

**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

Effects of dietary fat source on beef quality

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Thesis presented to obtain the degree of Doctor in Science.
Area: Food Science and Technology

**Piracicaba
2017**

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Título

DEDICATION

To my grandparents, Andralino & Benedita (*in memorian*)
To my grandparents, Edgar & Ignez
To my parents, Bira & Grace
To my sister Ariella
To my nephew Cauê
To my wife Thaís

I dedicate

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HEADING

“If I have seen further it is by standing on the shoulders of giants”

(Isaac Newton).

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RESUMO

Efeitos da fonte de gordura dietética na qualidade da carne bovina

De acordo com a literatura, a adição de resíduo de destilaria com solúveis em dietas de terminação de bovinos de corte aumenta a quantidade de ácidos graxos poliinsaturados (PUFA) na carne bovina. É sabido que quanto maior a concentração de PUFA na carne, maior será a probabilidade de ocorrência da oxidação lipídica e da mioglobina. Isso é importante porque o aumento da oxidação lipídica e da mioglobina acelera o desenvolvimento de odores indesejáveis e da descoloração da carne, reduzindo assim sua vida útil. O objetivo do primeiro estudo foi avaliar os efeitos do uso de diferentes resíduos de destilaria parcialmente desidratados com solúveis (MDGS) na vida útil da carne. Os resultados deste estudo sugerem que a inclusão de 40% de MDGS na dieta de terminação de bovinos pode reduzir a estabilidade lipídica e da cor, além de aumentar a quantidade de ácido linoléico (18:2) e PUFA da carne em comparação à carne de animais terminados com milho, reduzindo a vida útil da carne. No segundo estudo, nós trabalhamos com a hipótese de que a adição de elevadas quantidades de MDGS em dietas de terminação poderia aumentar a concentração de PUFA na membrana do retículo sarcoplasmático, e assim, alterar a integridade e permeabilidade dessa membrana, antecipando a liberação de cálcio postmortem. Com mais cálcio disponível, a proteólise muscular seria favorecida, aumentando a maciez da carne nos primeiros dias após o abate. Portanto, o objetivo do segundo estudo foi avaliar os efeitos da fonte de gordura dietética contida em diferentes tipos de MDGS na maciez da carne bovina. A inclusão de 40% de MDGS na dieta de terminação de novilhos aumentou a concentração de 18:2 e tendeu a aumentar a concentração de PUFA na membrana do retículo sarcoplasmático em comparação ao gado terminado com milho (sem inclusão de MDGS). Carne de animais terminados com MDGS apresentou maior quantidade de cálcio livre no sarcoplasma 48 horas após o abate. Carne de novilhos alimentados com MDGS desengordurado apresentou menor força de cisalhamento 48 horas após o abate quando comparada à carne de animais terminados com milho. Não houve diferença significativa entre nenhum dos tratamentos dietéticos 48 horas após o abate para comprimento de sarcômero e degradação de troponina T. Os resultados deste estudo sugerem que a inclusão de 40% de MDGS desengordurado na dieta de terminação de novilhos aumenta a maciez da carne 48 horas após o abate, possivelmente por aumentar a quantidade de cálcio livre no sarcoplasma. No entanto, o mecanismo pelo qual a fonte de gordura dietética acelerou a proteólise muscular ainda não está completamente elucidado.

Palavras-chave: Ácidos graxos; Cálcio livre; Maciez; Retículo sarcoplasmático; Vida útil

ABSTRACT

Effects of dietary fat source on beef quality

Feeding high levels of distillers grains increases polyunsaturated fatty acid (PUFA) levels in beef. It is well established that beef with higher concentrations of PUFA is more likely to have increased lipid and myoglobin oxidation. This is important because lipid and myoglobin oxidation lead to off-flavor development and discoloration of retail-displayed beef, reducing display life. In the first study, the effects of feeding different dietary fat sources with modified distillers grains plus solubles (MDGS) on beef display life were evaluated. Results suggest that feeding MDGS to cattle reduces color and lipid stability in addition to increasing C18:2 and PUFA content of beef in comparison to the corn diet. Thus, feeding MDGS to cattle has the potential to reduce beef display life. Perhaps, MDGS in feedlot diets increases PUFA concentration in the sarcoplasmic reticulum (SR) membrane, thereby altering membrane integrity, resulting in more rapid calcium leakage and improved tenderness. Therefore, the second study was dedicated to evaluate the effects of dietary fat source on the basic mechanism of beef tenderization. Feeding MDGS to cattle increased 18:2 and tended to increase PUFA concentration in the SR membrane, in addition to increase free calcium at d 2 postmortem in comparison to the corn diet. Beef from cattle finished on de-oiled MDGS and de-oiled MDGS plus oil had lower Warner-Bratzler shear force values than beef from cattle finished on corn at 2 d postmortem. No differences among dietary treatments were found for sarcomere length and troponin T degradation at 2 d postmortem. The results from this study suggest that feeding MDGS may increase tenderness, possibly by increasing free calcium in muscle early post-mortem. However, the true mechanism by which dietary fat source may accelerate the beef tenderization process is still unclear and should be further explored.

Keywords: Beef; Display life; Fatty acids; Free calcium; Sarcoplasmic reticulum; Tenderness

1. INTRODUCTION

This study seeks to understand the effects of dietary fat source with high concentration of modified distillers grains plus solubles (MDGS) on post-harvest changes in beef that may improve tenderness.

In 2016 ethanol production in the United States set a new high record producing 15.32 billion gallons of ethanol. Nebraska currently has approximately 25 operating ethanol plants and is the second largest ethanol producer in the US with a production capacity of 2.2 million gallons per year (RENEWABLE FUELS ASSOCIATION, 2017). Ethanol, made mostly from corn starch from kernels, is by far the most significant biofuel in the United States, accounting for 94 % of all biofuel production in 2012 (USDA, 2012). Corn ethanol production generates 1/3 ethanol, 1/3 distillers grains and 1/3 carbon dioxide (SAUNDERS; ROSENTRATER, 2009). As the demand for ethanol fuels has increased dramatically over the last 10 years, there is a proportional increase of byproducts available for cattle feeding in Nebraska. Over 90% of the cattle on feed in Nebraska utilize ethanol byproducts (WATERBURY et al., 2009).

When compared to corn, distillers grains are not only less expensive, but also contain more energy, pound-for-pound. Corn has a high carbohydrate content (70-72% weight on a dry matter basis is starch). During the ethanol production, starch is converted to glucose and through fermentation simple sugars are converted to ethanol (BOTHAST; SCHLICHER, 2005). Consequently, there is a concentration of other nutritional constituents such as protein, fat and fiber. Research has shown that distillers grains contain up to three times the levels of protein, fiber, and fat (KLOPFENSTEIN et al., 2007). Therefore, the nutritional value, large supply, and the price relative to corn make distillers grains an attractive feedstuff source in Nebraska.

The type and amount of distillers grains inclusion in cattle diets is greatly affected by the distance of the feedlot to the ethanol plant. Given the proximity of cattle feedlots to ethanol plants, Nebraska producers can feed distillers grains in the wet form. It is not necessary to dry the grains to minimize transportation costs. A survey with feedlot owners in Nebraska indicated a large shift away from dry distiller grains plus solubles (DDGS; 10% moisture), to more modified (MDGS; 50-55% moisture), and wet (WDGS; 65-70% moisture) distiller grains plus solubles. In 2010, DDGS were reported to be included at rate of 40% of a ration, but in 2015, none of the survey respondents indicated use of distillers in the dry form. Conversely, WDGS and MDGS inclusion levels on cattle diets doubled at the same period (BIRCH, 2015).

