypass the rumen and go directly into the small intestine where these are released into the blood stream and subsequently deposited in muscle (Enser *et al.,* 1996; Scollan *et al.,* 2006).

Recent researchers have started to look at lipid protection as a means to avoid biohydrogenation in the rumen. Initial attempts were made by the use of formaldehyde treatments (Wood *et al*,. 2008). A study conducted by Ladeira *et al.* (2014) looked at the use of rumen protected fat vs. ground soybean grains both with and without monensin. The rumen-protected fat or calcium salts from soybean oil release few fatty acids in the rumen, and thus mono-unsaturated fatty acids should be deposited in muscle. Monensin is an ionophore which will decrease biohydrogenation in the rumen in highly unsaturated fatty acid diets. As explained by Richardson *et al.* (1976) monensin causes a shift in rumen fermentation that result in increased proportions of propionate in relation to acetate and butyric acid. Ladeira *et al.* (2014) suggested that monensin increased arachidonic and linolenic acids in the *longissimus dorsi*. Greater linoleic acid was prevalent in the muscle of cattle exposed to ground soybean in comparison to cattle exposed to rumen protected fat. Ladeira *et al.* (2014) indicated that the differences were a result of greater linoleic acid in the actual diets (49.64% vs. 31.01%, respectively). On the other hand, despite greater linolenic acid in in the ground soybean diet (5.13%) in relation to the rumen-protected fat (2.02%), cattle fed rumen-protected fat had greater linolenic acid. Ladeira *et al.* (2014) indicated that the difference is a result of greater biohydrogenation of linolenic acid in relation to linoleic acid. There is however a limit to the amount of fatty acids that can be added in ruminants diets (no more than 60 g/kg DM basis) to ensure proper rumen function (Scollan *et al.*, 2006).

The efforts to promote greater unsaturated fatty acids in meat are considered beneficial to human health but can lead to greater lipid oxidation which in turn negatively

impact shelf life and sensory characteristics, particularly color and flavor (Ladeira *et al.*, 2014).

Lipid oxidation and the factors that promote it. Lipid oxidation is a non-microbial factor responsible for the deterioration of muscle foods (Descalzo *et al.*, 2005; Pradhan *et al.*, 2000). After slaughter, cells become damaged which favors the oxidative process and lipids, pigments, proteins, carbohydrates, vitamins and overall quality can be affected (Kanner, 1994). The deterioration in quality will be manifested in flavor, color, texture, nutritive value and production of undesired compounds (Kanner, 1994). However, the larger detriment of lipid oxidation is perceived in color and flavor (Greene, 1969).

Lipid oxidation is typically explained as a three step process: initiation, propagation and termination (Kanner, 1994; Morrissey *et al.*, 1998; Spallholz, 1989; Wong, 1989). Morrissey *et al.* (1998) detailed lipid oxidation *in vivo*. Initiation of lipid oxidation in muscle begins with the removal of hydrogen from a carbon chain of a fatty acid (Morrissey *et al.*, 1998). In animals, oxidation can be triggered by cigarette smoke, ozone, nitrogen oxides, chlorinated hydrocarbons, and heavy metals, among other things that can damage tissues (Spallholz, 1989). However, in live muscle, enzymes are capable of eliminating free radicals formed during oxidation through the formation of water (Morrissey *et al.*, 1998). Gray *et al.* (1996) and Morrissey *et al.* (1998) indicated that all the biochemical changes at death disrupt the balance of prooxidants and antioxidants of the living muscle favoring oxidation.

Wong (1989) defines initiation as the hemolytic abstraction of hydrogen to form a carbon-centered alkyl radical in the presence of an initiator. As described by Kanner

(1994), superoxide anion radicals ($O^{-}_{2}\cdot$), perhydroxyl radicals ($HO_2\cdot$), hydrogen peroxides (H_2O_2) and hydroxyl radicals ($HO\cdot$) are all initiators of lipid oxidation and they are formed via one-electron reduction of oxygen. In the presence of these initiators, free radicals are generated ($R\cdot$) that are very unstable because the unpaired electrons can now react with organic molecules (Spallholz, 1989). This initiation step has been said to begin at the phospholipid membranes (Gray *et al.*, 1996).

After initiation, a chain reaction is triggered where the free radicals react with oxygen to form peroxy radicals ($ROO\cdot$), which then react with unsaturated lipids forming hydroperoxides (ROOH). This step is known as propagation (Wong, 1989). During this chain reaction new species of free radicals can be generated ($R\cdot$, $RO\cdot$ and/or $\cdot OH$) having a strong additive and autocatalytic effect (Spallholz, 1989).

Finally, in the termination step, radicals react amongst one and other resulting in radical combinations ultimately terminating in non-radical products (Wong, 1989). The equations in figure 1 display the three phases of lipid oxidation (Fernández *et al.*, 1997):

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

Figure 1. Steps and reactions involved in lipid oxidation (Fernández *et al.*, 1997)

There are several factors that can promote lipid oxidation. Some of these factors include, but are not limited to: lighting (Djenane *et al.*, 2001), irradiation and high

pressure processing (Bolumar *et al.*, 2014; Ismail *et al.*, 2008; Kim *et al.*, 2009; Ma *et al.*, 2007), further processing (Gray *et al.*, 1996; Kanner, 1994; O'Grady *et al.*, 2000), addition of salt (Min and Ahu, 2005), and temperature and cookery (Crnjar, 1981; Kanner, 1994; Wong, 1989). Understanding the detrimental effects of pro-oxidants on meat quality is vital for the prevention of lipid oxidation and ultimately the prolongation of product shelf life.

Lipid oxidation's impact on meat color. Color is viewed by consumers as an indicator of freshness and a driver for consumer purchase (Gatellier *et al.*, 2001; Greene, 1969; O'Grady *et al.*, 2000; Renerre *et al.*, 1996). Consumer studies have reported a significant decline in purchasing decisions where with 20% discoloration on retail display, beef sales were reduced to 50% (Hood, 1973). The color of meat has been described as a balance between oxymyoglobin oxidation and metmyoglobin reduction (Gatellier *et al.*, 2001) and has been closely related to lipid oxidation (Greene, 1969). When oxidation of oxymyoglobin occurs to form metmyoglobin, intermediate radicals are generated that through propagation can further accelerate color oxidation as well as lipid oxidation (Faustman *et al.*, 2010).

Wong (1989) explained that metal ions can catalyze lipid oxidation. Wong indicated that Fe^{3+} can induce autoxidation and produce superoxide anions ($O^{-}_{2}\cdot$). In the presence of thiols, the thiyl radical can also result in superoxide anions ($O^{-}_{2}\cdot$) that then dismutates (simultaneously reduced and oxidized) to H_2O_2 or undergoes a one electron reduction of Fe^{3+} to Fe^{2+}. Subsequently, Fe^{2+} and H_2O_2 undergo the Fenton reaction where hydroxyl radicals ($\cdot OH$) are formed. (Fenton reaction = $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + H_2O + \cdot OH$) These hydroxyl radicals are considered active initiators of lipid oxidation.

The diagram presented in Figure 2 was proposed by Faustman *et al.* (2010) to try and explain the possible interaction between lipid and color oxidation. In accordance to Wong (1989), Faustman *et al.* (2010) indicates that the presence of oxygen catalyzes the formation of primary oxidative products that propagate to form secondary oxidative products that continue the oxidative chain reaction.

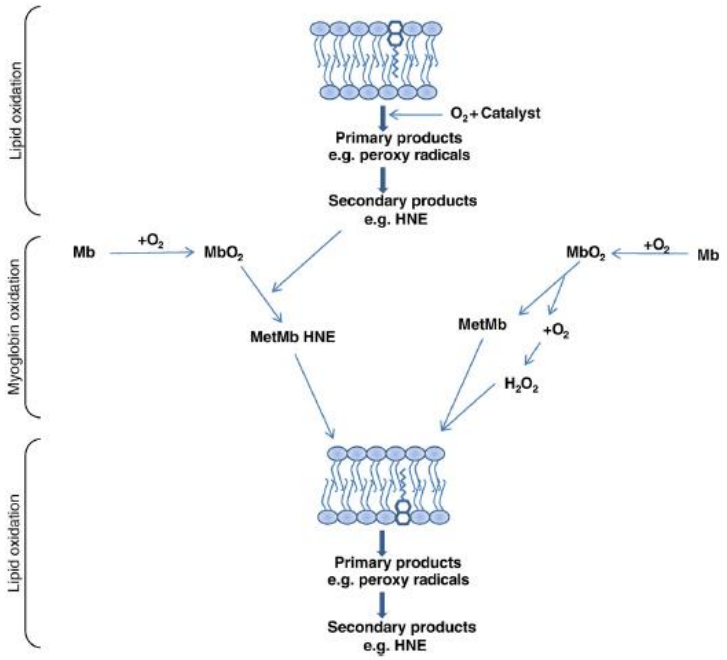

Figure 2. Interaction between lipid and color oxidation (Faustman *et al.*, 2010)

Lipid oxidation and meat flavor. Greene (1969) conducted an experiment where beef round samples were trimmed, ground and treated with three different antioxidants: butylated hydroxyanisol (BHA), propyl gallate (PG), or sodium tripolyphosphate (PP). Greene's study was designed to detect differences in rancid odors in raw and cooked meat samples via a trained panel. The results of the study indicated that samples with no antioxidants had greater detectable off-odors in relation to the samples treated with butylated hydroxyanisol (BHA) and propyl gallate (PG). These off-

odor differences were apparent in raw and cooked beef samples indicating that cooking did not destroy rancid odor in meats with elevated theobarbutiric reactive substances (TBARS). Greene's study was considered as one of the primary studies concerning the development of off-flavors in meat with oxidized pigments and lipids.

It has been well established in the literature that products generated as a result of lipid oxidation are responsible for the development of off-flavors in meat products. This is particularly true for PUFA's (Gatellier *et al.*, 2001). One of the principal products of oxidation is manohydroperoxides, which can undergo carbon-carbon cleavage on one of the two alkoxy radicals. As radicals continue reacting amongst themselves the formation of carbonyls, alcohols, esters and hydrocarbons arise resulting in the oxidized flavor of lipids (Wong, 1989).

A very interesting study was conducted by Campo *et al.* (2006) where the objective of their work was to assess the limit of rancidity in beef by relating sensory perception (taste panels) to traditional chemical methods (TBARS). In order to have a representative data set Campo *et al.* (2006) studied beef from a wide range of production systems particularly interested in obtaining differences in PUFA content. Campo *et al.* (2006) stored the samples under modified atmospheric packaging (MAP) (CO_2:O_2; 25:75) conditions intending to stimulate lipid oxidation with high atmospheric oxygen yet maintaining the myoglobin in its oxygenated form to decrease its activity as a prooxidant. The premise for using flavor perception over chemical measures was that in objective measures primary (ex. hexanal) and secondary (ex. malondialdehyde) products of oxidation are measured and these do not contribute to flavor (Campo *et al.*, 2006). Campo *et al.* (2006) determined that TBARS were good predictors of beef rancidity (R^2 =

0.84) and also indicated that beef flavor (R^2 = 0.93), absence of abnormal flavor (R^2 = - 0.88) and rancid flavor (R^2 = - 0.83) were all strong indicators of beef rancidity. Campo *et al.* (2006) concluded that a TBARS value of 2 can be considered as the limiting threshold of oxidized beef acceptability.

Mechanisms to delay or prevent lipid oxidation

Given the large impact and detrimental quality effects of lipid oxidation a substantial amount of research has been done to determine mechanisms to delay or prevent lipid oxidation. The majority of the research has looked at *ante-mortem* and *post-mortem* use of antioxidants, as well as packaging systems.

***Anti-mortem* use of antioxidants to extend shelf life.** Antioxidants were defined by Spallholz (1989) as molecules whose purpose is to prevent oxidation by either breaking free-radical chain reactions or preventing the cellular accumulation of toxic molecular forms of oxygen. Antioxidants protect the cells from oxidation by becoming oxidized themselves (donate an electron) and providing stability. The majority of the antioxidants used are vitamins; particularly vitamins A (β-carotene), C, and E. Vitamins A and E are lipid soluble and thus have an antioxidant effect on cell membranes. Vitamin C is water soluble and complements the lipid soluble antioxidants by trapping the radicals in the water portion of the cell (cytoplasm) (Spallholz, 1989).

Forage is considered an excellent source of vitamin E (α-tocopherol) (Descalzo *et al.*, 2005; Gatellier *et al.*, 2004; Warren *et al.*, 2008; Yang *et al.*, 2002). Cattle that are raised or finished on grass, rather than on concentrate diets, have been noted to have an increased PUFA content; yet these forages have sufficient amounts of natural

antioxidants to prevent or delay lipid oxidation associated with elevated PUFA's (Descalzo *et al.*, 2005). According to research done by Yang *et al.* (2002), supplementing cattle with 2500 IU vitamin E/hd/d for 132 days was sufficient to reduce lipid oxidation in grain-fed cattle compared with pasture-fed cattle despite having similar α-tocopherol contents (4-6 µg/g in muscle). Similarly, supplementing grain-fed cattle in Argentina with α-tocopherol at 500 units/hd/d, increased the vitamin E muscle content fourfold reaching equivalent concentrations to those of pasture-fed steers (Descalzo *et al*., 2005).

Vitamin E has been proven time and time again to function as a strong and effective antioxidant in meat. Spallholz (1989) explained that the mechanism through which vitamin E functions is through the acceptance of two free radicals, either from superoxide or an organic peroxy radical. A methyltocophenyl radical is produced after one electron oxidation of vitamin E, and it is typically reduced by cellular glutathione (antioxidant enzyme in muscle). After the second electron oxidation, tocophenly quinone is produced. If vitamin E is lacking, the cell will produce more gases (ethane and pentane), as well as malondialdehyde (Spallholz, 1989).

***Post-mortem* use of antioxidants to extend shelf life.** A series of approaches have been looked at to directly incorporate antioxidants to meat. Some of these approaches include injectable solutions, spray applications and dips with single or combined antioxidants. In the work of Djenane *et al.* (2003), fresh beef steaks were packaged (70% O_2 + 20% CO_2 + 10% N_2) and displayed under different lighting conditions (without illumination or with various fluorescent lights). Half of the steaks served as the control group (no surface spray) and the other half were sprayed with a

mixture of rosemary and vitamin C. Regardless of the lighting condition, the antioxidant spry of rosemary and vitamin C resulted in decreased metmyoglobin formation and lipid oxidation in comparison to the control steaks. The most favorable conditions for decreased lipid oxidation were obtained in the absence of UV radiation and with the addition of both antioxidants, resulting in a 10 d extension of shelf life.

Ismail *et al.* (2009) explored the effectiveness of incorporating different antioxidants either by mixing or spraying in irradiated ground beef. The four antioxidant treatments were: none, 0.05% ascorbic acid, 0.01% alpha-tocopherol + 0.01% sesamol, and 0.05% ascorbic acid + 0.01% alpha-tocopherol + 0.01% sesamol. In order to promote lipid oxidation the meat was irradiated (0 or 2.5 kGy) and placed under retail display conditions. Ismail *et al.* (2009) indicated that beef sprayed with antioxidants produced more hydrocarbons and alcohols than the mixing application. The mixing method was more effective at reducing lipid oxidation with the alpha-tocopherol + sesamol and ascorbic acid + alpha-tocopherol + sesamol treatments. Hence, mixing rather than spraying antioxidants was favored in irradiated beef.

Work in processed meats with pork sausage has also documented the successful use of rosemary extract, butylated hydroxy anisole (BHA), and butylated hydroxy toluene (BHT) in preventing lipid oxidation (Sebranek *et al.*, 2005). For a more complete overview of post-mortem use of antioxidants see the review of Ladikos and Lougovois (1990).

Packaging systems to extend shelf life. Another approach to reduce lipid oxidation in meat has been through different packaging systems. Zakrys *et al.* (2008) conducted a study to evaluate the effect of oxygen level in MAP beef samples (*M.*

longissimus dorsi), particularly on lipid oxidation. The steaks were packaged under 0%, 10%, 20%, 50% and 80% oxygen. As oxygen levels increased so did the TBARS values and the oxidative stability of the steaks decreased with prolonged storage times. These results were in accordance to those observed by O'Gray *et al.* (2000).

The idea that free oxygen exchange makes meat more susceptible to oxidation of lipids and color was established long ago (Greene, 1969). Greene (1969) stated that potential solutions for extending beef shelf life through packaging could include the use of antioxidants in anaerobic storage and expressed the need to provide color stability as well as lipid stability to entice consumer appeal. Since then, different packaging conditions and systems have been experimented with to minimize or delay the formation of oxidative products and thus extend shelf life.

Production of reactive oxygen species that lead to shorter shelf life

Reactive oxygen species (ROS) are oxygen derived compounds that in moderation serve physiological functions such as fighting off infections, killing invading organisms, as well as differentiation and proliferation. However, when ROS production is uncontrolled these can turn deadly and lead to cell damage, even cell death in more severe cases. The most affected components susceptible to oxygen-derived free radicals are lipids, DNA and proteins (Peng *et al*., 2014). The formation of ROS can be causes by a host of elements that can include: environmental factors such as ultraviolet or ionizing irradiation and pollutants (Halliwell, 1997), injured cells and pro-oxidative enzymes like lipoxygenase (Spiteller, 2001), presence of metal ions (Gutteridge and Halliwell, 1993), and in foods ROS can also result from lipid oxidation and exposure to light (Boff and

Min, 2002). As explained by Lee *et al.* (2004) the mitochondria consumes 90% of oxygen in aerobic organisms and it has been estimated that 1 – 5% of oxygen consumed by mitochondria will be converted to varicose forms of ROS (Ames *et al.*, 1993).

Endogenous enzymes acting as primary antioxidants

In order to offset the potential toxic effects of ROS production, cells have innate protection mechanisms to help regulate and mediate ROS toxic compounds through endogenous enzymes that turn superoxide ($O_2^{\cdot -}$) and hydrogen peroxides (H_2O_2) ions to less toxic forms of oxygen radicals (Cristiana *et al*., 2014). The three most important enzymatic systems protecting all cells against oxidative stress are superoxide dismutase, catalase, and glutathione peroxidase. Together, these three enzymes have antioxidant capabilities in all cells that help stabilize these highly active ROS that in terms of muscle foods result in oxidation, production of off-flavors and an overall reduction of shelf life. Very little research has explored the enzymatic activity of these mechanisms in muscle while much literature point to secondary antioxidant interventions (*pre* or *post-mortem*) to off-set oxidative damage in meat. However, having a better understanding of these primary antioxidant mechanisms and potential forms of stimulating these in muscle could provide huge benefits in terms of combating oxidation and ultimately prolonging meat shelf life.

Superoxide Dismutase (SOD). Superoxide dismutase is an enzyme that can have different metal cofactors (copper and zinc, manganese, or nickel ions). According to the site-of-action and metal cofactor, SOD can be classified into distinct families that include: SOD 1 or CuZnSOD (cytoplasm and nucleus with copper and zinc in reactive

site; 32 kDa), SOD 2 or MnSOD (mitochondria with manganese in reactive site; 88 kDa), and SOD 3 or ECSOD (extracellular with copper and zinc in reactive site; 155 kDa). The SOD with nickel in the reactive site is only found in prokaryotes (Cristiana *et al*., 2014; Weydert and Cullen, 2010). In general, the CuZnSOD makes up ~90% of total SOD activity in eukaryotic cells (Weydert and Cullen, 2010).

The role of SOD in cells is to remove superoxide ($O_2^{\cdot-}$) ions by causing dismutation (reaction with itself). As explained by Cristiana *et al.* (2014) the dismutation of superoxide by SOD is very efficient, in fact it is considered as the most catalytically effective enzyme having the largest k_{cat}/K_M of approximately 7 x 10^9 $M^{-1}.s^{-1}$. The dismutation reaction can be thought off as a two part reaction. The first reaction involves the reduction of a metal ion by one superoxide ion that results in the loss of an electron from one superoxide molecule to become molecular oxygen ($O_2^{\cdot-} \rightarrow O_2$). The second part of the reaction involves an electron "mutation" on the second superoxide ion in order to restore the initial oxidation state of the metal ion. This results in the generation of hydrogen peroxide (H_2O_2). The complete SOD reaction can be seen below as explained by Cristiana *et al.* (2014):

$$2\ O_2^{\cdot-} + 2H^+ \xrightarrow{\text{Superoxide dismutase}} O_2 + H_2O_2$$

In general, SOD is a ubiquitous enzyme that prevents the accumulation of ROS. It has been established that lack of SOD causes a shortage in lifespan and thus current human medical approaches trying to combat pro-inflammatory conditions are looking at the use of antioxidants that mimic the antioxidant capabilities of SOD to ward off such diseases and serve as therapeutic treatment alternatives (Cristiana *et al*., 2014).

Catalase. Catalase is an enzyme that has an iron molecule in the active site and is considered as one of the most effective redox enzymes (Peng *et al*., 2014). Working in conjunction with SOD, catalase converts hydrogen peroxide into oxygen and water and is found in the peroxisomes (subcellular organelles) and the cytoplasm (Weydert and Cullen, 2010).

$$H_2O_2 + H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

An excessive amount of hydrogen peroxide would lead to the conversion of hydrogen peroxide to hydroxyl radicals which is one of the most harmful radicals to living cells (Peng *et al*., 2014). In fact, rodent studies have been able to detect an adverse relationship between low catalase concentration and cancer metastasis (Nishikawa *et al*., 2002; Nelson *et al*., 2003).

Glutathione peroxidase. Different than SOD and catalase, glutathione peroxidase does require co-factors (reduced glutathione, NADPH, and glucose-6-phospahte) and other enzymes to function and also has five isoenzymes. Glutathione peroxidase is a selenium-containing enzyme found in several cellular compartments like the mitochondria and the nucleus and it has been linked to protecting hemoglobin from oxidative damage, suppressing apoptosis and aiding in reversing malignant cancer phenotypes. The capability of glutathione peroxidase to protect against oxidative damage lies on the auto-oxidation of glutathione, which can be reduced back by glutathione reductase. Much like catalase, glutathione also converts hydrogen peroxide to water (Peng *et al*., 2014; Weydert and Cullen, 2010).

$$H_2O_2 + 2GSH \xrightarrow{\text{Glutathione peroxidase}} 2H_2O + GSSG$$

The net result is that in conjunction with SOD, catalase and glutathione peroxidase all contribute to the cellular defense against excessive ROS accumulation. These have the capability of converting cytotoxic oxygen forms to water and the balance of the three enzymes will be key in the effective fight against oxidative stress (Weydert and Cullen, 2010).

OmniGen-AF as an antioxidant supplement for beef cattle

OmniGen-AF (Phibro Animal Health, Quincy, IL) is a patented nutritional supplement designed to augment and support the immune system of cattle. This nutritional supplement consists of live yeast (*Saccharomyces cerevisiae*) and premixes of vitamins and minerals that are carefully selected through the use of nutrigenomics (use of microarray technology) to aid in the nutrient modulation of genetic expression to promote cellular health (https://www.youtube.com/watch?v=ULj5t5tN7vo). In dairy cattle, OmniGen-AF is known to reduce somatic cell count and improve overall heard health (Ou *et al*., 2011) which has been linked to an augment of the innate immune response evidenced by increased neutrophils such as L-selectin and interlukin-1β in pathogen challenged ruminants (Wang *et al.*, 2004). In beef cattle, supplementation of OmniGen-AF has also proven to modulate the metabolic response during an immune challenge by preventing the breakdown of substrates such as proteins or fat as energy sources in times of an immune challenge (Burdick-Sanchez *et al*., 2014b) and at the same time priming the immune system to better respond to an immune challenge (Burdick-Sanchez *et al*., 2014a). Although OmniGen-AF is not classified as an antioxidant, the beef cattle industry could largely benefit from using this supplement if it posed the opportunity of

incorporating antioxidants into muscle foods to maintain meat quality over longer aging periods and retail display times.

Conclusion

Corn distillers grains have become a staple in feedlot rations as a source of protein and energy for cattle. Given the availability and the push toward ethanol energy production the forecast for the continued used of ethanol by-products looks bright. Innovations to further maximize the use and economic benefits of ethanol by-products open the door for modified feed alternatives available to producers such as de-oiled corn distillers grains. In the past, traditional or full-fat WDGS, although having superior performance in comparison to finishing diets not containing distillers grains, have resulted in beef with greater lipid oxidation due to increased PUFA content. However, to this point it is yet unknown if feeding de-oiled corn distillers grains would alter beef fatty acid composition and consequently beef shelf life. Thus, having a deeper understanding of the impacts of feeding de-oiled distillers grains on meat quality attributes is paradigm for the continued production of high quality beef in Nebraska. At the same time, a more profound understanding of the effectiveness of secondary antioxidants as well as primary antioxidant protection mechanisms against lipid oxidation could be valuable as a means to offset any potential detrimental effects of feeding corn distillers grains on beef shelf life.

MATERIALS AND METHODS

Study 1: Impact of Feeding De-Oiled Wet Distillers Grains Plus Solubles on Beef Shelf Life

University of Nebraska-Lincoln's Animal Care and Use Committee approved of all animal use protocols (IACUC # 902).

Cattle and dietary treatments

A total of 336 crossbred yearling steers (initial BW = 351.08 ± 19.05 kg) were fed (University of Nebraska feedlot at Mead, NE) one of seven finishing diets: an all corn control (1:1 blend of dry-rolled and high moisture corn with 12% corn silage), or 35%, 50%, or 65% inclusion of WDGS, either full-fat or de-oiled. Prior to the trial, steers were limited fed for five d (2% BW) and weighed in two consecutive days in order to identify initial BW for the trial. On d 1 all steers were implanted with Revalor-XS (Merck Animal Health, Summit, NJ). Body weight was used to block and stratify cattle before randomly assigning cattle to their respective pens (8 hd/pen with 6 replications for a total of 42 pens). All WDGS were from a single plant (KAAPA Ethanol, Minden, NE) that delivered the distillers grains every three weeks throughout the duration of the finishing period (147 d). All seven dietary treatments had 5% supplement inclusion which resulted in approximately 380 mg/steer/d of Rumensin® as well as 90 mg/steer/d of Tylan®. See Appendix I for the complete dietary composition.

Sample collection

At slaughter (October, 2012; Greater Omaha Packing, Omaha, NE) HCW was noted and 48 hours *post-mortem* carcass characteristics such as 12th rib fat thickness, LM area, marbling score, and USDA quality grades were recorded at the plant. The adjusted

final body weight was calculated after adjusting for a common dressing percentage (63%) based on the HCW. Prior to grading, carcasses were tagged with a university number that corresponded to the pen cattle were fed. Once carcasses were USDA graded, two to three low Choice carcasses within the same pen were selected for a total of fifteen low Choice carcasses within each treatment (n = 105). Selected carcasses were railed off to a separate rail at which time strip loins (*Longissimus lumborum*; IMPS # 180, NAMP, 2007) 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 new UNL meat lab number ranging from 1 to 105. Identified loins were vacuum packaged with corresponding laminated ID tags and transported to the Loeffel meat laboratory at the University of Nebraska-Lincoln. Strip loins were aged for 7 and 21 d (2°C) under dark storage.

Sample fabrication and preparation

Loins were fabricated anterior to posterior where a 1.27 cm steak was removed at both anterior and posterior ends to remove surfaces with outer exposure. After 7 d of aging, loins were fabricated into 2.54 cm steaks for visual discoloration and tenderness, and 1.27 cm steaks for fatty acid profile, proximate composition and lipid oxidation. The remaining portion of the loin was vacuum sealed (3 mil STD barrier, Prime Sources, St. Louis, MO) with a Multivac Packaging machine (Mutivac C500, Multivac, Kansas city, MO) and the same fabrication map was used at 21 d *post-mortem*. At both aging periods, samples for visual discoloration, tenderness and lipid oxidation were placed on foam trays (21.6 x 15.9 x 2.1 cm, Styro-Tech, Denver, CO), overwrapped with oxygen

permeable film (PVC-OW; PSM18, Prime Source, St. Louis, MO) and placed under retail display (RD) conditions for 4 and 7 d (2.7°C under white fluorescence lighting at 1000 to 1800 lux). Steaks used for fatty acid profile, proximate composition and 0 d RD were vacuum packaged and frozen for further analysis (-80°C). Later, samples free of subcutaneous fat for proximate analysis, fatty acids and lipid oxidation were frozen in liquid nitrogen and then powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT). Powdered samples were stored at -80°C.

Proximate analysis

Proximate analysis was performed to determine fat, moisture and ash; and protein determined by difference. Fat was quantified following the Soxhlet procedure (AOAC, 1990). Samples were measured in triplicate in Whatman #2 filter paper and fat was extracted with anhydrous ether (see step by step instructions in Appendix II). Briefly, after identifying and recording the weights of the folded filter papers with corresponding paper clips to hold packets closed, these were tared out and powdered meat samples (2 g) were weighed onto the center of the filter paper and these were then closed using the paper clip. Samples were then placed in Soxhlet tubes and the boiling flasks were filled with 400 mL of ether. Once in place, water was opened to enter the condensers and each individual burner was turned on. After 48 h, burners were turned off and allowed to cool completely. Samples were then air-dried under a fume hood for about two h at which time samples were transferred to a drying oven (105°C) overnight prior to recording final dry weight. In order to calculate final fat percentage the following equation was used:

% Fat = ((Pre-extraction wet weight with filter paper and paper clip – Post-extraction dry sample weight) / Sample weight) * 100) - % Moisture

Fat percentages were averaged per sample and eventually used to convert fatty acid percent data to mg/100 g tissue basis.

Moisture and ash (Appendix III) were determined with a LECO thermogravimetric analyzer (LECO Corporation, Model 604-100-400, St. Joseph, MI), and samples were measured in duplicates. Moisture was determined in nitrogen atmosphere with a start temperature of 25°C and an end temperature of 130°C (17 min ramp rate). Ash was determined in oxygen atmosphere with a start temperature of 130°C and an end temperature of 600°C (30 min ramp rate).

Fatty acid composition

Fatty acid profiles were obtained via gas chromatography as described by Folch *et al*. (1957) with modifications detailed by Morisson and Smith (1964) and Metcalfe *et al*. (1966). See full step by step instructions and gas chromatography settings in Appendix IV with pictures in Appendix V. Briefly, 1 g of powdered sample was weighed into a 15 mL conical tube to which 5 mL of 2:1 chloroform:methanol was added in order to separate the lipid fraction and vortexed for 5 s. After sitting for one hour at room temperature, samples were filtered through Whatman #2 filter paper onto a 13 x 150 mm glass screw cap tube. The volume was brought up to 10 ml with 2:1 chloroform:methanol using a water filled reference tube beside them. In order to separate any extracted proteins, 2 mL of 0.74% KCl were added and vortexed for 5 s. After centrifuging samples (1,000 x g for 5 min) the top aqueous layer was aspirated off and the remaining bottom layers containing the fatty acids were dried down completely on a heating block (60°C) under constant nitrogen purge. Then to achieve saponification, 0.5 mL of 0.5 M NaOH in methanol was added, vortexed (5 s) and heated (100°C) for 5 min.

Boron trifluoride in 14% methanol was added (0.5 ml), vortexed (5 s) and heated (100°C for 5 min) in order to methylate the fatty acids. Subsequently, 1 mL of saturated salt solution and 1 mL of hexane were added, vortexed (5 s) and samples were centrifuged (1,000 x g for 5 min). The top hexane layer was carefully pipetted into gas chromatography glass vials, nitrogen purged and lids were crimped on. Samples were stored at -80°C until analysis for approximately 2 months. Chromatography was done using a Chromopack CP-Sil 88 fused silica column (0.25 mm x 100 m, Santa Clara, CA) with an injector temperature of 270°C and a detector temperature of 300°C (Hewlett-Packard 6890 FID GC System; Agilent Technologies, Santa Clara, CA). The head pressure was set at 275kPa (40 psi) with a flow rate of 1.0 mL/min and total run time was 70 min. Individual fatty acids were identified by their retention times in relation to known standards and the percentage of fatty acids were determined by the peak areas in the chromatograph. Values were adjusted according to percent fat and values were converted to mg/100 tissue.

Objective color (L*, a*, b*)

Color measures were taken on a daily basis once samples were placed on retail display at all aging time points. Color was evaluated on steaks that were plastic overwrapped with oxygen permeable film (PVC-OW; PSM18, Prime Source, St. Louis, MO) and destined for tenderness assessment at the end of retail display. Objective color measures were collected using the L*, a*, b* scales with a Minolta CR-400 colorimeter (Minolta, Osaka, Japan). The handheld colorimeter reading device with an opening of 8mm was set to use a D65 illuminant and 2° observer. The colorimeter was set to record and print an average of 6 readings per steak. The calibration process was done on a daily

basis using a white ceramic tile (Calibration Plate, Serial No. 14933058, Konica Minolta, Japan) and the D65 settings were set as follows: Y = 93.13, x = 0.3164 and y = 0.3330 (Appendix VI). Once calibrated, the color space was selected to be the L*, a*, and b* scale; where, L* is a measure of lightness and has a range from 0 (black) to 100 (white), a* is a measure of redness and has a positive (red) to negative range (green), and b* is a measure of yellowness with a positive (yellow) to negative (blue) range. Color readings were recorded within a ± 2 hour window time frame for consistency and accuracy of data.

Subjective color (Discoloration)

Visual discoloration was assessed daily during RD with a trained five-person panel composed of graduate students from the University of Nebraska-Lincoln. Prior training consisted of becoming familiar with a color guide (Appendix VII) to be used as a reference to evaluate individual steaks under RD. A percentage scale was used were 0% meant no discoloration and 100% meant complete surface discoloration. Panelists were instructed to daily evaluate samples within a ±2 hour window time frame for consistency and accuracy of data. Samples were also randomly rotated daily to minimize any possible location effects.

Lipid oxidation

Lipid oxidation was determined with the 2-thiobarbituric acid reactive substances protocol (TBARS; Appendix VIII) as described by Ahn *et al*. (1998) which was modified from Beuge and Aust (1978). Briefly, five g of powdered sample were weighed into a 50 mL conical tube to which 14 mL of deionized distilled water and 1 mL of butylated hydroxyanisole (BHA) were added (10% BHA: 90% ethanol). The samples were then homogenized with a polytron (POLYTRON® Kinimatica CH-6010, Switzerland) for 15s.

The samples were then centrifuged (2,000 x g for 5 min). One mL of the supernatant was transferred to a 15 mL conical tube and 2 mL of TBA/TCA solution (15% TCA and 20 mM TBA in deionized distilled water) was added to each tube. Samples were then vortexed for 5 s and placed in a water bath (70°C for 30 min). During this incubation time samples developed the color that would indicate the magnitude of lipid oxidation in relation to the known concentration of the standards. After cooling for at least 10 min in a cold water bath, samples were centrifuged (2,000 x g for 15 min) and 200 μL of supernatant were transferred to 96-well plates in duplicate (Microtest III sterile 96 well flat bottomed microplate; Becton Dickinson & Company, Lincoln Park, NJ). All 96-well plates had standards made with 1, 1, 3, 3-tetraethoxypropane in order to calculate standard curves and ultimately determine the mg of malonaldehyde per kg of tissue. Plates were read at an absorbance of 540 nm (Model Epoch, Biotek, Winooski, VT). See step by step pictures in Appendix IX and a platting map in Appendix X.

Tenderness

Tenderness was measured via Warner-Bratzler Shear Force (WBSF). Samples were randomly sorted to cooking days making sure that all the samples from the same animal were cooked on the same day to minimize potential cooking day effects on tenderness data. Samples were thawed (4°C) 24 hours prior to cooking. Temperature was individually monitored for each steak using an insulated T thermocouple (5SC-TT-T-30-120, OMEGA Engineering, Inc., Stamford, CT) connected to a handheld thermometer (OMEGA 450-ATT, Engineering, Inc., Stamford, CT). The thermocouples were woven into the geometric center of each steak with large needles. Steaks were cooked on Hamilton Beach grills (Model 31605A, Proctor-Silex, Inc., Washington, NC)

until they reached an internal temperature of 35°C at which time they were flipped to continue cooking from the other side. The final internal temperature was 71°C. Cooked steaks were refrigerated for 24 hr and six cores (1.27 cm diameter) were taken parallel to the muscle fiber with a drill press and sheared using a portable Warner-Bratzler shear machine (3000, WBS 25 kg scale, 115 motor, ½ coring cutter, G-R Manufacturing Co., Manhattan, KS). All samples were sheared with the triangular WBSF attachment and the average of the 6 cores was calculated for statistical analysis.

Statistical analysis

Statistical analysis was done with SAS (version 9.2, Cary, NC, 2009). The experimental design was a 7 x 2 x 3 factorial where the main effects of dietary treatment, aging, retail display and their interactions were tested. Pen was considered a random variable in the model and pen variation was accounted for as an error term. Individual animal served as the experimental unit. The PROC MIXED procedure was used for the analysis of repeated measures of objective color and subjective visual discoloration where the most appropriate covariance structure was selected based on the best fit model. The PROC GLIMMIX procedure was used to evaluate all other variables measured. All means were separated with the LS MEANS statement and the TUKEY adjustment was used with an alpha level of 0.05.

Study 2: Impact of Feeding De-Oiled Dry Distillers Grains Plus Solubles on Beef Shelf Life

University of Nebraska-Lincoln's Animal Care and Use Committee approved of all animal use protocols (IACUC# 902).

Cattle and dietary treatments

A total of 448 crossbred yearling steers (initial BW = 363.78 ± 13.15 kg) were fed (University of Nebraska feedlot at Mead, NE) one of seven finishing diets that were part of a study looking at the effect of modifying different components of distillers grains on feedlot performance (Carlson *et al*., 2016). As part of that larger study, our group was interested in collecting samples from their negative control (diet not including any distillers grains plus solubles, rather 50% dry-rolled corn) and their positive control (replacement of 50% dry-rolled corn with 50% de-oiled dry distillers grains plus solubles) groups (Appendix XI). Prior to the trial, steers were limited fed for five d (2% BW) with a diet containing 50% Sweet Bran® (corn gluten feed; Cargill; Blair, NE) and 50% alfalfa hay. Steers were then weighed in two consecutive days in order to identify initial BW for the trial. On d 1 all steers were implanted with Ralgro® and re-implanted on d 36 or d 38 (evenly split between both days) with Revalor®-200 (Merck Animal Health, Summit, NJ). Body weight was used to block and stratify cattle before randomly assigning cattle to their respective pens (8 hd/pen with 8 replications for a total of 56 pens). All cattle were fed diets containing 31.5% high-moisture corn, 5.5% alfalfa hay, 5% liquid molasses, 5% supplement (formulated to have 30 g/ton Rumensin® and 90 mg/steer/day of Tylan®, on a DM basis from Elanco Animal Health), and 4% corn stillage. Cattle were harvested in two dates, fed for either 149 d or 156 d (based on cattle gain and harvest end point), in a commercial abattoir (Greater Omaha Pack, Omaha, NE).

Sample collection

Samples were identified and collected from cattle slaughter on the second kill date (November, 2014; Greater Omaha Packing, Omaha, NE). Therefore, the selection

process was conducted on 12 out of the 16 pens receiving the diets of interest (6 pens/treatment slaughtered on second date x 2 treatments of interest). At slaughter HCW was noted and 48 hours *post-mortem* carcass characteristics such as 12th rib fat thickness, LM area, marbling score, and USDA quality grades were recorded at the plant. The adjusted final BW was calculated after adjusting for a common dressing percentage (63%) based on the HCW. Prior to grading, carcasses were tagged with a university number that corresponded to the pen cattle were fed. Once carcasses began going through the grading rail four low Choice carcasses within the same pen were selected for a total of 24 low Choice carcasses within each treatment (n = 48). Selected carcasses were railed off to a separate rail at which time strip loins (*Longissimus lumborum*; IMPS # 180, NAMP, 2007), both from the right and left sides, were market 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 new UNL meat lab number ranging from 1 to 48 for left sides and 101 to 148 for right sides. Identified loins were vacuum packaged with corresponding food-grade ID tags and transported to the Loeffel meat laboratory at the University of Nebraska-Lincoln. Strip loins were aged for 2, 8, 14, and 21 d (2°C) under dark storage.

Sample fabrication and preparation

Loins were fabricated anterior to posterior where a 1.27 cm steak was removed at both anterior and posterior ends to remove surfaces with outer exposure. After 2 d of aging / collection day, all strip loins were opened and split in half at which time aging time was allotted as follows: anterior left = 2 d age, posterior left = 8 d age, anterior right

= 14 d age, and posterior right = 21 d age. Loin portions corresponding to 2 d age were fabricated into 1.27 cm steaks destined for fatty acid analysis, proximate composition, lipid oxidation, and 2.54 cm steaks for tenderness measures at 0 and 7 d of retail display. The remaining portion of the loins were vacuum sealed immediately (3 mil STD barrier, Prime Sources, St. Louis, MO) with a Multivac Packaging machine (Mutivac C500, Multivac, Kansas city, MO). The same fabrication map was used at 8, 14 and 21 d aged loins, with the exception of the fatty acid sample that was only taken from the loin portions aged for 2 d. Additional 1.27 cm steaks were also fabricated at each age time for mitochondria and sarcoplasmic reticulum extraction and analysis as part of a separate project.

