IMPACT OF FINISHING DIETS WITH DE-OILED DISTILLERS GRAINS OR ANTIOXIDANT CONTAINING SUPPLEMENT ON BEEF SHELF LIFE

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.*,