Research has shown that feeding distillers grains to cattle increases polyunsaturated fatty acid (PUFA) content of beef (MELLO et al., 2012; CHAO, 2015; DOMENECH-PÉREZ

et al., 2017). It is well established that beef with higher concentration of PUFA is more likely to have increased lipid and myoglobin oxidation. Not surprisingly, detrimental effects on beef color under retail display have been reported, especially when distillers grains were included in finishing cattle diets at high rates (ROEBER et al., 2005; DEPENBUSCH et al., 2009; LEUPP et al., 2009; SEGERS et al., 2011; MELLO et al., 2012; CHAO, 2015; DOMENECH-PÉREZ et al., 2017).

As ethanol plants look for ways to extract more value from distillers grains, the feed value of the byproduct has been changing. Improved extraction technologies in the ethanol industry has allowed for the increased removal of corn oil from distillers grains, reducing distillers fat content by about 30% on a dry matter basis. Currently, over half of Nebraska's ethanol plants are removing oil from distillers grains, and this percentage continues to increase (JOLLY, 2012). This is important because decreasing MDGS fat content from 12.0% to 7.2% decreased steer performance by 3.4% (BREMER, 2014). Not surprisingly, cattle feeders are concerned that removing oil from distillers grains would decrease its energy value and its subsequent feeding value in beef cattle diets. Hence, there is an interest in adding the oil back to de-oiled MDGS when economically feasible. However, it is still unknown if adding the oil back to de-oiled MDGS is equivalent to feeding full-fat distillers grains and/or de-oiled distillers grains.

A clear trend regarding the effects of feeding distillers grains on beef tenderness has not been established. Many authors have reported that the inclusion of distillers grains at different concentrations and feeding phases have no effect on meat tenderness measured via WBSF or sensory panel (ROEBER et al., 2005; GILL et al., 2008; LEUPP et al., 2009; KOGER et al., 2010; SEGERS et al., 2011; MELLO et al., 2012). On the other hand, results from many other studies indicated that meat from cattle fed distillers grains was more tender than the meat from cattle fed a regular corn diet (DEPENBUSCH et al., 2009; ALDAI et al., 2010; SENARATNE, 2012; CHAO et al., 2017).

Recent results from University of Nebraska suggest that oxidative stress in beef cattle may accelerate the beef tenderization process. Senaratne (2012) reported higher free calcium levels in the sarcoplasm when cattle were fed a high WDGS diet in comparison to cattle fed a corn diet. Then, Chao et al. (2017) observed that feeding 50% wet distillers grains to cattle altered the SR membrane composition, resulting in increased PUFA. These results triggered the materialization of this research and laid the foundation for our hypothesis. We hypothesize that high concentrations of PUFA in SR membrane could cause the membrane to quickly lose integrity due increased oxidation potential, leading to the early postmortem release of

previously sequestered calcium. The free calcium then interacts with calpains and accelerates early post-mortem protein degradation, making the meat more tender. Therefore, we want to know if a shift in dietary fat source can delay or accelerate the onset and/or the rate of oxidation, which could affect the beef tenderization mechanism.

1.1 Objectives

The general objectives of this research were to examine the effects of finishing diets containing various forms of MDGS and fat source on beef display life and tenderness. This manuscript was divided into two studies. The first study was designed to evaluate the effects of the dietary fat source with various forms of MDGS on beef display life, while the second study was focused on the effects of dietary fat source on the basic mechanism of beef tenderization.

More specifically, these studies aimed to complete the following objectives:

- 1) To examine the effects of finishing diets containing various forms of MDGS and fat source on beef color and lipid oxidation;
- 2) Quantify PUFA changes on muscle tissue and in the SR membrane from feeding various forms of MDGS to cattle;
- 3) Characterize the relationship between SR membrane fatty acid composition and lipid oxidation on calcium release early postmortem;
- 4) Determine the influence of SR membrane oxidation on calcium release.
- 5) Characterize the relationship between calcium release early postmortem and subsequent proteolysis of meat.

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. A deeper understanding of the dietary fat changes in corn byproducts could help improve beef tenderness, shelf life, and the way animals are fed.

References

ALDAI, N.; AALHUS, J.L.; DUGAN, M.E.R.; ROBERTSON, M.W.; McALLISTER, T.A.; WALTER, L.J.; McKINNON, J.J. Comparison of wheat-versus corn-based dried distillers grains with solubles on meat quality of feedlot cattle. **Meat Science**, Barking, v. 84, p. 569-577, 2010.

BIRCH, J.; BROOKS, K. (2015). **Nebraska feedyard: Labor costs benchmarks and historical trends** (Research Report/2015), Lincoln, NE, University of Nebraska - Extension, Institute of Agriculture and Natural Resources.

BREMER, M. **Energy value of de-oiled distillers grains plus solubles in beef cattle diets**. 2014. 110p. Dissertation (Master in Animal Science) University of Nebraska, Lincoln, 2014.

BOTHAST, R.J.; SCHLICHER, M.A. Biotechnological processes for conversion of corn into ethanol. **Applied Microbiology and Biotechnology**, Berlin, v. 67, p. 19-25, 2005.

CHAO, M.D. **Impact of wet distillers grains plus solubles and antioxidants on a basic mechanism of beef tenderization**. 2015. 184p. Thesis (Ph.D. in Animal Science) University of Nebraska, Lincoln, 2015.

CHAO, M.; DOMENECH-PÉREZ, K.I.; SENARATNE-LENAGALA, L.S.; CALKINS, C.R. Feeding wet distillers grains plus solubles contributes to sarcoplasmic reticulum membrane instability. **Animal Production Science**, Clayton South, v. Online Early, p. A-I, 2017a.

DEPENBUSCH, B.E.; COLEMAN, C.M.; HIGGINS, J.J.; DROUILLARD, J.S. Effects of increasing levels of dried corn distillers grains with solubles on growth performance, carcass characteristics, and meat quality of yearling heifers. **Journal of Animal Science**, Albany, v. 87, p. 2653–2663, 2009.

DOMENECH-PÉREZ, K.I.; CALKINS, C.R.; CHAO, M.D.; SEMLER, M.E.; VARNOLD, K.A.; ERICKSON, G.E. Impact of feeding de-oiled wet distillers grains plus solubles on beef shelf life. **Journal of Animal Science**, Albany, v. 95, p. 709–717, 2017.

GILL, R.K.; VanOVERBEKE, D.L.; DEOENBUSCH, B.; DROUILLARD, J.S.; DiCOSTANZO, A. Impact of beef cattle diets containing corn or sorghum distillers or beef color, fatty acid profiles, and sensory attributes. **Journal of Animal Science**, Albany, v. 86, p. 923-935, 2008.

JOLLY, M.L.; NUTTELMAN, B.L.; BURKEN, D.; SCHNEIDER, C.J.; KLOPFENSTEIN, T.J.; ERICKSON, G.E. Effects of modified distillers grains plus solubles and condensed distillers solubles with and without oil extraction on finishing performance. **Nebraska Beef Cattle Report**, Lincoln, v. 98, p. 64-65, 2013.