At all aging periods, samples for visual discoloration, tenderness and lipid oxidation were placed on foam trays (21.6 x 15.9 x 2.1 cm, Styro-Tech, Denver, CO), overwrapped with oxygen permeable film (PVC-OW; PSM18, Prime Source, St. Louis, MO) and placed under retail display (RD) conditions for 4 and 7 d (2.7°C under white fluorescence lighting at 1000 to 1800 lux). Steaks used for fatty acid profile, proximate composition and 0 d RD were vacuum packaged and frozen for further analysis (-80°C). Later, samples free of subcutaneous fat for proximate analysis, fatty acids and lipid oxidation were frozen in liquid nitrogen and then powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT). Powdered samples were stored at -80°C for about 2 months.

Proximate analysis

Proximate analysis was conducted on all samples as explained in study 1.

Fatty acid composition

Fatty acid profiles were obtained via gas chromatography as described by Folch *et al*. (1957) with modifications detailed by Morisson and Smith (1964) and Metcalfe *et al*. (1966) as explained in study 1.

Objective color (L*, a*, b*)

Color measures were taken on a daily basis once samples were placed on retail display at all aging time points as described in study 1.

Subjective color (Discoloration)

Visual discoloration was assessed daily during RD with a trained five-person panel composed of graduate students from the University of Nebraska-Lincoln as described in study 1.

Lipid oxidation

Lipid oxidation was determined with the 2-thiobarbituric acid reactive substances protocol (TBARS) as described by Ahn *et al*. (1998) which was modified from Beuge and Aust (1978) and is described in study 1.

Tenderness

Tenderness was measured via Warner-Bratzler Shear Force (WBSF) as described in study 1. However, rather than using the bench-top WBSF machine, the WBSF cores were measured using a texture analysis (model TMS-PRO, Food Technology Crop., Sterling, VA) fitted with a Warner-Bratzler blade. The texture analyzer was connected to a laptop that recorded all data points generated simultaneously. Data were subsequently averaged on a per sample basis and analyzed.

Statistical analysis

Statistical analysis was done with SAS (version 9.4, Cary, NC, 2009). The experimental design was a completely randomized design where the main effects of dietary treatment, aging, retail display and their interactions were tested. Pen was considered a random variable in the model and pen variation was accounted for as an error term. Individual animal served as the experimental unit. The PROC GLIMMIX procedure was used for the analysis of repeated measures of objective color and subjective visual discoloration where the most appropriate covariance structure was selected based on the best fit model. The PROC GLIMMIX procedure was also used to evaluate all other variables measured. All means were separated with the LS MEANS statement and the TUKEY adjustment was used with an alpha level of 0.05. When triple interactions were identified the SLICEBY option in PROC GLIMMIX was utilized to evaluate the response of two of the classifying variables after fixing a third classifying variable. In our case we fixed age and examined differences between retail display and dietary treatment within the four different aging times.

Study 3: Impact of Supplementing Cattle with OmniGen-AF at the Receiving or Finishing Phase on Beef Shelf Life and Superoxide Dismutase Activity

University of Nebraska-Lincoln's Animal Care and Use Committee approved of all animal use protocols (IACUC# 902).

Cattle and dietary treatments

A total of 288 calf-fed steers (initial BW = 263.54 ± 18.60 kg) were fed (University of Nebraska feedlot Mead, NE) one of three dietary treatments: a control diet not containing OmniGen-AF supplementation (CON), a diet containing 4 g/45.36kg BW/hd/d at the receiving phase (first 28 days at the feedlot; REC), and a diet containing 4 g/45.36kg BW/hd/d all throughout the finishing phase (FIN) that lasted 215 d. Prior to the trial, steers were allowed access to water, processed, weighed, and randomly sorted to treatments within 12 hours of arrival. Steers were grouped 8 hd/pen, with 12 pens per treatment, for a total of 36 pens. On a daily basis, OmniGen-AF was top dressed in pens assigned to either the REC or FIN supplementation groups. At the receiving phase, all cattle were fed a blend of alfalfa hay (30%) and dry rolled corn (30%), with 36% Sweet Bran® (corn gluten feed, Cargill; Blair, NE) plus 4% supplement. However, after the receiving phase (first 28 d), in order to determine an accurate weight prior to commencing the finishing phase, cattle were limited fed (50% alfalfa hay and 50% Sweet Bran®) for five d (2% BW) and weighed in two consecutive days in order to identify initial BW for the finishing phase. A transition period of 21 d was allowed between the receiving and finishing diets where alfalfa hay was replaced (27.5%, 20%, 12.5%, 5% and 0%) with high moisture corn (22.5%, 30%, 37.5%, 45%, and 50% for 3 d, 4 d, 7 d, and 7 d respectively). The finishing diet then consisted of 50% high moisture corn, 40% Sweet Bran®, 5% wheat straw, and 5% supplement (see both receiving and finishing diet composition in Appendix XII).

At the end of the receiving phase, all steers were implanted with Revelor® XS (Merck Animal Health, Summit, NJ) and the REC group was transferred to the CON diet

while the FIN group continued receiving OmniGen-AF supplementation all through the finishing phase. The OmniGen-AF supplementation amounts were re-calculated and re-adjusted every 30 d. The finishing phase lasted 215 d of which the last 28 d and all steers on the trial were fed 300 mg/hd/d of Optaflexx® (Elanco Animal Health).

Sample collection

At slaughter (May, 2014; Greater Omaha Packing, Omaha, NE) HCW was noted and 48 hours *post-mortem* carcass characteristics such as 12th rib fat thickness, LM area, marbling score, and USDA quality grades were recorded at the plant. The adjusted final BW was calculated after adjusting for a common dressing percentage (63%) based on the HCW. Prior to grading, carcasses were tagged with a university number that corresponded to the pen cattle were group in. Once carcasses began going through the grading rail two low Choice carcasses within the same pen were selected for a total of twenty-four low Choice carcasses within each treatment (n = 72). Selected carcasses were railed off to a separate rail at which time strip loins (*Longissimus lumborum*; IMPS # 180, NAMP, 2007) from both the left and right sides were market using food grade carcass crayons (Industrial markers Dixon No. 1530R Joseph Dixon Crucible Co., New Jersey, N. J.) and plastic carcass push pins (Beef brads, KMC-KK1075-064, Butchers and Packers supplies, Edmonton, AB) with new UNL meat lab numbers ranging from 1 to 72 for the left sides and 101-172 for the right sides. Identified loins were vacuum packaged with corresponding food grade ID tags and transported to the Loeffel meat laboratory at the University of Nebraska-Lincoln. Strip loins were aged for 8, 22 and 29 d (2°C) under dark storage.

Sample fabrication and preparation

Loins were fabricated anterior to posterior where a 1.27 cm steak was removed at both anterior and posterior ends to remove surfaces with outer exposure. After 8 d of aging, left side strip loins were opened where the anterior half of the loin was designated to 8 d age fabrication and the remaining posterior half of the strip loin was re-vacuum packaged (3 mil STD barrier, Prime Sources, St. Louis, MO) with a Multivac Packaging machine (Mutivac C500, Multivac, Kansas city, MO) to age for 22 d. The anterior half of the right side strip loins was used for 29 d aging. Loin portions corresponding to 8 d age were fabricated into 1.27 cm steaks destined for fatty acid analysis, proximate composition, lipid oxidation, and 2.54 cm steaks for tenderness measures at 0 and 7 d of retail display. An additional 1.27 cm steak was fabricated destined for superoxide dismutase activity measures as well as an additional 2.54 cm steak was reserved as an extra sample. The same fabrication map was used at 22 and 29 d aged loins, with the exception of the fatty acid sample that was only taken from the loin portions aged for 8 d.

At all aging periods, samples for visual discoloration, tenderness and lipid oxidation were placed on foam trays (21.6 x 15.9 x 2.1 cm, Styro-Tech, Denver, CO), overwrapped with oxygen permeable film (PVC-OW; PSM18, Prime Source, St. Louis, MO) and placed under retail display (RD) conditions for 4 and 7 d (2.7°C under white fluorescence lighting at 1000 to 1800 lux). Steaks used for fatty acid profile, proximate composition and 0 d RD were vacuum packaged and frozen for further analysis (-80°C). Later, samples free of subcutaneous fat for proximate analysis, fatty acids and lipid oxidation were frozen in liquid nitrogen and then powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT). Powdered samples were stored

at -80°C for about 2 months. Powdered meat samples were also used for the quantification of superoxide dismutase activity and protein concentration determination. These samples were stored for about 6 months (-80°C).

Proximate analysis

Proximate analysis was conducted on all samples as explained in study 1.

Fatty acid composition

Fatty acid profiles were obtained via gas chromatography as described by Folch *et al*. (1957) with modifications detailed by Morisson and Smith (1964) and Metcalfe *et al*. (1966) as explained in study 1.

Objective color (L*, a*, b*)

Color measures were taken on a daily basis once samples were placed on retail display at all aging time points as described in study 1.

Subjective color (Discoloration)

Visual discoloration was assessed daily during RD with a trained six-person panel composed of graduate students from the University of Nebraska-Lincoln as described in study 1.

Lipid oxidation

Lipid oxidation was determined with the 2-thiobarbituric acid reactive substances protocol (TBARS) as described by Ahn *et al*. (1998) which was modified from Beuge and Aust (1978) and is described in study 1.

Tenderness

Tenderness was measured via Warner-Bratzler Shear Force (WBSF) as described in study 1. However, rather than using the bench-top WBSF machine, the WBSF cores

were measured using a texture analysis (model TMS-PRO, Food Technology Crop., Sterling, VA) fitted with a Warner-Bratzler blade. The texture analyzer was connected to a laptop that recorded all data points generated simultaneously. Data were subsequently averaged on a per sample basis and analyzed.

Superoxide Dismutase activity

Superoxide dismutase (SOD) activity was determined with a colorimetric assay kit by Abcam capable of detecting SOD activity in tissues (ab65354; Cambridge, MA). The general principle of the kit is that through the use of a Water Soluble Tetrazolium Salt (WST-1) a water-soluble formazan dye is produced upon the reduction with the superoxide anion. The reduction is linearly related to the xanthine oxidase activity which is inhibited by SOD. Hence, the inhibition rate of SOD can be measured calorimetrically (the greater the color, the lower the SOD activity and vice versa). See detailed protocol with plating map in Appendix XIII.

Briefly, for the reagent preparation, the WST solution and the SOD enzyme solution were prepared from reagents included in the kit. The WST solution was made by combining 1 mL of WST solution with 19 mL of the SOD assay buffer. The SOD enzyme solution was made by combining 15 μL of SOD enzyme solution plus 2.5 mL of the dilution buffer. Next, the tissue sample preparation consisted of sonicating (Fisher Scientific Sonic Dismembrator Model 100) 10 mg of powdered meat sample in 150 μL of ice cold sample buffer (0.1M Tris/HCl at a pH of 7.4 containing 0.5% Triton X-100, 5 mM Beta-Mercaptoethanol [β-ME], 0.1 mg/mL Phenylmethylsulfonyl fluoride [PMSF]). After sonicating samples for about 15 s these were centrifuged at 14,000 x g for 5 min at 4°C (Eppendorf Model 5430; Eppendorf, Hamburg, Germany). Then, 100 μL of

supernatant was transferred to a clean 15 mL tube with 4.5 mL of sample buffer. Now that the sample has been diluted to the proper concentration, 20 μL of sample is transferred in duplicate to a 96 well plate (Microtest III sterile 96 well flat bottomed microplate; Becton Dickinson & Company, Lincoln Park, NJ). To the sample wells, 200 μL of WST solution and 20 μL of the SOD enzyme solution are added. Each sample well will also have a corresponding "Blank 2" well that is also plated in duplicate and consists of 20 μL of sample, 200 μL of WST solution and 20 μL of the dilution buffer. In order to calculate SOD activity two "Blank 1" and "Blank 3" wells are also filled with 20 μL of ddH_2O, 200 μL of WST solution and 20 μL of the SOD enzyme solution for "Blank 1" and 20 μL of ddH_2O, 200 μL of WST solution and 20 μL of the dilution buffer for "Blank 3." The addition of SOD enzyme solution must be done with a multi-channel repeater pipette (Thermo Scientific E1-ClipTip Electronic Pipette; Vantaa, Finland) to minimize reaction lag time and must be added at the end of the platting step. Once all wells are full (240 μL), the plate is placed in an incubator (Thermo-Shaker Grant-bio PHMP-4; Cambridgeshire, England) with a plate shaker set to 300 RPM's for 1 min. After shaking, the plate is allowed to incubate for 20 min at 37°C to develop color. Making sure the wells were bubble free (pop bubbles using a clean wooden toothpick per well) the plate absorbance was read at 450 nm (Model Epoch, Biotek, Winooski, VT). Finally, calculations for SOD activity were as follows:

$$\text{SOD activity (inhibition rate \%)} = [(A_{blank1} - A_{blank3}) - (A_{sample} - A_{blank2}) / (A_{blank1} - A_{blank3})] \times 100$$

The SOD activity was then converted to Units of activity with the following equation:

$$\text{SOD U} = [(\text{SOD \% Inhibition} / 50) / \text{Vol of assay sampled}] \times \text{Dilution factor}$$

Protein concentration determination

Protein concentration was determined using a detergent and reducing agent compatible protein kit (Bio-Rad RC DC Protein Assay) on the same samples used to measure SOD activity. Given that SOD activity was determined with meat in a buffer containing Triton X-100 (detergent) and Beta-Mercaptoethanol (reducing agent) the protein kit needed to meet these specifications. Briefly, utilizing the kit reagents, Reagent A' was made by mixing 5 µL of DC Reagent S to each 250 µL of DC Reagent A, where each standard or sample to be analyzed needed 127 µL of Reagent A' (calculated total amount of Reagent A' depended on the number of samples to be run that day). Then, the protein standard was made using bovine serum albumin in the same buffer as the SOD activity samples starting with an initial concentration of 1.45 mg/mL (standard 1). A serial delusion was made by transferring 1 mL of the first standard into 1 mL of buffer resulting in standard 2 with a concentration of 0.725 mg/mL. One mL from standard 2 was transferred to a new mL of buffer to generate standard 3 with a concentration of 0.3625 mg/ml. And lastly, 1 mL from standard 3 was transferred to a new mL of buffer to create standard 4 with a final concentration of 0.18125 mg/mL. Then, 25 µL of each standard and 5 µL of the supernatant left from the SOD activity protocol microfuge tube were transferred into a clean 2 mL microfuge tube. The RC Reagent I was then added (125 µL) to each tube, vortexed, and incubated at room temperature for 1 min. The RC Reagent II was added (125 µL) to each tube, vortexed, and centrifuged (Eppendorf Model 5430; Eppendorf, Hamburg, Germany) at 15,000 x g for 5 min. The supernatant was then discarded completely by inverting the tubes over

clean dry absorbent paper. Once tubes were dry, 127 μL of Reagent A' was added to all tubes. (NOTE: Reagent A' will start to precipitate and form crystals as it sits out; place Reagent A' mixture in a beaker with warn water and vortex until crystals go back into solution before using!) After the addition of Reagent A', samples were incubated at room temperature for 5 min. After incubating, tubes were vortexed and 1 mL on DC Reagent B was added to each tube, vortexed immediately and allowed to incubate at room temperature for 15 min to develop color. Using 96 well plates (Microtest III sterile 96 well flat bottomed microplate; Becton Dickinson & Company, Lincoln Park, NJ), wells were filled with 200 μL of supernatant in duplicate and absorbance was read at 750 nm (Model Epoch, Biotek, Winooski, VT). Using the standards and generating the best fit line allowed us to determine the protein concertation.

The SOD U of activity could then be normalized with the corresponding protein concertation values in order to present data in a SOD U/ mg protein basis. An example of these calculations is provided in Appendix XIV along with a plating map diagram.

Statistical analysis

Statistical analysis was done with SAS (version 9.4, Cary, NC, 2009). The experimental design was a completely randomized design where the main effects of dietary treatment, aging, retail display and their interactions were tested. Pen was considered a random variable in the model and pen variation was accounted for as an error term. Individual animal served as the experimental unit. The PROC GLIMMIX procedure was used for the analysis of repeated measures of objective color and subjective visual discoloration where the most appropriate covariance structure was

selected based on the best fit model. The PROC GLIMMIX procedure was also used to evaluate all other variables measured. All means were separated with the LS MEANS statement and the TUKEY adjustment was used with an alpha level of 0.05.

NOTE: Fabrication maps for all three studies are in Appendix XV

LITERATURE CITED

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.

Aldai, N., J. L. Aalhus, M. E. R. Dugan, W. M. Robertson, T. A. McAllister, L. J. Walter, and J. J. McKinnon. 2010. Comparison of wheat- versus corn-based dried distillers grains with solubles on meat quality of feedlot cattle. Meat Sci. 84:569-577.

Ames B. N., M. K. Shigenaga, and T. M. Hagen. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. Proc. Natl. Acad. Sci. 90:7915-7922.

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Atkinson, R. L., E. J. Scholljegerdes, S. L. Lake, V. Nayigihugu, B. W. Hess, and D. C. Rule. 2006. Site and extent of digestion, duodenal flow, and intestinal disappearance of total and esterified fatty acids in sheep fed a high-concentrate diet supplemented with high-linoleate safflower oil. J. Anim. Sci. 84:387-396.

Berger, L., and V. Singh. 2010. Changes and evolution of corn coproducts for beef cattle. J. Anim. Sci.88(E. Suppl.):143-150.

Beuge, J. A. and S. D. Aust. 1978. Microsomal lipid peroxidation. Methods Enzymol. 52: 302-310.

Boff, J. and D. B. Min. 2002. Chemistry and reaction of singlet oxygen in foods. Comp. Rev. Food Sci. 1:58-72.

Bolumar, T., M. L. Andersen, and V. Orlien. 2014. Mechanisms of radical formation in beef and chicken meat during high pressure processing evaluated by electron spin resonance detection and the addition of antioxidants. Food Chemistry 150:422-428.

Bothast, R. J., and M. A. Schlicher. 2005. Biotechnological processes foe conversion of corn into ethanol. Appl. Microbiol. Biotechnol. 67:19-25.

Buckner, C. D., T. J. Klopfenstein, G. E. Erickson, V. R. Bremer, and D. R. Mark. 2011. Economics for feeding distillers grains to finishing cattle. 2011 Nebraska Beef Cattle Report:37-39.

Burdick-Sanchez, N. C, J. O. Buntyn, J. A. Carroll, T. Wistuba, K. DeHaan, S. E. Sieren,

S. J. Jones, and T. B. Schmidt. 2014a. Enhancement of the acute phase response to lipopolysaccharide in feedlot steers supplemented with OmniGen-AF. J. Anim. Sci. 92: E-Suppl. 2:37-38.

Burdick-Sanchez, N. C, J. O. Buntyn, J. A. Carroll, T. Wistuba, K. DeHaan, S. E. Sieren, S. J. Jones, and T. B. Schmidt. 2014b. Supplementation of OmniGen-AF during the receiving period modulates the metabolic response to a lipopolysaccharide challenge in feedlot steers. J. Anim. Sci. 92: E-Suppl. 2:250.

Buttrey, E. K., K. H. Jenkins, J. B. Lewis, S. B. Smith, R. K. Miller, T. E. Lawrence, F. T. McCollum III, P. J. Pinedo, N. A. Cole, and J. C. MacDonald. (2013). Effects of 35% corn wet distillers grains plus solubles in stem-flaked and dry-rolled corn-based finishing diets on animal performance, carcass characteristics, beef fatty acid composition, and sensory attributes. J. Anim. Sci. 91:1850-1865.

Campo, M. M., G. R. Nute, S. I. Hughes, M. Enser, J. D. Wood, and R. I. Richardson. 2006. Flavour perception of oxidation in beef. Meat Sci. 72:303-311.

Carlson, Z. E., C. J. Bittner, D. B. Burken, G. E. Erickson, and J. C. MacDonald. 2016. Modifying different components of distillers grains and the impact on feedlot performance. 2016 Nebraska Beef Cattle Report pp. 128-131.

Chao, M. D. 2015. Impact of wet distillers grains plus solubles and antioxidants on a basic mechanism of beef tenderization. Diss, Univ. of Nebraska-Lincoln, Digital Commons.

Cristiana, F., Elena, A., and Nina, Z. 2014. Superoxide dismutase: Therapeutic targets in SOD related pathology. Health 6:975-988.

Crnjar, E. D., A. Witchwoot, and W. W. Nawar. 1981. Thermal Oxidation of a Series of Saturated Triacylglycerols. J. Agric. Food Chem. 29:39-42.

Depenbusch, B. E., C. M. Coleman, J. J. Higgins, and J. S. Drouillard. 2009. Effects of increasing levels of dried corn distillers grains with solubles on growth performance, carcass characteristics, and meat quality of yearling heifers. J. Anim. Sci. 87:2653-2663.

Descalzo, A. M. E. M. Insani, A. Biolatto, A. M. Sancho, P. T. García, N. A. Pensel, and J. A. Josifovich. 2005. Influence of pasture or grain-based diets supplemented with vitamin E on antioxidant/oxidative balance of Argentine beef. Meat Sci. 70:35-44.

Djenane, D., A. Sánchez-Escalante, J. A. Beltrán and P. Roncalés. 2001. Extension of the retail display life of fresh beef packaged in modified atmosphere by varying lighting conditions. J. of Food Sci. 66:181-186.

Djenane, D., A. Sánchez-Escalante, J. A. Beltrán, and P. Roncalés. 2003. Extension of the shelf life of beef steaks packaged in a modified atmosphere by treatment with rosemary and displayed under UV-free lighting. Meat Sci. 64:417-426.

Enser, M., K. Hallett, B. Hewitt, G. A. J. Fursey, and J. D. Wood. 1996. Fatty acid content and composition of english beef, lamb and pork at retail. Meat Sci. 42:443-456.

Erickson, G. E., C. D. Buckner, and T. J, Klopfenstein. 2010. Feeding corn milling co-products to feedlot cattle. 3rd Edition.

Faustman, C., Q. Sun, R. Mancini, and S. P. Suman. 2010. Myoglobin and lipid oxidation interactions: Mechanistic bases and control. Meat Sci. 86:86-94.

Fernández, J., J. A. Pérez-Álvarez, and J. A. Fernández-López. 1997. Thiobarbituric acid test for monitoring lipid oxidation in meat. Food Chemistry 59:345-353.

Folch, J., M. Lees, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-509.

Ganesan, V., K. A. Rosentrater, and K. Muthukumarappan. 2009. Physical and flow properties of regular and reduced fat distillers dried grains with solubles (DDGS). Food Bioprocess Technol. 2:156-166.

Gatellier, P., C. Hamelin, Y. Durand, and M. Renerre. 2001. Effect of a dietary vitamin E supplementation on colour stability and lipid oxidation of air- and modified atmosphere-packaged beef. Meat Sci. 59:133-140.

Gatellier, P., Y. Mercier, and M. Renerre. 2004. Effect of diet finishing mode (pasture or mixed diet) on antioxidant status of Charolais bovine meat. Meat Sci. 67:385-394.

Gill, R. K., D. L. VanOverbeke, B. Deoenbusch, J. S. Drouillard, and A. DiCostanzo. 2008. Impact of beef cattle diets containing corn or sorghum distillers or beef color, fatty acid profiles, and sensory attributes. J. Anim. Sci. 86:923-935.

Gordon, C. M., J. S. Drouillard, R. K. Phebus, K. A. Hachmeister, M. E. Dikeman, J. J. Higgins, and A. L. Reicks. 2002. The effect of Dakota Gold®-brand dried distillers grains with solubles of varying levels on sensory and color characteristics of ribeye steaks. Pages 72-74 in Cattlemen's Day, Manhattan, KS. Kansas State Univ., Manhattan.

Gray, J. I., E. A. Gomaa, and D. J. Buckley. 1996. Oxidative quality and shelf life of meats. Meat Sci. 43, Supplement 1:111-123.

Greene, B. E. 1969. Lipid Oxidation and Pigment Changes in Raw Beef. J. of Food Sci. 34:110-113.

Gunn, P. J., A. D. Weaver, R. P. Lemenager, D. E. Gerrard, M. C. Claeys, and S. L. Lake. 2009. Effects of dietary fat and crude protein on feedlot performance, carcass characteristics, and meat quality in finishing steers fed differing levels of dried distillers grains with solubles. J. Anim. Sci. 87:2882-2890.

Gutteridge, J. M. C. and B. Halliwell. 1993. Transition metal ions and antioxidant proteins in extracellular fluids. Atmos. Oxid. Antiox. 3:71-99.

Halliwell, B. 1997. Antioxidants and human disease: a general introduction. Nutr. Rev. 55:44-52.

Ham, G. A., R. A. Stock, T. J. Klopfenstein, E. M. Larson, D. H. Shain, and R. P. Huffman. 1994. Wet corn distillers byproducts compared with dried corn distillers grains with solubles as a source of protein and energy for ruminants. J. Anim. Sci. 72:3246-3257.

Hood, D. E. R., E. B. Reordan. 1973. Discolouration in pre-packaged beef: measurement by reflectance spectrophotometry and shopper discrimination. J. of Food Technol. 8:333-343.

Ismail, H. A., E. J. Lee, K. Y. Ko, and D. U. Ahn. 2008. Effects of aging time and natural antioxidants on the color, lipid oxidation and volatiles of irradiated ground beef. Meat Sci. 80:582-591.

Ismail, H. A., E. J. Lee, K. Y. Ko, and D. U. Ahn. 2009. Effect of antioxidant application methods on the color, lipid oxidation, and volitiles of irradiated ground beef. J. Food. Sci. 74:25-32.

Jenschke, B. E., J. R. Benton, C. R. Calkins, T. P. Carr, K. M. Eskridge, T. J. Klopfenstein, and G. E. Erickson. 2008. Chemical and sensory properties of beef of known sources and finishing on wet distillers grains diets containing varying types and levels of roughage. J. Anim. Sci. 86:949-959.

Jenschke, B. E., J. M. James. K. J. Vander Pol, T. J. Klopfenstein, and C. R. Calkins. 2007. Wet distillers grains plus solubles do not increase liver-like off-flavors in cooked beef from yearling steers. J. of Muscle Foods 18:341-348.

Kanner, J. 1994. Oxidative processes in meat and meat products: Quality implications. Meat Sci. 36:169-189.

Kim, Y. H., J. T. Keeton, S. B. Smith, J. E. Maxim, H. S. Yang, and J. W. Savell. 2009. Evaluation of antioxidant capacity and colour stability of calcium lactate

enhancement on fresh beef under highly oxidising conditions. Food Chemistry 115:272-278.

Kim, Y., N. S. Mosier, R. Hendrickson, T. Ezeji, H. Blaschek, B. Dien, M. Cotta, B. Dale, and M. R. Ladisch. 2008. Composition of corn dry-grind ethanol by-products: DDGS, wet cake, and thin stillage. Bioresource Technology 99:5165–5176.

Klopfenstein, T. J., G. E. Erickson, and V. R. Bremer. 2008. Board-Invited Review: Use of distillers by-products in the beef cattle feeding industry. J. Anim. Sci. 86:1223-1231.

Koger, T. L., D. M. Wulf, A. D. Weaver, C. L. Wright, K. E. Tjardes, K. S. Mateo, T. E. Engle, R. J. Maddock, and A. J. Smart. 2010. Influence of feeding various quantities of wet and dry distillers grains to finishing steers on carcass characteristics, meat quality, retail-case life of ground beef, and fatty acid profile of longissimus muscle. J. Anim. Sci. 88:3399-3408.

Kunze, E.K., N. H. Herrera, K. I. Domenech-Pérez, M. D. Chao, C. R. Calkins. 2016. The influence of diet on calcium flux in fresh beef. American Meat Science Association 69th Reciprocal Meats Conference (Abstract).

Ladeira, M. M. L. C.Santarosa ,M. L.Chizzotti, E. M.Ramos, O. R. Machado Neto, D. M. Oliveira, J. R. R. Carvalho,L. S. Lopes, and J. S. Ribeiro. 2014. Fatty acid profile, color and lipid oxidation of meat from young bulls fed ground soybean or rumen protected fat with or without monensin. Meat Sci. 96:597-605.

Ladikos, D., V. Lougovois. 1990. Lipid oxidation in muscle foods: A review. Food Chemistry 35:295-314.

Larson, E. M., R. A. Stock, T. J. Klopfenstein, M. H. Sindt, and R. P. Huffman. 1993. Feeding value of wet distillers byproducts for finishing ruminants. J. Anim. Sci. 71:2228-2236.

Lee. J, N. Koo, and D. B. Min. 2004. Reactive oxygen species, aging, and antioxidant nutraceuticals. Comprehensive Reviews in Food Science and Food Safety 3:21-33.

Leupp, J. L., G. P. Lardy, M. L. Bauer, K. K. Karges, M. L. Gibson, J. S. Caton, and R. J. Maddock. 2009. Effects of distillers dried grains with solubles on growing and finishing steer intake, performance, carcass characteristics, and steak color and sensory attributes. J. Anim. Sci. 87:4118-4124.

Ma, H. J., D. A. Ledward, A. I. Zamri, R. A. Frazier, and G. H. Zhou. 2007. Effects of high pressure/thermal treatment on lipid oxidation in beef and chicken muscle.

Food Chemistry 104:1575-1579.

Mathews, K. H. Jr., and M. J. McConnell. 2009. Ethanol co-product use in U.S. cattle feeding: Lessons learned and considerations. Economic Research Service / USDA. http://www.ers.usda.gov/media/147398/fds09d01.pdf

Mello, A. S., C. R. Calkins, B. E. Jenschke, T. P. Carr, M. E. R. Dugan, and G. E. Erickson. 2012a. Beef quality of calf-fed steers finished on varying level of corn-based wet distillers grains plus solubles. J. Anim. Sci. 90:4625-4633.

Mello, A. S., B. E. Jenschke, L. S. Senaratne, T. P. Carr, G. E. Erickson, and C. R. Calkins. 2012b. Effects of feeding modified distillers grains plus solubles on marbling attributes, proximate composition, and fatty acid profile of beef. J. Anim. Sci. 90:4634-4640.

Metcalfe, L. D., A. A. Schmitz, and J. R. Pelka. 1966. Rapid separation of fatty acid esters from lipids for gas chromatography analysis. Anal. Chem. 38: 514-515.

Min,B. and D. U. Ahu. 2005. Mechanism of lipid peroxidation in meat and meat products – A review. Food Sci. Biotechnol. 14:152-163.

Morisson W. R. and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5: 600-608.

Morrissey, P. A., P. J. A. Sheehy, K. Galvin, J. P. Kerry, and D. J. Buckley. 1998. Lipid stability in meat and meat products. Meat Sci. 49, Supplement 1:S73-S86.

NAMP. 2007. The meat buyers guide. North American Meat Processors Association, Reston, VA.

Nishikawa, M., A. Tamada, H. Kumai, F. Yamashita, and M. Hashida. 2002. Inhibition of experimental pulmonary metastasis by controlling biodistribution of catalase in mice. Int. J. Cancer 99:474-479.

Nelson, K. K., A. C. Ranganathan, J. Mansouri, A. M. Rodriguez, K. M. Providence, J. L. Rutter, K Pumiglia, J. A. Bennett, and J. A. Melendez. 2003. Elevated Sod2 activity augments matrix metalloproteinase expression: evidence for the involvement of endogenous hydrogen peroxide in regulating metastasis. Clinical Cancer Research 9:424-432.

O'Grady, M. N., F. J. Monahan, R. M. Burke, and P. Allen. 2000. The effect of oxygen level and exogenous α-tocopherol on the oxidative stability of minced beef in modified atmosphere packs. Meat Sci. 55:39-45.

Ou, B. R., Y. Q. Wang, N.E. Forsberg. 2011. Effects of feeding OmniGen-AF to rats on gastrointestinal gene expression: Microarray analysis. J. Anim. Sci. 89 E-Suppl. 1:270-271.

Peng, C., X. Wang, J. Chen, R. Jiao, L. Wang, Y. Man Li, Y. Zuo, Y. Liu, L. Lei, K. Ying Ma, Y. Huang, and Z. Y. Chen. 2014. Biology of aging and role of dietary antioxidants. BioMed Research International: Volume 2014, Article ID 831841, 13 pages http://dx.doi.org/10.1155/2014/831841

Plascencia, A., G. D. Mendoza, C. Vásquez, and R. A. Zinn. 2003. Relationship between body weight and level of fat supplementation on fatty acid digestion in feedlot cattle. J. Anim. Sci. 81:2653-2659.

Pradhan, A. A., K. S. Rhee, and P. Hernández. 2000. Stability of catalase and its potential role in lipid oxidation in meat. Meat Sci. 54:385-390.

Renerre, M., F. Dumont, and P. Gatellier. 1996. Antioxidant enzyme activities in beef in relation to oxidation of lipid and myoglobin. Meat Sci. 43:111-121.

Renewable Fuels Association. 2007. Ethanol Industry Outlook 2007: Building New Horizons.

Renewable Fuels Association. 2015. Ethanol Industry Statistics. http://www.ethanolrfa.org/ Accessed March 24, 2015.

Richardson, L. F., A. P. Raun, E. L. Potter, C. O. Cooley, and R. P. Rathmacher. 1976. Effect of monensin on rumen fermentation in vitro and in vivo. J. Anim. Sci. 43:657-664.

Roeber, D. L., R. K. Gill, and A. DiCostanzo. 2005. Meat quality responses to feeding distillers grains to finishing Holstein steers. J. Anim. Sci. 83:2455-2460.

Saunders, J. A., and K. A. Rosentrater. 2009a. Properties of solvent extracted low-oil corn distillers dried grains with solubles. Biomass and Bioenergy 33:1486-1490.

Saunders, J. A., and K. A. Rosentrater. 2009b. Survey of US fuel ethanol plants. Bioresource Technology 100:3277-3284.

Scollan, N. D., N. J. Choi, E. Kurt, A. V. Fisher, M. Enser, and J. D. Wood. 2001. Manipulating of fatty acid composition of muscle and adipose tissue in beef cattle. British J. of Nutr. 85:115-124.

Scollan, N., Jean-François Hocquette, Karin Nuernberg, Dirk Dannenberger, Ian Richardson, and Aidan Moloney. 2006. Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. Meat Sci. 74:17-33.

Sebranek, J. G., V. J. H. Sewalt, K. L. Robbins, and T. A. Houser. 2005. Comparison of natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. Meat Sci. 69:289-296.

Segers, J. R., R. L. Stewart Jr., C. A. Lents, T. D. Pringle, M. A. Froetschel, B. K. Lowe, R. O. McKeith, and A. M. Stelzleni. 2011. Effect of long-term corn by-product feeding on beef quality, strip loin fatty acid profiles, and shelf life. J. Anim. Sci. 89:3792-3802.

Senaratne, L. S. 2012. Mechanism and control of beef toughening during retail display in high oxygen modified atmosphere packages. Diss, Univ. of Nebraska-Lincoln, Digital Commons.

Shapouri H. and P. Gallagher. 2005. USDA's 2002 Ethanol Cost-of-Production Survey. Agricultural Economic Report Number 841. http://ethanolrfa.3cdn.net/6dfd76a7273de6109c_uwm6bh2wy.pdf

Spallholz, J. E. 1989. Nutrition: Chemistry & Biology. Prentic-Hall, Inc., New Jersey.

Spiteller, G. 2001. Lipid oxidation in aging and age-dependent disease. Exp. Gerontol. 36:1425-1457.

Tonsor, G. T. 2006. Feed cost savings of distillers grains in feedlot rations. Michigan State University. http://www.agmanager.info/livestock/budgets/production/beef/DGSFeedCostSavings(Feedlot)WriteUp.pdf

U.S. Grains Council. 2012. Sulfur Concerns and Benefits in DDGS. A Guide to Distiller's Dried Grains with Solubles (DDGS), 3rd Ed., U.S. Grains Council, Washington, DC. Pages 146-150.

Vander Pol, K. J., G. E. Erickson, T. J. Klopfenstein, M . A. Greenquist, and T. Robb. 2006. Effect of dietary inclusion of wet distillers grains on feedlot performance on finishing cattle and energy value relative to corn. 2006 Nebraska Beef Cattle Report: 51-53.

Vander Pol, K. J., M. K. Luebbe, G. I. Crawford, G. E. Erickson, and T. K. Klopfenstein. 2009. Performance and digestibility characteristics of finishing diets containing distillers grains, composites of corn processing coproducts, or supplemental corn oil. J. Anim. Sci. 87:639-652.

Vannessa, S. J., T. J. Klopfenstein, G. E. Erickson, and K. K. Karges. Sulfur in Distillers Grains. 2009 Nebraska Beef Cattle Report:79-80.

Wang, Y. Q., S. B. Puntenney, and N. E. Forsberg. 2004. Identification of the

mechanisms by which OmniGen-AF, a nutritional supplement, augments immune function in ruminant livestock. J. Anim. Sci. 82(Suppl. 2):128-129.

Warren, H. E. N. D. Scollan, M. Enser, S. I. Hughes, R. I. Richardson, and J. D. Wood. 2008. Effects of breed and a concentrate or grass silage diet on beef quality in cattle of 3 ages. I: Animal performance, carcass quality and muscle fatty acid composition. Meat Sci. 78:256-269.

Waterbury, J. A., D. R. Mark, S. M. Thomas, G. E. Erickson, and T. J. Klopfenstein. 2009. An Economic Overview of Ethanol Co-product Utilization in Nebraska. University of Nebraska-Lincoln Extension Publication EC858. http://ianrpubs.unl.edu/live/ec858/build/ec858.pdf

Watkins, C. 2007. Two fuels from one kernel. Inform 18:714-717.

Waydert, J. C. and Cullen J. J. 2010. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissues. Nature Protocols 5:51-66.

Winkler-Moser, J. K., and L. Breyer. 2011. Composition and oxidative stability of crude oil extracts of corn germ and distillers grains. Industrial Crops and Products 33:572-578.

Wong, D. W. S. 1989. Mechanism and Theory in Food Chemistry. Van Nostrand Reinhold, New York.

Wood, J. D. M. Enser, A. V. Fisher, G. R. Nute, P. R. Sheard, R. I. Richardson, S. I. Hughes, F. M. Whittington. 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Sci. 78:343-358.

Yang, A., M. C. Lanari, M. Brewster, and R. K. Tume. 2002. Lipid stability and meat colour of beef from pasture- and grain-fed cattle with or without vitamin E supplement. Meat Sci. 60:41-50.

Zakrys, P. I, S. A. Hogan, M. G. O'Sullivan, P. Allen, J. P. Kerry. 2008. Effects of oxygen concentration on the sensory evaluation and quality indicators of beef muscle packaged under modified atmosphere. Meat Sci. 79:648-655.

Zinn, R. A., S. K. Gulati, A. Plascencia, and J. Salinas. 2000. Influence of ruminal biohydrogenation on the feeding value of fat in finishing diets for feedlot cattle. J. Anim. Sci. 78:1738-1746.

Impact of Feeding De-Oiled Wet Distillers Grains Plus Solubles on Beef Shelf Life

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ABSTRACT

Research was conducted to determine the effect of feeding de-oiled wet distillers grains plus solubles (WDGS) on beef fatty acid profile, retail shelf life and development of oxidation products during retail display (RD). A total of 336 crossbred yearling steers (initial BW = 351.08 ± 19.05 kg) were fed one of seven dietary treatments: an all corn control (1:1 blend of dry rolled and high moisture corn), 35%, 50%, or 65% inclusion of WDGS, either full-fat or de-oiled. Within each treatment 15 low Choice carcasses were randomly selected (n = 105), strip loins were obtained, aged 7 and 21 d, and representative steaks from each strip loin were placed in RD conditions for 7 d. Fatty acid profiles were determined and differences ($P \leq 0.05$) were found in the C16:1, C18:1T, C18:2 and polyunsaturated fatty acids (PUFA) among dietary treatments. Palmitoleic acid (C16:1) was predominant ($P < 0.0001$) in the corn control group, intermediate in the 35% de-oiled WDGS group, but no differences were observed between all other diets. Elaidic acid (C18:1T) was greater ($P = 0.01$) in the 65% full-fat WDGS group, least for the corn control group, and intermediate for all other diets. Linoleic acid (C18:2) was greater ($P = 0.0001$) in all three full-fat WDGS groups and 65% de-oiled WDGS group (290.98 mg/100 g, on average), intermediate in the 50% and 35% de-oiled WGDS groups (231.08 and 227.16 mg/100 g, respectively) and least for the corn control group (177.70 mg/100 g). The PUFA content was greater ($P < 0.01$) in all three full-fat WDGS groups and 65% de-oiled WDGS group (337.13 mg/100 g, on average), intermediate in the 50% and 35% de-oiled WGDS groups (274.77 and 273.84 mg/100 g, respectively) and least for the corn control group (223.98 mg/100 g). Dietary treatment did not alter discoloration ($P = 0.30$) or lipid oxidation ($P = 0.36$). Tenderness

increased with age and RD ($P < 0.0001$) but dietary treatment had no effect on shear force ($P = 0.93$). In general, feeding 35% and 50% de-oiled WDGS had intermediate PUFA content relative to a corn control or full-fat WDGS diet. Feeding de-oiled WDGS did not seem to increase beef shelf life and does not negatively alter beef quality parameters in relation to full-fat WDGS.