KLOPFENSTEIN, T.J.; ERICKSON, G.E.; BREMER, V.R. Board-invited review: Use of distillers by-products in the beef cattle feeding industry. **Journal of Animal Science**, Albany, v. 86, p. 1223–1231, 2008.

KOGER, T.J.; WULF, D.M.; WEAVER, A.D.; WRIGHT, C.L.; TJARDES, K.E.; MATEO, K.S.; ENGLE, T.E.; MADDOCK, R.J.; SMART, A.J. 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. **Journal of Animal Science**, Albany, v. 88, p. 3399–3408, 2010.

LEUPP, J.L.; LARDY, G.P.; BAUER, M.L.; KARGES, K.K.; GIBSON, M.L.; CATON, J.S.; MADDOCK, R.J. Effects of distillers dried grains with solubles on growing and finishing steer intake, performance, carcass characteristics, and steak color and sensory attributes **Journal of Animal Science**, Albany, v. 87, p. 4118-4124, 2009.

MELLO, A.S.; CALKINS, C.R.; JENSCHKE, B.E.; CARR, T.P.; DUGAN, M.E.R.; ERICKSON, G.E. Beef quality of calf-fed steers finished on varying level of corn-based wet distillers grains plus solubles. **Journal of Animal Science**, Albany, v. 90, p. 4625-4633, 2012.

RENEWABLE FUELS ASSOCIATION. **Statistics**. 2017. Disponível em: <<http://www.ethanolrfa.org/pages/statistics>>. Acesso em: 14 Mar. 2017.

ROEBER, D.; GILL, R.; DiCOSTANZO, A. Meat quality responses to feeding distiller's grains to finishing Holstein steers. **Journal of Animal Science**, Albany, v. 83, p. 2455-2460, 2005.

SAUNDERS, J.A.; ROSENTRATER, K.A. Survey of US fuel ethanol plants. **Bioresource Technology**, New York, v. 100, p. 3277-3284, 2009.

SEGERS, J.R.; STWART, R.L.; LENTS, C.A.; PRINGLE, T.D.; FROETSCHER, M.A.; LOWE, B.K.; McKEITH, R.O.; STELZLENI, A.M. Effect of long-term corn by-product feeding on beef quality, strip loin fatty acid profiles, and shelf life. **Journal of Animal Science**, Albany, v 89, p. 3792-3802 2011.

SENARATNE, L.S. **Mechanism and control of beef toughening during retail display in high oxygen modified atmosphere packages**. 2012. 238p. Thesis (Ph.D. in Animal Science) University of Nebraska, Lincoln, 2012.

USDA. **Economic Research Service: U.S. Bioenergy Statistics**. 2012.

Disponível em: <<https://www.ers.usda.gov/data-products/us-bioenergy-statistics/>>. Acesso em: 15 set. 2017.

2 EFFECTS OF DIETARY FAT SOURCE ON BEEF DISPLAY LIFE

Abstract

The effects of feeding different dietary fat sources with modified distillers grains plus solubles (MDGS) on beef display life were evaluated. Steers (n=256) were fed for 134 d on either a corn, 40% full-fat MDGS, 40% de-oiled MDGS, or 38% de-oiled MDGS plus 2% corn oil diet. Twenty-four Choice carcasses (3 head/pen) were randomly selected within each dietary treatment and strip loins were collected and aged for 2, 9, 16, or 23 d. Steaks from each aging period were placed under retail display (RD) conditions for 0, 4 and 7 d. Stearic acid was predominant (C18:0; $P = 0.03$) in beef from the de-oiled MDGS plus oil treatment in comparison with all other dietary treatments. Feeding MDGS increased linoleic acid (C18:2; $P < 0.0026$) and polyunsaturated fatty acids (PUFA; $P = 0.01$) in comparison to the corn diet. The de-oiled MDGS plus oil group had greater C18:3 content ($P = 0.03$) when compared to corn, but no differences were observed between all other diets. There were no difference among dietary treatments for L* and b* values. The de-oiled MDGS group had lower a* values than all other treatments ($P = 0.0054$) at d 5 of RD. The corn treatment had greater a* values ($P \leq 0.05$) than de-oiled MDGS and de-oiled MDGS plus oil at d 6 and 7 of RD. Beef from cattle fed full-fat MDGS tended to have lower a* values ($P = 0.10$) than cattle fed corn at d 7 of RD. Feeding de-oiled MDGS results in greater discoloration ($P \leq 0.05$) at d 5, 6 and d 7 of RD when compared to corn. Steaks from the de-oiled MDGS plus oil and full-fat MDGS groups had greater discoloration scores at d 7 of RD in comparison to corn ($P \leq 0.05$). Beef from cattle fed corn tended to have lower TBARS values ($P \leq 0.10$) in comparison to de-oiled MDGS and de-oiled MDGS plus oil at d 7 of RD. Results suggest that feeding MDGS to cattle reduces color and lipid stability in addition to increasing C18:2 and PUFA content of beef. Addition of corn oil to de-oiled MDGS decreased redness and increased discoloration and lipid oxidation in comparison to corn diets.

Keywords: Beef quality; Distillers grains; Fatty acid composition; Oxidation; Shelf life

2.1 Introduction

Over the last 10 years, a significant increase in ethanol production has occurred in the United States, from 6.49 billion gallons in 2006 to 14.80 billion in 2015 (RENEWABLE FUELS ASSOCIATION, 2017). Hence, greater amounts of distillers grains have been available for cattle feeding. Interestingly, even after the fermentation of corn for ethanol production, which removes starch, distillers grains have more energy per kg on a dry matter basis than regular corn due increased protein from about 10 to 30% and fat from 4 to 12% (KLOPFENSTEIN et al., 2008). Research has shown that feeding distillers grains to cattle increases the concentration of polyunsaturated fatty acids (PUFA) in meat (MELLO et al., 2012a, b). It is well established that beef with higher concentrations of PUFA is more likely to have increased lipid and myoglobin oxidation. This is important because lipid and myoglobin

oxidation lead to off-flavor development and discoloration of retail-displayed beef, reducing display life (FAUSTMAN, et al., 2010). The ethanol industry appears to have evolved in removal of oils from distillers grains. According to Jolly et al. (2013), approximately 50% of the ethanol plants removed oil from distillers grains in 2012, reducing the amount of energy per kg on a dry matter basis. There is an interest in adding the oil back to cattle diets when economically feasible. It is unknown if adding corn oil is equivalent to feeding full-fat or de-oiled distillers grains. A deeper understanding of the dietary fat changes in corn byproducts could help improve beef shelf-life and the way animals are fed. Research was conducted to determine the effect on beef display life as a result of feeding de-oiled modified distillers grains plus solubles (MDGS) with corn oil added back.

2.2 Materials and Methods

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

2.2.1 Cattle and Dietary Treatments

Initially, 256 Angus crossbreed finishing steers (initial BW = 412 kg, SD = 24) were fed for 134 d on either a corn, 40% full-fat MDGS, 40% de-oiled MDGS, or 38% de-oiled MDGS plus 2% corn oil diet. Cattle were blocked by body weight and randomly assigned 8 per pen for a total of 32 pens (8 pens/treatment). All dietary treatments are presented in Table 1.

Table 1. Diet composition (% of DM basis) fed to finishing steers receiving either a corn diet, 40% Full-fat MDGS, 40% De-oiled MDGS or 38% De-oiled MDGS plus corn oil.

Feed ingredient	Dietary treatment			
	Corn	40% MDGS Full-fat	40% MDGS De-oiled	38% MDGS Plus Oil
Ingredient, % of DM				
DRC ¹	42.75	24.00	24.00	24.00
HMC ¹	42.75	24.00	24.00	24.00
MDGS De-oiled ¹	-	-	40.00	38.00
MDGS Full-fat ¹	-	40.00	-	-
Corn Oil	-	-	-	2.0
Alfalfa	3.0	3.0	3.0	3.0
Sorghum Silage	4.0	4.0	4.0	4.0
Supplement ²	6.165	3.66	3.66	3.66
Tallow	0.125	0.125	0.125	0.125
FGC	1.20	1.20	1.20	1.20

¹DRC = Dry rolled corn; HMC = High moisture corn; MDGS = Modified distillers grains plus solubles; FGC = Fine ground corn.

²Formulated to contain 383mg/hd per d of Rumensin and 90mg/hd per d of Tylan.

2.2.2 Sample Collection and Fabrication

At harvest (Greater Omaha Packing, Omaha, NE), 24 Choice carcasses (3 hd/pen) were randomly selected within each treatment (n=96) and strip loins (*Longissimus lumborum*) from both sides were collected. Loins were transferred to the University of Nebraska Meat Laboratory. 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. Then, both left and right strip loins were divided in half, and each of the four sections per animal were randomly assigned to one of the four aging periods (2, 9, 16, or 23 d). Loins sections assigned for 2 d of aging were immediately trimmed of subcutaneous fat, and fabricated into three steaks (2.54 cm thickness) for proximate composition, fatty acid profile, objective color, visual discoloration, and lipid oxidation (1 steak for objective color and discoloration, 1 steak was split in half for fatty acid profile, proximate composition and lipid oxidation for 0 d RD, 1 steak was split in half for 4 and 7 d RD lipid oxidation). At d 2, steaks used for fatty acid profile and proximate composition were vacuum packaged and frozen (-80°C) until further analysis.

After fabrication, steaks used for color analysis 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 (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO;

oxygen transmission rate = 2.25 ml/cm²/24 hr at 23° C and 0% relative humidity; water vapor transfer rate = 496 g/m²/24 hr at 37.8° C and 90% relative humidity) and placed under RD conditions for 7 d (under white fluorescence lighting at 1000 to 1800 lux) at 3°C. After RD, samples were frozen in liquid nitrogen, powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT) and stored at -80°C until further analysis. The same fabrication scheme was used at 9, 16 and 23 d postmortem, with the exception of proximate composition and fatty acid profile, which were analyzed only at d 2 postmortem.

2.2.3 Proximate Composition

Moisture and ash (%) were quantified with a LECO Thermogravimetric Analyzer in duplicate (Model 604-100-400, LECO Corporation, St. Joseph, MI). Fat content was quantified in triplicate by ether extraction according to the Soxhlet procedure (AOAC, 1990). Protein content was calculated by difference.

2.2.4 Fatty Acid Analysis

Total lipid was extracted following the procedure described by Folch et al. (1957). After extraction, lipids were converted to fatty acid methyl esters according to Morrison and Smith (1964) and Metcalfe et al. (1966). One g of powdered sample was homogenized with 5 mL of 2:1 chloroform:methanol. After 1 h in room temperature (23°C), samples were filtered through filter paper (Whatman #2). The final volume was brought up to 10 mL with 2:1 chloroform:methanol and then vortexed for 5 s with 2 mL of 0.74% KCl. Samples were centrifuged (1,000 x g for 5 min) and the top layer phase was aspirated off. After centrifugation, samples were dried on a heating block at 60°C under nitrogen purge. After drying, one half mL of 0.5 M NaOH in methanol was added to the samples, vortexed and heated for 5 min at 100°C. Subsequently, 0.5 mL of boron trifluoride in 14% methanol was mixed and reheated for 5 min at 100°C. Then, samples were homogenized with 1 mL of saturated NaCl solution and 1 mL of hexane and centrifuged at 1,000 x g for 5 min. The top hexane layer was removed and analyzed using gas chromatography (Hewlett-Packard 6890 FID GC System; Agilent Technologies, Santa Clara, CA). Fatty acids were separated using a Chrompack CP-Sil 88 capillary column (0.25 mm by 100 m; injector temperature: 270°C detector temperature: 300°C; pressure: 40 psi; flow rate: 1.0 mL/min) and identified by their retention times in relation to known commercial standards (NU-Check Prep, Inc., Elysian, MN; # GLC-68D, GLC-79, GLC-

87, GLC-455 and GLC-458). The percentage of fatty acids were determined by the peak areas in the chromatograph. Then, values were adjusted according to percent fat and converted to mg/100 g tissue by the following equation:

$$\text{Fatty acid mg/100 g tissue} = (\text{Fatty acid peak area} * \text{fat of sample}) * 1000$$

2.2.5 Instrumental Color Evaluation

Objective color measurements were taken using the L*, a*, b* scales with a Minolta CR-400 colorimeter (Illuminant D65, 8 mm diameter aperture, 2° standard observer; Minolta, Osaka, Japan). Six measurements from different areas of the steak surface were taken through the overwrap film once daily at a standardized time from d 0 to 7 of RD.

2.2.6 Subjective Color (Discoloration)

Trained visual color panelists (n = 5) evaluated surface discoloration from d 0 to 7 of RD once daily. Ten steak images ranging from 0% to 100% surface discoloration with increments of 10% were used as a reference for panelist training. Panelists evaluated surface discoloration at 24-h intervals using a percentage scale where 0% meant no discoloration and 100% meant complete surface discoloration. Steaks were randomly rotated daily to minimize any possible location effects within the display.

2.2.7 Lipid Oxidation (TBARS)

Thiobarbituric acid reactive substance values (TBARS) were measured for each aging period at 0, 4 and 7 d of RD according to the procedure of Ahn et al. (1998). From each steak, 5 g of sample was blended with 1 mL of butylated hydroxyanisole (BHA) solution (10%) and 14 mL of distilled water. Samples were homogenized using a Polytron (Kinematica AG, Lucern, Sui) for 15 s and centrifuged (2,000 x g for 5 min). One mL of supernatant was mixed with 2 mL of thiobarbituric acid/trichloroacetic acid (TBA/TCA) solution (15% TCA and 20 mM TBA in deionized distiller water) and placed in a water bath at 70°C for 30 min. Samples were cooled in a water bath at 20°C for 10 min and centrifuged (2,000 x g for 5 min). After centrifugation, duplicate 200 µL of supernatant were transferred to 96-well plates and the absorbance at 540 nm was measured using a microplate spectrophotometer (Model Epoch, Biotek, Winooski, VT). The results were expressed in mg of malonaldehyde per kg of tissue.