Keywords: beef, de-oiled wet distillers grains plus solubles, fatty acid profile, oxidation, retail display

INTRODUCTION

Feeding wet distillers grains plus solubles (WDGS) is a common practice in the state of Nebraska as WDGS are a by-product generated by ethanol production from corn that decreases beef production cost while providing great nutritional value and is widely available to producers. During ethanol production, by-products such as distillers grains and carbon dioxide are generated (Sauders and Rosentrater, 2009a, b). Previous research done at the University of Nebraska-Lincoln has found that feeding WDGS increases the PUFA content of beef, resulting in greater lipid oxidation (Mello *et al.*, 2008a, b).

More recently, the ethanol industry has been extracting soluble fats from WDGS via centrifugation to maximize profits (Berger and Singh, 2010). As explained by Winker-Moser and Breyer (2011), the oil removal process can occur at different processing stages. Oil removed from the corn prior to fermentation is primarily utilized for human consumption, while oil removed after fermentation is utilized for cattle feed, biodiesels, and potentially for human consumption if food grade oil quality parameters are met (Winker-Moser and Breyer, 2011). In 2012 over 50% of ethanol plants were removing the soluble fat portion of WDGS, and this percentage continues to increase (Jolly *et al.*, 2013).

Given the growing availability of de-oiled WDGS in the market, it is imperative to understand how their inclusion in feedlot diets will affect beef quality. The working hypothesis is that the reduction of the oil fraction in WDGS could alter the fatty acid composition of beef by diminishing the PUFA content and consequently aiding shelf stability. Thus, the objectives of this study were to evaluate how feeding de-oiled WDGS affected fatty acid profiles, lipid oxidation, and shelf life of beef in comparison to full-fat

WDGS and corn control diet consisting of a 1:1 blend of dry rolled corn and high moisture corn.

MATERIALS AND METHODS

University of Nebraska-Lincoln's Animal Care and Use Committee approved of all animal use protocols (IACUC# 902).

Cattle and dietary treatments

A total of 336 crossbred yearling steers (initial BW = 351.08 ± 19.05 kg) were fed (University of Nebraska feedlot Mead, NE) one of seven finishing diets: an all corn control (1:1 blend of dry-rolled and high moisture corn), 35%, 50%, or 65% inclusion of WDGS, either full-fat or de-oiled. Upon arrival at the feedlot (d 1) steers were implanted with Revalor-XS (Merck Animal Health, Summit, NJ). Steers were blocked by body weight and randomly assigning to pens (8 hd/pen with 6 replications for a total of 42 pens). All WDGS were produced from a single ethanol plant (KAAPA Ethanol, Minden, NE) and steers were finished for 147 d.

All dietary treatments are presented in Table 1. As inclusion of WDGS increased, for both full-fat and de-oiled diets, the percentage of dry rolled corn and high moisture corn (1:1) were adjusted accordingly to formulate all diets with equal amounts of corn silage (12% DM basis) and supplement (5% DM basis). The supplement was formulated to provide approximately 380 mg/steer/d of Rumensin® as well as 90 mg/steer/d of Tylan® throughout the feeding period, irrespective of dietary treatment.

Sample collection and fabrication

At harvest (Greater Omaha Packing, Omaha, NE), fifteen low Choice carcasses were randomly selected within each treatment (n = 105). Strip loins (*Longissimus*

lumborum) were collected, vacuum packaged, and aged for 7 and 21 d (2°C) under dark storage. After 7 d of aging, loins were fabricated into 2.54 cm steaks for visual discoloration and tenderness. A 1.27 cm steak was utilized for fatty acid profile, proximate composition and lipid oxidation. The remaining portion of the loin was vacuum sealed (3mil STD barrier, Prime Sources, St. Louis, MO) with a Multivac Packaging machine (Mutivac C500, Multivac, Kansas city, MO) and the same fabrication scheme was used at 21 d *post-mortem*. For both aging periods, steaks for visual discoloration, tenderness and lipid oxidation were placed on foam trays (21.6 x 15.9 x 2.1 cm, Styro-Tech, Denver, CO), overwrapped with oxygen permeable film (PVC-OW; PSM18, Prime Source, St. Louis, MO) and placed under retail display (RD) conditions for 4 and 7 d (2.7°C under white fluorescence lighting at 1000 to 1800 lux). Steaks used for fatty acid profile, proximate composition and 0 d RD were vacuum packaged and frozen for further analysis (-80°C). Samples trimmed of all subcutaneous fat for proximate analysis, fatty acids, and lipid oxidation were frozen in liquid nitrogen and powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT). Powdered samples were stored at -80°C.

Proximate analysis

Proximate analysis was conducted to determine fat, moisture and ash content; and protein content was determined by difference. Fat was quantified following the Soxhlet procedure (AOAC, 1990). Samples were measured in triplicate in Whatman #2 filter paper and fat was extracted with ether. Fat percentages were averaged per sample and used to convert fatty acid percent data to mg/100 g tissue basis. Moisture and ash were determined with a LECO thermogravimetric analyzer (LECO Corporation, Model 604-

100-400, St. Joseph, MI), and samples were measured in duplicate. Moisture was determined in nitrogen atmosphere with a start temperature of 25°C and an end temperature of 130°C (17 min ramp rate). Ash was determined in oxygen atmosphere with a start temperature of 130°C and an end temperature of 600°C (30 min ramp rate).

Fatty acid composition

Fatty acid profiles were determined via gas chromatography as described by Folch *et al.* (1957) with modifications detailed by Morisson and Smith (1964) and Metcalfe *et al.* (1966). Briefly, 1 g of powdered sample was weighed into a 15 mL conical tube to which 5 mL of 2:1 chloroform:methanol was added and vortexed for 5 s. After one hour, samples were filtered through Whatman #2 filter paper onto a 13 x 150 mm glass screw cap tube, volume was brought up to 10 mL with 2:1 chloroform:methanol and 2 mL of KCl were added and vortexed. After centrifuging samples (1,000 x g for 5 min) the top organic matter layer was aspirated off and samples were dried down completely on a heating block (60°C) under constant nitrogen purge. One half mL of 0.5 M NaOH in methanol was added, vortexed and heated (100°C) for 5 min. Then, boron trifluoride in 14% methanol (0.5 mL) was added, vortexed and heated (100°C for 5 min). Subsequently, 1 mL of saturated salt solution and 1 mL of hexane were added and samples were centrifuged (1,000 x g for 5 min). The top hexane layer was carefully pipetted into gas chromatography glass vials, nitrogen purged and lids were crimped on. Chromatography was done using a Chromopack CP-Sil (0.25 mm x 100 m) column with an injector temperature of 270°C and a detector temperature of 300°C (Hewlett-Packard 6890 FID GC System; Agilent Technologies, Santa Clara, CA). The head pressure was set at 40 psi with a flow rate of 1.0 mL/min. The fatty acids were

identified by their retention times in relation to known standards and the percentage of fatty acids were determined by the peak areas in the chromatograph. Values were adjusted according to percent fat and values were converted to mg/100 g tissue.

Subjective color (Discoloration)

Visual discoloration was assessed daily during retail display with a trained five-person panel. Panelists were provided with a visual discoloration guide to use as a reference. A percentage scale was used were 0% meant no discoloration and 100% meant complete discoloration. Panelists were instructed to perform the evaluation at the same time each day to minimize variation. Samples were randomly rotated daily to minimize any possible location effects.

Lipid oxidation

Lipid oxidation was determined with the 2-thiobarbituric acid reactive substances protocol (TBARS) as described by Ahn *et al.* (1998). Briefly, 5 g of powdered sample were weight into a 50 mL conical tube to which 14 mL of deionized distilled water and 1 mL of BHA (10% BHA: 90% ethanol) were added. After polytroning for 15 s the samples were centrifuged (2,000 x g for 5 min). One mL of the supernatant was transferred to a 15 mL conical tube and 2 mL of TBA/TCA solution (15% TCA and 20 mM TBA in deionized distilled water) was added and vortexed before placing samples in a water bath (70°C for 30 min). After cooling, samples were centrifuged (2,000 x g for 5 min) and 200 μL of supernatant were transferred to 96-well plates. All 96-well plates had standards to calculate standard curves and ultimately mg of malonaldehyde per kg of tissue read at 540 nm.

Tenderness

Tenderness was measured via Warner-Bratzler Shear Force (WBSF). Samples were thawed (4°C) 24 h prior to cooking and internal temperature was monitored with a thermocouple (5SC-TT-T-30-120, OMEGA Engineering, Inc., Stamford, CT) inserted in the geometric center of each steak. Steaks were cooked on Hamilton Beach grills (Model 31605A, Proctor-Silex, Inc., Washington, NC) until an internal temperature of 35°C was achieved at which time steaks were flipped to continue cooking until a final internal temperature of 71°C. Following cooking steaks were refrigerated for 24 h and six cores (1.27 cm diameter) were taken parallel to the muscle fiber with a drill press and sheared using a portable Warner-Bratzler shear machine (3000, WBS 25 kg scale, 115 motor, ½ coring cutter, G-R Manufacturing Co., Manhattan, KS). All samples were sheared with the triangular WBSF attachment and the average of the 6 cores was calculated for statistical analysis.

Statistical analysis

Data were analyzed as a 7 x 2 x 3 factorial (7 dietary treatments x 2 aging times x 3 retail display times) with SAS (version 9.2, Cary, NC, 2009). The main effects of dietary treatment, aging, retail display and their interactions were tested. Individual animal served as the experimental unit while pen was considered a random variable. The PROC MIXED procedure was used for repeated measures of visual discoloration and the most appropriate covariance structure was selected based on the best fit model. The PROC GLIMMIX procedure was utilized to evaluate all other variables measured. Means were separated with the LS MEANS statement and the TUKEY adjustment was used with an alpha level of 0.05.

RESULTS AND DISCUSSION

Proximate analysis

Finishing diet had no effect ($P > 0.05$) on moisture (71.70%), protein (20.26%), fat (6.48%), or ash (1.56%) content in beef. Given that de-oiled WDGS is more recently available as an ethanol by-product for cattle feed, there is limited information on their impact on nutritional composition of beef in the literature. However, one study examined feeding full-fat WDGS at 0%, 15% and 30% of inclusion on corn based diets indicated that the finishing diet did not affect the moisture, fat, or ash content of *Infraspinatus* (top blade) and *Psoas major* (tenderloin) steaks (Mello *et al.*, 2012b). A similar observation was made by Mello *et al.* (2012a) in a study with six diets containing varying levels (0% - 50% DM basis) of modified wet distillers grains plus solubles (MWDG: distillers grains with partial drying for a moisture level of 50% - 54%). Although these diets included full-fat WDGS these did not affect moisture, fat, or ash content of beef. In addition, the fat percentage reported ranged from 7.43 to 8.68%, slightly higher than that observed in the current study. On the other hand, Buttrey *et al.* (2013) reported that after finishing crossbred steers with 35% WDGS, steaks tended ($P < 0.10$) to increase total fat content compared to diets without WDGS.

Fatty acid composition

Table 2 provides the fatty acid profiles of all the dietary treatments reported in mg/100 g of tissue basis. No differences ($P > 0.05$) were observed in the amounts of mono-unsaturated fatty acids (MUFA), saturated fatty acids (SFA), unsaturated fatty acids (UFA), the SFA:UFA ratio, or the total amount of fatty acids. Similarly, Mello *et al.* (2012b) reported that these fatty acids were unaffected in beef from cattle fed diets

containing WDGS vs. cattle finished on a corn control diet. Buttrey *et al.* (2013) reported a decreased ratio of MUFA:SFA in the *longissimus* muscle of cattle feed 35% WDGS vs. cattle with no inclusion of WDGS. Caution is advised regarding direct comparisons to the fatty acid data reported by previously published data as these are reported on a percent of fatty acid composition basis and not mg/100 g of tissue basis, as in the current study.

In the current study, differences ($P < 0.05$) were found in the C16:1, C18:1T, C18:2 and PUFA content among dietary treatments. Palmitoleic acid (C16:1) was predominant ($P < 0.0001$) in beef from cattle on the corn control diet, intermediate in beef from cattle on the 35% de-oiled WDGS diet, with lower values found in cattle on all other dietary treatments (Figure 1). These results are consistent with the findings of Buttrey *et al.* (2013) that explain that palmitoleic acid is the desaturated product of palmitic acid (C16:0) and that feeding corn WDGS decreases palmitoleic acid ($P \leq 0.01$). In a similar study conducted by Mello *et al.* (2012b), decreases in both C16:0 and C16:1 contents were observed in the *Longissimus thoracis*, *Psoas major* and *Infraspinatus* muscles as inclusion levels of WDGS increased from 0, 15 to 30% on a percentage basis. These decreases in C16:0 and C16:1 were also observed with increasing concentrations of modified wet distillers grains plus solubles (MGDS; Mello *et al.*, 2012a). Plascencia *et al.* (2003) has also shown that ruminal digestion of C16:0 is decreased with increasing concentrations of fat in cattle diets.

Elaidic acid (C18:1T) content was greatest ($P = 0.01$) in beef from cattle finished on 65% full-fat WDGS, least for the corn control fed cattle, and intermediate in beef from all other dietary treatments (256.20 mg/100 g, 120.12 mg/100 g, and 210.36 mg/100 g,

respective average values; Figure 2). Similar results were also reported by Mello *et al.* (2012b) which reflected an increase in C18:1T content with increasing levels of WDGS in the *Longissimus thoracis* and *Infraspinatus* muscles. However, this difference was not observed in the *Psoas major*. Research conducted by Vander Pol *et al.* (2009) using ruminal and duodenal cannulated steers aimed to evaluate differences in dietary lipids comparing corn distillers grains versus corn finishing diets. Vander Pol *et al.* (2009) indicated that cattle fed WDGS had greater total fat digestion as well as a greater amount of unsaturated fatty acids reaching the duodenum ($P < 0.10$). These findings suggest a plausible explanation for the increased availability and ultimately deposition of unsaturated fatty acids in meat of cattle fed WDGS.

Linoleic acid (C18:2) was greater ($P = 0.0001$) in beef from cattle finished on the three full-fat WDGS and 65% de-oiled WDGS diets (290.98 mg/100 g, on average), intermediate in cattle fed 50% and 35% de-oiled WGDS (231.08 and 227.16 mg/100 g, respectively) and least for meat from the corn control group (177.70 mg/100 g; Figure 3). Enser *et al.* (1996) indicated that linoleic acid is an essential fatty acid which means that it is mostly derived from the diet and not synthesized in the body. In monogastric animals, linoleic acid is unchanged as it passes through the stomach and is absorbed into the blood stream and is deposited in muscle tissue. In the case of ruminants, this fatty acid can be degraded into MUFA's and SFA's because of biohydrogenation of ruminal bacteria. Around 10% of dietary linoleic acid is available to be deposited in muscle tissue in ruminants (Enser *et al.*, 1996). However, Vander Pol *et al.* (2009) observed that when feeding WDGS there is an increased concentration of linoleic acid in the duodenum, indicating a protection phenomenon when WDGS are fed. This would then

suggest less ruminal biohydrogenation of linoleic acid, thus increasing linoleic acid's availability to be deposited in muscle.

The PUFA content of beef was different ($P = 0.0003$) between dietary treatments. Beef from cattle fed either of the three full-fat WDGS diets or the 65% de-oiled WDGS diet had the greatest amount of PUFA (337.13 mg/100 g, on average), cattle on the 50% and 35% de-oiled WGDS diets had intermediate amounts (274.77 and 273.84 mg/100 g, respectively), while beef from cattle on the corn control diet had the least amount of PUFA (223.98 mg/100 g; Figure 4).

Biohydrogenation occurs following lipolysis and is dependent on ruminal pH (Plascencia *et al.*, 1999). Grain inclusions in cattle diets, particularly in elevated proportions, create a more acidic ruminal environment which in turn suppresses lipolysis and hence microbial biohydrogenation of fatty acids is inhibited (Atkinson *et al.*, 2006; Plascencia *et al.*, 1999). As fatty acids pass the rumen unchanged, these fatty acids reach the duodenum where bile salt micelles form and increase fat digestibility (Zinn *et al.*, 2000).

Wood *et al.* (2008) suggested that monogastric animals have the ability of depositing the PUFA's from the diet directly into muscle mass; whereas ruminants, due to bacterial biohydrogenation, can only deposit a limited amount of PUFA's in muscle. Although containing limited fat, ruminant diets are rich in PUFA's; this is particularly true when considering WDGS in relation to regular corn based diets (Vander Pol *et al.*, 2009). Yet due to biohydrogenation, SFA are dominant in muscle of ruminants in relation to PUFA's (Ham *et al.*, 1994; Warren *et al.*, 2008). However, contemplating the low ruminal pH environment (Plascencia *et al.*, 1999) in diets with greater grain

inclusions and the bile salt formation (Zinn *et al.*, 2000) in the duodenum there is a feasible explanation for the more elevated PUFA deposition in cattle finished on higher levels of WDGS.

Particularly for ruminants the use of unprotected lipids has been explored as well as protected lipids. Incorporation of unprotected lipids such as plant and fish oils has been attempted with some degree of success (Scollan *et al.*, 2001). Although unprotected from the harsh environment of the rumen, some PUFA's can bypass the rumen and go directly into the small intestine where these are released into the blood stream and subsequently deposited in muscle (Enser *et al.*, 1996; Scollan *et al.*, 2014).

Subjective color (Discoloration)

Dietary treatment had no effect on discoloration in samples aged for 7 d (P = 0.69) or on samples aged for 21 d (P = 0.30; Table 3). As expected, discoloration at both aging periods increased as retail display time increased, irrespective of dietary treatment ($P < 0.0001$). At 21 d aging, discoloration was greatest ($P < 0.0001$) at 7 d of retail display (64.32%), followed by 6 d of retail display (33.43%), followed by 5 d of retail display (11.14%), while no significant difference was observed on d 0 to 4 of retail display (0.94%, on average; Table 3).

Consumer studies have reported a significant decline in purchasing decisions with 20% surface discoloration on retail displayed beef, resulting in sale reductions of up to 50% (Hood and Riordan, 1973). Data from this study suggest that after 21 d of aging, the 20% discoloration threshold was first met by steaks from corn control fed cattle at d 5 of retail display. However, samples aged 21 d with 6 d of retail display, all surpassed the 20% discoloration threshold except the samples from cattle having been fed 35% de-oiled

WDGS (Table 3 & Figure 5). The fact that at d 5 of retail display the corn control samples reached this threshold first was unexpected as one would anticipate these samples to have greater color stability due to the lower PUFA content in relation to cattle fed WDGS. Typically, color of meat is a balance between oxymyoglobin oxidation and metmyoglobin reduction (Gatellier *et al.* 2001) and has been closely related to lipid oxidation (Greene, 1969) which should be increased with increased PUFA content. When oxidation of oxymyoglobin occurs to form metmyoglobin, intermediate radicals are generated that, through propagation, can further accelerate color oxidation as well as lipid oxidation (Faustman *et al.*, 2010).

Lipid oxidation

Lipid oxidation, measured by the amount of thiobarbituric acid reactive substances (TBARS), indicated there was an age by retail display interaction ($P < 0.0001$) where the rate of lipid oxidation was greater in samples aged 21 d vs. those aged for 7 d, particularly at 4 and 7 d of RD (Figure 6). Dietary treatment did not significantly alter ($P = 0.36$) lipid oxidation values (Table 4).

It has been established in the literature that increased unsaturated fatty acid content in meat products will greatly diminish shelf life, particularly due to less color stability and lipid rancidity (Ladeira *et al.*, 2014). By-products generated as a result of lipid oxidation are responsible for the development of off-flavors in meat products (Gatellier *et al.*, 2001). Campo *et al.* (2006) concluded that a TBARS value of 2 can be considered as the limiting threshold for oxidized beef acceptability. According to the current study, beef from the corn control, 65% and 35% full-fat WDGS treatment groups reached this threshold first by the 4th d of retail display when aged for 21 d (Table 4).

These results also suggest a faster reduction in beef shelf life in several of the full-fat WDGS in relation the de-oiled WDGS. Interestingly though, the corn control steaks presented decreased color and lipid stability, despite their decreased PUFA content.

A potential oxidation protection phenomenon could be taking place with cattle fed WDGS due to the sulfur concentration of the diets (Chao, 2015). One of the limiting factors in the inclusion levels of WDGS is the sulfur content given that levels at or above 0.40% could result in a condition called polioencephalomalomalacia which is caused by the accumulation of hydrogen sulfide in the rumen resulting in sulfur toxicity (U.S. Grains Council, 2012). However, it has been demonstrated that sulfur concentrations up to 0.50% are feasible without the risk of sulfur toxicity if roughage close to 15% is provided in the finishing diet (U.S. Grains Council, 2012); standards met in the finishing diets of the current study. In pork, an increase in sulfur content in the diet is associated with an increase in sulfur containing amino acids which have been known for their antioxidant capabilities (Song *et al.*, 2013). This may explain why the corn control diet in this study presented greater TBARS values in comparison to several finishing diets containing WDGS despite the more elevated PUFA content of the WDGS diets.

Tenderness

There was an increase in tenderness from 7 to 21 d of aging ($P < 0.0001$) and as retail display time progressed ($P < 0.0001$), dietary treatment had no effect on WBSF ($P = 0.93$). These results are in agreement with those observed by Mello *et al.* (2012b) with beef aged for 7 d that were finished with 0%, 15% or 30% corn WDGS. On the other hand, a subset group of samples from the current study were used by Chao (2015) looking to compare beef from cattle fed the corn control diet vs. 50% WDGS to evaluate

tenderness at 2, 7, 14 and 21 d postmortem. Chao (2015) stated that at 2 d of aging, steaks from cattle finished on WDGS were more tender than the corn control steaks ($P < 0.01$). These differences in tenderness did not persist with increased aging time. Chao (2015) hypothesizes that at early postmortem there is greater membrane instability of the sarcoplasmic reticulum given the increased PUFA content associated with feeding WDGS. The greater membrane fluidity results in greater calcium flux released from the sarcoplasmic reticulum and ultimately increases early proteolysis.

CONCLUSION

Feeding lower inclusion levels (35% and 50%; DM basis) of de-oiled WDGS resulted in intermediate PUFA content relative to a corn control diet and full-fat WDGS. At 7 and 21 d of aging, steaks increased surface discoloration regardless of dietary treatment. Just as well, lipid oxidation increased with increasing aging and retail display time.

The de-oiling process poses an economical advantage for ethanol plants and producers alike as ethanol plants can further diversify corn distillers by-products and at the same time producers can obtain greater feed shelf life. The removal of the soluble oil fraction of WDGS does not seem to augment beef shelf life and does not negatively affect basic beef quality parameters relative to full-fat WDGS.

LITERATURE CITED

Ahn, D. U., D. G. Olson, C. Jo, X. Chen,C. Wu, J. I. Lee. 1998. Effect of muscle type, packaging, and irradiation on lipid oxidation, volatile production, and color in raw pork patties. Meat Sci. 47:27-39.

AOAC. 1990. Official methods of analysis of AOAC (Association of Official Analytical Chemists) international. 18th edition. AOAC International, Arlington, VA, USA.

Atkinson, R. L., E. J. Scholljegerdes, S. L. Lake, V. Nayigihugu, B. W. Hess and D. C. Rule. 2006. Site and extent of digestion, duodenal flow, and intestinal disappearance of total and esterified fatty acids in sheep fed a high-concentrate diet supplemented with high-linoleate safflower oil. J. Anim. Sci. 84:387-396.

Berger, L and V. Singh. 2010. Changes and evolution of corn coproducts for beef cattle. J. Anim. Sci. 88:143-150.

Buttrey, E. K., K. H. Jenkins, J. B. Lewis, S. B. Smith, R. K. Miller, T. E. Lawrence, F. T. McCollum III, P. J. Pinedo, N. A. Cole and J. C. MacDonald. 2013. Effects of 35% corn wet distillers grains plus solubles in stem-flaked and dry-rolled corn-based finishing diets on animal performance, carcass characteristics, beef fatty acid composition, and sensory attributes. J. Anim. Sci. 91:1850-1865.

Campo, M. M., G.R. Nute, S.I. Hughes, M. Enser, J.D. Wood and R.I. Richardson. 2006. Flavour perception of oxidation in beef. Meat Sci. 72:303-311.

Chao, M. D. 2015. Impact of wet distilers grains plus solubles and antioxidants on a basic mechanism of beef tenderization. Diss., Univ. of Nebr.-Lincoln, DigitalCommons.

Enser, M., K. Hallett, B. Hewitt, G. A. J. Fursey, and J. D. Wood. 1996. Fatty acid content and composition of english beef, lamb and pork at retail. Meat Sci. 42:443-456.

Faustman, C., Q. Sun, R. Mancini, and S. P. Suman. 2010. Myoglobin and lipid oxidation interactions: Mechanistic bases and control. Meat Sci. 86:86-94.

Folch, J., M. Lees, G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissue. J. Biol. Chem. 226:497-509.

Gatellier, P., C. Hamelin, Y. Durand, and M. Renerre. 2001. Effect of a dietary vitamin E supplementation on colour stability and lipid oxidation of air- and modified atmosphere-packaged beef. Meat Sci. 59:133-140.

Greene, B. E. 1969. Lipid Oxidation and Pigment Changes in Raw Beef. J. Food Sci. 34:110-113.

Ham, G., R. Stock, T. Klopfenstein, E. Larson, D. Shain, and R. Huffman. 1994. Wet corn distillers byproducts compared with dried corn distillers grains with solubles as a source of protein and energy for ruminants. J. Anim. Sci. 72:3246-3257.

Hood, D. E. R. and E. B. Riordan. 1973. Discolouration in pre-packaged beef: measurement by reflectance spectrophotometry and shopper discrimination. J. Food Technol. 8:333-343.

Jolly, M. L., B. L. Nuttelman, D. Burken,C. J. Schneider, T. J. Klopfenstein, G. E. Erickson. 2013. Effects of Modified Distillers Grains Plus Solubles and Condensed Distillers Solubles With and Without Oil Extraction on Finishing Performance. Nebraska Beef Cattle Report, pp. 64-65.

Ladeira, M. M., L. C. Santarosa, M. L. Chizzotti , E. M. Ramos, O. R. Machado Neto, D. M. Oliveira, J. R. R. Carvalho, L. S. Lopes and J. S. Ribeiro. 2014. Fatty acid profile, color and lipid oxidation of meat from young bulls fed ground soybean or rumen protected fat with or without monensin. Meat Sci. 96:597-605.

Mello, A. S, B. E. Jenschke, C. R Calkins. 2008a. Influence of Feeding Wet Distillers Grains on Fatty Acid Composition of Beef. Nebraska Beef Cattle Report, pp. 108-109.

Mello, A. S, B. E. Jenschke, C. R. Calkins. 2008b. Wet Distillers Grains Plus Solubles Affect Lipid Oxidation and Objective Color of Beef Steaks. Nebraska Beef Cattle Report, pp. 110-111.

Mello, A. S., B. E. Jenschke, L. S. Senaratne, T. P. Carr, G. E. Erickson and C. R. Calkins. 2012a. Effect of feeding modified distillers grains plus solubles on marbling attributes, proximate composition, and fatty acid profile of beef. J. Anim. Sci. 90:4634-4640.

Mello, A. S., C. R. Calkins, B. E. Jenschke, T. P. Carr, M. E. R. Dugan and G. E. Erickson. 2012b. Beef quality of calf-fed steers finished on varying levels of corn-based wet distillers grains plus solubles. J. Anim. Sci. 90:4625-4633.

Metcalfe, L. D., A. A. Schmitz, J. R. Pelka. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem. 38:514-515.

Morrison, W. R. and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5:600-608.

Plascencia, A., G. D. Mendoza, C. Vázquez and R. A. Zinn. 2003. Relationship between body weight and level of fat supplementation on fatty acid digestion in feedlot cattle. J. Anim. Sci. 81:2653-2659.

Plascencia, A., M. Estrada and R. A. Zinn. 1999. Influence of free fatty acid content on the feeding value of yellow grease in finishing diets for feedlot cattle. J. Anim. Sci. 77:2603-2609.

Saunders, J. A and K. A. Rosentrater. 2009. Properties of solvent extracted low-oil corn distillers dried grains with solubles. Biomass and Bioenergy 33:1486-1490.

Saunders, J. A and K. A. Rosentrater. 2009. Survay of US fuel ethanol plants. Bioresource Technology 100:3277-3284.

Scollan, N. D., D. Dannenberger, K. Nuernberg, I. Richardson, S. MacKintosh, J. F. Hocquette, and A. P. Moloney. 2014. Enhancing the nutritional and health value of beef lipids and their relationship with meat quality. Meat Sci. 97:384-394.

Scollan, N. D., N. J. Choi, E. Kurt, A. V. Fisher, M. Enser, and J. D. Wood. 2001. Manipulating of fatty acid composition of muscle and adipose tissue in beef cattle. British J. Nutrition 85:115-124.

Song, R., C. Chen, L. Wang, L. Johnston, B. Kerr, T. Weber, and G. Shurson. 2013. High sulfur content in corn dried distillers grains with solubles protects against oxidized lipids by increasing sulfur-containing antioxidants in nursery pigs. J. Anim. Sci. 91:2715-2728.

U.S. Grains Council. 2012. Sulfur Concerns and Benefits in DDGS. Pages 146-150 in A Guide to Distiller's Dried Grains with Solubles (DDGS), 3rd Ed., U.S. Grains Council, Washington, DC.

Vander Pol, K., M. Luebbe, G. I. Crawford, G. Erickson, and T. Klopfensteine. 2009. Performance and digestibility characteristics of finishing diets containing distillers grains, composites of corn processing coproducts, or supplemental corn oil. J. Anim. Sci. 87:639-652.

Warren, H. E., N.D. Scollan, M. Enser, S.I. Hughes, R.I. Richardson and J.D. Wood. 2008. Effects of breed and a concentrate or grass silage diet on beef quality in cattle of 3 ages. I: Animal performance, carcass quality and muscle fatty acid composition. Meat Sci. 78:256-269.

Winker-Moser, J. K. and L. Breyer. 2011. Composition and oxidative stability of crude oil extracts of corn germ and distillers grains. Industrial Crops and Products 33:572-578.

Wood, J. D., M. Enser, A.V. Fisher, G.R. Nute, P.R. Sheard, R.I. Richardson, S.I. Hughes, F.M. Whittington. 2008. Fat deposition, fatty acid composition and meat quality: A review. Meat Sci. 78:343-358.

Zinn, R. A., S. K. Gulati, A. Plascencia and J. Salinas. 2000. Influence of ruminal biohydregenation on the feeding value of fat in finishing diets for feedlot cattle. J. Anim. Sci. 78:1738-1746.

Table 1. Diet composition (DM basis) fed to finishing steers receiving either 35%, 50%, or 65% De-oiled or Full-fat WDGS or a corn control diet

	Control	35% WDGS		50% WDGS		65% WDGS	
		De-Oiled	Full-Fat	De-Oiled	Full-Fat	De-Oiled	Full-Fat
Ingredient, % of DM							
DRC[1]	41.5	24	24	16.5	16.5	9	9
HMC[1]	41.5	24	24	16.5	16.5	9	9
WDGS: De-Oiled[1]	-	35	-	50	-	65	-
WDGS: Full-Fat[1]	-	-	35	-	50	-	65
Corn Silage	12	12	12	12	12	12	12
Supplement [2]	5	5	5	5	5	5	5
Analyzed Composition, %							
Fat	4.5	5.5	7.1	6.0	8.2	6.4	9.3
CP	11.4	15.2	14.8	18.7	18.1	22.1	21.4
Sulfur	0.09	0.32	0.31	0.42	0.41	0.52	0.51
NDF	13.5	26.6	27.8	32.3	34.0	38.0	40.2

[1]DRC = Dry rolled corn; HMC = High moisture corn; WDGS = Wet distillers grains plus solubles
[2]Formulated to contain 383 mg/hd/d of Rumensin and 90 mg/hd/d of Tylan

Table 2. Amount[1] of fatty acids from steers feed different inclusion levels of De-oiled or Full-fat WDGS (*L. lumborum*)

	Treatment								
	Corn	De-oiled WDGS			Full-Fat WDGS				
Fatty Acid	Control	35%	50%	65%	35%	50%	65%	*SEM*	*P*-value
C14:0	180.61	156.49	139.36	150.35	171.89	155.00	162.89	14.82	0.47
C14:1	40.66	33.03	29.98	28.01	33.35	27.36	30.37	3.43	0.11
C15:0	31.90	32.17	26.78	29.08	32.30	31.71	30.59	2.62	0.71
C15:1	33.55	35.15	28.32	30.45	30.27	29.01	30.73	5.29	0.53
C16:0	1,679.52	1,588.06	1,364.32	1,501.57	1,706.01	1,543.98	1,609.64	107.87	0.31
C16:1	194.26^{a}	149.67^{ab}	132.58^{b}	115.32^{b}	145.11^{b}	120.71^{b}	128.23^{b}	11.14	< 0.0001
C17:0	98.83	103.35	86.42	93.81	103.89	104.32	98.18	8.03	0.67
C17:1	80.07	73.43	64.38	59.95	66.60	65.26	61.78	5.81	0.18
C18:0	927.89	1,017.22	874.61	1,029.27	1,126.37	1,109.31	1,119.00	15.21	0.12
C18:1T	120.12^{b}	156.98^{ab}	170.91^{ab}	227.49^{ab}	248.40^{ab}	248.03^{ab}	256.20^{a}	31.04	0.01
C18:1	2,590.88	2,514.37	2,180.72	2,243.01	2,697.93	2,383.59	2,514.37	179.47	0.35
C18:1V	318.34	252.13	245.10	256.12	268.89	255.38	288.60	24.41	0.17
C18:2	177.70^{b}	227.16^{ab}	231.08^{ab}	287.89^{a}	294.87^{a}	279.78^{a}	301.36^{a}	19.49	0.0001
C18:3	0.00	0.00	5.59	8.63	10.06	12.03	8.94	2.28	0.52
C20:1	30.89	31.94	26.69	28.58	38.48	30.85	33.51	3.56	0.22
C20:4	46.29	47.62	42.39	45.41	45.33	42.77	45.83	2.34	0.60
C22:0	12.04	18.31	14.51	14.83	16.10	14.39	16.09	2.36	0.44
Total	6,545.69	6,414.15	5,637.85	6,134.96	7,005.20	6,427.48	6,692.61	443.02	0.42
SFA	2,947.00	2,901.25	2,494.55	2,811.00	3,151.19	2,947.00	3,024.60	201.02	0.38
UFA	3,624.53	3,512.90	3,143.30	3,323.96	3,854.01	3,480.48	3,668.00	248.10	0.45
SFA:UFA	0.81	0.82	0.79	0.85	0.82	0.85	0.83	0.02	0.25
MUFA	3,400.54	3,238.13	2,869.46	2,988.93	3,512.47	3,156.32	3,320.22	228.91	0.38
PUFA	223.98^{b}	273.77^{ab}	273.84^{ab}	335.03^{a}	341.54^{a}	324.15^{a}	347.79^{a}	20.75	0.0003

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

[a,b]Means in the same row with different superscripts differ ($P < 0.05$)

Table 3. Discoloration (%) of strip loin steaks (*L. lumborum*) aged for 21 d from steers fed different inclusion levels of De-oiled or Full-fat WDGS or a corn control diet

	Days on retail display							
Treatment	0	1	2	3	4	5	6	7
35% De-oiled WDGS	0.12	0.32	0.33	0.88	1.53	4.35	17.75	52.98
50% De-oiled WDGS	0.50	0.88	1.07	1.73	3.10	15.42	39.50	67.75
65% De-oiled WDGS	0.28	0.60	0.75	1.00	3.43	9.38	40.20	69.88
35% Full-fat WDGS	0.38	0.80	1.02	1.73	2.50	4.48	25.83	67.67
50% Full-fat WDGS	0.17	1.05	0.33	0.55	1.87	11.95	31.30	57.30
65% Full-fat WDGS	0.50	1.50	1.15	1.67	3.75	14.98	50.30	76.72
Corn control	0.38	1.56	1.17	2.22	6.87	20.03	31.77	60.60
Average of all dietary treatments	**0.33^{d}**	**0.96^{d}**	**0.83^{d}**	**1.40^{d}**	**3.29^{d}**	**11.51^{c}**	**33.81^{b}**	**64.70^{a}**

$^{a-d}$ Means in the same row with different superscripts differ ($P \leq 0.05$)
SEM = 1.80

Table 4. Lipid oxidation (TBARS) means by aging period and retail display times from steers fed different levels of either De-oiled or Full-fat WDGS or a corn control diet

	7 d Age			21 d Age			**Overall Average**	*SEM*
Retail display (d)	0	4	7	0	4	7		
Dietary treatment								
35% De-oiled WDGS	0.04	0.53	1.38	0.15	1.47	3.21	**1.15**	0.32
50% De-oiled WDGS	0.11	0.36	1.26	0.50	1.52	3.03	**1.21**	0.32
65% De-oiled WDGS	0.06	0.58	1.30	0.14	1.36	3.85	**1.07**	0.32
35% Full-fat WDGS	0.32	1.16	2.01	0.8	2.21	4.16	**1.70**	0.35
50% Full-fat WDGS	0.30	0.72	1.65	0.23	1.35	2.84	**1.24**	0.32
65% Full-fat WDGS	0.61	1.09	1.88	0.65	2.23	4.23	**1.88**	0.32
Corn Control	0.59	1.47	1.89	1.59	2.58	3.74	**1.78**	0.32

Figure Legends

Figure 1. C16:1 differences ($P < 0.0001$; $SEM = 11.14$) of beef from steers fed either 35%, 50%, or 65% De-oiled or Full-fat WDGS or a corn control diet

[a-b]Different superscripts indicate differences ($P \leq 0.05$)

Figure 2. C18:1T differences ($P = 0.01$; $SEM = 31.04$) of beef from steers fed either 35%, 50%, or 65% De-oiled or Full-fat WDGS or a corn control diet

[a-b]Different superscripts indicate differences ($P \leq 0.05$)

Figure 3. C18:2 differences ($P = 0.0001$; $SEM = 19.49$) of beef from steers fed either 35%, 50%, or 65% De-oiled or Full-fat WDGS or a corn control diet

[a-b]Different superscripts indicate differences ($P \leq 0.05$)

Figure 4. Polyunsaturated fatty acid differences ($P = 0.0003$; $SEM = 20.75$) of beef from steers fed either 35%, 50%, or 65% De-oiled or Full-fat WDGS or a corn control diet

[a-b]Different superscripts indicate differences ($P \leq 0.05$)

Figure 5. Discoloration changes of beef aged for 21 d under 5, 6 and 7 d of retail display from steers fed either 35%, 50%, or 65% De-oiled or Full-fat WDGS or a corn control diet

Figure 6. Age by retail display interaction ($P < 0.0001$; $SEM = 0.15$) for lipid oxidation of samples aged 7 and 21 d with 0, 4 and 7 d retail display

[a-b]Different superscripts indicate differences within the same retail display time ($P \leq 0.05$)

Figure 1.

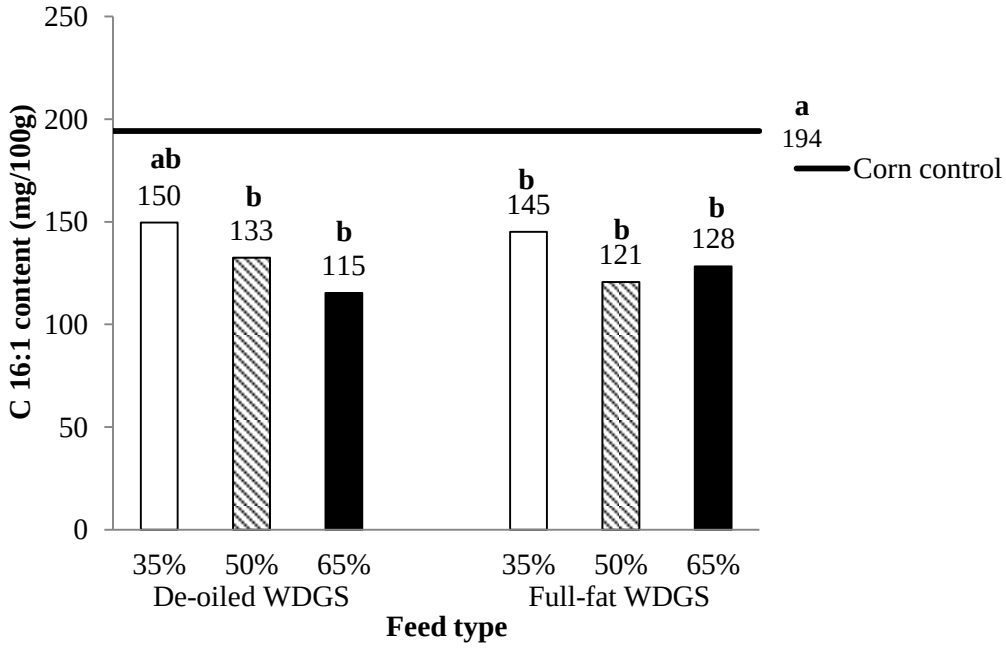

250
200
150
100
50
0
C 16:1 content (mg/100g)
ab
150
b
133
b
115
b
145
b
121
b
128
a
194
Corn control
35%
50%
65%
35%
50%
65%
De-oiled WDGS
Full-fat WDGS
Feed type

Figure 2.