2.2.8 Statistical Analysis

Objective and subjective color data were analyzed as a split-split-plot repeated measures design with dietary fat source as the whole-plot, aging period as the split-plot and retail display time as the repeated measures. Lipid oxidation data were analyzed as a split-split-plot design with dietary fat source as the whole-plot, aging period as the split-plot and retail display time as the split-split-plot. Fatty acid composition was analyzed as a completely randomized design. In this experiment, pen was considered the experimental unit. Data were analyzed using the PROC GLIMMIX procedure of SAS. All means were separated with the LS MEANS and DIFF functions and the TUKEY adjustment was used with $\alpha = 0.05$. Alpha values between 0.05 and 0.10 were considered as trends.

2.3 Results and Discussion

2.3.1 Proximate composition

In the present study dietary treatments did not affect proximate composition of beef ($P > 0.05$). The overall averages for the nutritional constituents were: 71.91% \pm 0.33 moisture, 19.77% \pm 0.27 protein, 6.96% \pm 0.07 fat, and 1.36% \pm 0.01 ash. No differences in marbling scores were found among dietary treatments ($P > 0.05$).

2.3.2 Fatty acid profile

Fatty acid values for all dietary treatments are presented in Table 2. Differences ($P < 0.05$) were found in the amount of stearic acid (C18:0), linoleic acid (C18:2), α -linolenic acid (C18:3) and PUFA among dietary treatments. Stearic acid (C18:0) was predominant in beef from cattle fed de-oiled MDGS plus oil ($P = 0.03$) in comparison with all other dietary treatments. Samples from cattle fed corn, full-fat MDGS and de-oiled MDGS did not differ from each other. Van der Pol et al. (2009) indicated that cattle receiving diets supplemented with corn oil had greater proportions of C18:0 reaching the duodenum when compared to cattle receiving WDGS. Therefore, greater amounts of stearic acid would be available to be deposited in muscle. This could explain the greater C18:0 concentration found in beef from cattle fed de-oiled MDGS supplemented with corn oil in this study.

In our study, feeding MDGS resulted in greater linoleic acid (C18:2) values in strip loins ($P = 0.0026$). The C18:2 content was lowest for beef from cattle fed corn (406.60 mg/100 g) in comparison to all MDGS dietary treatments (Table 2), which did not differ from each other (549.61 mg/100 g for the full-fat MDGS, 555.89 mg/100 g for the de-oiled MDGS, and 565.68 mg/100 g for the de-oiled MDGS plus oil treatment).

The α -linolenic (C18:3) content was least for steaks from cattle fed corn (13.59 mg/100 g) and greatest for the de-oiled MDGS plus oil (17.97 mg/100 g). Full-fat MDGS and de-oiled MDGS had intermediate values (15.99 mg/100 g and 14.05 mg/100 g, respectively) and did not differ from either (Table 2).

Type of diet influenced PUFA content ($P = 0.01$; Table 2). Beef from cattle fed corn had the lowest amount of PUFA (577.41 mg/100 g) in comparison to all MDGS dietary treatments (729.68 mg/100 g for the full-fat MDGS, 731.75 mg/100 g for the de-oiled MDGS, and 751.96 mg/100 g for the de-oiled MDGS plus oil group).

These results matches the results from previous studies, which also reported increases of C18:2 and PUFA content in the *Longissimus* muscle when beef cattle were fed finishing diets containing distillers grains compared with those consuming a diet without distillers grains (DOMENECH-PÉREZ et al., 2017; MELLO et al., 2012a, b). These results suggest that the fatty acid composition of beef is dependent on the dietary fat source. Particularly, the levels of linoleic acid and PUFA in beef have been recognized to be affected by diet.

In ruminant animals, a high proportion of unsaturated fatty acids are biohydrogenated to saturated fatty acids in the rumen, limiting PUFA deposition in muscle (JENKINS et al., 2008). In a review, Wood et al. (2008) indicated that only a small proportion, around 10% of dietary C18:2 is still available for incorporation into tissue after ruminal biohydrogenation. Increased ruminal biohydrogenation of 18-carbon unsaturated fatty acids for diets with higher lipid content was reported by Duckett et al. (2002). In the same study, authors indicated that feeding higher lipid diets increased duodenal flow of palmitic, stearic, oleic, linoleic, and arachidonic acids by more than 30%. Similarly, Vander Pol et al. (2009) also noted greater amount of PUFA reaching the duodenum when cattle were fed WDGS compared with corn.

Even though ruminant diets contains modest level of fat, the final concentration of fat is increased in diets with high amounts of ethanol byproducts (KLOPFENSTEIN et al., 2008). According to Ham et al. (1994), wet distillers grains plus solubles has double the amount of PUFA compared to corn. Therefore, the greater PUFA deposition observed in muscular tissue when corn was substituted for distillers grains in this study could be from increased dietary

supply of PUFA in cattle diets, and increased intestinal supply of PUFA due to higher lipid content in cattle rations.

Table 2. Amount¹ of fatty acids from *L. lumbarum* muscle of steers fed either a corn diet, 40% Full-fat MDGS, 40% De-oiled MDGS or 38% De-oiled MDGS plus corn oil.

Fatty Acids	Corn	Full-fat MDGS	De-oiled MDGS	De-oiled MDGS plus oil	P-value
C14:0	251.39	242.1	254.82	287.61	0.37
C14:1	70.61	65.31	70.99	75.81	0.74
C15:0	40.46	32.71	32.23	41.27	0.13
C15:1	115.99	134.60	87.21	87.10	0.14
C16:0	1828.8	1715.3	1868.5	2036.3	0.14
C16:1	236.26	220.69	242.3	250.14	0.70
C17:0	81.61	66.41	61.75	68.97	0.13
C17:1	67.11	61.92	75.92	79.04	0.42
C18:0	866.3 ^b	959.2 ^{ab}	946.2 ^{ab}	1100.0 ^a	0.03
C18:1T	10.23	22.44	23.77	23.93	0.07
C18:1	2246.0	2239.0	2229.0	2526.0	0.22
C18:1V	135.1	138.5	122.14	158.95	0.07
C18:2	406.6 ^b	549.6 ^a	555.8 ^a	565.6 ^a	0.002
C18:3	13.59 ^b	15.99 ^{ab}	14.05 ^{ab}	17.97 ^a	0.03
C20:4	104.38	96.61	98.82	91.22	0.72
Total	6636.9	6685.7	6830.9	7649.3	0.12
SFA	3139.7	3078.8	3242.2	3627.89	0.10
UFA	3497.1	3615.1	3588.7	4021.4	0.16
MUFA	2913.5	2885.5	2856.9	3627.89	0.18
PUFA	577.41 ^b	729.68 ^{ab}	731.75 ^a	751.96 ^a	0.011

¹Amount (mg/100 g tissue) of fatty acid in powdered strip loin sample.

^{a-b} Means in the same row with different superscripts differ ($P \leq 0.05$).

2.3.3 Objective color

For all three color scales, age by RD time interactions were detected ($P < 0.0001$). In general, L* values increased and a* and b* values decreased as aging and retail display time increased, regardless the dietary fat source.