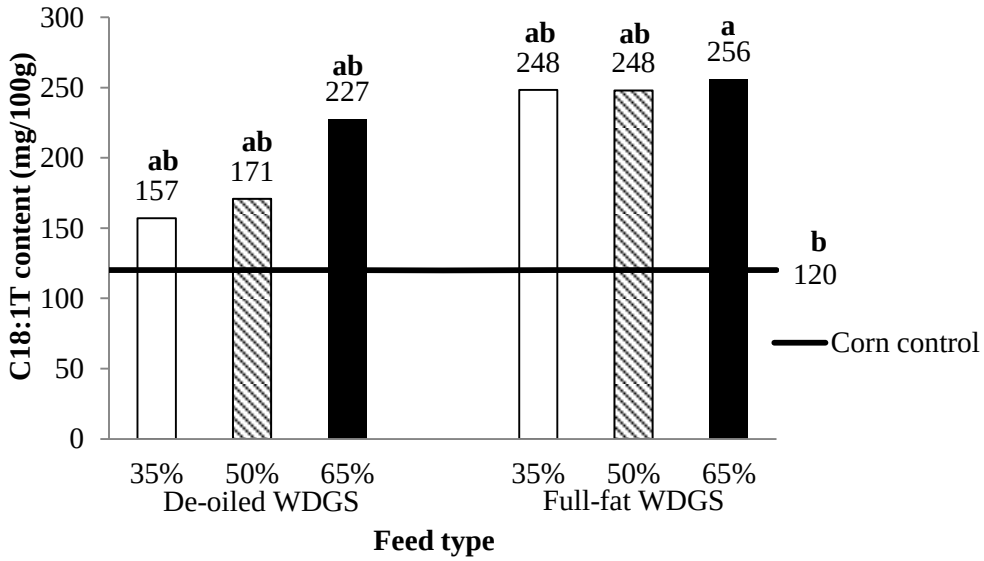

300
250
200
150
100
50
0
C18:1T content (mg/100g)
ab
157
ab
171
ab
227
ab
248
ab
248
a
256
b
120
Corn control
35%
50%
65%
35%
50%
65%
De-oiled WDGS
Full-fat WDGS
Feed type

Figure 3.

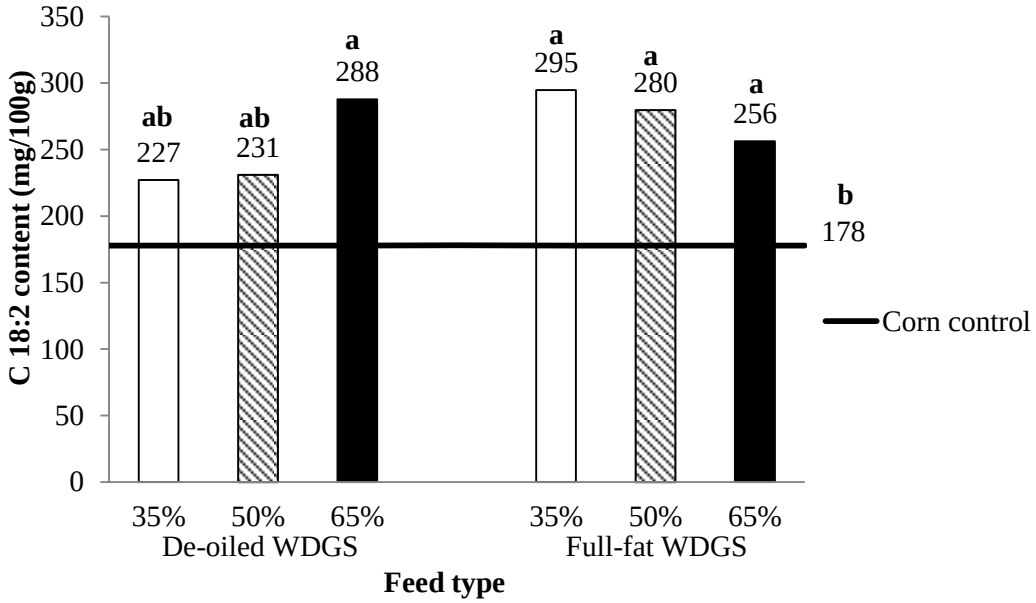

350
300
250
200
150
100
50
0
C 18:2 content (mg/100g)
ab
227
ab
231
a
288
a
295
a
280
a
256
b
178
Corn control
35%
50%
65%
35%
50%
65%
De-oiled WDGS
Full-fat WDGS
Feed type

Figure 4.

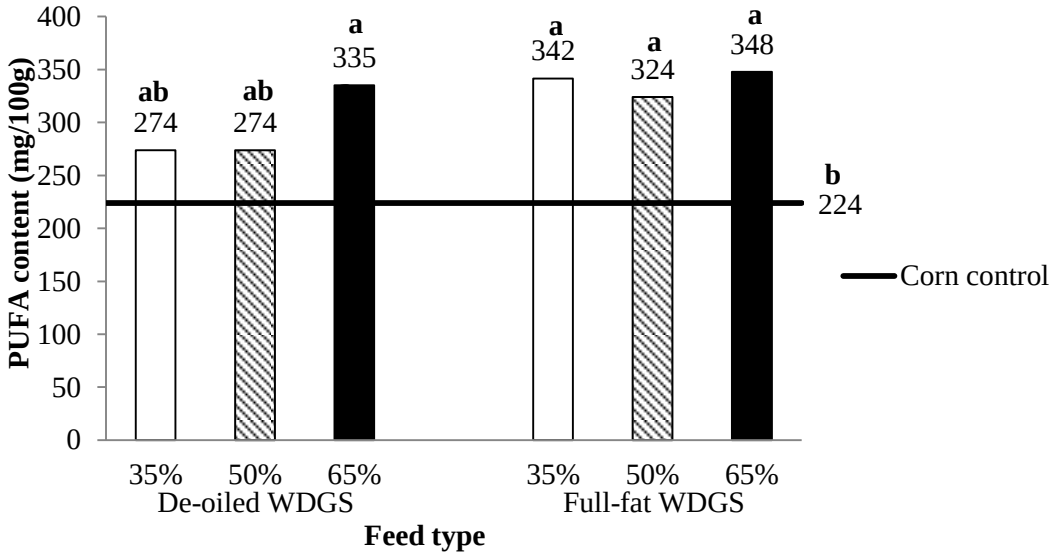

400
350
300
250
200
150
100
50
0
PUFA content (mg/100g)
ab
274
ab
274
a
335
a
342
a
324
a
348
b
224
Corn control
35%
50%
65%
35%
50%
65%
De-oiled WDGS
Full-fat WDGS
Feed type

Figure 5.

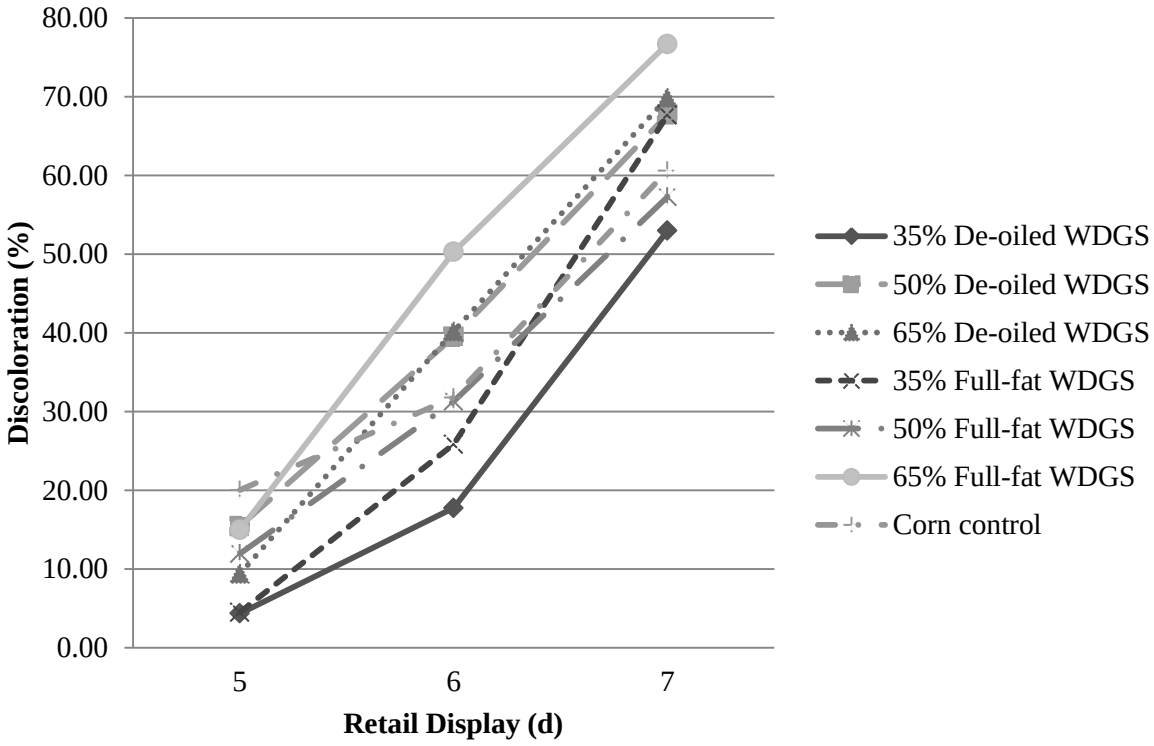
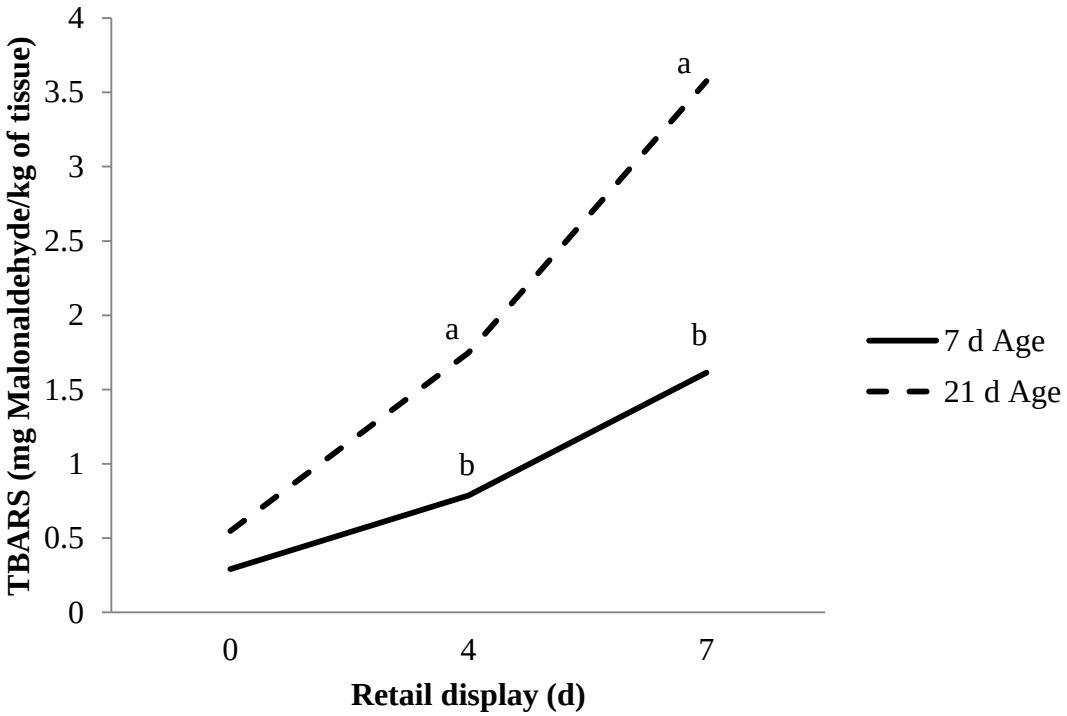

80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Discoloration (%)
5
6
7
Retail Display (d)
35% De-oiled WDGS
50% De-oiled WDGS
65% De-oiled WDGS
35% Full-fat WDGS
50% Full-fat WDGS
65% Full-fat WDGS
Corn control

Figure 6.


TBARS (mg Malonaldehyde/kg of tissue)
4
3.5
3
2.5
2
1.5
1
0.5
0
a
a
b
b
0
4
7
Retail display (d)
7 d Age
21 d Age

Impact of Feeding De-Oiled Dry Distillers Grains Plus Solubles on Beef Shelf Life

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ABSTRACT

Two finishing dietary treatments, a corn control and a 50% de-oiled dry distiller grains plus solubles (de–oiled DDGS, DM basis) diet, were evaluated to determine their effect on beef fatty acid profiles, retail shelf life and lipid oxidation during retail display (RD). Steers were fed for 156 d and at harvest, 24 low Choice carcasses were randomly selected within each treatment (n = 48) and strip loins (*Longissimus lumborum*) were collected. Loins were vacuum packaged, aged (2, 8, 14, and 21 d), and placed under RD conditions for 7 d at each aging period. Differences ($P < 0.05$) were found in the C15:0, C16:1, C17:0, C17:1, C18:1T, C18:2, C20:3ω6, trans, and polyunsaturated fatty acids (PUFA). The C15:0, C16:1, C17:0, and C17:1 fatty acids were greater in beef from the corn control cattle, while C18:1T, C18:2, C20:3ω6, trans, and PUFA content were greater for beef from cattle receiving the 50% de-oiled DDGS diet (509.37 vs. 304.57 mg/100 g tissue for PUFA content; $P <0.0001$). A three way interaction between dietary treatment, aging, and RD was observed ($P < 0 .0001$) for discoloration. The triple interaction was evident at 21 d of age with extended RD time where the magnitude of discoloration of steaks from cattle fed 50% de-oiled DDGS was greater than that of the corn control steaks. These differences were not seen with shorter ageing periods. A triple interaction between dietary treatment, aging, and RD was also observed ($P < 0 .0001$) for a* values that closely parallel the pattern seen with discoloration. An age by RD interaction ($P < 0.0001$) was noted where the rate of lipid oxidation was greater in samples aged for 21 d and least for 2 d aged samples (particularly at 4 and 7 d of RD). A dietary treatment by RD interaction was observed for lipid oxidation ($P < 0.0001$). Steaks from cattle having

been fed 50% de-oiled DDGS had greater (P <0.0001) lipid oxidation at 4 and 7 d of RD (2.67 vs. 2.16 mg/malonaldehyde/kg tissue and 4.99 vs. 3.37 mg/malonaldehyde/kg tissue, respectively), but no differences were observed for 0 d RD (0.77 vs. 0.87 mg/malonaldehyde/kg tissue). The de-oiling of DDGS resulted in elevated beef PUFA content, negatively impacting beef shelf life. Techniques such as strategic use of antioxidants should be explored when utilizing de-oiled DDGS in finishing feedlot rations.

Keywords: beef, de-oiled dry distillers grains plus solubles, fatty acid profile, oxidation, retail display

INTRODUCTION

The ethanol industry is constantly evolving in order to maximize by-product utilization and increase profitability. As a result, numerous modified versions of distillers grains have emerged that are available for cattle and these new or modified feed ingredients merit evaluation in terms of cattle performance and beef quality. This is particularly the case for states such as Nebraska which is one of the largest ethanol producing states. Previous economic studies have stated that 91.2% of cattle on feed in Nebraska utilize ethanol co-products (Waterbury *et al.*, 2009). More recently, ethanol plants have been extracting soluble fats in distillers grains by centrifugation (Berger and Singh, 2010) for uses such as human consumption, biofuels and commercial feed production (Saunders and Rosentrater, 2009b; Watkins, 2007; Winkler-Moser and Breyer, 2011).

Fat content of full-fat DDGS has been reported to range anywhere from 3 to 13%, but in most cases the fat content is closer to 8-9% fat (Ganesan *et al.*, 2009; Saunders and Rosentrater, 2009a; Winkler-Moser and Breyer, 2011). With new de-oiling techniques the fat content of de-oiled distillers grains has been reduced to as low as 2.1% fat on a DM basis (Ganesan *et al.*, 2009). Not only are the new de-oiled distillers grains a more attractive feed source for ruminants due to an increase in protein concentration, these modified versions of corn distillers grains are considered superior due to the extension of ethanol by-product shelf life (Watkins, 2007).

Previous research conducted at the University of Nebraska-Lincoln has found that feeding full-fat wet distillers grains plus solubles (WDGS; 65-70% moisture) increases the polyunsaturated fatty acid (PUFA) content of beef, resulting in greater oxidation

(Mello *et al.*, 2008a, b). Subsequent research evaluated the impact of feeding de-oiled WDGS vs. full-fat WDGS or a corn-based control diet and determined that the reduction in soluble fat from the feed decreases the total PUFA content of beef compared to the full-fat WDGS (Domenech *et al.*, 2014). However, the question still remains if the reduction in beef PUFA content and the extension of shelf life would also be obtained by feeding de-oiling dry distillers grains plus solubles (DDGS; 10-12% moisture). Thus, the objectives of this study were to determine if there are any changes in beef fatty acid profiles and shelf life associated with feeding 50% de-oiled DDGS compared to a corn-based control diet.

MATERIALS AND METHODS

University of Nebraska-Lincoln's Animal Care and Use Committee approved of all animal use protocols (IACUC# 902).

Cattle and dietary treatments

A total of 448 crossbred yearling steers (initial BW = 363.78 ± 13.15 kg) were fed (University of Nebraska feedlot Mead, NE) one of seven finishing diets that were part of a study looking at the effect of modifying different components of distillers grains on feedlot performance (Carlson *et al.*, 2016). Samples from the positive (replacement of 50% dry-rolled corn with 50% de-oiled dry distillers grains plus solubles) and negative control (diet not including any distillers grains plus solubles, rather 50% dry-rolled corn) of this larger study were utilized for this study. On d 1 all steers were implanted with Ralgro® and re-implanted on d 36 or d 38 (evenly split between both d) with Revalor®-200 (Merck Animal Health, Summit, NJ). Steers were blocked by body weight and randomly assigning to pens (8 hd/pen with 8 replications for a total of 56 pens). Cattle

were fed diets containing 31.5% high-moisture corn, 5.5% alfalfa hay, 5% liquid molasses, 5% supplement (formulated to have 30 g/ton Rumensin® and 90 mg/steer/day of Tylan®, on a DM basis from Elanco Animal Health), and 4% corn stillage. The corn control group received 50% dry rolled corn in place of the 50% de-oiled DDGS (Table 1). Steers were fed for 156 d and harvested in a commercial abattoir (Greater Omaha Pack, Omaha, NE).

Sample collection and fabrication

At harvest, 24 low Choice carcasses were randomly selected within each treatment (n = 48) and strip loins (*Longissimus lumborum*), both from the left and right sides, were collected. Loins were vacuum packaged and aged for 2, 8, 14, and 21 d (2°C) under dark storage. After 2 d of aging, all strip loins were opened and split in half at which time aging time was allotted as follows: anterior left = 2 d age, posterior left = 8 d age, anterior right = 14 d age, and posterior right = 21 d age. Loin portions corresponding to 2 d age were fabricated into 1.27 cm steaks destined for fatty acid analysis, proximate composition, lipid oxidation, and 2.54 cm steaks for tenderness measures at 0 and 7 d of retail display. The remaining portions of the loins were vacuum sealed immediately (3 mil STD barrier, Prime Sources, St. Louis, MO) with a Multivac Packaging machine (Mutivac C500, Multivac, Kansas city, MO). The same fabrication protocol was used at 8, 14 and 21 d aged loins, with the exception of the fatty acid sample which was only taken from the loin portions aged for 2 d.

At all aging periods, steaks for visual discoloration, tenderness and lipid oxidation were placed on foam trays (21.6 x 15.9 x 2.1 cm, Styro-Tech, Denver, CO), overwrapped with oxygen permeable film (PVC-OW; PSM18, Prime Source, St. Louis, MO) and

placed under retail display (RD) conditions for 4 and 7 d (2.7°C under white fluorescence lighting at 1000 to 1800 lux). Steaks used for fatty acid profile, proximate composition and 0 d RD were vacuum packaged and frozen for further analysis (-80°C). Later, samples trimmed of all subcutaneous fat for proximate analysis, fatty acid profile, and lipid oxidation were frozen in liquid nitrogen and powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT). Powdered samples were stored at -80°C.

Proximate analysis

Proximate analysis was performed to determine fat, moisture and ash content; protein content was determined by difference. Fat was quantified following the Soxhlet procedure (AOAC, 1990). Samples were measured in triplicate in Whatman #2 filter paper and fat was extracted with ether. Fat percentages were averaged per sample and used to convert fatty acid percent data to mg/100 g tissue basis. Moisture and ash were determined with a LECO thermogravimetric analyzer (LECO Corporation, Model 604-100-400, St. Joseph, MI), and samples were measured in duplicates. Moisture was determined in nitrogen atmosphere with a start temperature of 25°C and an end temperature of 130°C (17 min ramp rate). Ash was determined in oxygen atmosphere with a start temperature of 130°C and an end temperature of 600°C (30 min ramp rate).

Fatty acid composition

Fatty acid profiles were obtained via gas chromatography as described by Folch *et al.* (1957) with modifications detailed by Morisson and Smith (1964) and Metcalfe *et al.* (1966). Briefly, 1 g of powdered sample was weighed into a 15 mL conical tube to which 5 mL of 2:1 chloroform:methanol was added and vortexed for 5 s. After one hour,

samples were filtered through Whatman #2 filter paper onto a 13 x 150 mm glass screw cap tube, volume was brought up to 10 mL with 2:1 chloroform:methanol and 2 mL of KCl were added and vortexed. After centrifuging samples (1,000 x g for 5 min) the top organic matter layer was aspirated off and samples were dried down completely on a heating block (60°C) under constant nitrogen purge. One half mL of 0.5 M NaOH in methanol was added, vortexed and heated (100°C) for 5 min. Then, boron trifluoride in 14% methanol (0.5 mL) was added, vortexed and heated (100°C for 5 min). Subsequently, 1 mL of saturated salt solution and 1 mL of hexane were added and samples were centrifuged (1,000 x g for 5 min). The top hexane layer was carefully pipetted into gas chromatography glass vials, nitrogen purged and lids were crimped on. Chromatography was done using a Chromopack CP-Sil (0.25 mm x 100 m) column with an injector temperature of 270°C and a detector temperature of 300°C (Hewlett-Packard 6890 FID GC System; Agilent Technologies, Santa Clara, CA). The head pressure was set at 40 psi with a flow rate of 1.0 mL/min. The fatty acids were identified by their retention times in relation to known standards and the percentage of fatty acids were determined by the peak areas in the chromatograph. Values were adjusted according to percent fat and values were converted to mg/100g tissue.

Subjective color (Discoloration)

Visual discoloration was assessed daily during retail display with a trained five-person panel. Panelists were provided with a visual discoloration guide to use as a reference. A percentage scale was used were 0% meant no discoloration and 100% meant complete discoloration. Panelists were instructed to perform the evaluation at the

same time each day to minimize variation. Samples were randomly rotated daily to minimize any possible location effects.

Objective color (L*, a*, b*)

Color measures were taken on a daily basis once samples were placed on retail display at all aging time points. Color was evaluated on steaks that were plastic overwrapped with oxygen permeable film and destined for tenderness assessment at the end of retail display. Objective color measures were taken with the L*, a*, b* scales with a Minolta CR-400 colorimeter (Minolta, Osaka, Japan). The handheld colorimeter reading device with an opening of 8mm was set to use a D65 illuminant and 2° observer. The colorimeter was set to record and print an average of 6 readings per steak. The calibration process was done on a daily basis using a white tile and the D65 settings were set as follows: Y = 93.13, x = 0.3164 and y = 0.3330. Once calibrated, the color space was selected to be the L*, a*, and b* scale; where, L* is a measure of lightness and has a range from 0 (black) to 100 (white), a* is a measure of redness and has a positive (red) to negative range (green), and b* is a measure of yellowness with a positive (yellow) to negative (blue) range. Color readings were taken within a ± 2 hour window time frame for consistency and accuracy of data.

Lipid oxidation

Lipid oxidation was determined with the 2-thiobarbituric acid reactive substances protocol (TBARS) as described by Ahn *et al.* (1998). Briefly, five g of powdered sample were weight into a 50 mL conical tube to which 14 mL of deionized distilled water and 1 mL of BHA (10% BHA: 90% ethanol) were added. After polytroning for 15 s the samples were centrifuged (2,000 x g for 5 min). One mL of the supernatant was transferred to a 15 mL conical tube and 2

mL of TBA/TCA solution (15% TCA and 20 mM TBA in deionized distilled water) was added and vortexed before placing samples in a water bath (70°C for 30 min). After cooling, samples were centrifuged (2,000 x g for 5 min) and 200 μL of supernatant were transferred to 96-well plates. All 96-well plates had standards to calculate standard curves and ultimately mg of malonaldehyde per kg of tissue read at 540 nm.

Tenderness

Tenderness was measured via Warner-Bratzler Shear Force (WBSF). Steaks were thawed (4°C) 24 h prior to cooking and internal temperature was monitored with a thermocouple (5SC-TT-T-30-120, OMEGA Engineering, Inc., Stamford, CT) inserted in the geometric center of each steak. Steaks were cooked on Hamilton Beach grills (Model 31605A, Proctor-Silex, Inc., Washington, NC) until an internal temperature of 35°C was achieved at which time they were flipped to continue cooking. The final internal temperature was 71°C. Cooked steaks were refrigerated for 24 h and six cores (1.27 cm diameter) were taken parallel to the muscle fiber with a drill press and sheared using a texture analysis (model TMS-PRO, Food Technology Crop., Sterling, VA) fitted with a Warner-Bratzler blade. The average of the 6 cores was calculated for statistical analysis.

Statistical analysis

Data were analyzed as a completely randomized design where the main effects of dietary treatment, aging, retail display and their interactions were evaluated with SAS (version 9.4, Cary, NC, 2009). Individual animal served as the experimental unit while pen was used as a random variable. The PROC GLIMMIX procedure was used for the analysis of repeated measures of objective color and subjective visual discoloration where the most appropriate covariance structure was selected based on the best fit model.

The PROC GLIMMIX procedure was also used to evaluate all other variables measured. All means were separated with the LS MEANS statement and the TUKEY adjustment was used with an alpha level of 0.05. When triple interactions were identified the SLICEBY option in PROC GLIMMIX was utilized to evaluate the response of two of the classifying variables after fixing a third classifying variable. In our case we fixed age and examined differences between retail display and dietary treatment within the four different aging times.

RESULTS AND DISCUSSION

Proximate analysis

Finishing diet had no effect ($P > 0.05$) on moisture (72.08%), protein (19.31%), fat (7.31%), or ash (1.31%) content in beef. These results are in accordance to those reported by Domenech *et al.* (2014) where cattle receiving several inclusion levels of full-fat or de-oiled WDGS and a corn control diet did not differ in proximate composition in *Longissimus lumborum* samples. The average values reported for proximate composition were 71.70% moisture, 20.26% protein, 6.48% fat, and 1.56% ash (Domenech *et al.*, 2014). Similarly, Mello *et al.* (2012a) conducted a study with six diets containing varying levels (0% - 50% DM basis) of modified wet distillers grains plus solubles (MWDG: distillers grains with partial drying for a moisture level of 50% - 54%) and found no alterations in proximate composition due to dietary treatment. Also, from evaluating 0%, 15% and 30% inclusion of full-fat WDGS, Mello *et al.* (2012b) indicated there were no compositional changes in moisture, fat, or ash content of *Infraspinatus* (top blade) and *Psoas major* (tenderloin) steaks. Observations made by Buttrey *et al.* (2013) reported that after finishing crossbred steers with 35% WDGS, steaks tended ($P < 0.10$)

to increase in total intramuscular fat content compared to diets without WDGS. Conversely, Segers *et al.* (2011) detected a trend ($P = 0.07$) where steaks from cattle fed 25% DDGS had greater moisture values than cattle on corn gluten feed with no effects on protein or lipid concentration ($P \geq 0.13$). Aldai *et al.* (2010) compared the proximate composition from cattle fed 20% or 40% corn or wheat DDGS versus a control barley-based finishing diet and report a slight increase in protein concertation in the control group, intermediate values for the corn DDGS group, and lowest values for the wheat DDGS group (21.6%, 21.3%, 21.1%, respectively; $P < 0.05$). Fat and moisture content in the previous study were not affected by dietary treatment, however.

Fatty acid composition

Fatty acid profiles of all the dietary treatments are reported in Table 2 on a mg/100 g of tissue basis. No differences ($P > 0.05$) were seen in the total, saturated fatty acids (SFA), unsaturated fatty acids (UFA), SFA:UFA ratio, or monounsaturated fatty acids (MUFA). Previous research has also noted that inclusion of varying levels of full-fat or de-oiled WDGS in comparison to a corn control diet did not alter these fatty acid profiles in strip loin steaks (Domenech *et al.*, 2014). Similarly, Mello *et al.* (2012b) reported that these fatty acids were unaffected in beef from cattle fed diets containing WDGS vs. cattle finished on a corn control diet. On the other hand, Buttrey *et al.* (2013) reported that there was a decreased ratio of MUFA:SFA in the *longissimus* muscle of cattle feed 35% WDGS vs. cattle with no inclusion of WDGS.

In the current study, differences ($P < 0.05$) were observed in the C15:0, C16:1, C17:0, C17:1, C18:1T, C18:2, C20:3ω6, as well as total trans, and polyunsaturated fatty

acids (PUFA). Generally, the shorter chain fatty acids (C15:0, C16:1, C17:0, and C17:1) were greater in beef from the corn control cattle in relation to beef from cattle fed the 50% de-oiled DDGS diet. The longer chain (C18:1T, C18:2, and C20:3ω6) fatty acids, the trans fatty acids, and the PUFA contents were greater for steaks from cattle receiving the 50% de-oiled DDGS diet in relation to the corn control fed cattle.

Although in previous studies no mention has been made regarding changes in C15:0, C17:0, C17:1 or trans fatty acids, associated to the feeding of various forms of corn distillers grains, a handful of studies have identified changes to the C16:1, C18:1T, C18:2 and PUFA content with these diets. In the current study the palmitoleic acid (C16:1) content ranged from 216.31 to 175.88 mg/100 g tissue in the corn control and 50% de-oiled DDGS groups, respectively ($P = 0.01$). As reported by Domenech-Pérez *et al.* (2016), C16:1 has been found to be predominant in beef from cattle fed a corn-based control diet (194.26 mg/100 g tissue), in comparison to diets having greater inclusion levels of both de-oiled and full-fat WDGS (ranging from 115.32 to 145.11; $P < 0.0001$). These results are consistent to those reported by Buttrey *et al.* (2013) that explain that palmitoleic acid is the desaturated product of palmitic acid (C16:0) and that feeding corn WDGS decreases palmitoleic acid ($P \leq 0.01$). In a similar study conducted by Mello *et al.* (2012b) decreases in both C16:0 and C16:1 contents were observed in the *Longissimus thoracis*, *Psoas major* and *Infraspinatus* muscles as inclusion levels of WDGS increased from 0, 15 to 30% on a percentage basis. Decreases in C16:0 and C16:1 were also observed with increasing levels of modified wet distillers grains plus solubles (MGDS; Mello *et al.*, 2012a).

Elaidic acid (C18:1T) content was greatest (P = 0.0015) in beef from cattle finished on 50% de-oiled DDGS versus the corn control group (191.80 vs. 128.91 mg/100g tissue). Similarly, data from Domenech-Pérez *et al.* (2016) reported that cattle fed 65% full-fat WDGS possessed greater proportion of C18:1T (256.20 mg/100 g tissue), comparted to the corn control fed cattle (120.12 mg/100 g tissue), with all other inclusion levels of de-oiled WDGS and full-fat WDGS having intermediate values (210.36 mg/100 g tissue, on average; P = 0.01). Similar results were also noted by Mello *et al.* (2012b) which reflected an increase in C18:1T content with increasing levels of WDGS in the *Longissimus thoracis* and *Infraspinatus* muscles. This difference was not observed in the *Psoas major* however. Vander Pol *et al.* (2009) attempted to further understand fatty acid digestion in ruminants fed corn distillers grains in comparison to corn control fed cattle by using ruminal and duodenal cannulated steers. Vander Pol *et al.* (2009) indicated that cattle fed WDGS had greater total fat digestion and greater amount of unsaturated fatty acids reaching the duodenum (P < 0.10). The greater concentration of unsaturated fatty acids reaching the duodenum points to the fact that cattle subjected to diets containing corn distillers grains diets have greater propensity for depositing these fatty acids in muscle tissue (Atkinson *et al.*, 2006; Vander Pol *et al.*, 2009).

Linoleic acid (C18:2) was greater (P <0.0001) in meat from cattle finished on the 50% de-oiled DDGS (389.39 mg/100 g tissue) than the cattle finished on the corn control diet (194.79 mg/100 g tissue). Similarly, Domenech-Pérez *et al.* (2016) reported that beef from cattle fed 35%, 50%, 65% full-fat WDGS, and 65% de-oiled WDGS diets had greater C18:2 content (290.98 mg/100 g, on average), intermediate amounts were found

for cattle fed 50% and 35% de-oiled WGDS (231.08 and 227.16 mg/100 g, respectively) with the corn control group having the lowest amount of C18:2 (177.70 mg/100 g; P = 0.0001). Linoleic acid is an essential fatty acid, meaning that it is derived from the diet (Enser *et al.*, 1996). In the case of ruminants, this fatty acid can be degraded into MUFA's and SFA's because of biohydrogenation by ruminal bacteria and it has been suggested that around 10% of dietary linoleic acid is available for muscle deposition in ruminant tissues (Enser *et al.*, 1996). However, Vander Pol *et al.* (2009) observed that when feeding WDGS there is an increased concentration of linoleic acid in the duodenum. This indicates that there is a protection phenomenon when WDGS are fed which causes less biohydrogenation of linoleic acid, thus increasing its availability to be deposited in muscle. Dugan *et al.* (2010) also reported that when cattle were fed wheat DDGS there was a linear increase in percentage of C18:2 (P = 0.001).

The PUFA content was greater (P <0.0001) for meat from cattle fed 50% de-oiled DDGS (509.37 mg/100 g tissue) in comparison to that of the corn control group (304.57 mg/100 g tissue). This also coincides with findings reported by Domenech-Pérez *et al.* (2016) where beef from cattle fed various levels of full-fat WDGS or a 65% de-oiled WDGS diet had greater (P < 0.01) amounts of PUFA (337.13 mg/100 g tissue, on average), cattle on 50% and 35% de-oiled WGDS diets had intermediate amounts (274.77 and 273.84 mg/100 g tissue, respectively), while beef from cattle on a corn control diet had the least amount of PUFA (223.98 mg/100 g tissue). The PUFA content is of particular interest given that it has been well established in the literature that increased unsaturated fatty acid content in meat products will greatly diminish shelf life,

particularly due to less color stability and lipid rancidity (Gatellier *et al.*, 2001; Ladeira *et al.*, 2014).

Grain inclusions in cattle diets, particularly in elevated proportions, create a more acidic ruminal environment, thus suppressing lipolysis and inhibiting microbial biohydrogenation of fatty acids (Atkinson *et al.*, 2006; Plascencia *et al.*, 1999; Scollan *et al.*, 2014). Once fatty acids pass the rumen unchanged these then reach the duodenum where bile salts form micelles resulting in greater fatty acid absorption (Zinn *et al.* 2000). Traditionally, ruminant diets, although containing little fat, are rich in PUFA's; this is particularly true when considering WDGS in relation to regular corn based diets (Vander Pol *et al.*, 2009). However, contemplating the low ruminal pH environment in diets with greater grain inclusions (Plascencia *et al.*, 1999) and the bile salt formation (Zinn *et al.*, 2000) in the duodenum there is a feasible explanation for the more elevated PUFA deposition in cattle finished on higher levels of WDGS. Based on our observations, it seems as though the de-oiling process with both DDGS and WDGS (Domenech-Pérez *et al.*, 2016) does not alter the greater fatty acid digestion associated with full-fat corn distillers grains. Hence, de-oiled DDGS or WDGS still augment the PUFA content in muscle tissue relative to a corn control diet.

Subjective color (Discoloration)

A three way interaction between dietary treatment, aging time and retail display time was identified ($P < 0.0001$; Figure 1A-D; Appendix XVI). An increase in surface discoloration as retail display time increased was seen at all aging periods and both dietary treatments. Steaks from cattle fed 50% de-oiled DDGS had greater discoloration in comparison to cattle having been fed the corn control diet at extended retail display

time at 21 d of age. It is at 21 d age were the triple interaction is evident given that the magnitude of discoloration of steaks with 6 and 7 d of retail display was greater in beef from cattle fed 50% de-oiled DDGS. This was not observed in steaks aged for shorter aging periods (Figure 1D vs. 1A-C).

Consumer studies have reported a significant decline in purchasing decisions where with 20% discoloration on retail display, beef sales were reduced to 50% (Hood and Riordan, 1973). According to our data, samples aged for 8 and 14 d reached the 20% cutoff point by 7 d retail display at both aging times but only for the samples from cattle on the 50% de-oiled DDGS diet. In the case of 21 d aged beef, the 20% discoloration mark was met and surpassed after 6 d of retail display by the samples from the 50% de-oiled DDGS group and by d 7 of retail display both dietary treatments surpassed this threshold (57.40% in the 50% de-oiled DDGS group vs. 25.73% in the corn control group).

Objective color (L, a*, b*)*

Objective color data indicated that dietary treatment had an effect ($P = 0.0008$) on L* values, where meat from the 50% de-oiled DDGS cattle had lighter meat (47.11 vs. 45.36). Also, a triple interaction ($P = 0.0011$) between dietary treatment, age, and retail display time was found for a* values (Figure 2 A-D; Appendix XVI). The patterns seen with a* values closely resembles those seen with steak discoloration data discussed earlier. It is evident that a* values decrease with a greater slope with more extended aging and retail display times (Figure 2D vs. 2A-C). Starting at 8 d age, steaks with longer retail display times indicate that beef from cattle fed 50% de-oiled DDGS had

lower redness than did the steaks from corn control fed cattle. The magnitude of these differences increases with increased aging time.

It has been previously recorded that inclusion of WDGS seems to have an effect on beef color (Mello *et al.*, 2012a; Roeber *et al.*, 2005), which appears to be a dose dependent response. Studies have suggested that inclusion of 15 or 30% WDGS are sufficient to decrease redness (lower a* values) of strip loin steaks under retail display conditions after 7 d of display ($P < 0.05$; Mello *et al.*, 2012a). Similar findings have been reported by Buttrey *et al.* (2013), Depenbusch *et al.* (2009), Gordon *et al.* (2002), Leupp *et al.* (2009), and Segers *et al.* (2011) who have indicated that inclusion of corn WDGS or DDGS causes a reduction in a* values. Similar observations were noted by Roeber *et al.* (2005) where they state that inclusions greater than 40% distillers grains can result in decreased color stability in a retail setting whereas inclusion of up to 25% distillers grains can be included with little to no effect on retail display color stability.

Lipid oxidation

Lipid oxidation, measured by the amount of thiobarbituric acid reactive substances (TBARS), indicated there was an age by retail display interaction ($P < 0.0001$; Figure 3). At 0 d retail display, no differences were found between aging times. However, at 4 d retail display, steaks aged for 2 d had the least ($P \leq 0.05$) amount of lipid oxidation (1.72 mg malonaldehyde/kg tissue), steaks aged for 21 d had the greatest amount of oxidation (3.22 mg malonaldehyde/kg tissue), while 8 and 14 d ages steaks had intermediate oxidation levels (2.36 mg malonaldehyde/kg tissue, on average). At 7 d retail display, steaks aged for 2 d had the least ($P \leq 0.05$) amount of lipid oxidation (3.14 mg malonaldehyde/kg tissue), steaks aged for 21 d had the greatest amount of oxidation

(5.30 mg malonaldehyde/kg tissue), followed by steaks aged for 8 d (4.48 mg malonaldehyde/kg tissue) and 14 d ages steaks being intermediate between the 8 d and 2 d aged samples (3.82 mg malonaldehyde/kg tissue, on average). Hence, the rate of lipid oxidation was greater in samples aged for 21 d and least for 2 d aged samples, particularly at 4 and 7 d of RD, regardless of dietary treatment.