There were no significant interactions that included L* and b* among dietary treatments. However, a two-way interaction between RD and dietary treatment was found for a* values ($P < 0.001$; Table 3). Specifically, lower a* values ($P \leq 0.05$) were found for beef from cattle fed de-oiled MDGS in comparison to all other dietary treatments at d 5 of RD. Steaks from

steers fed corn had greater a^* values than steaks from cattle fed de-oiled MDGS and de-oiled MDGS plus oil at d 6 of RD. However, full-fat MDGS did not differ from corn and de-oiled MDGS plus oil group at d 6 of RD. Greater a^* values ($P \leq 0.05$) were found for beef from cattle fed corn in comparison to beef from cattle fed de-oiled MDGS and de-oiled MDGS plus oil at d 7 of RD. Moreover, beef from cattle fed full-fat MDGS tended to have lower a^* values ($P = 0.10$) than cattle fed corn at d 7 of RD.

Meat purchasing decisions are mainly influenced by color because consumers use discoloration as an indicator of freshness. Myoglobin is the protein responsible for the bright cherry red color of beef. When myoglobin is oxidized to metmyoglobin, discoloration occurs, which leads consumers to discriminate against such products in the retail market. This results in product being either discounted or discarded (MANCINI; HUNT, 2005).

Many factors affect metmyoglobin formation. These include oxygen partial pressure, temperature, pH, mitochondrial oxygen consumption, metmyoglobin reducing activity, and lipid oxidation. Previous studies have reported that lipid oxidation enhances meat discoloration. The mechanisms by which lipid oxidation enhance myoglobin oxidation have been explained due the propagation of oxidation by PUFA (FAUSTMAN et al., 2010). This study showed that finishing diets including distillers grains at high rates (40%; DM basis) significantly decreased color stability of strip loins steaks when compared to a corn diet, as other studies have noted (MELLO et al., 2012a; ROEBER et al., 2005). Therefore, the reduced color stability of beef from cattle supplemented with MDGS is likely due to the propagation of oxidation caused by higher PUFA content observed in these samples. In general, these results suggest that at the onset of RD, MDGS had no effect on a^* values. However, as RD progressed, beef from cattle fed MDGS was less red than beef from cattle fed corn.

Table 3. Objective redness (a^* values) of strip loin steaks (*Longissimus lumborum*) from steers fed either a corn diet, 40% Full-fat MDGS, 40% De-oiled MDGS or 38% De-oiled MDGS plus 2% corn oil through 7 d of retail display (SEM = 0.23).

Treatment	Days on retail display								
	0	1	2	3	4	5	6	7	
Corn	21.24	21.40	20.71	20.08	19.37	18.13 ^a	16.20 ^a	13.79 ^a	
Full-fat MDGS	21.60	21.87	21.14	20.38	19.52	18.15 ^a	15.57 ^{ab}	13.05 ^{ab}	
De-oiled MDGS	21.86	21.82	21.04	20.32	18.80	17.04 ^b	14.54 ^c	12.33 ^b	
De-oiled MDGS +oil	22.00	21.93	21.16	20.31	19.07	17.91 ^a	15.22 ^{bc}	12.48 ^b	

^{a-c} Means in the same column with different superscripts differ ($P \leq 0.05$).

2.3.4 Discoloration

A two-way interaction between aging time and RD for discoloration was observed ($P < 0.0001$; Figure 1). At all aging periods, discoloration increased as retail display time increased, regardless the dietary fat source. A significant decline in purchasing decisions with 20% surface discoloration on retail displayed beef has been reported by Hood and Riordan (1973). In this study, the 20% discoloration threshold for steaks aged for 9 d was met by steaks from the de-oiled MDGS treatment at d 6 of RD, and at d 7 for the de-oiled MDGS plus oil and full fat MDGS treatments. Steaks from animals fed corn aged for 9 d had 14.72% discoloration at d 7 and therefore did not reach the discoloration threshold.

Steaks aged for 16 d met the 20% discoloration threshold at d 6 of RD for all dietary treatments. Steaks aged for 23 d met the discoloration threshold at d 6 of RD for all dietary treatments, except for de-oiled MDGS, which met the discoloration threshold at d 5 of RD.

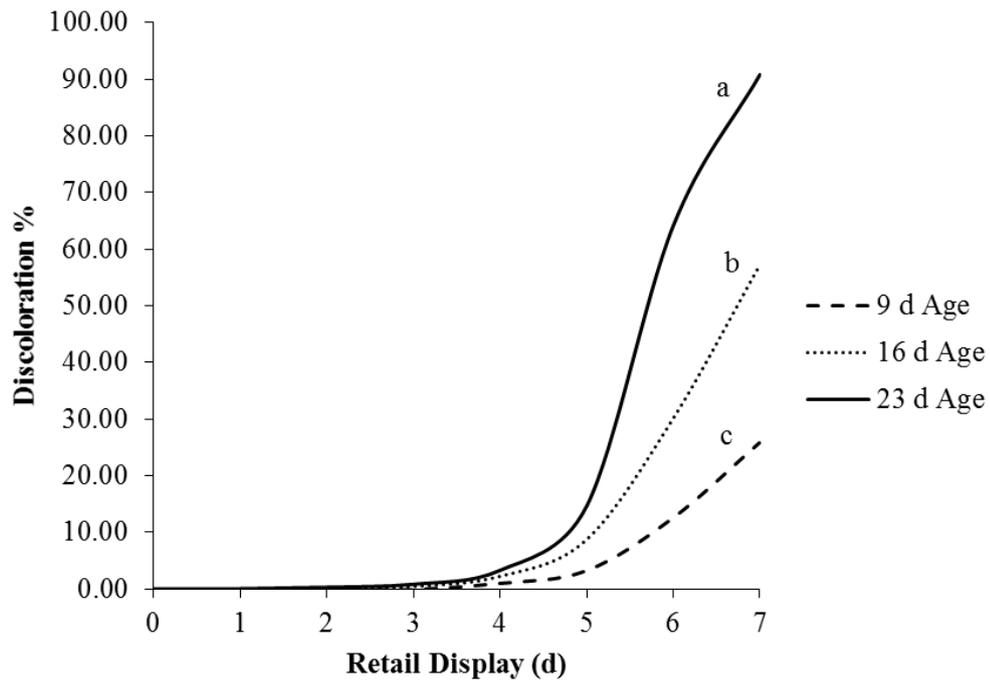


Figure 1. Age by retail display interaction ($P = 0.0001$) for subjective discoloration of samples aged 9, 16 and 23 d from 0 to 7 d retail display. ^{a-c} Different superscripts indicate differences within the same retail display time ($P \leq 0.05$).

A two-way interaction between dietary treatment and RD for discoloration was found ($P = 0.0006$). Surface discoloration scores of strip loin steaks at prolonged retail display are presented in Table 4. Beef from cattle fed de-oiled MDGS had greater discoloration ($P \leq 0.05$) than beef from any other dietary treatment at d 5 and 6 of RD. Beef from cattle fed full-fat MDGS tended to have more discoloration than beef from cattle fed corn ($P = 0.0614$) at d 6 of RD.

Table 4. Discoloration (%) of strip loins steaks (*Longissimus lumborum*) from steers fed either a corn diet, 40% Full-fat MDGS, 40% De-oiled MDGS or 38% De-oiled MDGS plus 2% corn oil through 7 d of retail display (SEM = 1.00).