A dietary treatment by retail display interaction for TBARS was also identified ($P < 0.0001$; Figure 4). In this instance, samples from cattle having been fed the 50% de-oiled DDGS diet had greater lipid oxidation values at 4 and 7 d of retail display in relation to cattle on the corn control treatment (2.67 vs. 2.16 mg/malonaldehyde/kg tissue and 4.99 vs. 3.37 mg/malonaldehyde/kg tissue, respectively). This difference due to dietary treatment was not seen at 0 d retail display samples (0.77 vs. 0.87 mg/malonaldehyde/kg tissue). At greater retail display times the de-oiled DDGS diet resulted in beef with lower lipid stability in relation to the corn control treatment (2.81 vs. 2.14 mg/malonaldehyde/kg tissue; $P = 0.0013$).

Campo *et al.* (2006) concluded that a TBARS value of 2 can be considered as the limiting threshold of oxidized beef acceptability. According to our results, beef from both the corn control and de-oiled DDGS diets reached or surpassed this threshold by d 4 of retail display at 8, 14 and 21 d of aging.

Previous research has reported that inclusion of WDGS in finishing diets causes an increase in steak lipid oxidation (TBARS) under retail display conditions, thus limiting beef shelf life (Buttrey *et al.*, 2013; Koger *et al.*, 2010). Similarly, Mello *et al.* (2012b) determined that beef cattle fed 30% WDGS (DM basis) resulted in increased TBARS values for top blade and strip loin steaks ($P < 0.01$) in comparison to 0% or 15%

inclusion of WDGS; yet, no differences were found for tenderloin steaks ($P = 0.19$). On the contrary, other researchers have indicated that lipid oxidation of beef was unaffected by dietary treatments including DDGS (Depenbusch *et al*., 2009; Gunn *et al*., 2009). Gill *et al.* (2008) reported mixed results when comparing corn DDGS and WDGS, where treatments including DDGS had greater TBARS values than did the beef from cattle fed WDGS ($P < 0.05$).

Tenderness

An age by retail display interaction ($P < 0.0001$) was detected for tenderness (Figure 5). The rate of tenderization observed with steaks aged for 2 d was greater than that of all other aging periods. Dietary treatment had no effect on WBSF ($P = 0.95$), nor was dietary treatment involved in any interactions. These results are in agreement with those observed by Mello *et al.* (2012b) with beef aged for 7 d that were finished with 0%, 15% or 30% corn WDGS. On the other hand, Chao (2015) compared beef from cattle fed a corn control diet vs. 50% WDGS and found that at 2 d of aging steaks from cattle finished on WDGS were more tender than the corn control steaks ($P < 0.01$). These differences in tenderness did not persist with increased aging time.

CONCLUSION

Feeding cattle with 50% de-oiled DDGS did alter several fatty acid profiles, but most importantly the PUFA content was increased in relation to cattle on a corn control finishing diet. In this study, it was evident that the more elevated PUFA content in beef from cattle fed 50% de-oiled DDGS caused greater color and lipid instability at longer aging and retail display times. Based on our observations it seems as though the de-

oiling process in DDGS still results in elevated meat PUFA content even after the removal of part of the soluble fat portion in the feed relative to a corn control diet.

Findings from the current study indicate that much like full-fat corn distillers grains, de-oiled DDGS may also have a biohydrogenation protection in the rumen that allows for greater muscle uptake of PUFA. This would indicate that even when the deposition of PUFA in de-oiled corn distillers grains is relatively less than that of full-fat corn distillers grains, PUFA content would still be superior to that of cattle finished on diets not containing corn distillers grains, irrespective of feed moisture content. Therefore, in order to offset the potential reduction in beef shelf life associated with elevated PUFA content, techniques such as strategic use of antioxidants should be explored.

LITERATURE CITED

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.

Aldai, N., J. L. Aalhus, M. E. R. Dugan, W. M. Robertson, T. A. McAllister, L. J. Walter, and J. J. McKinnon. 2010. Comparison of wheat- versus corn-based dried distillers grains with solubles on meat quality of feedlot cattle. Meat Sci. 84:569-577.

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Atkinson, R. L., E. J. Scholljegerdes, S. L. Lake, V. Nayigihugu, B. W. Hess, and D. C. Rule. 2006. Site and extent of digestion, duodenal flow, and intestinal disappearance of total and esterified fatty acids in sheep fed a high-concentrate diet supplemented with high-linoleate safflower oil. J. Anim. Sci. 84:387-396.

Berger, L., and V. Singh. 2010. Changes and evolution of corn coproducts for beef cattle. J. Anim. Sci.88(E. Suppl.):143-150.

Buttrey, E. K., K. H. Jenkins, J. B. Lewis, S. B. Smith, R. K. Miller, T. E. Lawrence, F. T. McCollum III, P. J. Pinedo, N. A. Cole and J. C. MacDonald. 2013. J. Anim. Sci. 91:1850-1865.

Carlson, Z. E., C. J. Bittner, D. Burken, G. E. Erickson, and J. C. MacDonald. 2016. Modifying Different Components of Distillers Grains and the Impact on Feedlot Performance. 2016 Nebraska Beef Cattle Report:128-131.

Chao, M. D. 2015. Impact of wet distillers grains plus solubles and antioxidants on a basic mechanism of beef tenderization. Diss, Univ. of Nebraska-Lincoln, Digital Commons.

Depenbusch, B. E., C. M. Coleman, J. J. Higgins, and J. S. Drouillard. 2009. Effects of increasing levels of dried corn distillers grains with solubles on growth performance, carcass characteristics, and meat quality of yearling heifers. J. Anim. Sci. 87:2653-2663.

Domenech, K. I., K. A. Varnold, M. E. Semler, M. D. Chao, T. F. Jones, G. E. Erickson, and C. R. Calkins. 2014. Effect of Feeding De-oiled Wet Distillers Grains Plus Solubles on Beef Fatty Acid Profiles. 2014 Nebraska Beef Cattle Report:116-118.

Domenech-Pérez, K. I., M. D. Chao, M. E. Semler, K. A. Varnold, G. E. Erickson, and C.

R. Calkins. 2016. Impact of Feeding De-Oiled Wet Distillers Grains Plus Solubles on Beef Shelf Life. In preparation.

Dugan, M. E. R., N. Aldai, J. K. G. Kramer, D. J. Gibb, M. Juárez, and T. A. McAllister. 2010. Feeding wheat dried distillers grains with solubles improves beef trans and conjugated linoleic acid profiles. J. Anim. Sci. 88:1842-1847.

Folch, J., M. Lees, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Ganesan, V., K. A. Rosentrater, and K. Muthukumarappan. 2009. Physical and flow properties of regular and reduced fat distillers dried grains with solubles (DDGS). Food Bioprocess Technol. 2:156-166.

Gatellier, P., C. Hamelin, Y. Durand, and M. Renerre. 2001. Effect of a dietary vitamin E supplementation on colour stability and lipid oxidation of air- and modified atmosphere-packaged beef. Meat Sci. 59:133-140.

Gill, R. K., D. L. VanOverbeke, B. Deoenbusch, J. S. Drouillard, and A. DiCostanzo. 2008. Impact of beef cattle diets containing corn or sorghum distillers or beef color, fatty acid profiles, and sensory attributes. J. Anim. Sci. 86:923-935.

Gordon, C. M., J. S. Drouillard, R. K. Phebus, K. A. Hachmeister, M. E. Dikeman, J. J. Higgins, and A. L. Reicks. 2002. The effect of Dakota Gold®-brand dried distillers grains with solubles of varying levels on sensory and color characteristics of ribeye steaks. Pages 72-74 in Cattlemen's Day, Manhattan, KS. Kansas State Univ., Manhattan.

Gunn, P. J., A. D. Weaver, R. P. Lemenager, D. E. Gerrard, M. C. Claeys, and S. L. Lake. 2009. Effects of dietary fat and crude protein on feedlot performance, carcass characteristics, and meat quality in finishing steers fed differing levels of dried distillers grains with solubles. J. Anim. Sci. 87:2882-2890.

Hood, D. E. R. and E. B. Riordan. 1973. Discolouration in pre-packaged beef: measurement by reflectance spectrophotometry and shopper discrimination. Journal of Food Technology 8:333-343.

Koger, T. L., D. M. Wulf, A. D. Weaver, C. L. Wright, K. E. Tjardes, K. S. Mateo, T. E. Engle, R. J. Maddock, and A. J. Smart. 2010. Influence of feeding various quantities of wet and dry distillers grains to finishing steers on carcass characteristics, meat quality, retail-case life of ground beef, and fatty acid profile of longissimus muscle. J. Anim. Sci. 88:3399-3408.

Ladeira, M. M., L. C. Santarosa, M. L. Chizzotti , E. M. Ramos, O. R. Machado Neto, D. M. Oliveira, J. R. R. Carvalho, L. S. Lopes and J. S. Ribeiro. 2014. Fatty acid

profile, color and lipid oxidation of meat from young bulls fed ground soybean or rumen protected fat with or without monensin. Meat Sci. 96:597-605.

Leupp, J. L., G. P. Lardy, M. L. Bauer, K. K. Karges, M. L. Gibson, J. S. Caton, and R. J. Maddock. 2009. Effects of distillers dried grains with solubles on growing and finishing steer intake, performance, carcass characteristics, and steak color and sensory attributes. J. Anim. Sci. 87:4118-4124.

Mello, A. S., C. R. Calkins, B. E. Jenschke, T. P. Carr, M. E. R. Dugan, and G. E. Erickson. 2012a. Beef quality of calf-fed steers finished on varying level of corn-based wet distillers grains plus solubles. J. Anim. Sci. 90:4625-4633.

Mello, A. S., B. E. Jenschke, L. S. Senaratne, T. P. Carr, G. E. Erickson, and C. R. Calkins. 2012b. Effects of feeding modified distillers grains plus solubles on marbling attributes, proximate composition, and fatty acid profile of beef. J. Anim. Sci. 90:4634-4640.

Metcalfe, L. D., A. A. Schmitz, and J. R. Pelka. 1966. Rapid separation of fatty acid esters from lipids for gas chromatography analysis. Anal. Chem. 38:514-515.

Morisson W. R. and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5:600-608.

Plascencia, A., M. Estrada, and R. A. Zinn. 1999. Influence of fatty acid content on the feeding value of yellow grease in finishing diets for feedlot cattle. J. Anim. Sci. 77:2603-2609.

Roeber, D. L., R. K. Gill, and A. DiCostanzo. 2005. Meat quality responses to feeding distillers grains to finishing Holstein steers. J. Anim. Sci. 83:2455-2460.

Saunders, J. A., and K. A. Rosentrater. 2009a. Properties of solvent extracted low-oil corn distillers dried grains with solubles. Biomass and Bioenergy 33:1486-1490.

Saunders, J. A., and K. A. Rosentrater. 2009b. Survey of US fuel ethanol plants. Bioresource Technology 100:3277-3284.

Scollan, N. D., D. Dannenberger, K. Nuernberg, I. Richardson, S. MacKintosh, J. F. Hocquette, and A. P. Moloney. 2014. Enhancing the nutritional and health value of beef lipids and their relationship with meat quality. Meat Sci. 97:384-394.

Segers, J. R., R. L. Stewart Jr., C. A. Lents, T. D. Pringle, M. A. Froetschel, B. K. Lowe, R. O. McKeith, and A. M. Stelzleni. 2011. Effect of long-term corn by-product feeding on beef quality, strip loin fatty acid profiles, and shelf life. J. Anim. Sci. 89:3792-3802.

Vander Pol, K. J., M. K. Luebbe, G. I. Crawford, G. E. Erickson, and T. K. Klopfenstein. 2009. Performance and digestibility characteristics of finishing diets containing distillers grains, composites of corn processing coproducts, or supplemental corn oil. J. Anim. Sci. 87:639-652.

Waterbury, J. A., D. R. Mark, S. M. Thomas, G. E. Erickson, and T. J. Klopfenstein. 2009. An Economic Overview of Ethanol Co-product Utilization in Nebraska. University ofNebraska-Lincoln Extension Publication EC858. http://ianrpubs.unl.edu/live/ec858/build/ec858.pdf

Watkins, C. 2007. Two fuels from one kernel. Inform 18:714-717.

Winkler-Moser, J. K., and L. Breyer. 2011. Composition and oxidative stability of crude oil extracts of corn germ and distillers grains. Industrial Crops and Products 33:572-578.

Table 1. Diet composition on a DM basis of steers fed either a corn control diet or 50% de-oiled DDGS

	Dietary treatment	
	Control[1]	De-oiled DDGS
Ingredient, % of DM		
HMC[2]	31.5	31.5
DRC[2]	50.0	-
De-Oiled DDGS[2]	-	50.0
Alfalfa hay	5.5	5.5
Corn silage	4.0	4.0
Liquid molasses	5.0	5.0
Supplement [3]	4.0	4.0
Nutrient Composition, %		
DM	81.7	82.2
OM	94.4	92.3
CP	15.5	22.7
NDF	12.2	25.8
ADF	8.2	12.8
Fat	3.7	6.1
Ca	0.8	1.0
P	0.3	0.6
K	0.7	1.2
S	0.2	0.3

[1]Supplemented with urea at 1.36% of diet to meet the degradable intake protein (DIP) requirements

[2]HMC = High moisture corn; DRC = Dry rolled corn; De-oiled DDGS = De-oiled dry distillers grains plus solubles

[3]Supplement included: Limestone (1.43%) Urea (1.36%), Fine ground corn (0.73%), Salt (0.30%), Tallow (0.10%), Beef trace minerals (0.05%), Vitamin A-D-E (0.02%), Rumensin-90® (0.02%), and Tylan-40® (0.01%). Formulated to contain 30 g/ton Rumensin® and 90 mg/steer/day of Tylan®.

Table 2. Fatty acid[1] composition of beef from cattle finished on 50% de-oiled DDGS vs. a corn-based control diet (*L. lumborum*)

Fatty acid	De-oiled DDGS	Corn Control	*SEM*	*P*-value
C4:0	4.59	25.41	19.44	0.42
C10:0	5.53	4.79	0.62	0.45
C12:0	5.28	4.93	0.54	0.71
C14:0	179.94	187.25	10.99	0.63
C14:1	45.24	46.01	2.87	0.85
C15:0	30.58[b]	37.68[a]	1.97	0.01
C15:1	37.96	38.55	1.88	0.81
C16:0	1,736.43	1,841.70	74.71	0.32
C16:1T	22.54	22.87	1.49	0.86
C16:1	175.88[b]	216.31[a]	10.62	0.01
C17:0	83.12[b]	116.92[a]	6.05	0.0003
C17:1	56.57[b]	90.51[a]	4.02	<0.0001
C18:0	1,044.80	1,033.62	52.81	0.88
C18:1T	191.80[a]	128.91[b]	13.05	0.0015
C18:1	2,647.65	2,920.66	105.34	0.07
C18:1V	514.33	452.46	29.25	0.14
C18:2TT	12.28	11.56	2.61	0.86
C18:2	389.39[a]	194.79[b]	12.85	<0.0001
C18:3ω6	7.87	6.31	0.56	0.08
C18:3ω3	30.75	26.12	3.06	0.28
C19:0	9.08	9.12	0.97	0.97
C20:1	15.09	17.48	3.25	0.53
C20:3ω6	18.77[a]	15.48[b]	0.98	0.03
C20:4ω6	55.15	50.76	2.87	0.28
C22:4	10.86	11.17	0.69	0.67
C22:5	9.84	10.79	0.92	0.41
Total	7,281.87	7,461.55	259.56	0.62
Other	66.04	67.03	6.0	0.84
SFA	3,081.63	3,231.64	134.07	0.42
UFA	4,200.25	4,229.91	139.29	0.88
SFA:UFA	0.73	0.76	0.02	0.32
MUFA	3,676.23	3,904.54	128.91	0.21
PUFA	509.37[a]	304.57[b]	18.85	<0.0001
Trans	210.13[a]	152.86[b]	14.66	0.0083

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

[a,b]Means in the same row with different superscripts are different ($P < 0.05$)

Figure legends

Figure 1 A-D. Discoloration triple interaction ($P < 0.0001$) for dietary treatment by age by retail display of strip loin steaks aged for 2 d (Figure 1A), 8 d (Figure 1B), 14 d (Figure 1C), 21 d (Figure 1D) under 7 d retail display

SEM = 10.14

Figure 2 A-D. a* triple interaction ($P = 0.0011$) for dietary treatment by age by retail display of strip loin steaks aged for 2 d (Figure 2A), 8 d (Figure 2B), 14 d (Figure 2C), 21 d (Figure 2D) under 7 d retail display

SEM = 0.40

Figure 3. Lipid oxidation measures (TBARS) of the age by retail display interaction ($P < 0.0001$; *SEM* = 0.18) for strip loins steaks placed under retail display conditions for 7 d from cattle fed both dietary treatments

[a-c]Different superscripts within the same retail display time indicated defenses within aging times ($P \leq 0.05$)

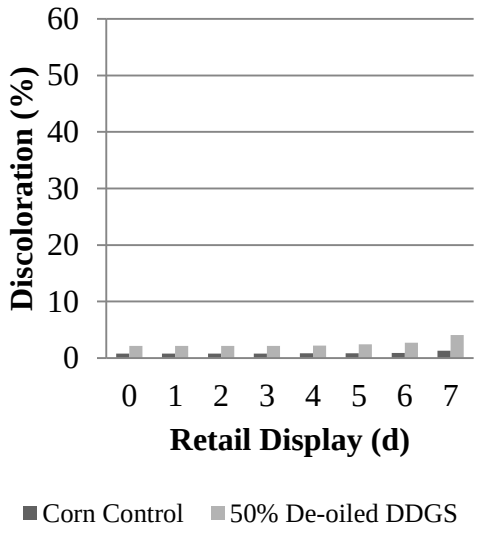
Figure 4. Lipid oxidation measures (TBARS) of the dietary treatment by retail display interaction ($P < 0.0001$; *SEM* = 0.17) for strip loin steaks placed under retail display conditions for 0, 4 or 7 d

[a-b]Different superscripts indicate differences among dietary treatments under the same retail display time ($P \leq 0.05$)

Figure 5. Tenderness data (WBSF) of the age by retail display interaction ($P < 0.0001$; *SEM* = 0.11) for strip loin steaks placed under retail display for 0 or 7 d

[a-b]Different superscripts within the same retail display time indicated defenses within aging times ($P \leq 0.05$)

Figure 1A.



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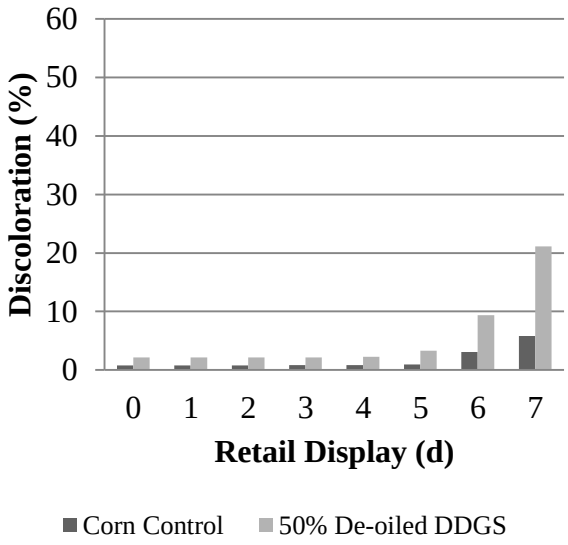

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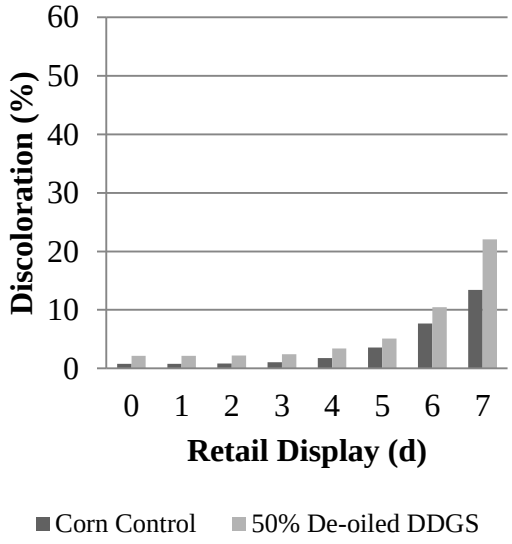


Figure 1D.

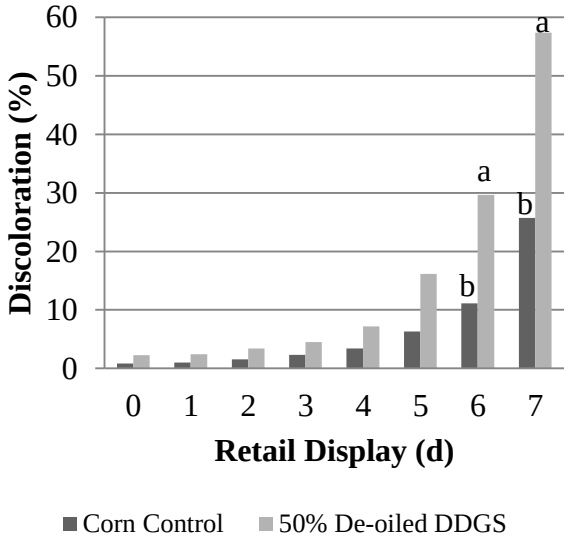
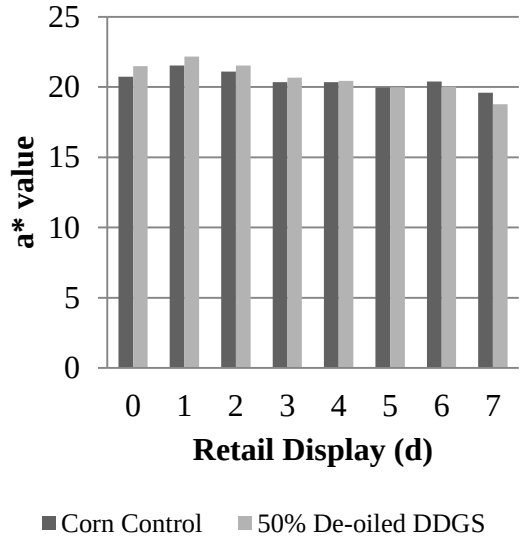


Figure 2A.



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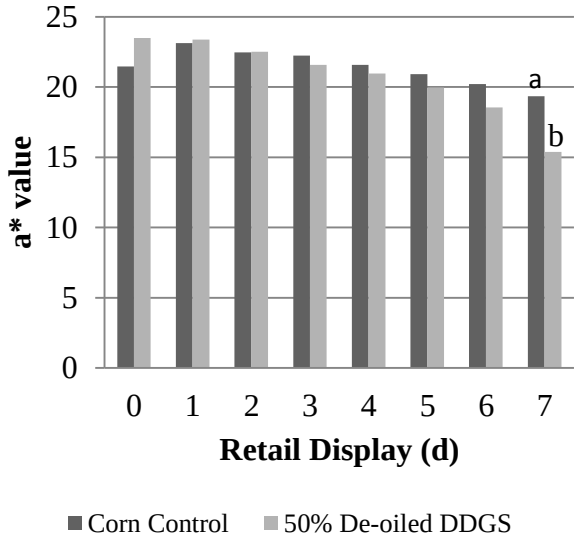

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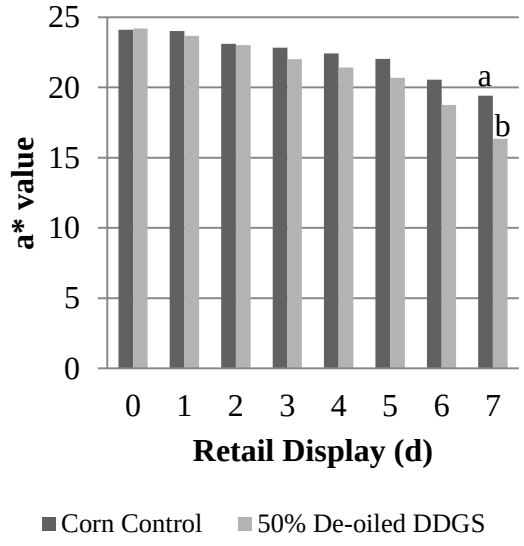

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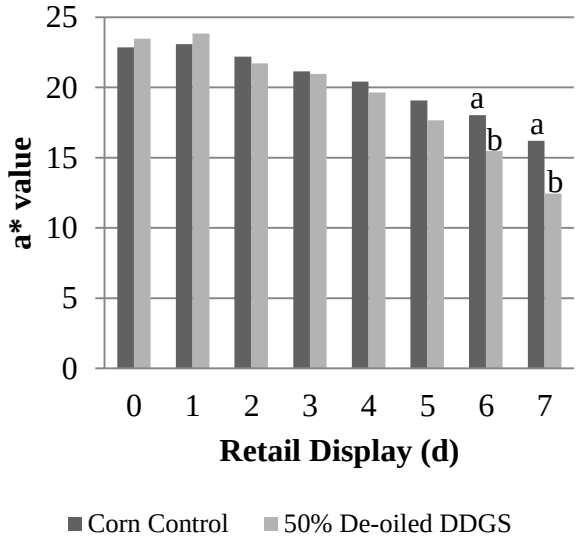

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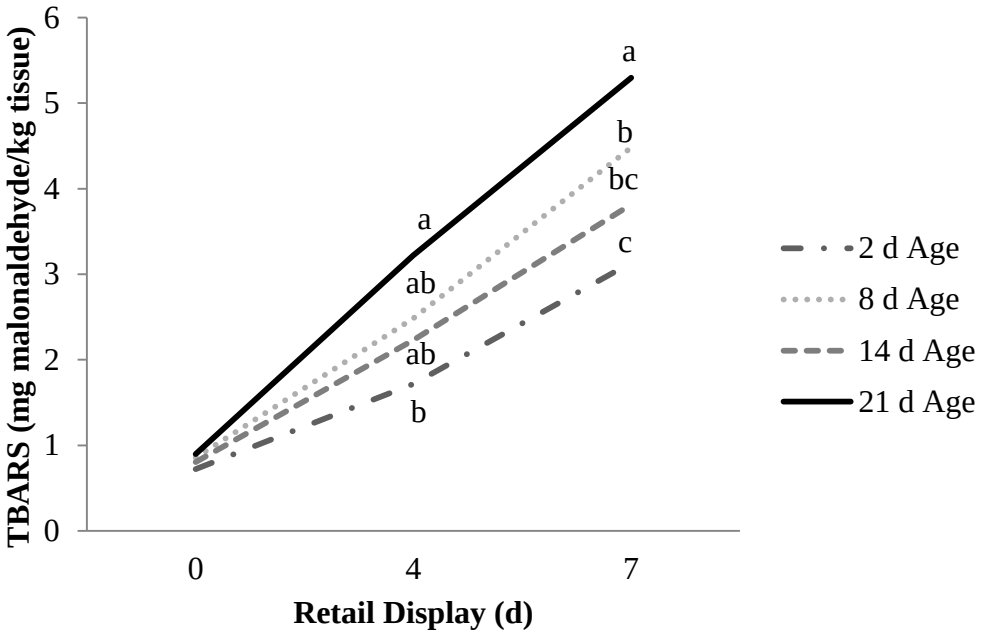

TBARS (mg malonaldehyde/kg tissue)
0
1
2
3
4
5
6
0
4
7
Retail Display (d)
a
b
bc
c
a
ab
ab
b
2 d Age
8 d Age
14 d Age
21 d Age

Figure 4.

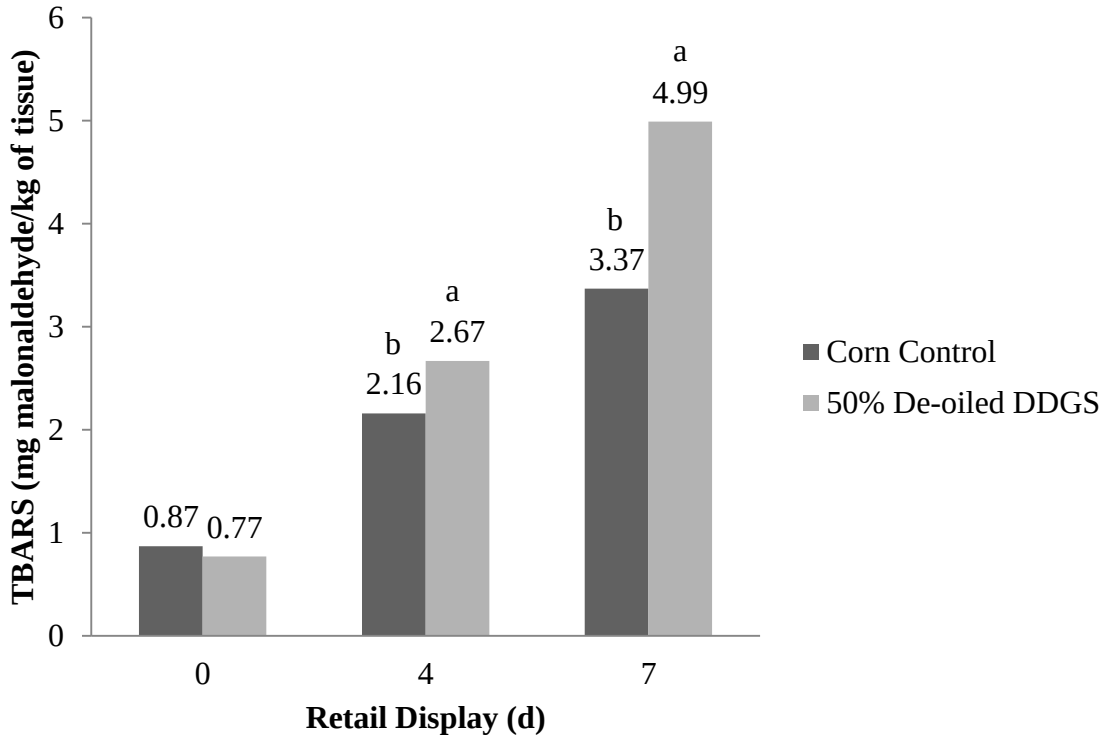
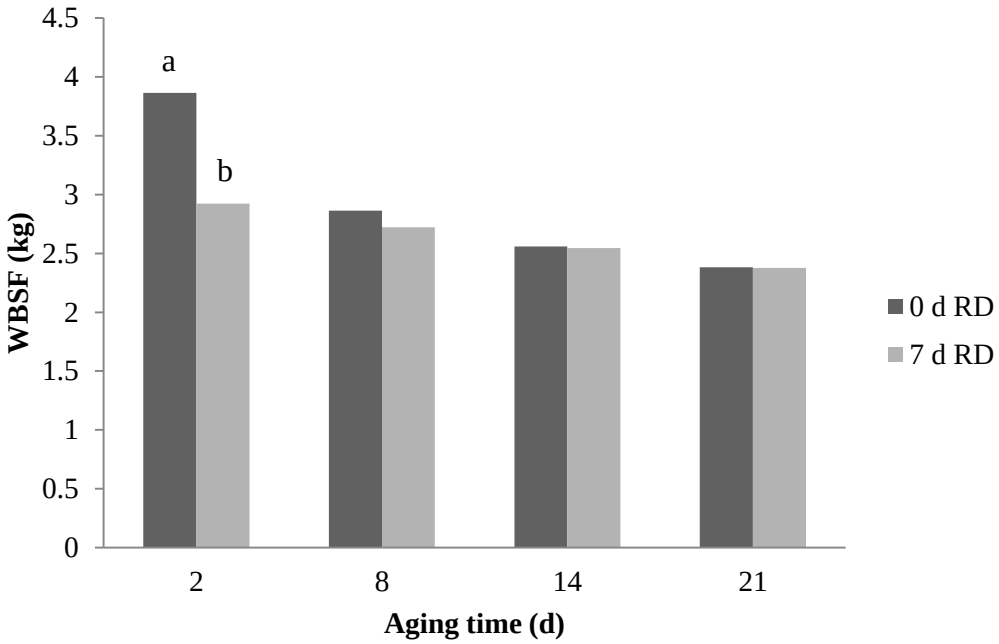

TBARS (mg malonaldehyde/kg of tissue)
6
5
4
3
2
1
0
0.87
0.77
b
2.16
a
2.67
b
3.37
a
4.99
0
4
7
Retail Display (d)
Corn Control
50% De-oiled DDGS

Figure 5.



Impact of Supplementing Cattle with OmniGen-AF at the Receiving or Finishing Phase on Beef Shelf Life and Superoxide Dismutase Activity

The author acknowledges the assistance of M. D. Chao, J. O. Buntyn, T. B. Schmidt, and C. R. Calkins in the research discussed in the following chapter.

ABSTRACT

A proprietary feed additive designed to augment the innate immune function in cattle, OmniGen-AF (Phibro Animal Health Cooperation, Teaneck, NJ), might extend beef shelf life by incorporating antioxidants via phenolic-rich compounds. Thus, the objective of this study was to evaluate the effect of supplementing cattle at the receiving or finishing phase on beef steak shelf life after 8, 22, and 29 days of aging. Three treatment groups included a control group with no supplementation (CON), OmniGen-AF supplementation (4g/45.36kg BW/hd/d) for 28 d after receiving (REC), and supplementation for 215 d during finishing (FIN). The study had a total of 288 crossbred steers (initial BW = 263.54 ± 18.60 kg) that were randomly assigned to one of the three treatment groups (96 hd/treatment) which were randomly sorted into groups of 8 steers for a total of 12 pens/treatment. At harvest, 24 USDA Choice carcasses were randomly selected within each dietary treatment (n = 72) and left and right side *Longissimus lumborum* samples were collected for analysis. Fat content was found to be greater (P = 0.03) for the FIN group, intermediate for the CON group, and least for the REC group. The C18:1, C19:0, C18:2, C20:5ω3, total, UFA and MUFA were greater ($P \leq 0.05$) in beef from the FIN group, lowest in cattle in the REC group, and intermediate for the CON cattle. The SFA:UFA ratio was found to be greater (P = 0.05) for the REC group, intermediate for the CON group, and lowest for the FIN group. The PUFA content was greatest (P = 0.01) for the FIN and CON groups and lower for the REC group (610.07 and 598.34 mg/100 g tissue vs. 526.60 mg/100 g tissue, respectively. Age by retail display interactions were found for subjective and objective color measures ($P < 0.0001$),

where samples aged for 22 d had greater discoloration, greater L* values and lower a* and b* values than did the other two aging groups after 4 d of retail display. However, lipid oxidation data indicated that samples aged for 29 d had greater oxidation ($P = 0.04$) than samples aged for 22 or 8 d. Dietary treatment did not have an impact on meat tenderness, color measures, lipid oxidation or superoxide dismutase activity ($P > 0.05$). Feeding OmniGen-AF had no detrimental effects on basic beef quality parameters and the supplementation does not seem to effectively prolong beef shelf life.

Keywords: beef, OmniGen-AF, fatty acid profile, oxidation, superoxide dismutase activity

INTRODUCTION

OmniGen-AF (Phibro Animal Health, Quincy, IL) is a patented nutritional supplement designed to augment and support the immune system of cattle. This nutritional supplement consists of live yeast and premixes of vitamins and minerals that are carefully selected through the use of nutrigenomics (use of microarray technology) to aid in the nutrient modulation of genetic expression to promote cellular health (https://www.youtube.com/watch?v=ULj5t5tN7vo). In dairy cattle, OmniGen-AF is known to reduce somatic cell count and improve overall heard health (Ou *et al*., 2011) which has been linked to an augment of the innate immune response evidenced by increased neutrophils such as L-selectin and interlukin-1β in pathogen challenged ruminants (Wang *et al.*, 2004). In beef cattle, supplementation of OmniGen-AF also has proven to modulate the metabolic response during an immune challenge by preventing the breakdown of substrates such as proteins or fat as energy sources in times of an immune challenge (Burdick-Sanchez *et al*., 2014b) and at the same time priming the immune system to better respond to an immune challenge (Burdick-Sanchez *et al*., 2014a).

Although originally designed with dairy cattle in mind, the beef cattle industry could possibly benefit from using this supplement to further improve the immune response of cattle under stress as well as potentially incorporating antioxidants into muscle foods to maintain meat quality over longer aging periods and retail display times. The extension of beef shelf life ultimately benefits the beef industry as a whole as it promotes greater color and lipid stability with greater aging times. These have proven to

be strong drivers for consumer purchasing decisions (Campo *et al.* 2006; Hood and Riordan, 1973).

Thus, the objectives of this research were to assess the impact of feeding OmniGen-AF either at the receiving or throughout the finishing phases of production on beef fatty acid profiles, color and lipid stability under retail display conditions as well as attempting to decipher a mechanism of added oxidative stability by quantifying superoxide dismutase activity.

MATERIALS AND METHODS

University of Nebraska-Lincoln's Animal Care and Use Committee approved of all animal use protocols (IACUC# 902).

Cattle and dietary treatments

A total of 288 calf-fed steers (initial BW = 263.54 ± 18.60 kg) were fed (University of Nebraska feedlot Mead, NE) one of three dietary treatments: a control diet not containing OmniGen-AF supplementation (CON), a diet containing 4 g/45.36kg /hd/d at the receiving phase (first 28 days at the feedlot; REC), and a diet containing 4 g/45.36kg /hd/d all throughout the finishing phase (FIN). Steers were grouped 8 hd/pen, with 12 pens per treatment, for a total of 36 pens. On a daily basis, OmniGen-AF was top dressed in pens assigned to either the REC or FIN supplementation groups. At the end of the receiving period cattle on the REC group were treated as the CON group while the FIN group continued receiving OmniGen-AF through the entirety of the finishing period. For the FIN group the OmniGen-AF amount was adjusted every 30 days.

At the receiving phase, all cattle were fed a blend of alfalfa hay (30%) and dry rolled corn (30%), with 36% Sweet Bran® (corn gluten feed; Cargill; Blair, NE) plus 4%

supplement. A transition period of 21 d was allowed between the receiving and finishing diets where alfalfa hay was replaced (27.5%, 20%, 12.5%, 5% and 0%) with high moisture corn (22.5%, 30%, 37.5%, 45%, and 50% for 3 d, 4 d, 7 d, and 7 d respectively). The finishing diet then consisted of 50% high moisture corn, 40% Sweet Bran®, 5% wheat straw, and 5% supplement (see both receiving and finishing diet composition in Table 1).

At the end of the receiving phase, all steers were implanted with Revelor® XS (Merck Animal Health, Summit, NJ) and the REC group was transferred to the CON diet while the FIN group continued receiving OmniGen-AF supplementation throughout the finishing phase. The OmniGen-AF supplementation amounts were re-calculated and re-adjusted every 30 d. The finishing phase lasted 215 d of which the last 28 d all steers were fed 300 mg/hd/d of Optaflexx® (Elanco Animal Health).

Sample collection and fabrication

At harvest (Greater Omaha Packing, Omaha, NE), two low Choice carcasses within each pen were selected for a total of twenty-four low Choice carcasses within each treatment (n = 72). Strip loins (*Longissimus lumborum*) from both the left and right sides were vacuum packaged and aged for 8, 22 and 29 d. Loins were fabricated anterior to posterior where a 1.27 cm steak was removed at both anterior and posterior ends to remove surfaces with outer exposure. After 8 d of aging, left side strip loins were opened where the anterior half of the loin was designated to 8 d age fabrication and the remaining posterior half of the strip loin was re-vacuum packaged (3 mil STD barrier, Prime Sources, St. Louis, MO) with a Multivac Packaging machine (Mutivac C500, Multivac, Kansas city, MO) to age for 22 d. The anterior half of the right side strip loins was used

for 29 d aging. Loin portions corresponding to 8 d age were fabricated into 1.27 cm steaks destined for fatty acid analysis, proximate composition, lipid oxidation, and 2.54 cm steaks for tenderness measures at 0 and 7 d of retail display. An additional 1.27 cm steak was fabricated and designated for superoxide dismutase activity measures as well as an additional 2.54 cm steak was reserved as an extra sample. The same fabrication map was used at 22 and 29 d of aging, with the exception of the fatty acid sample that was only taken from the loin portions aged for 8 d.

At all aging periods, samples for visual discoloration, tenderness and lipid oxidation were placed on foam trays (21.6 x 15.9 x 2.1 cm, Styro-Tech, Denver, CO), overwrapped with oxygen permeable film (PVC-OW; PSM18, Prime Source, St. Louis, MO) and placed under retail display conditions for 4 and 7 d (2.7°C under white fluorescence lighting at 1000 to 1800 lux). Steaks used for fatty acid profile, proximate composition and 0 d RD were vacuum packaged and frozen for further analysis (-80°C). Later, samples free of subcutaneous fat for proximate analysis, fatty acids and lipid oxidation were frozen in liquid nitrogen and then powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT). Powdered samples were stored at -80°C. Powdered meat samples were also used for the quantification of superoxide dismutase activity and protein concentration determination.