Treatment	Days on retail display							
	0	1	2	3	4	5	6	7
Corn	0	0	0	0.15	1.11	5.70 ^b	31.39 ^b	49.82 ^c
Full-fat MDGS	0	0.05	0.26	0.76	2.06	7.13 ^b	35.85 ^{ab}	58.08 ^b
De-oiled MDGS	0	0.02	0.10	0.54	4.04	14.63 ^a	41.32 ^a	65.16 ^a
De-oiled MDGS +oil	0	0	0.06	0.24	1.57	7.99 ^b	33.76 ^b	58.64 ^b

^{a,c} Means in the same column with different superscripts differ ($P \leq 0.05$).

At d 7 RD, discoloration scores were least for cattle fed corn, intermediate for cattle fed full-fat MDGS and de-oiled MDGS plus oil, and greatest for the de-oiled MDGS. These results suggest that as retail display progressed, discoloration progressed at slower rates in beef from cattle fed corn in relation to cattle fed MDGS. Thus, lower PUFA concentration in steaks from cattle fed corn in relation to cattle fed MDGS may have allowed greater color stability during display.

2.3.5 Lipid oxidation

There was an age by RD interaction for lipid oxidation ($P < 0.001$). As expected, lipid oxidation was favored by long-term storage and increased TBARS values were seen as aging and RD progressed (Figure 2). Greater lipid oxidation values were observed in samples aged 23 d, followed by samples aged for 16 and 9 d, which did not differ from each other. As expected, samples aged for 2 d had the lowest lipid oxidation values. Within all aging periods, greater TBARS values were seen as RD progressed from d 0 to d 4 and d 7.

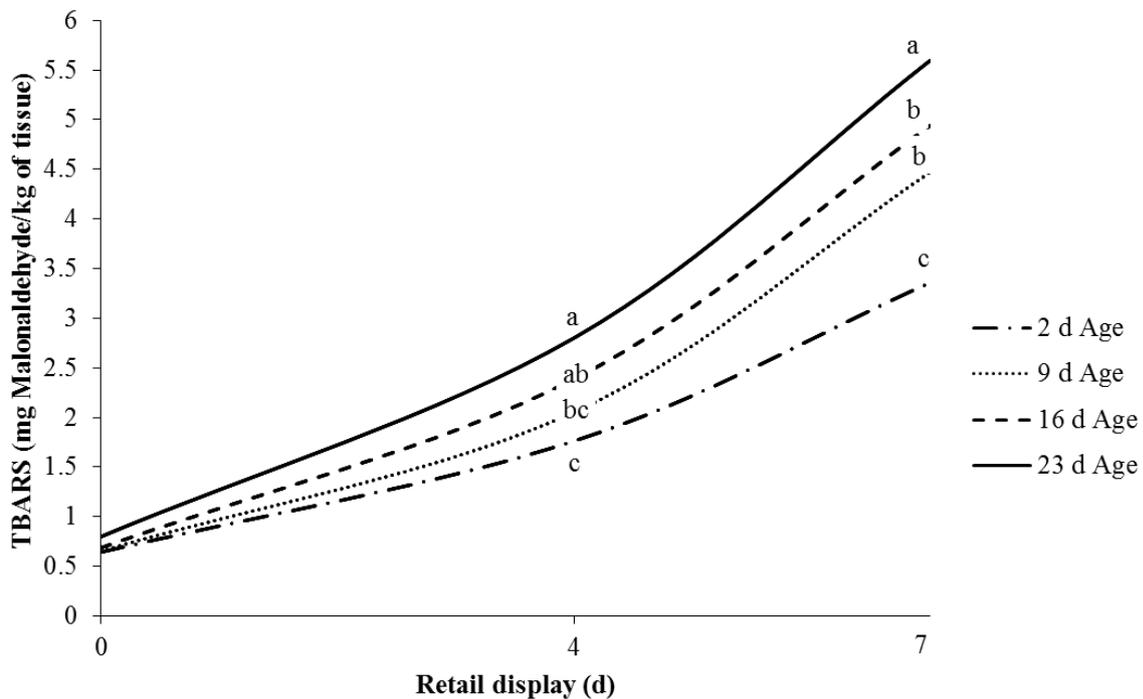


Figure 2. Age by retail display interaction ($P = 0.0010$; SEM = 0.17) for lipid oxidation of samples aged 2, 9, 16 and 23 d with 0, 4 and 7 d retail display. ^{a-c} Different superscripts indicate differences within the same retail display time ($P \leq 0.05$).

A significant interaction between dietary treatment and RD was found for lipid oxidation ($P = 0.001$; Table 5). No differences in lipid oxidation were found among dietary treatments at 0 and 4 d of RD. Beef from cattle fed corn tended to have lower lipid oxidation ($P \leq 0.10$) when compared with de-oiled MDGS and de-oiled MDGS plus oil (3.90 vs 4.94 and 4.90 mg malonaldehyde/kg of meat, respectively) at d 7 of RD. Cattle fed full-fat MDGS had intermediate TBARS values (4.45 mg malonaldehyde/kg of meat) and did not differ from any other dietary treatment at d 7 of RD (Table 5).

Table 5. Lipid oxidation value (TBARS; mg malonaldehyde /kg of meat) of strip loin steaks (*longissimus lumborum*) from steers fed either a corn diet, 40% Full-fat MDGS, 40% De-oiled MDGS or 38% De-oiled MDGS plus 2% corn oil with 0, 4 and 7 d retail display.

	Dietary treatments					SEM	P-value
	d	Corn	Full-fat MDGS	De-oiled MDGS	De-oiled MDGS + oil		
TBARS						0.28	0.001
	0	0.75 ^a	0.75 ^a	0.72 ^a	0.76 ^a		
	4	2.07 ^a	2.16 ^a	2.44 ^a	2.43 ^a		
	7	3.98 ^b	4.45 ^{ab}	4.94 ^a	4.90 ^a		

^{a,b} Means in the same row with different superscripts differ ($P \leq 0.10$).

In this study, feeding MDGS to cattle increased PUFA content of beef ($P = 0.011$). Fatty acid stability is dependent on the number of double bonds (HORWITT, 1986). Unsaturated fatty acids are more easily oxidized in comparison to saturated fatty acids (ZHANG et al., 2007). Therefore, a probable reason for the increase in rancidity could be due to the fact that steers fed de-oiled MDGS and de-oiled MDGS plus oil had greater PUFA content, which are more susceptible to lipid oxidation.

Lipid oxidation that occurs during beef storage can affect beef color, flavor, aroma, and consequently, shelf life (MORRISSEY et al., 1994; GRAY et al., 1996; LADEIRA et al., 2014). Campo et al. (2006) suggested that a TBARS value of 2.28 mg/kg could be considered as the limiting threshold for acceptability of oxidation in beef because at this point the perception of rancidity overpowers beef flavor. In this study, beef from cattle fed corn and full fat MDGS remained below this limiting threshold (2.07 and 2.16 mg malonaldehyde/kg, respectively) after 4 d under RD condition. Beef from de-oiled MDGS and de-oiled MDGS plus oil treatments groups overcame this acceptability threshold at d 4 of RD (2.44 and 2.43 mg malonaldehyde/kg,

respectively). All dietary treatments overcame this threshold for oxidized beef acceptability at d 7 of RD.

Lipid and myoglobin oxidation in meat often appear to be linked. Oxidation of one these leads to the formation of species that can accelerate oxidation of the other (FAUSTMAN et al., 2010). In our study, lipid oxidation values followed a similar pattern with the subjective and objective color scores. These results suggest that feeding MDGS to cattle may lead to faster reduction in beef display life.