Proximate analysis

Proximate analysis was done to determine fat and moisture. Fat was quantified following the Soxhlet procedure (AOAC, 1990). Samples were measured in triplicate in Whatman #2 filter paper and fat was extracted with ether. Fat percentages were averaged per sample and used to convert fatty acid percent data to mg/100 g tissue basis. Moisture

was determined with a LECO thermogravimetric analyzer (LECO Corporation, Model 604-100-400, St. Joseph, MI) and samples were measured in duplicates. Moisture was determined in nitrogen atmosphere with a start temperature of 25°C and an end temperature of 130°C (17 min ramp rate).

Fatty acid composition

Fatty acid profiles were obtained via gas chromatography as described by Folch *et al.* (1957) with modifications detailed by Morisson and Smith (1964) and Metcalfe *et al.* (1966). Briefly, 1 g of powdered sample was weighed into a 15 mL conical tube to which 5 mL of 2:1 chloroform:methanol was added and vortexed for 5 s. After one hour, samples were filtered through Whatman #2 filter paper onto a 13 x 150 mm glass screw cap tube, volume was brought up to 10 mL with 2:1 chloroform:methanol and 2 mL of KCl were added and vortexed. After centrifuging samples (1,000 x g for 5 min) the top organic matter layer was aspirated off and samples were dried down completely on a heating block (60°C) under constant nitrogen purge. One half mL of 0.5 M NaOH in methanol was added, vortexed and heated (100°C) for 5 min. Then, boron trifluoride in 14% methanol (0.5 mL) was added, vortexed and heated (100°C for 5 min). Subsequently, 1 mL of saturated salt solution and 1 mL of hexane were added and samples were centrifuged (1,000 x g for 5 min). The top hexane layer was carefully pipetted into gas chromatography glass vials, nitrogen purged and lids were crimped on. Chromatography was done using a Chromopack CP-Sil (0.25 mm x 100 m) column with an injector temperature of 270°C and a detector temperature of 300°C (Hewlett-Packard 6890 FID GC System; Agilent Technologies, Santa Clara, CA). The head pressure was set at 40 psi with a flow rate of 1.0 mL/min. The fatty acids were identified by their

retention times in relation to known standards and the percentage of fatty acids were determined by the peak areas in the chromatograph. Values were adjusted according to percent fat and values were converted to mg/100 g tissue.

Subjective color (Discoloration)

Visual discoloration was assessed daily during retail display with a trained five-person panel. Panelists were provided with a visual discoloration guide to use as a reference. A percentage scale was used were 0% meant no discoloration and 100% meant complete discoloration. Panelists were instructed to perform the evaluation at the same time each day to minimize variation. Samples were randomly rotated daily to minimize any possible location effects.

Objective color (L*, a*, b*)

Color measures were taken on a daily basis once samples were placed on retail display at all aging time points. Color was evaluated on steaks that were plastic overwrapped with oxygen permeable film and destined for tenderness assessment at the end of retail display. Objective color measures were taken with the L*, a*, b* scales with a Minolta CR-400 colorimeter (Minolta, Osaka, Japan). The handheld colorimeter reading device with an opening of 8mm was set to use a D65 illuminant and 2° observer. The colorimeter was set to record and print an average of 6 readings per steak. The calibration process was done on a daily basis using a white tile and the D65 settings were set as follows: Y = 93.13, x = 0.3164 and y = 0.3330. Once calibrated, the color space was selected to be the L*, a*, and b* scale; where, L* is a measure of lightness and has a range from 0 (black) to 100 (white), a* is a measure of redness and has a positive (red) to negative range (green), and b* is a measure of yellowness with a positive (yellow) to

negative (blue) range. Color readings were taken within a ±2 hour window time frame for consistency and accuracy of data.

Lipid oxidation

Lipid oxidation was determined with the 2-thiobarbituric acid reactive substances protocol (TBARS) as described by Ahn *et al.* (1998). Briefly, five g of powdered sample were weight into a 50 ml conical tube to which 14 ml of deionized distilled water and 1 mL of BHA (10% BHA: 90% ethanol) were added. After polytroning for 15 s the samples were centrifuged (2,000 x g for 5 min). One mL of the supernatant was transferred to a 15 mL conical tube and 2 mL of TBA/TCA solution (15% TCA and 20 mM TBA in deionized distilled water) was added and vortexed before placing samples in a water bath (70°C for 30 min). After cooling, samples were centrifuged (2,000 x g for 5 min) and 200 μL of supernatant were transferred to 96-well plates. All 96-well plates had standards to calculate standard curves and ultimately mg of malonaldehyde per kg of tissue read at 540 nm.

Tenderness

Tenderness was measured via Warner-Bratzler Shear Force (WBSF). Samples were thawed (4°C) 24 hours prior to cooking and internal temperature was monitored with a thermocouple (5SC-TT-T-30-120, OMEGA Engineering, Inc., Stamford, CT) inserted in the geometric center of each steak. Steaks were cooked on Hamilton Beach grills (Model 31605A, Proctor-Silex, Inc., Washington, NC) until they reached an internal temperature of 35°C at which time they were flipped to continue cooking. The final internal temperature was 71°C. Cooked steaks were refrigerated for 24 hours and six cores (1.27 cm diameter) were taken parallel to the muscle fiber with a drill press and sheared using a texture analysis (model TMS-PRO, Food Technology Crop., Sterling,

VA) fitted with a Warner-Bratzler blade. The average of the 6 cores was calculated for statistical analysis.

Superoxide dismutase activity

Superoxide dismutase (SOD) activity was determined with a colorimetric assay kit (ab65354; Abcam, Cambridge, MA). Samples utilized for this assay were from the CON and FIN groups aged for 29 d with 7 d retail display. Briefly, through the use of a Water Soluble Tetrazolium Salt (WST-1) a water-soluble formazan dye is produced upon the reduction with the superoxide anion. The reduction is linearly related to the xanthine oxidase activity which is inhibited by SOD. Hence, the inhibition rate of SOD can be measured colorimetrically (the greater the color, the lower the SOD activity and vice versa). The percent inhibition data was then normalized by determining the protein concentration of the samples used for the SOD analysis. In this case given that our buffers have both a detergent and a reducing agent, the protein concentration kit required must be both detergent and reducing agent compatible (Bio-Rad RC DC Protein Assay). Once the protein concertation was determined per sample the SOD units of activity could be reported over mg of protein (SOD U/mg protein).

Statistical analysis

Data were analyzed as a completely randomized design where the main effects of dietary treatment, aging, retail display and their interactions were evaluated with SAS (version 9.4, Cary, NC, 2009). Individual animal served as the experimental unit while pen served as a random variable. The PROC GLIMMIX procedure was used for the analysis of repeated measures of objective color and subjective visual discoloration where the most appropriate covariance structure was selected based on the best fit model.

The PROC GLIMMIX procedure was also used to evaluate all other variables measured. All means were separated with the LS MEANS statement and the TUKEY adjustment was used with an alpha level of 0.05.

RESULTS AND DISCUSSION

Proximate analysis

Fat content was found to be greater ($P = 0.03$) for the FIN group, intermediate for the CON group, and least for the REC group (12.23%, 11.68%, and 11.22% respectively). These results were not expected given that all cattle selected for the study graded as USDA low Choice. However, it could be argued that the range of fat percentages could all still qualify as low Choice carcasses and that perhaps the low standard error (0.27) speaks to the consistency of the data that allowed statistical differences within the same grade.

Fatty acid composition

Dietary treatment altered the following fatty acids: C18:1, C19:0, C18:2, C20:5ω3, total, UFA, SFA:UFA ratio, MUFA, and PUFA ($P \leq 0.05$; Table 2). The C18:1, C19:0, C18:2, C20:5ω3, total, UFA and MUFA were found to be greater in beef from cattle supplemented OmniGen-AF all through the finishing phase, lowest in cattle fed OmniGen-AF at the receiving phase, and intermediate for the control cattle. The SFA:UFA ratio was found to be greater ($P = 0.05$) for the REC group, intermediate for the CON group, and lowest for the FIN group. Lastly, the PUFA content was greatest ($P = 0.01$) for the FIN and CON groups and lower for the REC group (610.07 and 598.34 mg/100 g tissue vs. 526.60 mg/100 g tissue, respectively).

Subjective color (Discoloration)

An age by retail display interaction ($P < 0.0001$) was found for visual assessment of discoloration of steaks under retail display (Figure 1). It was evident that under retail display conditions, samples aged for 22 d discolored at a greater rate than did the samples aged for 8 and 29 d. The greater differences were noted past 4 d of retail display. At 5 d retail display, no differences were found between discoloration of steaks aged for 8 and 29 d, whereas samples aged for 22 d had greater discoloration (0.77 and 1.56% vs. 9.12%, respectively). At 6 d retail display, 8 d aged samples had the least amount of discoloration, 29 d aged samples had intermediate discoloration, and 22 d aged samples had the most discoloration (4.84 % vs. 9.27% vs. 33.47%, respectively). Lastly, at 7 d retail display, the least discolored samples were those aged for 8 d, followed by samples aged for 29 d, and 22 d aged samples being the most discolored (11.85% vs. 19.34% vs. 58.74%, respectively).

Logically one would expect that samples would discolor at a greater rate as aging time increases. In the current study, samples of intermediate aging time (22 d age) discolored faster than those aged for 29 d, which seemed to have discolored at a rate more similar than the samples aged for 8 d. One potential explanation for this discrepancy could be that due to the fabrication of the left side loins versus the right sided loins. In our study the anterior left loins were fabricated at 8 d age and the remaining posterior portions of the left loins were re-vacuum packaged for fabrication at 22 d of age. The samples fabricated for 29 d age were under commercial packaging conditions and came from the anterior portion of the right sided loins.

As explained by Mancini (2009), levels of residual oxygen in anaerobic packages for beef must be less than 0.05% to limit metmyoglobin formation at low partial pressure and also proper oxygen consumption must occur for proper bloom and oxygenation once beef packages are opened for retail display. Ledward (1992) also indicated that oxygen consumption rate tends to decrease as aging time increases. Both residual oxygen and the longer aging time could have contributed to lower myoglobin stability under retail display in relation to the samples that were always under commercial vacuum packaging. Similarly, Robertson *et al.* (2007) pointed out that re-vacuum packaging for a second time increases residual oxygen and negatively impacts re-blooming of meat. This was also in accordance to findings published by Avilés *et al.* (2014) who mentioned that with vacuum packaging there is an initial formation of metmyoglobin that slowly changes to deoxymyoglobin. This process is referred to as the "seasoning period" and takes approximately 5 d. However, this conversion to deoxymyoglobin and the subsequent blooming to form oxymyoglobin once re-exposed to oxygen can be affected by many factors including animal age, muscle, and enzymatic activity, all of which can be largely unpredictable and uncontrollable. Avilés *et al.* (2014) also indicated that repeated vacuum packaging should be limited.

Objective color (L, a*, b*)*

For all three color scales, age by retail display time interactions were detected (Figures 2 – 4; $P < 0.0001$). In general, meat aged for 22 d had greater L* values and lower a* and b* values than did the samples aged for 8 and 29 d. Much like the subjective discoloration data, the a* value data indicated that particularly after 4 d of retail display, samples aged for 22 d had significantly reduced redness in comparison to

the other two aging periods. Just as the discoloration panel indicated at 6 d retail display, 8 d aged samples had the greatest redness, 29 d aged samples had intermediate a* values, and 22 d aged samples had the lowest a* values (18.86 vs. 16.20 vs. 13.85, respectively). Also, at 7 d retail display, the greatest a* values corresponded to samples aged for 8 d, followed by samples aged for 29 d, and 22 d aged samples having the lowest a* values, indicative of greater discoloration (17.30 vs. 14.73 vs. 11.28, respectively).

Lipid oxidation

An age by retail display interaction was found (P = 0.04; Figure 5) for lipid oxidation. No significant changes in lipid oxidation were noted at 0 d of retail display. However, at 4 and 7 d of retail display, 29 d aged samples had greater oxidation values (3.45 and 5.66 mg malonaldehyde/kg tissue, respectively) in comparison to samples aged for 8 d having the least oxidation (2.64 and 4.73 mg malonaldehyde/kg tissue, respectively), with samples aged for 22 d having intermediate oxidation amounts (3.21 and 5.19 mg malonaldehyde/kg tissue, respectively).

Tenderness

Tenderness values decreased as aging time increase (3.28 kg at 8 d age, 2.92 kg at 22 d age, and 2.68 kg at 29 d age; $P < 0.0001$) and as retail display time increased (3.06 kg at 0 d retail display, and 2.87 kg at 7 d retail display; $P < 0.0001$). Dietary treatment did not have an impact on meat tenderness (P = 0.31).

Superoxide dismutase activity

No differences (P = 0.19) in superoxide dismutase (SOD) inhibition percent were determined due to dietary treatment (62.29% for the CON group and 58.58% for the FIN group). After calculating enzymatic units and adjusting for protein concentration, no

difference (P = 0.92) was found for SOD activity between dietary treatments (19.11 SOD U/mg protein for the CON group and 18.98 SOD U/mg protein for the FIN group).

OmniGen-AF is developed using nutrigenomics and each ingredient is selectively chosen to upregulate certain genetic factors to promote cellular health. Given that part of the proprietary blend includes premixes of vitamins that can serve as antioxidants, the possibility exists that the supplement could also upregulate innate antioxidant enzymes such as SOD. As explained by Cristiana *et al*. (2014) under stressful conditions with increased amounts of reactive oxygen species which negatively impact cells if uncontrolled, enzymes work in conjunction to protect cells against oxidative stress. There are three particular enzymes that can turn superoxide ($O_2^{\cdot-}$) and hydrogen peroxides (H_2O_2) ions (the most toxic oxygen radicals) to less toxic forms of oxygen radicals (Cristiana *et al*., 2014). The three most important enzymatic systems protecting all cells against oxidative stress are superoxide dismutase, catalase, and glutathione peroxidase. Together, these three enzymes have antioxidant capabilities in all cells that help stabilize these highly active ROS that in terms of muscle foods result in oxidation, production of off-flavors and an overall reduction of shelf life.

Very little research has explored the enzymatic activity of these mechanisms in muscle while much literature point to secondary antioxidant interventions (*pre* or *post-mortem*) to off-set oxidative damage in meat (Figure 6A-B). However, having a better understanding of these primary antioxidant mechanisms and potential forms of stimulating these in muscle could provide huge benefits in terms of combating oxidation and ultimately prolonging meat shelf life.

In the current study we were unable to detect differences in SOD activity. We speculate that this could be due to a number of factors, amongst which we could consider: (A) the amount of antioxidants in OmniGen-AF fed at this concentration is not sufficient to cause an upregulation of SOD, (B) perhaps there could be differences in downstream enzymes such as catalase and/or glutathione peroxidase, or (C) more fine-tuning is required to perfect the accurate quantification of SOD activity in muscle tissue samples. We believe that in order to better understand oxidative stability of meat, determining the activity and mechanisms to up-regulate these antioxidant enzymes are key in order to extend beef shelf life.

CONCLUSION

Several fatty acids were altered with the supplementation of OmniGen-AF, the most relevant difference being an increase in PUFA content in the FIN group relative to the REC group. Despite the increased PUFA content, lipid oxidation and color stability data indicated that dietary treatment had no effect on these variables of interest. Similarly, SOD activity values did not differ from the FIN and the CON groups. Therefore, feeding OmniGen-AF had no detrimental effects on basic meat quality parameters and the supplementation does not seem to effectively prolong beef shelf life. In order to consider OmniGen-AF as a potential antioxidant source for beef cattle the supplement may need to be fed at a greater concentration.

LITERATURE CITED

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.

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Avilés, C. M. Juárez, I. L. Larsen, A. Rodas-González, and J. L. Aalhus. 2014. Effect of multiple vacuum packs on color development and stability in beef steaks. Can. J. Anim. Sci. 94:63-69.

Burdick-Sanchez, N. C, J. O. Buntyn, J. A. Carroll, T. Wistuba, K. DeHaan, S. E. Sieren, S. J. Jones, and T. B. Schmidt. 2014a. Enhancement of the acute phase response to lipopolysaccharide in feedlot steers supplemented with OmniGen-AF. J. Anim. Sci. 92: E-Suppl. 2:37-38.

Burdick-Sanchez, N. C, J. O. Buntyn, J. A. Carroll, T. Wistuba, K. DeHaan, S. E. Sieren, S. J. Jones, and T. B. Schmidt. 2014b. Supplementation of OmniGen-AF during the receiving period modulates the metabolic response to a lipopolysaccharide challenge in feedlot steers. J. Anim. Sci. 92: E-Suppl. 2:250.

Campo, M. M., G. R. Nute, S. I. Hughes, M. Enser, J. D. Wood, and R. I. Richardson. 2006. Flavour perception of oxidation in beef. Meat Sci. 72:303-311.

Cristiana, F., Elena, A., and Nina, Z. 2014. Superoxide dismutase: Therapeutic targets in SOD related pathology. Health 6:975-988.

Folch, J., M. Lees, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497-509.

Hood, D. E. R. and E. B. Riordan. 1973. Discolouration in pre-packaged beef: measurement by reflectance spectrophotometry and shopper discrimination. J. of Food Technol. 8:333-343.

Ledward, D. A. 1992. Color of raw and cooked meat. Pages 128-144 in D. A. Johnston, M. K. Knight, and D. A. Ledward, eds. The chemistry of muscle-based foods. Royal Society of Chemistry, Cambridge, UK.

Mancini, R. A. 2009. Meat color. Pages 217-255 in M. Du and R. J. McCormick, eds. Applied muscle biology ans meat science. CRC Press, Taylor and Francis Group, New York, NY.

Metcalfe, L. D., A. A. Schmitz, and J. R. Pelka. 1966. Rapid separation of fatty acid

esters from lipids for gas chromatography analysis. Anal. Chem. 38:514-515.

Morisson W. R. and L. M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5:600-608.

Ou, B. R., Y. Q. Wang, N.E. Forsberg. 2011. Effects of feeding OmniGen-AF to rats on gastrointestinal gene expression: Microarray analysis. J. Anim. Sci. 89 E-Suppl. 1:270-271.

Robertson, W., T. Veale, H. Kirbyson, S. Landry, and J. Aalhus. 2007. Can ultimate pH of the longissimus muscle help differntiate borderline dark-cutting beef carcasses from slow blooming carcasses? Canadian Meat Science Association Newsletter. Canadian Meat Science Edmonton, AB. Pages 16-22.

Wang, Y. Q., S. B. Puntenney, and N. E. Forsberg. 2004. Identification of the mechanisms by which OmniGen-AF, a nutritional supplement, augments immune function in ruminant livestock. J. Anim. Sci. 82(Suppl. 2):128-129.

Table1. Diet Composition (DM basis) fed to steers with no OmniGen-AF supplementation or OmniGen-AF supplemented at either the receiving or finishing phases of production[1]

	Composition (%)
Receiving phase (first 28 d)	
Sweet Bran®	36
Alfalfa hay	30
Dry rolled corn	30
Supplement	4
Finishing phase (all 215 d)	
High moisture corn	50
Sweet Bran®	40
Wheat straw	5
Supplement	5

[1]Steers on the supplementation treatments (both at the receiving and finishing phases) were fed OmniGen-AF top dressed on a daily basis (4 g/45.36kg /hd/d). For the finishing group, OmniGen-AF amounts were re-calculated and adjusted every 30 d.

Table 2. Fatty acid[1] composition of beef from cattle supplemented with OmniGen-AF at the receiving or finishing phase (*L. lumborum*)

Fatty acid	Control[2]	OmniGen-AF at Receiving[2]	OmniGen-AF at Finishing[2]	*SEM*	*P*-value
C4:0	40.68	40.18	55.84	13.61	0.64
C10:0	6.48	7.39	6.64	1.23	0.57
C12:0	7.50	7.38	7.78	0.52	0.82
C13:0	7.29	6.42	7.58	0.78	0.48
C14:0	328.19	309.13	324.29	15.76	0.68
C14:1	97.61	98.07	96.90	6.78	0.99
C15:0	62.36	61.35	60.70	4.22	0.94
C15:1	62.03	52.88	61.18	3.56	0.14
C16:0	2,823.89	2,766.27	2,889.82	74.91	0.51
C16:1T	54.08	40.47	45.83	7.45	0.44
C16:1	429.65	414.61	443.86	19.58	0.58
C17:0	156.40	150.63	159.87	6.66	0.61
C17:1	134.23	124.41	131.46	7.27	0.65
C18:0	1,471.92	1,469.44	1,582.63	49.94	0.19
C18:1T	230.04	220.63	254.45	14.85	0.23
C18:1	4,290.04[ab]	4,141.40[b]	4,546.06[a]	113.48	0.05
C18:1V	725.56	625.80	763.83	44.97	0.09
C18:2TT	24.45	16.27	15.67	2.89	0.08
C18:2	414.88[ab]	376.39[b]	443.09[a]	15.40	0.01
C18:3ω6	8.68	8.57	9.41	0.41	0.35
C18:3ω3	18.09	17.40	19.64	0.70	0.08
C19:0	16.66[ab]	16.20[b]	18.26[a]	0.62	0.05
C20:1	56.28	57.96	62.03	3.33	0.46
C20:2	3.89	3.83	6.77	2.30	0.39
C20:3ω6	27.50	22.97	27.84	1.62	0.07
C20:4ω6	80.23	67.05	78.89	4.94	0.13
C20:5ω3	7.92[a]	6.04[b]	7.50[ab]	0.54	0.05
C22:5	34.38	17.13	19.54	7.68	0.24
C24:1	13.23	11.80	12.29	0.67	0.31
Total	11,570.06[ab]	11,112.04[b]	12,107.25[a]	263.78	0.04
Other	113.44	103.14	123.68	12.72	0.52
SFA	4,895.38	4,826.70	5,082.91	127.02	0.34
UFA	6,674.69[ab]	6,285.33[b]	7,024.34[a]	158.61	0.01
SFA:UFA	0.74[ab]	0.77[a]	0.73[b]	0.01	0.05
MUFA	6,076.35[ab]	5,758.73[b]	6,414.27[a]	146.87	0.01
PUFA	598.34[a]	526.60[b]	610.07[a]	20.05	0.01
Trans	213.25	245.05	312.04	21.17	0.09
ω6	113.10	97.84	110.89	5.85	0.14
ω3	22.41	21.34	23.22	1.16	0.54
ω6: ω3	5.28	4.73	4.71	0.31	0.32

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

[2]Control group was never supplemented; Receiving group was supplemented for 28 d; Finishing group was supplemented for 215 d. Both at the receiving and finishing phases OmniGen-AF was fed at 4g/45.36kg BW/hd/d.

[a,b]Means in the same row with different superscripts are different ($P \leq 0.05$)

Figure legends

Figure 1. Age by retail display interaction ($P < 0.0001$; $SEM = 1.97$) for discoloration of strip loin steaks aged for 8, 22 or 29 days of age under 7 day retail display

[a-c]Different superscripts within the same retail display day indicate differences ($P \leq 0.05$)

Figure 2. Age by retail display interaction ($P < 0.0001$; $SEM = 0.29$) for L* value of strip loin steaks aged for 8, 22 and 29 days of age under 7 day retail display

Figure 3. Age by retail display interaction ($P < 0.0001$; $SEM = 0.25$) for a* value of strip loin steaks aged for 8, 22 and 29 days of age under 7 day retail display

[a-c]Different superscripts within the same retail display day indicate differences ($P \leq 0.05$)

Figure 4. Age by retail display interaction ($P < 0.0001$; $SEM = 0.12$) for b* value of strip loin steaks aged for 8, 22 and 29 days of age under 7 day retail display

Figure 5. Age by retail display interaction ($P = 0.04$; $SEM = 0.20$) for lipid oxidation measures (TBARS) of strip loin steaks aged for 8, 22 and 29 days with 0, 4 and 7 day retail display

[a-b]Different superscripts within the same retail display time indicate defenses within aging times ($P \leq 0.05$)

Figure 6A. Primary versus secondary antioxidants as they relate to oxidative stability of meat

SOD = Superoxide dismutase activity; Cat = Catalase; Gpx = Glutathione peroxidase

Figure 6B. Primary versus secondary free radicals and the role of innate antioxidant enzymes to combat most reactive oxygen forms (greater toxicity to cells toward top of pyramid)

Note: With TBARS we quantify malonaldehyde (a secondary free radical) that is generated from primary free radicals

Figure 1.

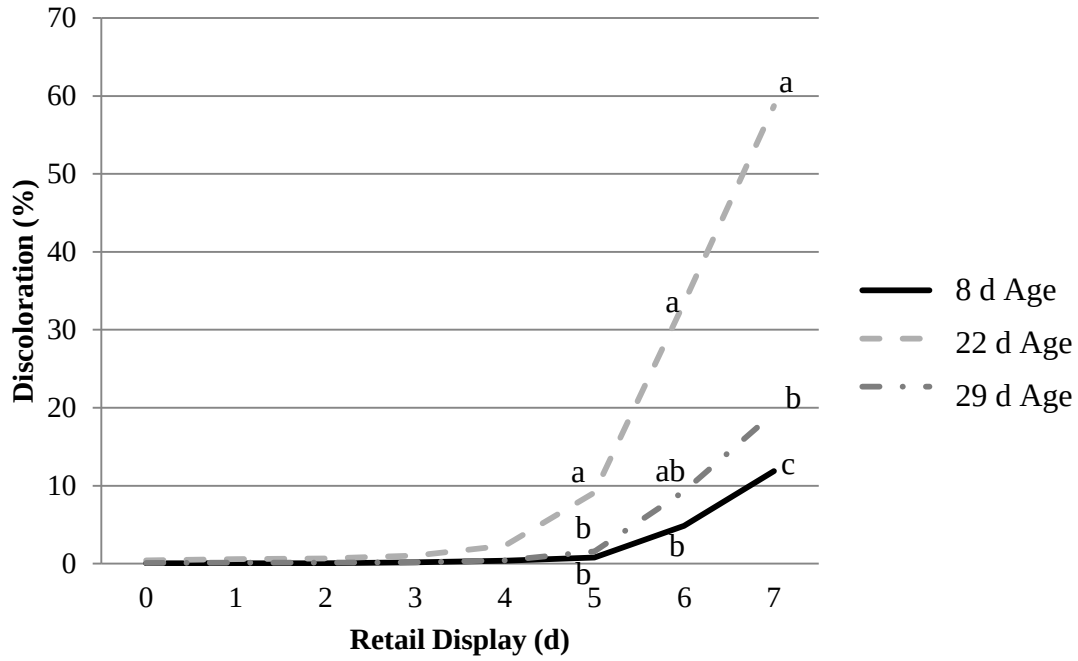

70
60
50
40
30
20
10
0
Discoloration (%)
0
1
2
3
4
5
6
7
Retail Display (d)
a
a
b
a
ab
c
b
b
b
8 d Age
22 d Age
29 d Age

Figure 2.

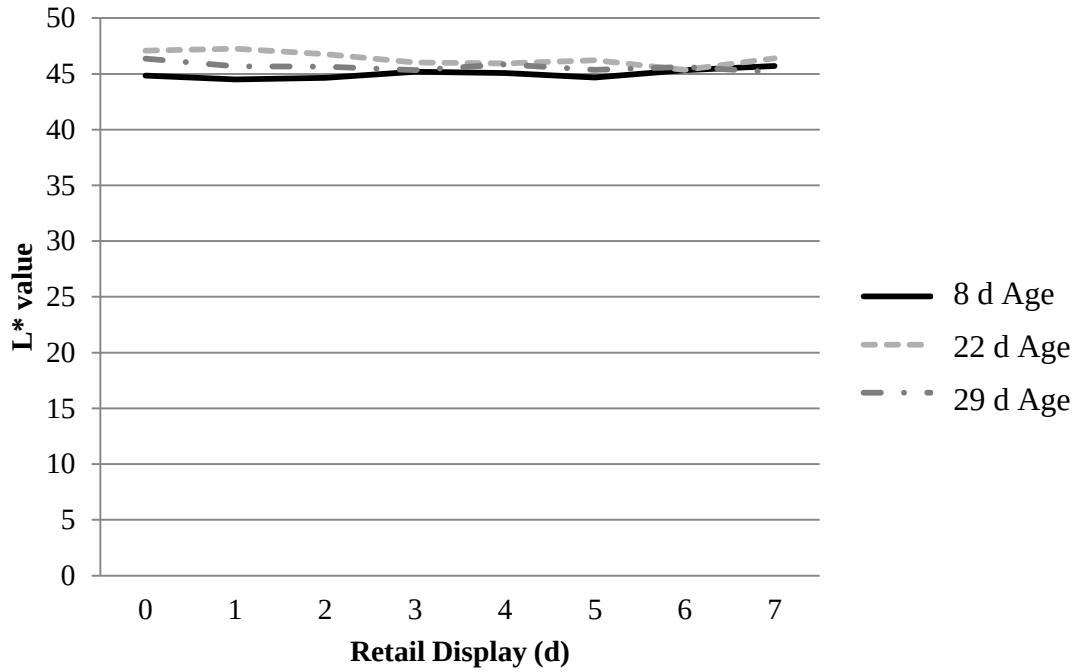

50
45
40
35
30
25
20
15
10
5
0
L* value
0
1
2
3
4
5
6
7
Retail Display (d)
8 d Age
22 d Age
29 d Age

Figure 3.

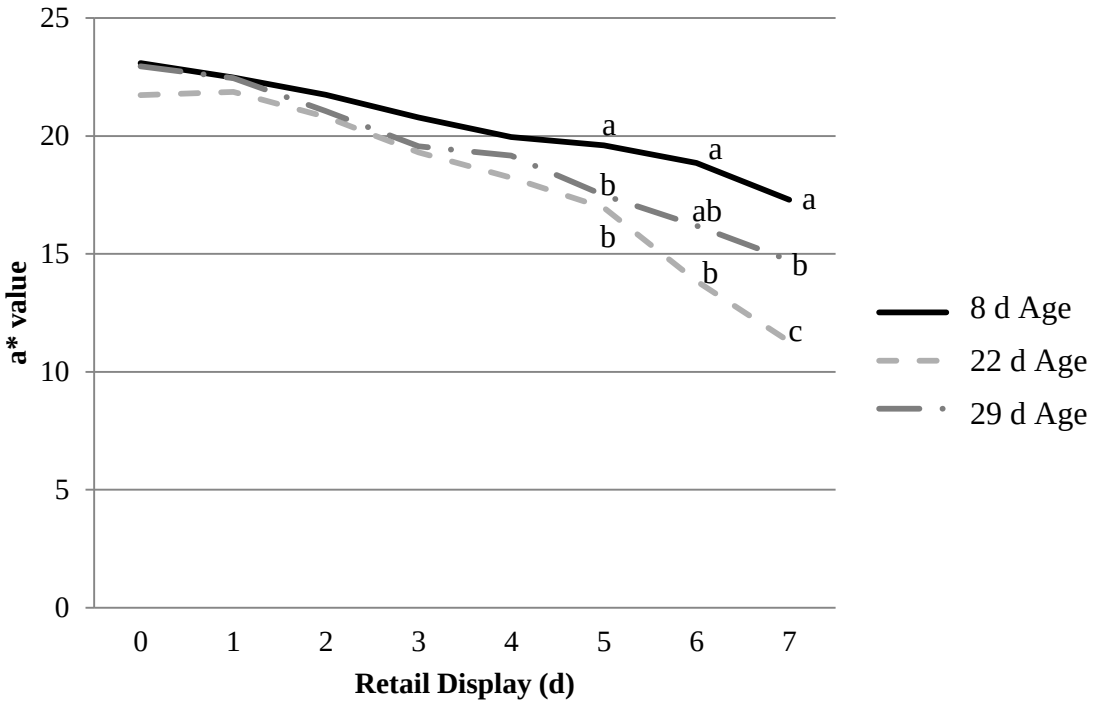

25
20
15
10
5
0
a* value
0
1
2
3
4
5
6
7
Retail Display (d)
a
a
a
b
ab
b
b
b
c
8 d Age
22 d Age
29 d Age

Figure 4.

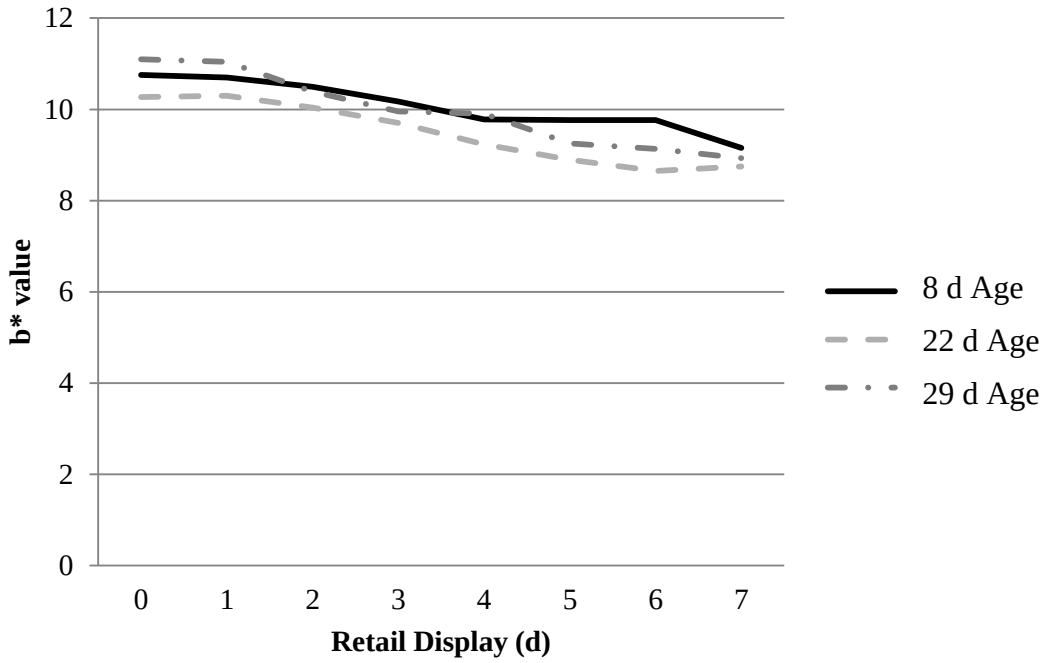

12
10
8
6
4
2
0
b* value
0
1
2
3
4
5
6
7
Retail Display (d)
8 d Age
22 d Age
29 d Age

Figure 5.

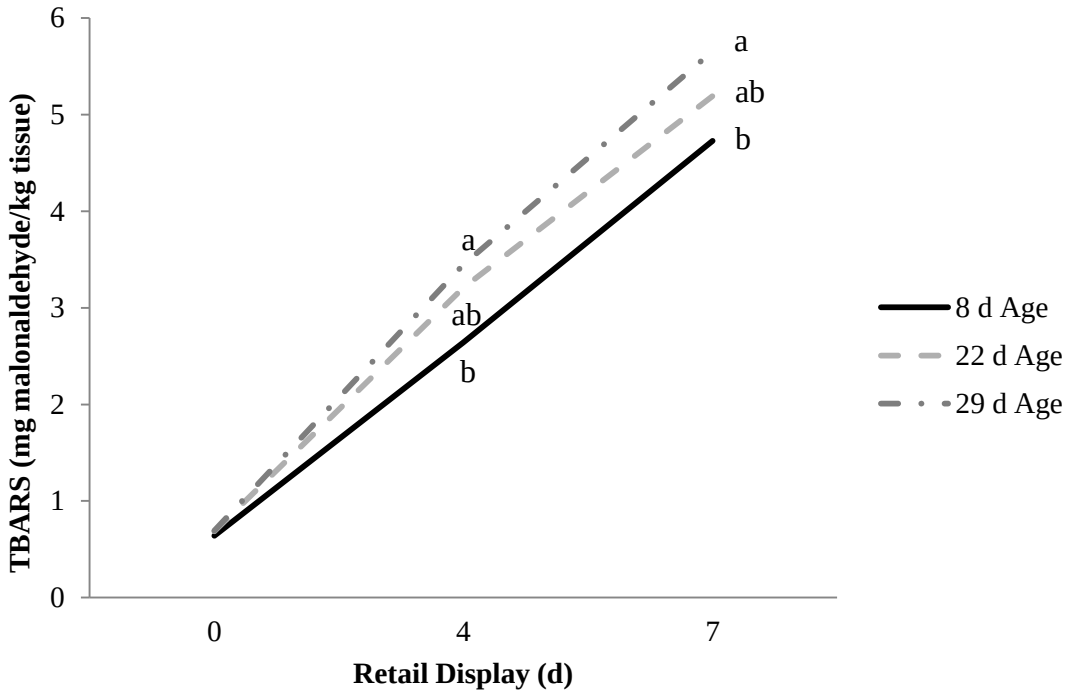

6
5
4
3
2
1
0
TBARS (mg malonaldehyde/kg tissue)
a
ab
b
a
ab
b
0
4
7
Retail Display (d)
8 d Age
22 d Age
29 d Age

Figure 6A.

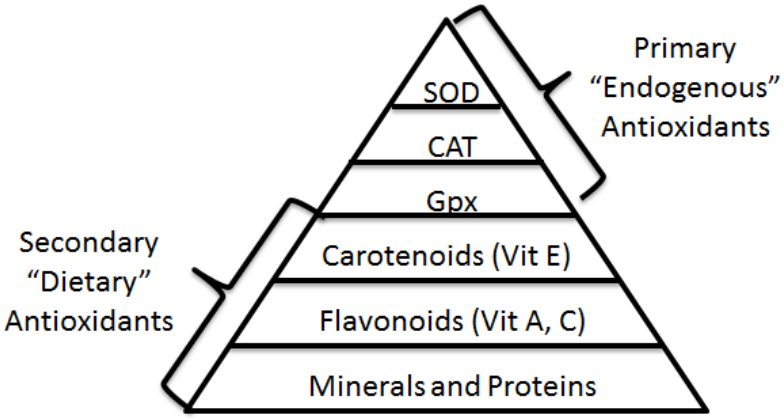
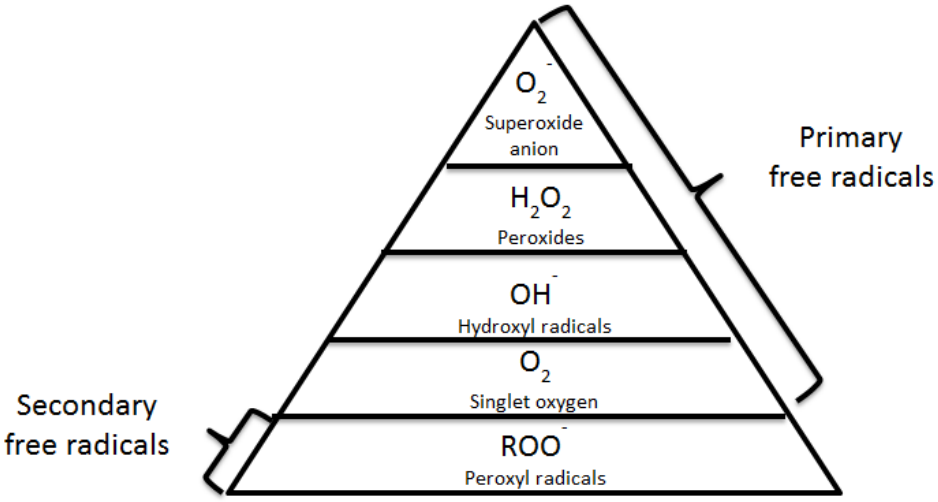


Figure 6B.



$$2\ O_2^{\cdot -} + 2H^+ \xrightarrow{\text{Superoxide dismutase}} O_2 + H_2O_2$$

$$H_2O_2 + H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

$$H_2O_2 + 2GSH \xrightarrow{\text{Glutathione peroxidase}} 2H_2O + GSSG$$

RECOMMENDATIONS FOR FUTURE RESEARCH

The state of Nebraska has been historically characterized as the beef state. Factors such as climate conditions, genetics, nutrition, industry focus and organization, research and extension efforts, along with availability of feed and water, have all contributed in the fortification and the establishment of high quality standards typical of Nebraska beef. In order to continue living up to or surpassing such standards it is imperative that academic research efforts address current issues that could alter or somehow impact meat quality. The scope of this work has evaluated the current feed of preference used by beef producers in Nebraska, de-oiled forms of corn distillers grains, as well as a feed supplement, OmniGen-AF, in hopes of functioning as an antioxidant source to extend beef shelf life.

In conjunction, we have learned that inclusion levels below 50% of de-oiled WDGS have a reduced content of PUFA's in relation to full-fat WDGS which would be beneficial given the lower propensity for lipid oxidation under retail display conditions. Despite the reduction in PUFA content of the lower inclusion levels of de-oiled WDGS, these were still greater than those of cattle fed a corn control diet. While evaluating a diet containing 50% DDGS we observed that the PUFA content was greater than that of cattle fed a corn control diet. However, this last comparison did not include a group having been fed 50% full-fat DDGS. Consequently, it is hard to decipher the magnitude of differences seen with the de-oiling process in DDGS vs. WDGS. Therefore, a future study should contemplate the addition of a full-fat DDGS diet to fully address the potential differences that may exist due to the additional draying of corn distillers grains.

In general, both de-oiled WDGS and de-oiled DDGS had greater PUFA content than a corn control diet. As a result, if the majority of Nebraska beef producers will continue formulating finishing diets using significant inclusions of de-oiled corn distillers grains research must contemplate the incorporation of antioxidants to offset potential detrimental effects due to lipid oxidation. Two potential antioxidants worth exploring would be resveratrol, a powerful antioxidant found in dessert plants, and alpha-tocotrienol, an isoform of vitamin E that has begun to get great attention in the human medical field to treat inflammation associated with chronic human conditions. Both of these antioxidants are known for their great potency but have yet to be evaluated in animal agriculture.

Although the supplementation with OmniGen-AF was evaluated as a potential antioxidant source, dietary treatment did not seem to alter lipid oxidation. However, cattle supplemented all through the finishing phase had increased PUFA content. Therefore, it could be speculated that perhaps increasing the antioxidant components of the supplement or increasing the amount of supplementation could potentially reduce lipid oxidation.

A portion of this work was also dedicated to the quantification of superoxide dismutase. Although no differences were detected in the samples used, there is still great value in having a deeper understanding of how innate antioxidant enzymes aid in the reduction of lipid oxidation. This is surprisingly an area of little to no research in meat science that I believe merits attention. Understanding the activity of superoxide dismutase, catalase, and glutathione peroxidase can shed light in understanding oxidative stability from a new and different perspective at the cellular level.

In summary, based on the findings of the current studies some suggestions for future research can include:

1. Conduct a feed trial that parallels the treatments in the first study using DDGS in place of WDGS to have a more accurate comparison of how the moister level in distillers grains alters beef shelf life after the de-oiling process at several inclusion levels.
2. Explore the use of more potent antioxidants like resveratrol or alpha-tocotrienol in finishing diets that include de-oiled WDGS or de-oiled DDGS.
3. Collaborative work with the private industry could explore the possibility of reformulating the antioxidant components of OmniGen-AF to have greater efficacy in retarding lipid oxidation. Through the use of nutrigenomics this supplement can more directly target the upregulation of antioxidant enzymes. This supplement should then be evaluated with cattle receiving de-oiled WDGS or de-oiled DDGS.
4. Determining appropriate methods of quantifying the three most important antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) would be of valuable insight to have a deeper and clearer understanding of antioxidant protection at the cellular level.

APPENDIX I:

Finishing diet composition (Study 1)

Diet Composition on a DM basis fed to finishing steers

	Control	35% WDGS		50% WDGS		65% WDGS	
		De-Oiled	Normal	De-Oiled	Normal	De-Oiled	Normal
Ingredient, % of DM							
DRC[1]	41.5	24	24	16.5	16.5	9	9
HMC[1]	41.5	24	24	16.5	16.5	9	9
WDGS: De-Oiled[1]	-	35	-	50	-	65	-
WDGS: Normal Fat[1]	-	-	35	-	50	-	65
Corn Silage	12	12	12	12	12	12	12
Supplement [2]	5	5	5	5	5	5	5
Analyzed Composition, %							
Fat	4.5	5.5	7.1	6.0	8.2	6.4	9.3
CP	11.4	15.2	14.8	18.7	18.1	22.1	21.4
Sulfur	0.09	0.32	0.31	0.42	0.41	0.52	0.51
NDF	13.5	26.6	27.8	32.3	34.0	38.0	40.2

[1]DRC = Dry rolled corn; HMC = High moisture corn; WDGS = Wet distillers grains plus solubles
[2]Formulated to contain 383 mg/hd/d of Rumensin® and 90 mg/hd/d of Tylan®

APPENDIX II:

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 BEOND 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 and approved container for reuse or discarding. <u>DO NOT LEAVE ETHER OUT OF THE HOOD OR THE FLAMMABLE CABINET.</u>
11. Place samples in drying oven (105°C) for about four hours or overnight before weighing back.

Calculation: ([(Original weight including filter paper and paper clip – Fat extracted sample weight) / Sample weight] * 100) - % Moisture = % Fat

APPENDIX III:

Proximate Analysis – Minerals and Ash determination

1. Place crucible in drying oven at 100°C for 4 h and then in a desiccator.
2. Place 2 gr of pulverized muscle tissue into the crucible in duplicates.
3. Moisture and ash are determined with the following program:

Name	Covers	Ramp Rate	Ramp Time	Start Temp	End Temp
Moisture	Off	6 d/m	17 min	25°C	130°C
Ash	Off	20 d/m	30 min	130°C	600°C

Name	Atmosp	Flow Rate	Hold Time	Const. Wt.	Const. Wt. Time
Moisture	N	High	00 min	0.05%	09 min
Ash	O	High	00 min	0.05%	09 min

Crucible density and cover density were set at 3.00, while sample density was set at 1.00.

The calculations to determine moisture and ash are as follows:

Moisture = ([Initial weight – Dry weight)] / Initial weight) * 100

Ash = (Weight of ash / Initial weight) * 100

APPENDIX IV:

Fatty acid determination

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

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

GC Settings

Column- Chrompack CP-Sil 88 (0.25 mm x 100 m)
Injector Temp- 270°C
Detector Temp- 300°C
Head Pressure-40 psi
Flow Rate-1.0 mL/min

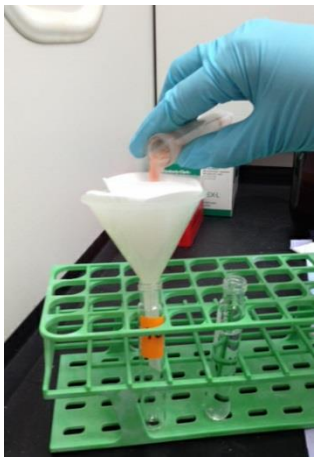
Temperature Program- Start at 140°C and hold for 10 min. Following 10 min, raise temperature 2°C/min until temperature reaches 220°C. At 220°C, hold for 20 min.

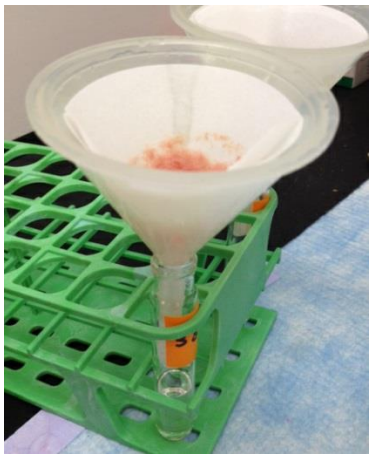
APPENDIX V:

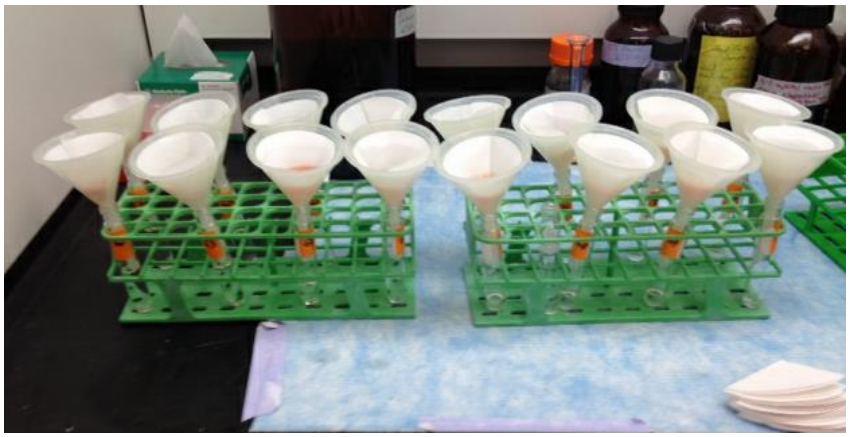
Fatty acid protocol step by step pictures

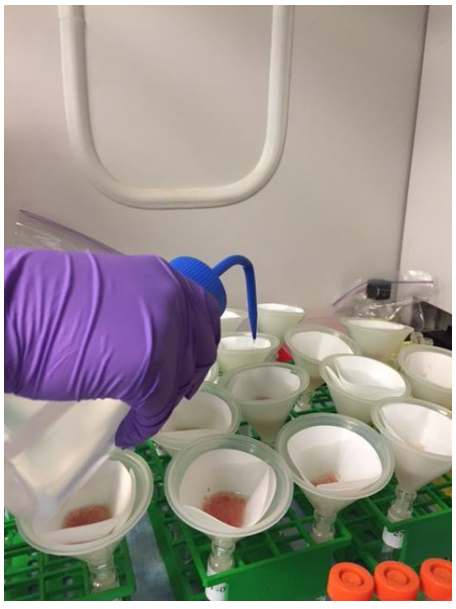
1. Add 5 mL of 2:1 chloroform:methanol with bottle-top dispenser and vortex (5 s).

2. Filter after sitting for 1 hour through Whatman #2 filter papers. Rinse two to three times with more 2:1 chloroform:metanol as they dry.

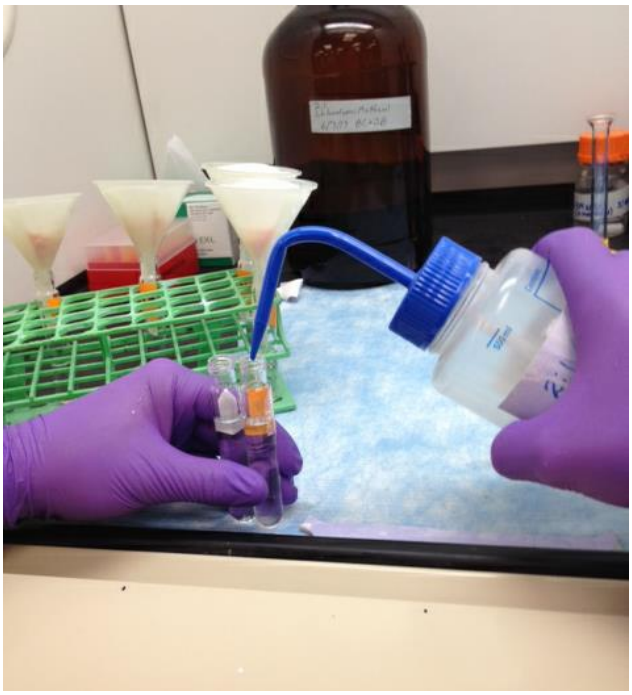




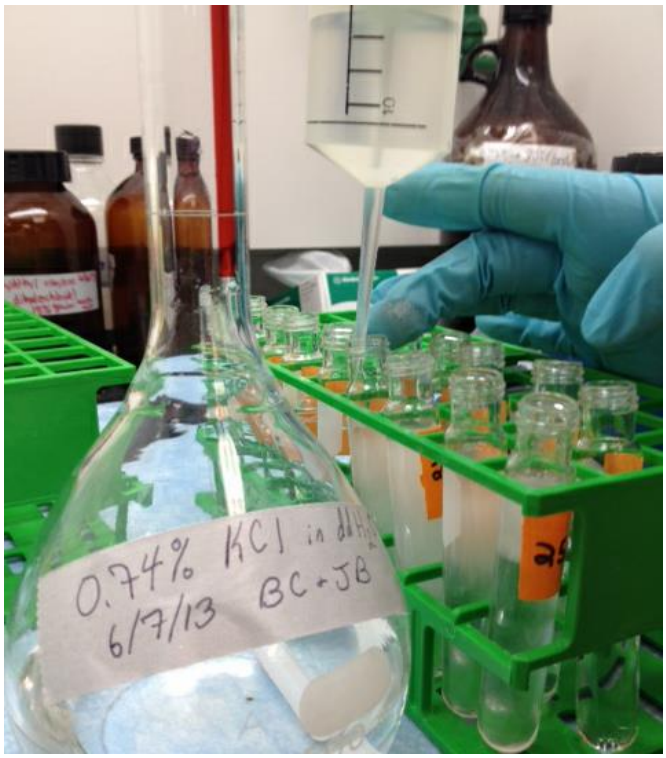




3. Complete the volume to 10 mL with 2:1 chloroform:meathanol using a water reference tube as a guide.

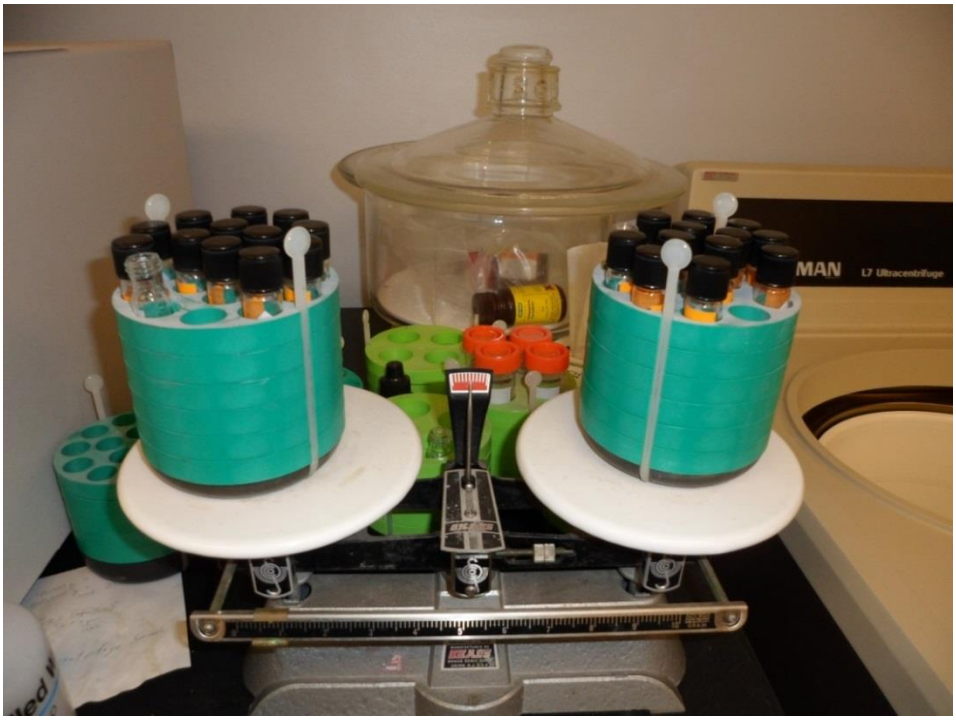


4. Add 2 mL of 0.74% KCl using a repeat pipetter and vortex with the caps on.

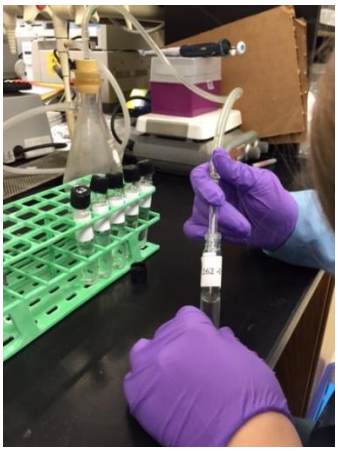


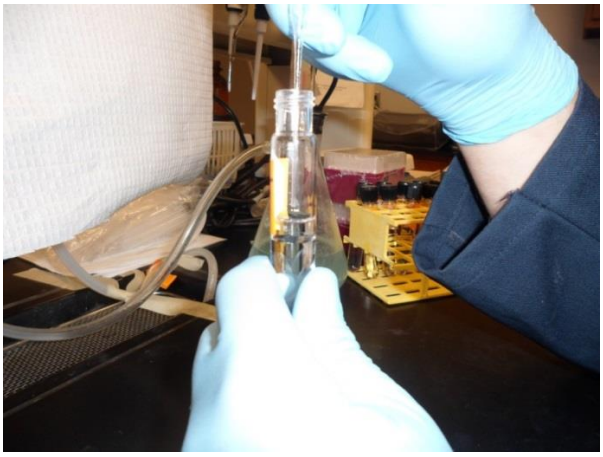
5. If stopping at this point nitrogen purge (remove ambient air with nitrogen) the vials and store overnight (-80°C).

6. Balance out the load and prior to centrifuging for 5 min at 1000 x g.

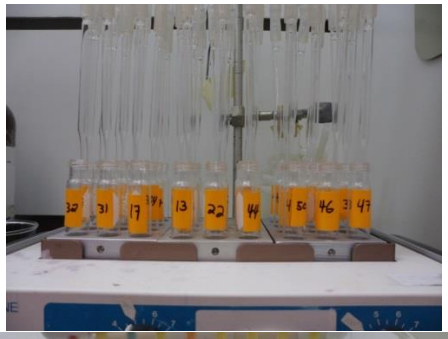


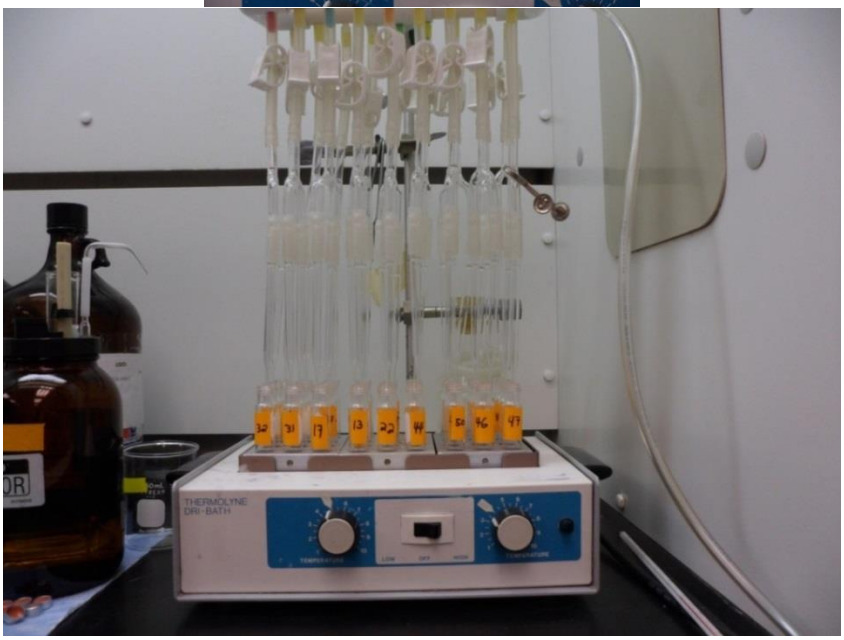
7. After the centrifugation, with a suction system aspire off the aqueous top layer without disrupting the bottom layer that contains the fatty acids. This top layer will be discarded.



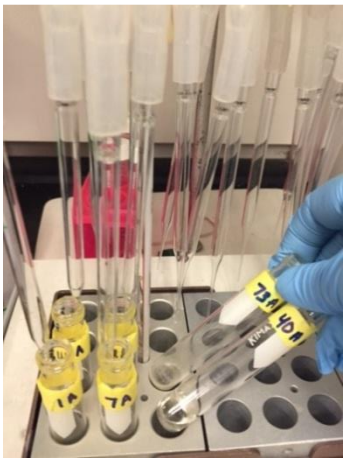


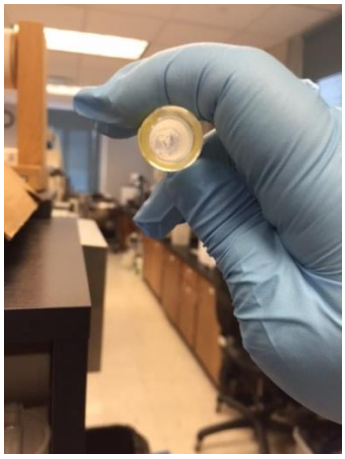
8. Place under hood on heating block (60°C) under continuous nitrogen flow.





9. Monitor the temperature of the heating block and the nitrogen flow during the drying process. Make sure that the tubes are completely dry and have no residual odor of any alcohol.

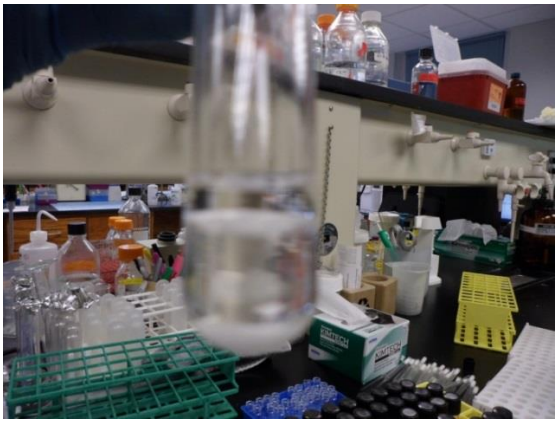




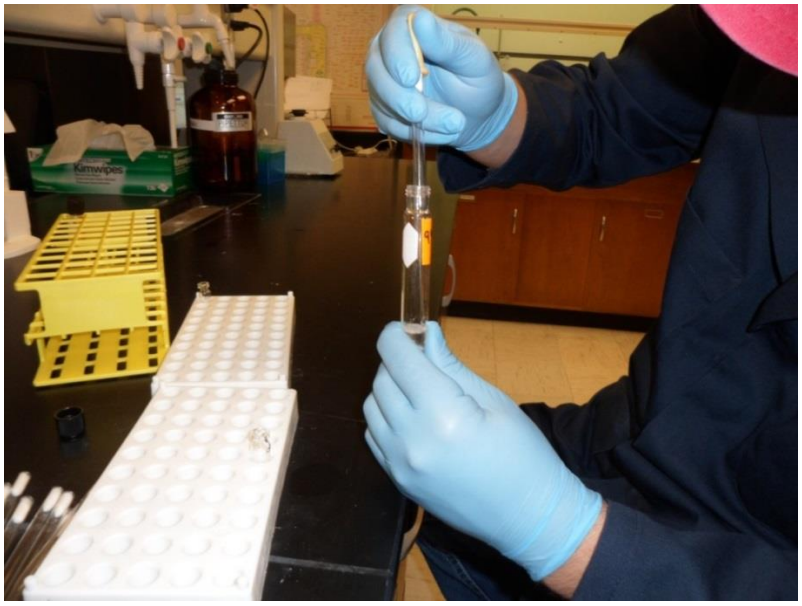
10. Add 0.5 mL of 0.5 M NaOH and vortex for 5 s, nitrogen purge and place samples in a beaker to heat for 5 min a 100°C.

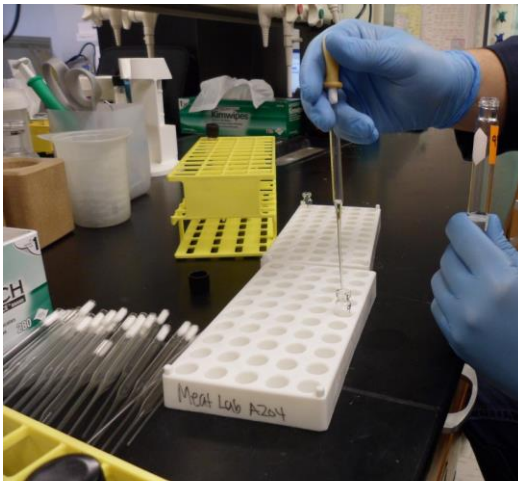
11. Under a well ventilated hood, add 0.5 mL of boron trifluoride in 14% methanol and vortex for 5 s, nitrogen purge and place samples in a beaker to heat for 5 min a 100°C.

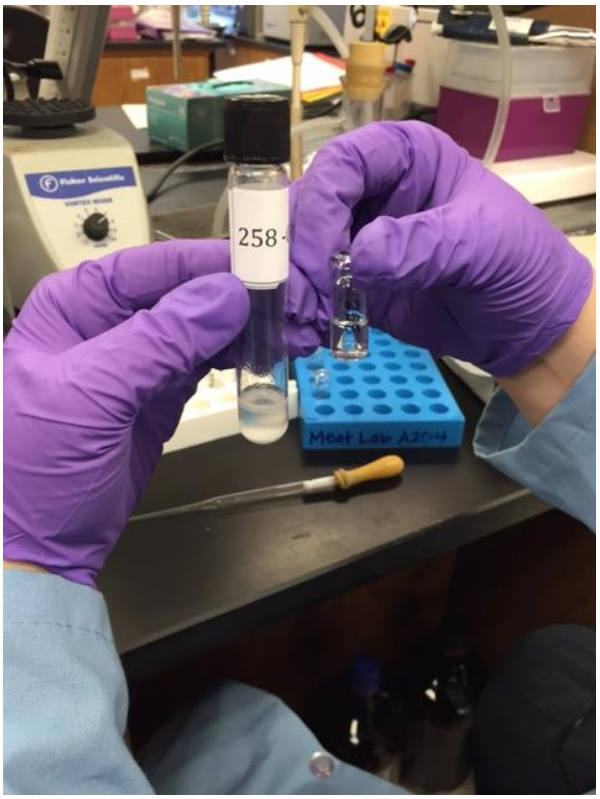
12. Add 1 mL of saturated salt solution and 1 mL of hexane. Vortex for 5 s and centrifuge (1000 x g for 5 min). At this point two distinct phases will separate out in the tubes (the top layer contains the hexane with the fatty acids).

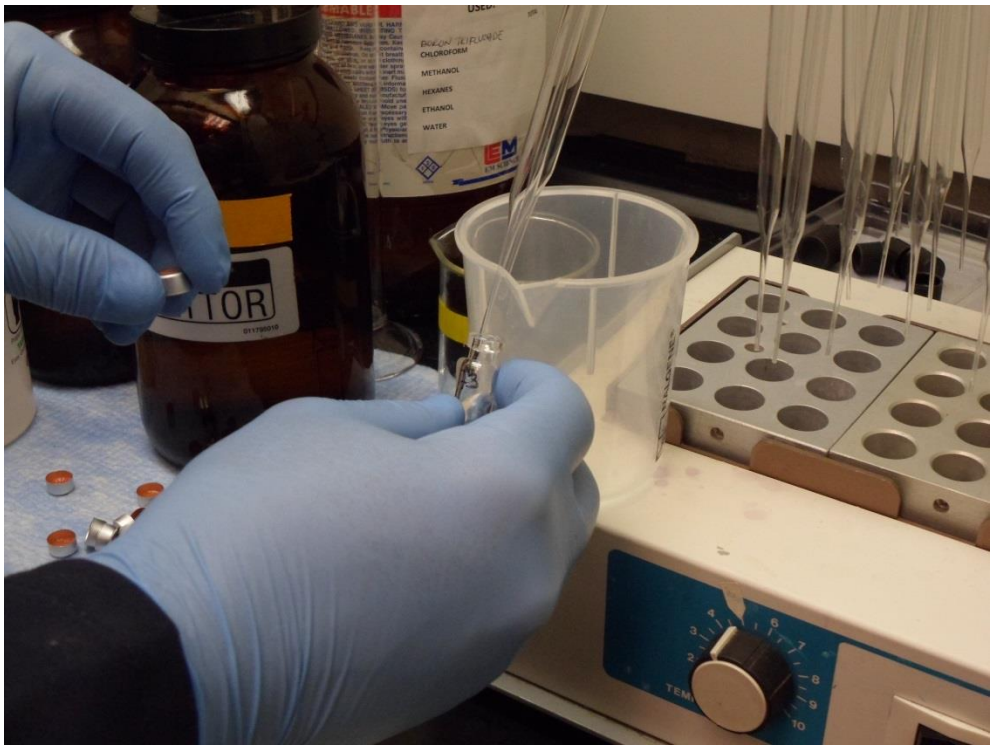
13. Using disposable glass pipettes remove the top layer and transfer to the GC vials making sure NONE of the bottom layer solution gets transferred.



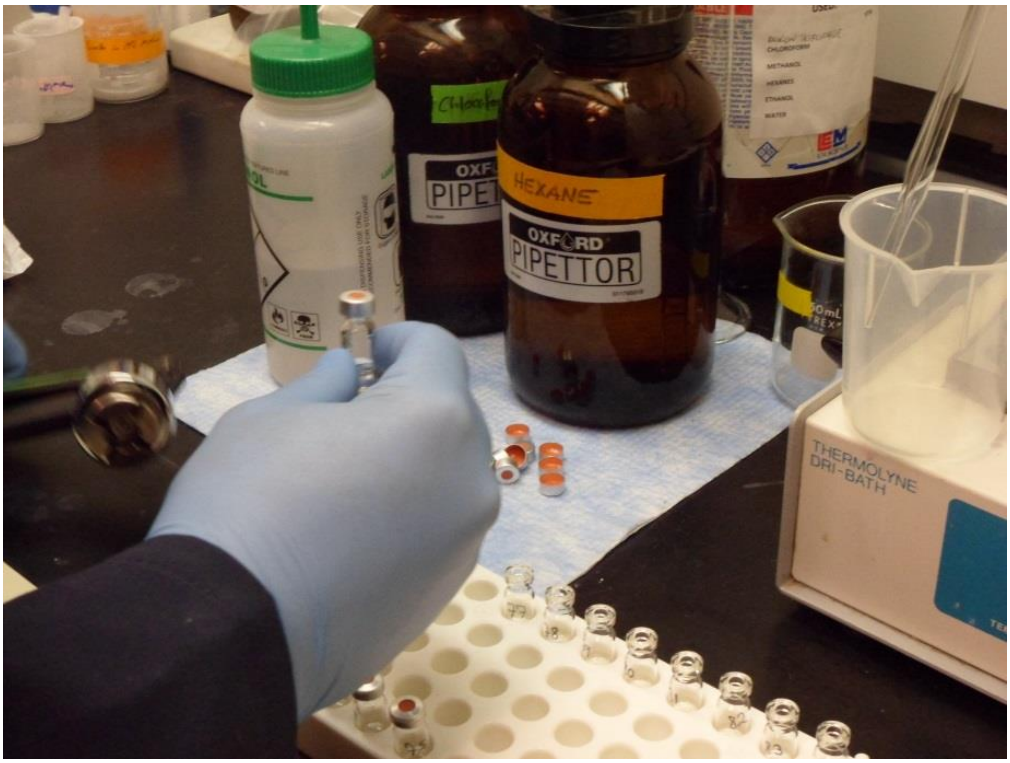


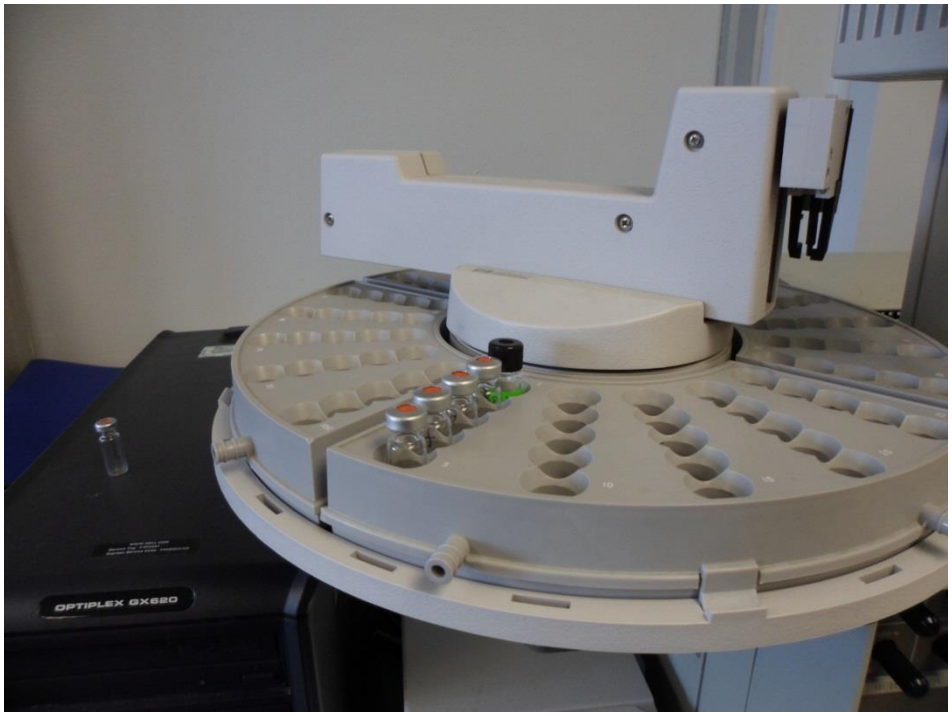


14. With very low nitrogen pressure (to make sure nothing spills) purge the GC vials and crimp the lids on making sure they are on tight and they don't leak.



15. Once GC vials are done these can be stored (-80°C) until needed for analysis.





APPENDIX VI

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 <u>OR</u>
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, ***<u>ONLY turn on the power buttons</u>***. ***<u>DO NOT hold down the [DELETE/UNDO] key at the same time</u>***. 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.

<u>Minolta Helpful Tips</u>

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 2001st reading from being taken so you cannot accidently 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 accidently take a reading, hitting the [DELETE/UNDO] key will delete the last reading.
- If you accidently 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:
    - Color space → On
    - Disp. Limit → press the [Measure Enter] key to select this option
    - 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**

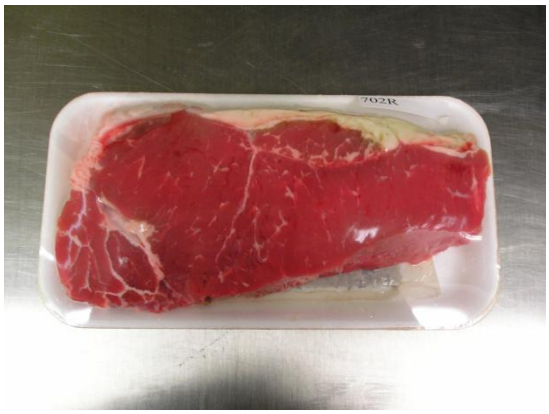
If you get done measuring and realize that you meant to measure in a different color space (For example: measured everything using Yxy and meant to use L*a*b*), you can correct it using these steps:

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**.
    - 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**
    - 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.

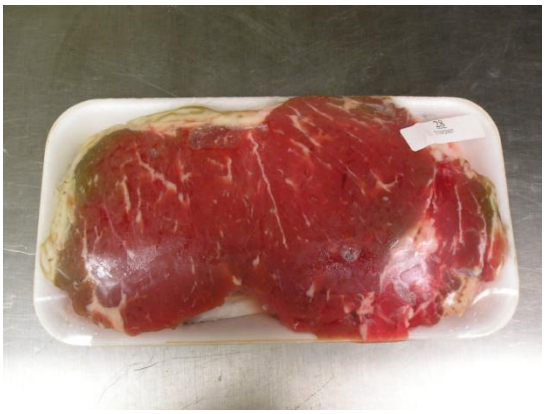
APPENDIX VII

Visual guide for percentage surface discoloration

0%



5%



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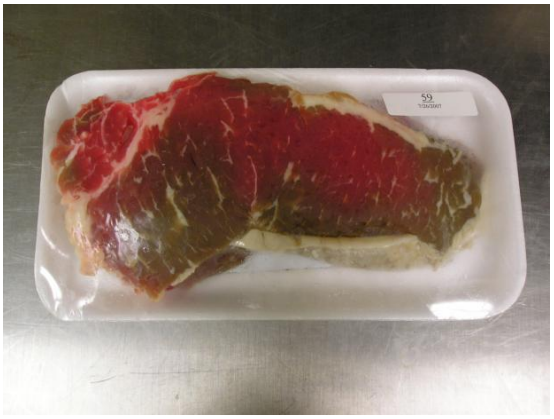

50%

60%

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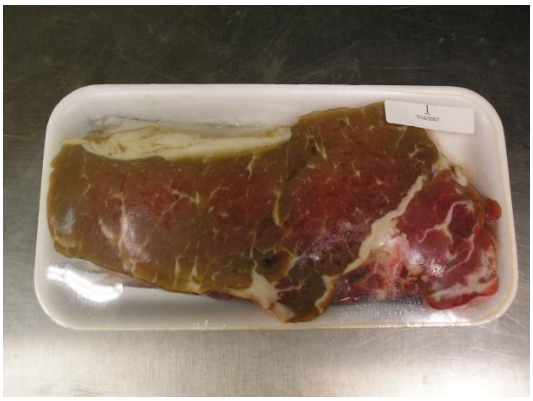


80%

90%

100%

APPENDIX VIII

Lipid Oxidation Thiobarbituric Acid Assay Protocol

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

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

Stock Solution**:** Dilute 99 µl TEP (97%) bring volume to 100 mL ddH_20

Working Solution: Dilute stock solution to 1:3 (TEP Solution:ddH20) ($1x10^{-3}$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_20.

Dissolve 2.88 g TBA in warm ddH_20 first, then add TCA (150g) and ddH_20 to 1L

BHA (ButylatedHydroxyAnisole) Stock Solution:

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

10g BHA dissolved in 90 mL ethanol (90%) + 5mL ddH_20

Standards: In duplicate

		Moles of TEP
Blank:	1 ml ddH_20	
Standard 5:	100 µl working TEP + 1.90 mL ddH_20	($5x10^{-5}$M)
Standard 4:	1 mL Std. 5 + 1 mL ddH_20	($2.5x10^{-5}$M)
Standard 3:	1 mL Std. 4 + 1 mL ddH_20	($1.25x10^{-5}$M)
Standard 2:	1 mL Std. 3 + 1 mL ddH_20	($.625x10^{-5}$M)
Standard 1:	1 mL Std. 2 + 1 ml ddH20	($.3125x10^{-5}$M)

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

Procedure

- Mix all reagents and standards before beginning.
- Transfer 5 g of powdered sample into a 50 ml conical tube, add 14 ml of ddH_2O and 1.0 mL of BHA (Butylated hydroxyanisole).
- Homogenize for 15 sec with a polytron
- Centrifuge for 2000xg for 5 minutes.
- Transfer 1 ml of homogenate or standard to 15 ml conical tube
- Add 2 ml of TBA/TCA solution, vortex.
- Incubate in a 70ºC water bath for 30 min to develop color.
- Cool samples in a cold water bath for 10 min.
- Centrifuge tubes at 2000×g for 15 min.
- Transfer duplicate aliquots of 200 µl from each tube into wells on a 96 well plate.
- Read absorbance at 540nm.

Calculations: mgs of malonaldehyde/kg of tissue

K(extraction)=(S/A) x MW x (10^6/E) x 100

Where S=Standard concentration ($1x10^{-8}$ moles 1,1,3,3-tetraethoxypropane)/5ml.

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

E= sample equivalent (1) P=Percent recovery

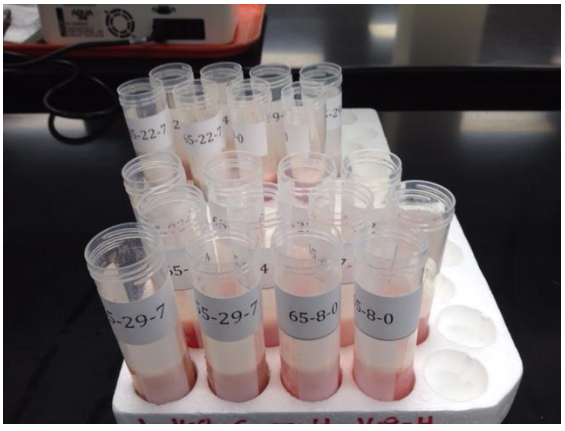
Final calculation: .012 x concentration x $72.063x10^6$ = mgs Malonaldehyde/kg of tissue

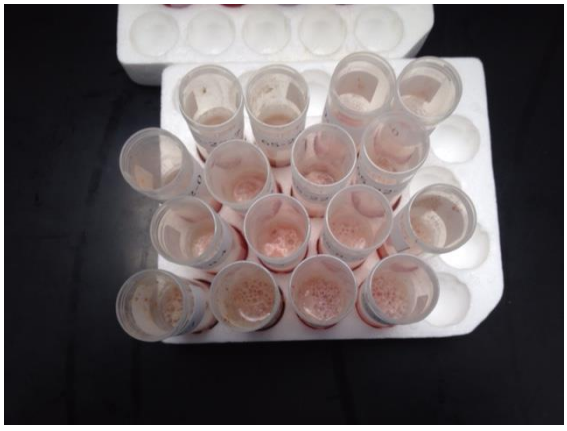
Reagents (Sigma): TBA- T5500; TCA- T9159; TEP- T9889; BHA- B1253

APPENDIX IX:

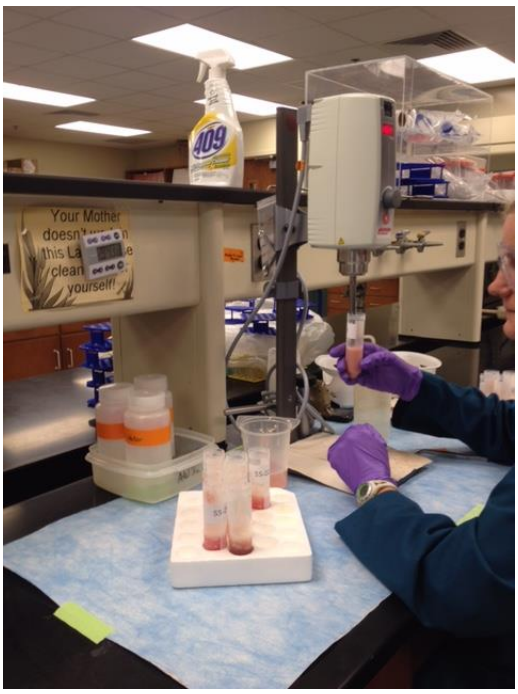
Lipid oxidation (TBARS) protocol step by step pictures

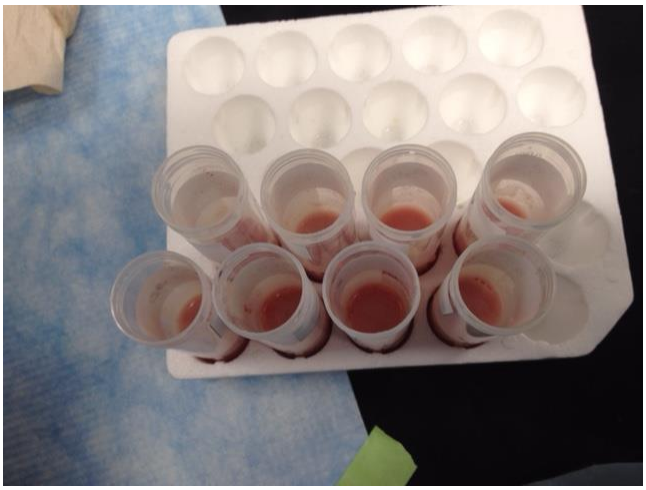
1. Remove 50 mL tubes containing 5 g of powdered meat sample and add 14 mL of ddH_2O with bottle top-dispenser and 1.0 mL of BHA with a repeat pipetter.





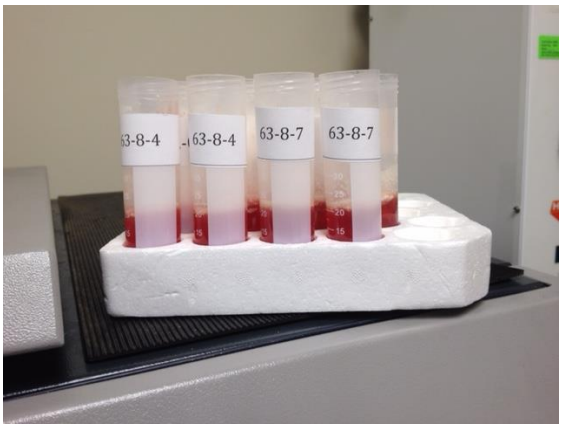
2. Homogenize completely by polytroning for 15 s at medium to high speed making sure you don't spill part of your sample. Make sure you rinse and clean the polytron thoroughly with ddH_2O and tweezers, and then pat it dry prior to polytroning the next sample.

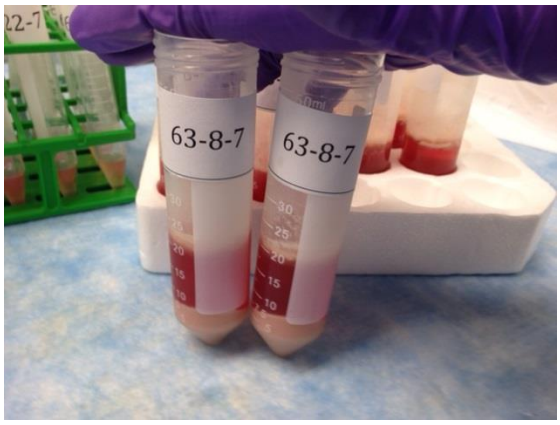


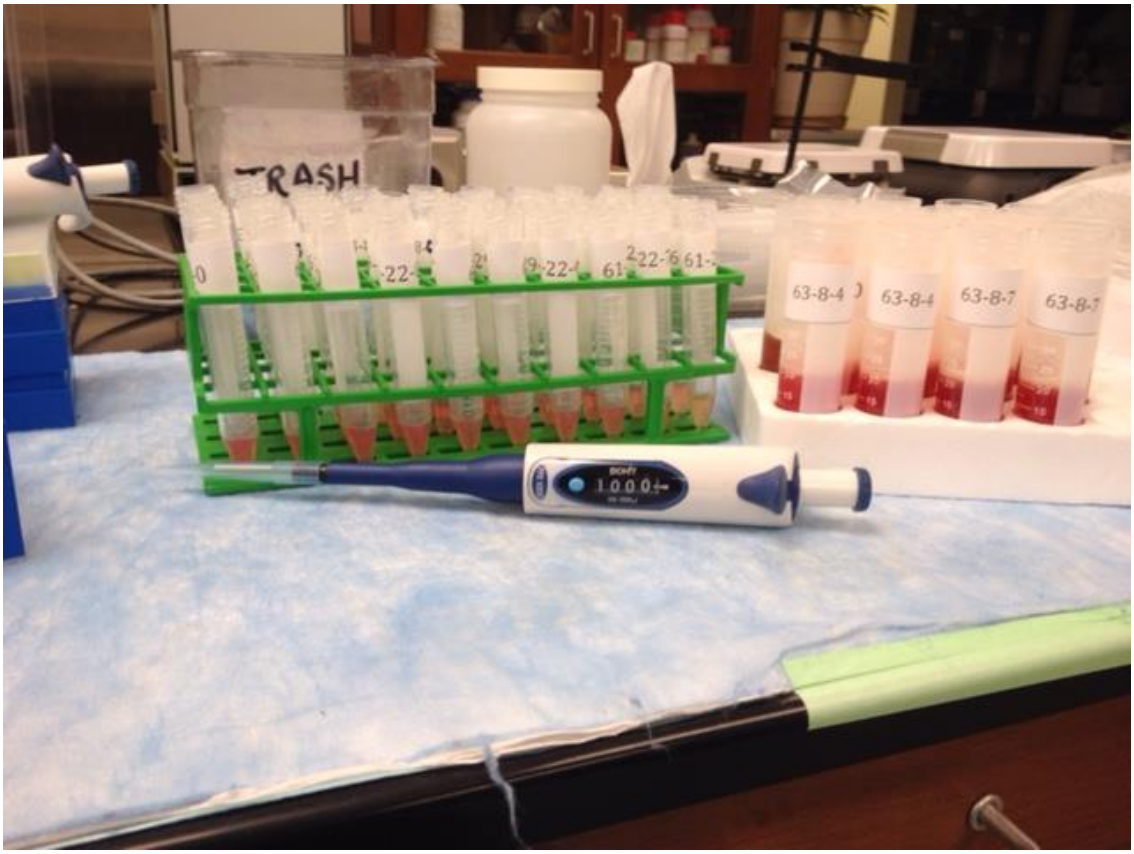


3. Once homogenized load the centrifuge and set it to run for 5 min at 2,000 x g at about 10°C.

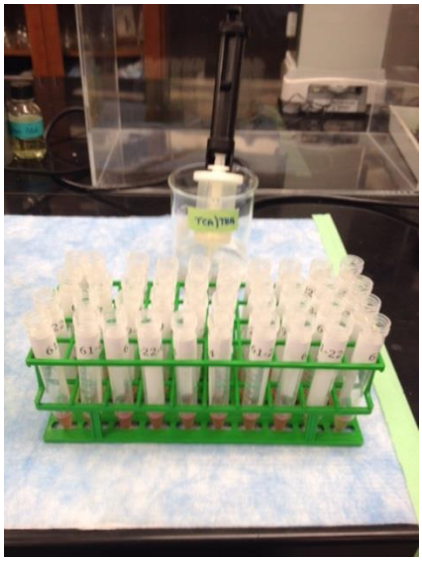
4. Once samples are centrifuged, 1 mL of the supernatant was transferred to a new 15 mL tube (each sample had a duplicate 50 mL tube, therefore we started with duplicate samples and ended with 4 smaller tubes per sample).

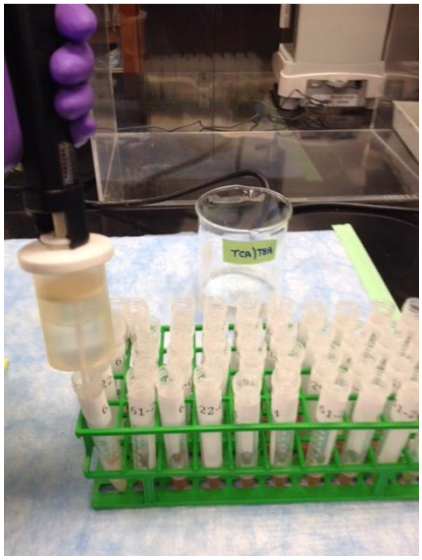


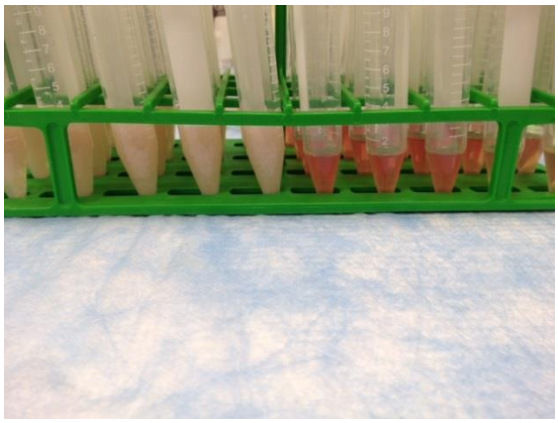




5. Then, 2 mL of TCA/TBA solution were added with a repeat pipetter to each tube and vortexed for 5 s.

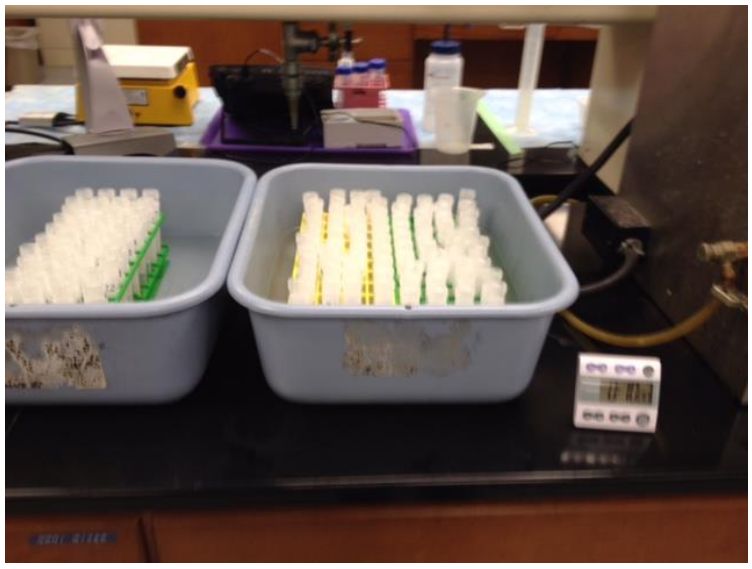




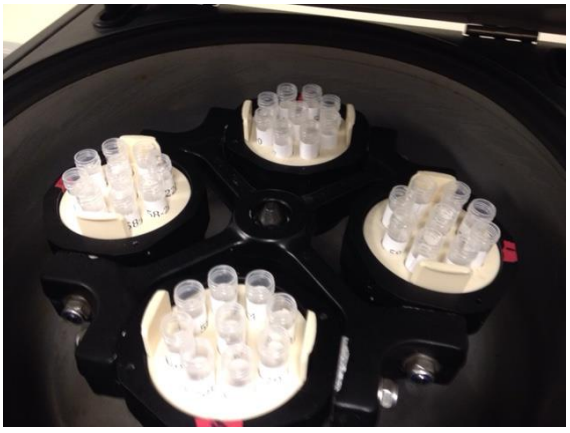


6. Standards and samples were placed in water bath (70°C) for 30 min to develop color.

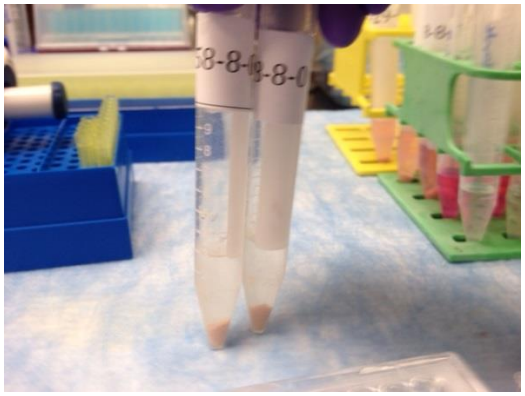
7. Paste the 30 min in the water bath, samples were placed on a cold water bath for 10 min.

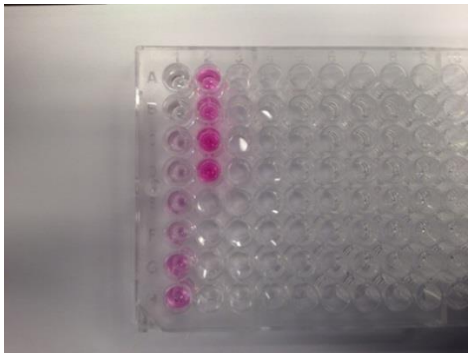


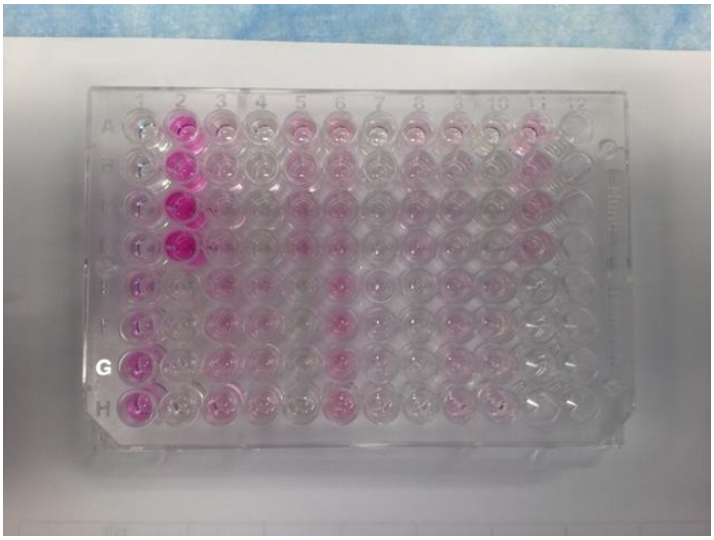
8. Once cooled, sample tubes were centrifuged for 15 min at 2,000 x g.



9. Then, 200 µL of supernatant of the standards and the samples get transferred onto a 96 well plate closely following a platting map. Make sure that the supernatant is clear and that no particles from the pellet get transferred onto the plate as this will dramatically alter your results. Plates can now be read at an absorbance of 540 nm.

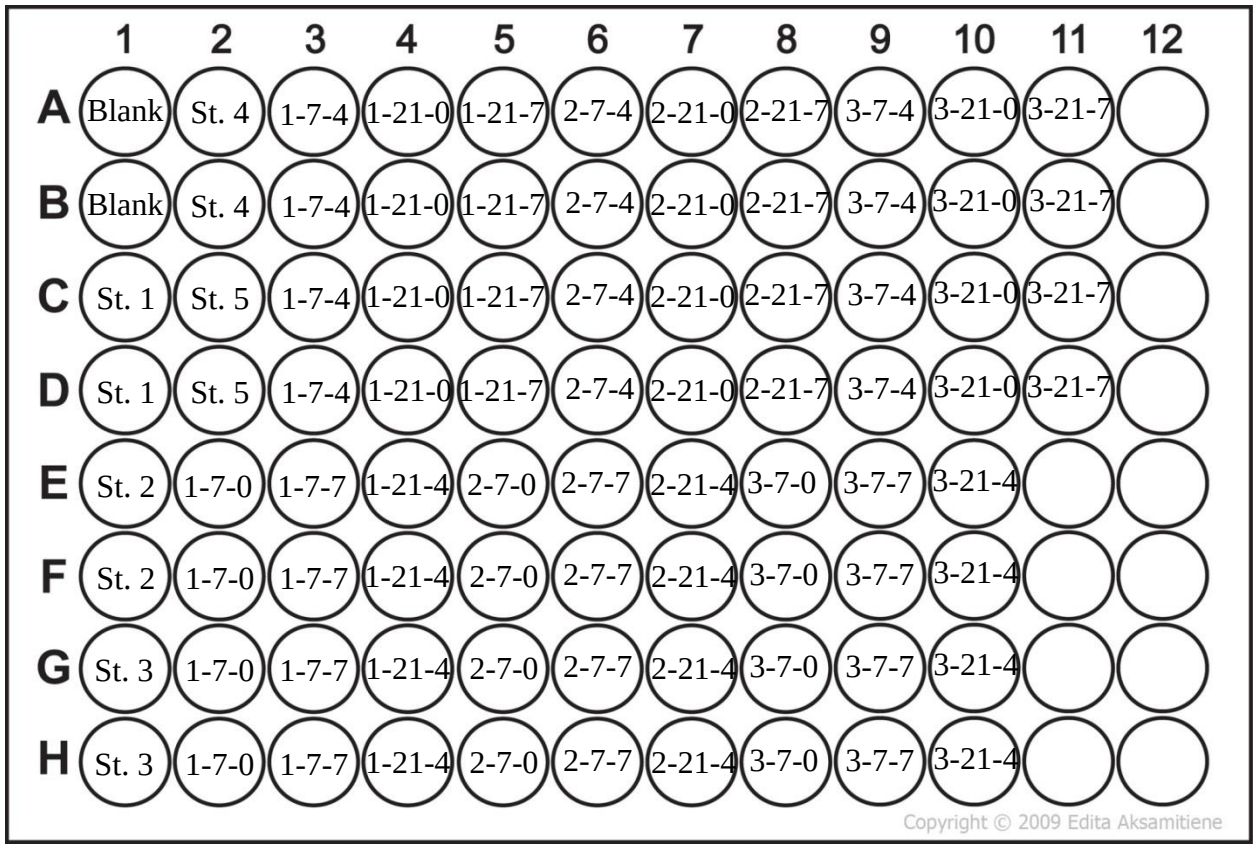






APPENDIX X:

Lipid oxidation (TBARS) platting map



Key:

Blank = 1 mL ddH_20
St. 1 = Standard 1: 1 mL St. 2 + 1 mL ddH_2O (0.3125×10^{-5} M TEP)
St. 2 = Standard 2: 1 mL St. 3 + 1 mL ddH_2O (0.625×10^{-5} M TEP)
St. 3 = Standard 3: 1 mL St. 4 + 1 mL ddH_2O (1.25×10^{-5} M TEP)
St. 4 = Standard 4: 1 mL St. 5 + 1 mL ddH_2O (2.5×10^{-5} M TEP)
St. 5 = Standard 5: 100 µL working TEP + 1.90 mL ddH_2O (5×10^{-5} M TEP)

Three digit codes (#-#-#) = Sample number-Age-Retail display
(For example: 1-7-0 = Sample 1, Aged 7d, RD 0d)

Note:

Study 1 = Had 2 aging times: 7 and 21 d
Study 2 = Had 4 aging times: 2, 8, 14, and 21 d
Study 3 = Had 3 aging times: 8, 22, and 29 d
*All studies had 0, 4 and 7 d RD times

APPENDIX XI:

Finishing diet composition (Study 2)

Diet Composition on a DM basis fed to finishing steers

	Dietary treatment	
	Control[1]	De-Oiled DDGS
Ingredient, % of DM		
HMC[2]	31.5	31.5
DRC[2]	50.0	-
De-Oiled DDGS[2]	-	50.0
Alfalfa hay	5.5	5.5
Corn silage	4.0	4.0
Liquid molasses	5.0	5.0
Supplement[3]	4.0	4.0
Nutrient Composition, %		
DM	81.7	82.2
OM	94.4	92.3
CP	15.5	22.7
NDF	12.2	25.8
ADF	8.2	12.8
Fat	3.7	6.1
Ca	0.8	1.0
P	0.3	0.6
K	0.7	1.2
S	0.2	0.3

[1]Supplemented with urea at 1.36% of diet to meet the degradable intake protein (DIP) requirements

[2]HMC = High moisture corn; DRC = Dry rolled corn; De-oiled DDGS = De-oiled dry distillers grains plus solubles

[3]Supplement included: Limestone (1.43%) Urea (1.36%), Fine ground corn (0.73%), Salt (0.30%), Tallow (0.10%), Beef trace minerals (0.05%), Vitamin A-D-E (0.02%), Rumensin-90® (0.02%), and Tylan-40® (0.01%). Formulated to contain 30 g/ton Rumensin® and 90 mg/steer/day of Tylan®.

APPENDIX XII:

Diet compositions of the receiving and finishing phases in the OmniGen-AF study (Study 3)

Diet Composition on a DM basis fed to steers[1]

	Composition (%)
Receiving phase (first 28 d)	
Sweet Bran®	36
Alfalfa hay	30
Dry rolled corn	30
Supplement	4
Finishing phase (all 215 d)	
High moisture corn	50
Sweet Bran®	40
Wheat straw	5
Supplement	5

[1]Steers on the supplementation treatments (both at the receiving and finishing phases) were fed OmniGen-AF top dressed on a daily basis (4 g/45.36kg/hd/d). For the finishing group, OmniGen-AF amounts were re-calculated and adjusted every 30 d.

APPENDIX XIII:

Superoxide Dismutase Activity Kit Protocol (ab65354) and Plating Map Example

*Kit must be stored at 4°C in the dark upon arrival and is good for 1 year

*Reconstituted components are stable for 2 months; except the Enzyme working solution that is stable for only 3 weeks

1. **Reagent preparation**: **Briefly centrifuge small vials at low speed prior to opening**
    a. **SOD Assay Buffer**: Ready to use – Equilibrate to room temperature before use (Store aliquots at 4°C)
    b. **WST Solution**:
        - **1 mL** WST solution + **19 mL** of SOD Assay buffer (Aliquot according to # of assays and store at 4°C)
    c. **SOD Dilution Solution**: Ready to use – Equilibrate to room temperature before use (Store aliquots at 4°C)
    d. **SOD Enzyme Solution**: **Centrifuge enzyme solution for 5 seconds and mix by pipetting**
        - **15 µL** SOD Enzyme Solution + **2.5 mL** of Dilution buffer = diluted enzyme solution (Aliquot according to # of assays, store at 4°C)

2. **Sample preparation**: *For tissue samples
    a. Harvest tissue (recommended to start = ***10 mg***)
    b. Add and homogenize with ice cold 0.1M Tris/HCl, pH 7.4 containing 0.5% Triton X-100, 5mM β-ME, 0.1mg/mL PMSF (***Add 150 µL and homogenize with sonicator***)
        - For 50 mL of solution: (If doing one full plate, which can hold up to 23 samples, you must multiply buffer amounts by 3)
            1. **50 mL** of ddH_2O
            2. **0.788 g** Tris/HCl (*Verify pH after adding Tris/HCl to about 30 mL of ddH_20, pH should be 7.4; if too high adjust with HCl / too low adjust with NaOH, complete volume with ddH_20 at the end)
            3. **25 µL** Triton X-100
            4. **17.44 µL** β-ME
            5. **5 mg** PMSF (must be solubilized in 200 μL of ethanol prior to adding to the buffer)

c. Centrifuge at 14,000 x g for 5 min at 4°C
d. Collect **100 *µL*** of supernatant and transfer to clean 15 mL tube with 4.5 mL of buffer
e. *OPTIONAL*: If collected supernatant is not clear you could centrifuge a second time at 14,000 x g for 5 min at 4°C
f. *Keep samples on ice

3. **Assay procedure and detection**: *See table 1 below as template for pipetting as well as 96 well plate example
    a. Add **20 µL** of **sample** to the Sample and Blank 2 wells in duplicates
    b. Add **20 µL** of **ddH_2O** to Blank 1 and Blank 3
    c. Add **200 µL** of **WST Working Solution** to each well
    d. Add **20 µL** of **Dilution Buffer** to Blank 2 and Blank 3
    e. Add **20 µL** of **Enzyme Working Solution** to Blank 1 and each sample well ***USE MULTIPLE CHANNEL PIPETTE* here to avoid Rx time lag**
    f. Mix with a plate shaker at **300 RPM's** for 1 min (*With higher RPM's samples will bubble, spill, and mix within your plate)
    g. Incubate at 37°C for 20 min
    h. Measure on microplate reader at OD 450 nm

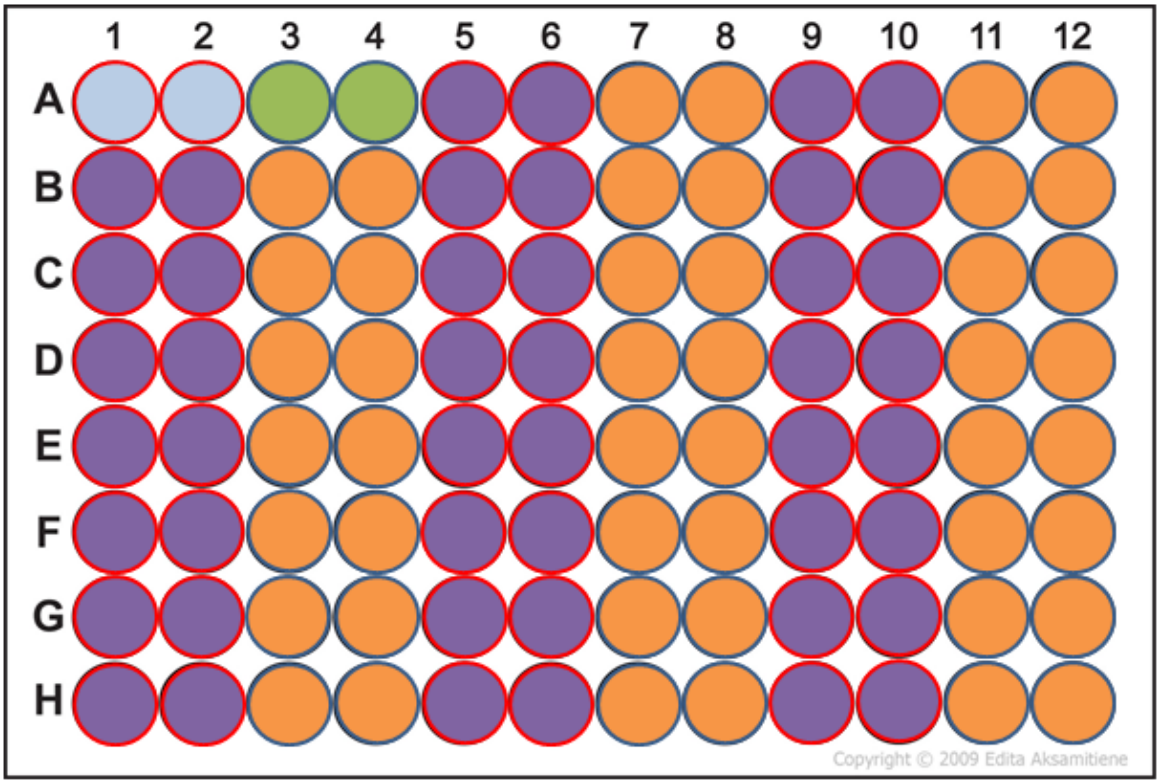
4. **Calculations**:
    a. Calculate SOD activity (inhibition rate %) with the following equation:

$$\text{SOD activity (inhibition rate \%)} = \left[\frac{(A_{blank1} - A_{blank3}) - (A_{sample} - A_{blank2})}{(A_{blank1} - A_{blank3})} \right] \times 100$$

Table 1. Amount of solution in each well

	Sample	Blank 1	Blank 2	Blank 3
Sample Solution	20 µl	--	20 µl	--
ddH_2O	--	20 µl	--	20 µl
WST Working Solution	200 µl	200 µl	200 µl	200 µl
Enzyme Working Solution	20 µl	20 µl	--	--
Dilution Buffer	--	--	20 µl	20 µl

96 well plate example (example for full plate n = 23 samples)



Blank 1 = 20 µl ddH_2O + 200 µl WST Working Soln. + 20 µl Enzyme Working Soln.

Blank 3 = 20 µl ddH_2O + 200 µl WST Working Soln. + 20 µl Dilution Buffer

Sample = 20 µl Sample + 200 µl WST Working Soln. + 20 µl Enzyme Working Soln.

Blank 2 = 20 µl Sample + 200 µl WST Working Soln. + 20 µl Dilution Buffer

THIS KIT ACCOUNTS FOR SOD ACTIVITY, FOR AN ACTUAL MEASURE OF SOD UNITS ONE MUST QUANTIFY PROTEIN CONCENTRATION WITH A PROTEIN KIT COMPATIBLE TO REDUCING COMPOUNDS AS WELL AS DETERGENTS SUCH AS THE BIO-RAD RC DC PROTEIN ASSAY (CATALOG # 500-0122)

APPENDIX XIV:

Example of SOD U/mg protein calculations and platting map

Step 1: SOD activity (% Inhibition) calculation

SOD activity (% Inhibition) =$[(A_{blank1} - A_{blank3}) - (A_{sample} - A_{blank2}) / (A_{blank1} - A_{blank3})]$ x 100

Sample X SOD % Inhibition = [(0.41-0.1565) – (0.2805-0.176)] / (0.41-0.1565)] x 100

Sample X SOD % Inhibition = 58.78%

Step 2: SOD U of activity calculation

SOD U = [(SOD % Inhibition / 50) / Vol of assay sampled] x Dilution factor

Sample X SOD U = [(58.78% / 50) / 0.02] x 0.022

Sample X SOD U = 1.29 U

Step 3: Protein concentration determination using 5 µL of SOD activity supernatant

Protein standard curve equation: $y = 0.1671x + 0.118$ ($R^2 = 0.98$)

Absorbance = 0.266

Sample X protein concentration calculation = [(Absorbance – Intercept) / Slope]

Sample X protein concentration calculation = [(0.266 – 0.118) / 0.1671]

Sample X protein concentration calculation = 0.89 mg/mL

Step 4: Protein concentration adjustment based on amounts used for both SOD activity and protein concentration determination

Given that we used 20 µL of the supernatant in the SOD activity kit wells and we only used 5µL of that same supernatant for protein determination we must adjust for that

difference. Hence, we multiply the protein concertation x 4 to account for the amount of protein in the samples used for SOD activity.

First adjustment for protein concentration = Protein concertation x 4

First adjustment for protein concentration = 0.89 mg/mL x 4

First adjustment for protein concentration = 3.56 mg/mL

Step 5: Use of conversion factor to correct for protein concertation differences between SOD activity and protein concentration determination

Given that we diluted 100 µL of the SOD activity supernatant (that were in 150 µL of buffer) in 4.5 mL of buffer yet we pulled 5 µL of that supernatant that was in 150 µL of buffer we had to adjust for the protein dilution factor differences. After calculating the conversion factor to account for these differences (44.99999944), this factor was used as a constant to for all samples.

Second protein adjustment = First adjustment for protein concertation / Conversion factor

Second protein adjustment = 3.56 mg/mL / 44.99999944

Second protein adjustment = 0.08 mg/mL

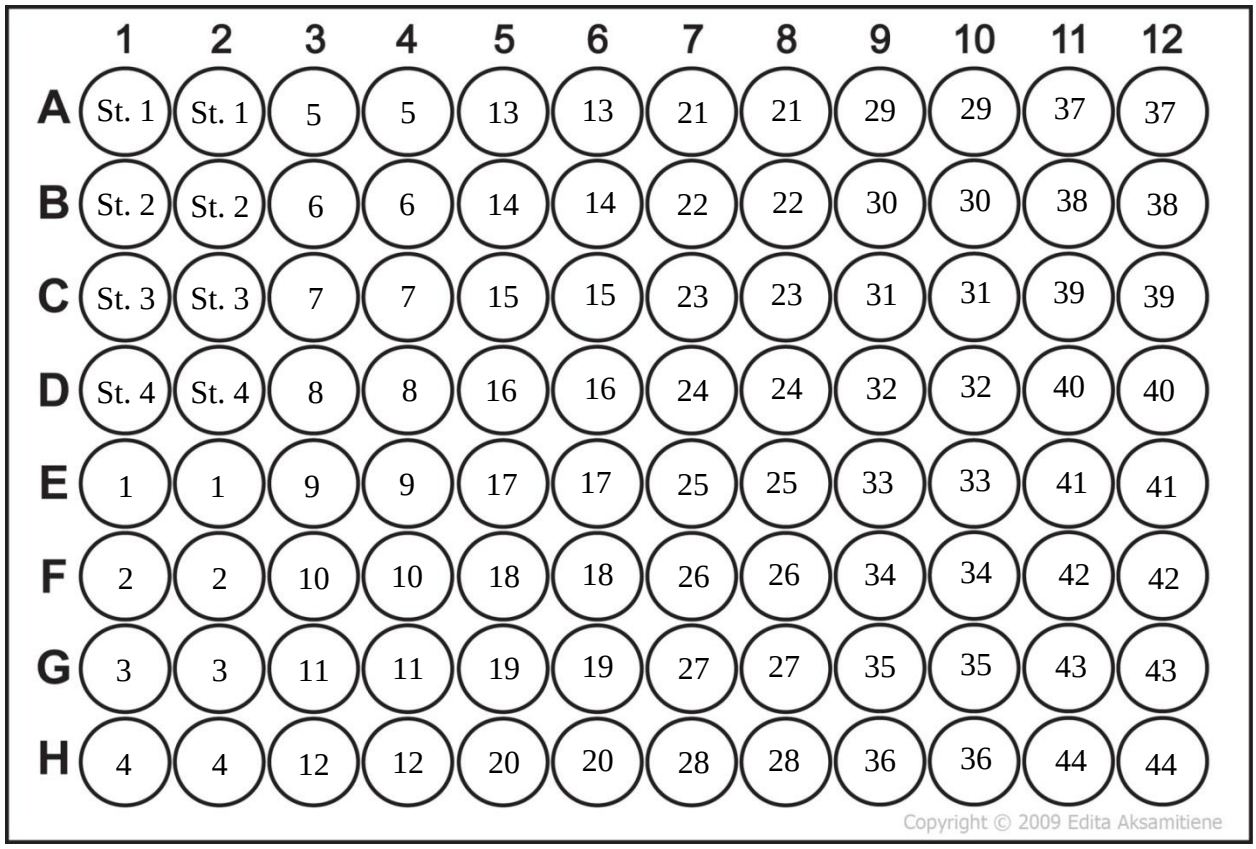
Step 6: Final SOD U/mg protein calculation

Final calculation = SOD U / Second protein adjusted value

Final calculation = 1.29 U / 0.08 mg

Final calculation = 16.13 SOD U / mg of protein

Plating map example for protein determination with the RC DC protein assay kit:



Key:

St. 1 = Standard 1: 1.45 mg/mL of bovine serum albumin
St. 2 = Standard 2: 0.725 mg/mL of bovine serum albumin
St. 3 = Standard 3: 0.3625 mg/mL of bovine serum albumin
St. 4 = Standard 4: 0.18125 mg/mL of bovine serum albumin

Same digits in duplicate = Same sample plated side by side

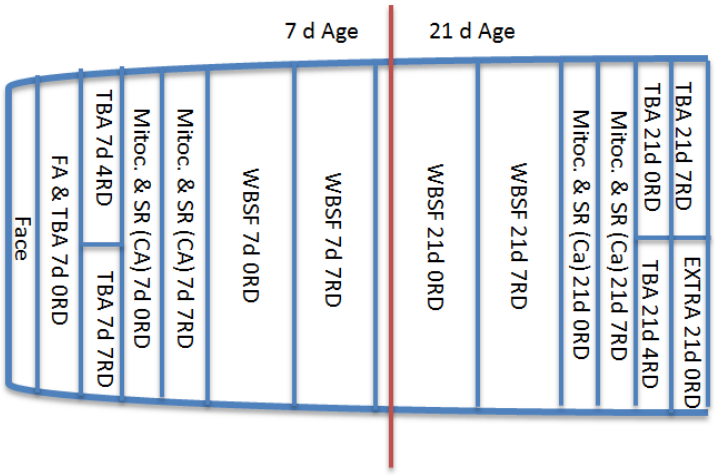
APPENDIX XV:

Fabrication map for all three studies

Study 1:

NOTES:

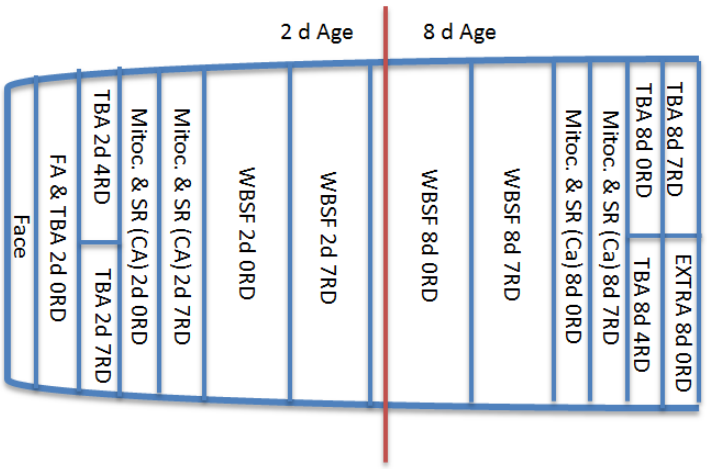
- WBSF steaks will be 1”
- All other steaks will be ½”



Study 2:

NOTES:

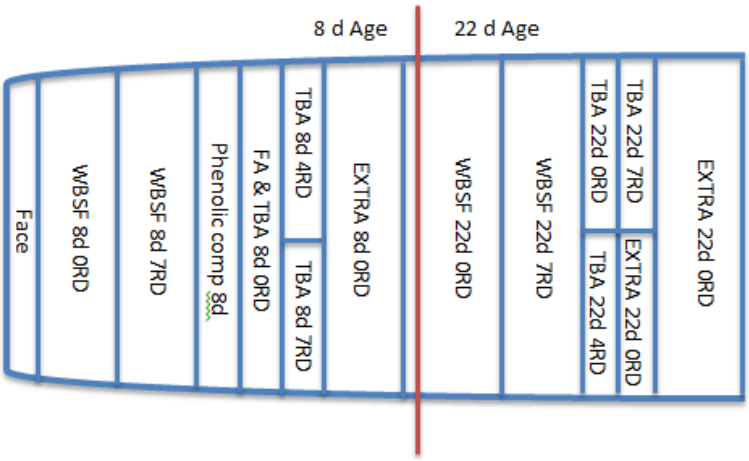
- Fabrication map for 14 and 21 d age will be the same as 8 d age on the right strip loin
- WBSF steaks will be 1”
- All other steaks will be ½”



Study 3:

NOTES:

- Fabrication map for 29 d age will be the same as 22 d age on the right strip loin + sample for Phenolic compounds
- WBSF and EXTRA steaks will be 1"
- All other steaks will be ½"



Note: On study 3 the samples labeled for Phenolic composition were that samples used for SOD determination.

APPENDIX XVI:

Tables for discoloration (A) and a* (B) triple interactions in Study 2

A

Triple interaction ($P < 0.0001$) between age, retail display and dietary treatment for discoloration (%) for strip loin steaks from cattle fed a corn control vs. 50% de-oiled DDGS diet

Retail display (d)	2 d Age		8 d Age		14 d Age		21 d Age	
	Corn control	50% De-oiled DDGS	Corn control	50% De-oiled DDGS	Corn control	50% De-oiled DDGS	Corn control	50% De-oiled DDGS
0	0.80	2.17	0.80	2.17	0.81	2.17	0.85	2.28
1	0.80	2.17	0.80	2.17	0.81	2.17	1.02	2.40
2	0.80	2.17	0.80	2.17	0.82	2.18	1.55	3.39
3	0.81	2.17	0.81	2.17	1.07	2.41	2.31	4.48
4	0.82	2.19	0.83	2.28	1.76	3.43	3.41	7.21
5	0.82	2.40	0.96	3.30	3.59	5.09	6.30	16.14
6	0.92	2.70	3.08	9.35	7.66	10.48	11.10^{a}	29.64^{b}
7	1.29	4.07	5.83	21.14	13.42	22.03	25.73^{a}	57.40^{b}

[a-b]Different superscripts indicate differences within aging time between dietary treatment ($P \leq 0.05$)
SEM = 10.14

B

Triple interaction ($P = 0.0011$) between age, retail display and dietary treatment for a* (redness) for strip loin steaks from cattle fed a corn control vs. 50% de-oiled DDGS diet

Retail display (d)	2 d Age		8 d Age		14 d Age		21 d Age	
	Corn control	50% De-oiled DDGS	Corn control	50% De-oiled DDGS	Corn control	50% De-oiled DDGS	Corn control	50% De-oiled DDGS
0	20.75	21.49	21.47	23.49	24.12	24.21	22.85	23.48
1	21.54	22.17	23.13	23.38	24.02	23.67	23.08	23.84
2	21.10	21.53	22.48	22.53	23.11	23.02	22.19	21.72
3	20.34	20.66	22.25	21.59	22.84	22.01	21.14	20.96
4	20.35	20.45	21.58	20.98	22.42	21.43	20.43	19.65
5	19.97	19.98	20.92	19.97	22.05	20.70	19.07	17.66
6	20.40	20.04	20.22	18.56	20.55	18.75	18.02^{a}	15.48^{b}
7	19.61	18.78	19.35^{a}	15.39^{b}	19.41^{a}	16.35^{b}	16.22^{a}	12.44^{b}

[a-b]Different superscripts indicate differences within aging time between dietary treatment ($P \leq 0.05$)
SEM = 0.40