2.4 Conclusion

Feeding MDGS resulted in increased PUFA content of the meat in comparison to corn finishing diet. Results suggest that with prolonged aging periods and retail display, feeding MDGS to cattle has the potential to reduce color and lipid stability compared to corn and thus reduce shelf life. Addition of corn oil to de-oiled MDGS decreased redness and increased discoloration and lipid oxidation in comparison to corn diets.

References

AHN, D.U.; OLSON, D.G.; JO, C.; CHEN, X.; WU, C.; LEE, J.I. Effect of muscle type, packaging, and irradiation on lipid oxidation, volatile production, and color in raw pork patties. **Meat Science**, Barking, v. 47, p. 27-39, 1998.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. **Official methods of analysis of the AOAC International**. 18th ed. Arlington, 1990. 2200p.

CAMPO, M.M.; NUTE, G.R.; HUGHES, S.I.; ENSER, M.; WOOD, J.D.; RICHARDSON, R.I. Flavour perception of oxidation in beef. **Meat Science**, Barking, v. 72, p. 303-311, 2006.

DOMENECH-PÉREZ, K.I.; CALKINS, C.R.; CHAO, M.D.; SEMLER, M.E.; VARNOLD, K.A.; ERICKSON, G.E. Impact of feeding de-oiled wet distillers grais plus solubles on beef shelf life. **Journal of Animal Science**, Albany, v. 95, p. 709–717, 2017.

DUCKETT, S.K.; ANDRAE, J.G.; OWENS, F.N. Effect of high-oil corn or added corn oil on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. **Journal of Animal Science**, Albany, v. 80, p. 3353–3360, 2002.

FAUSTMAN, C.; SUN, Q.; MANCINI, R.; SUMAN, S.P. Review: Myoglobin and lipid oxidation interactions: Mechanistic bases and control. **Meat Science**, Barking, v. 86, p. 86-94, 2010.

FOLCH, J.; LEES, M.; STANLEY, G.H.S. A simple method for the isolation and purification of total lipids from animal tissue. **Journal of Biological Chemistry**, Rockville, v. 226, p. 497-509, 1957.

GRAY, J.I.; GOMAA, E.A.; BUCKLEY, D.J. Oxidative quality and shelf life of meats. **Meat Science**, Barking, v. 43, p. 11-23, 1996.

HAM, G.; STOCK, R.; KLOPFENSTEIN, T.; LARSON, E.; SHAIN, D.; HUFFMAN, R. Wet corn distillers byproducts compared with dried corn distillers grains with solubles as a source of protein and energy for ruminants. **Journal of Animal Science**, Albany, v. 72, p. 3246–3257, 1994.

HOOD, D.E.R.; RIORDAN, E.B. Discoloration in prepackaged beef: Measurement by reflectance spectrophotometry and shopper discrimination. **International Journal of Food Science and Technology**, Edinburgh, v. 8, p. 333–343, 1973.

HORWITT, M.K. Interpretations of requirements for thiamin, riboflavin, niacin-tryptophan, and vitamin E plus comments on balance studies and vitamin B-6. **The American Journal of Clinical Nutrition**, Rockville, v. 44, p. 973-985, 1986.

JENKINS, T.C.; WALLACE, R.J.; MOATE, P.J.; MOSLEY, E.E. Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. **Journal of Animal Science**, Albany, v. 86, p. 397–412, 2008.

JOLLY, M.L.; NUTTELMAN, B.L.; BURKEN, D.; SCHNEIDER, C.J.; KLOPFENSTEIN, T.J.; ERICKSON, G.E. Effects of modified distillers grains plus solubles and condensed distillers solubles with and without oil extraction on finishing performance. **Nebraska Beef Cattle Report**, Lincoln, v. 98, p. 64-65, 2013.

KLOPFENSTEIN, T.J.; ERICKSON, G.E.; BREMER, V.R. Board-invited review: Use of distillers by-products in the beef cattle feeding industry. **Journal of Animal Science**, Albany, v. 86, p. 1223–1231, 2008.

LADEIRA, M.M.; SANTAROSA, L.C.; CHIZZOTTI, M.L.; RAMOS, E.M.; MACHADO NETO, O.R.; OLIVEIRA, D.M.; CARVALHO, J.R.R.; LOPES, L.S.; RIBEIRO, J.S. Fatty acid profile, color, lipid oxidation of meat from Young bulls fed soybean or rumen protected fat with or without monensin. **Meat Science**, Barking, v. 96, p. 597-605, 2014.

MANCINI, R.A.; HUNT, M.C. Current research in meat color. **Meat Science**, Barking, v. 71, p. 100-121, 2005.

MELLO, A.S.; CALKINS, C.R.; JENSCHKE, B.E.; CARR, T.P.; DUGAN, M.E.R.; ERICKSON, G.E. Beef quality of calf-fed steers finished on varying level of corn-based wet distillers grains plus solubles. **Journal of Animal Science**, Albany, v. 90, p. 4625-4633, 2012a.

MELLO, A.S.; JENSCHKE, B.E.; SENARATNE, L.S.; CARR, T.P.; ERICKSON, G.E.; CALKINS, C.R. Effects of feeding modified distillers grains plus solubles on marbling attributes, proximate composition, and fatty acid profile of beef. **Journal of Animal Science**, Albany, v. 90, p. 4634-4640, 2012b.

METCALFE, L.D.; SCHMITZ, A.A.; PELKA, J.R. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. **Analytical Chemistry**, Moscow, v. 38, p. 514–515, 1996.

MORRISON, W.R.; SMITH, L.M. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. **Journal of Lipid Research**, Rockville, v. 5, p. 600–608, 1964.

MORRISSEY, P.A.; BUCKLEY, D.J.; SHEEHY, P.J.; MONAHAN, F.J. Vitamin E and meat quality. **Proceedings of the Nutrition Society**, Cambridge, v. 53, p. 289-295, 1994.

VANDER POL, K.; LUEBBE, M.; CRAWFORD, G.I.; ERICKSON, G.E.; KLOPFENSTEIN, T.J. Performance and digestibility characteristics of finishing diets containing distillers grains, composites of corn processing coproducts, or supplemental corn oil. **Journal of Animal Science**, Albany, v. 87, p. 639–652, 2009.

Renewable Fuels Association. Statistics. 2017. Available at: <<http://www.ethanolrfa.org/pages/statistics>>. Accessed on: 14 March 2017.

ROEBER, D.L.; GILL, R.K.; DiCONSTANZO, A. Meat quality responses to feeding distillers grains to finishing Holstein steers. **Journal of Animal Science**, Albany, v. 83, p. 2455–2460, 2005.

WOOD, J.D.; ENSER, M.; FISHER, A.V.; NUTE, G.R.; SHEARD, P.R.; RICHARDSON, R.I.; HUGHES, S.I.; WHITTINGTON, F.M. Fat deposition, fatty acid composition and meat quality: A review. **Meat Science**, Barking, v. 78 p. 343–358, 2008.

ZHANG, W.; SHI, B.; SHI, J. A theoretical study on autoxidation of unsaturated fatty acids and antioxidant activity of phenolic compounds. **Journal of the American Leather Chemists Association**, Lubbock, v. 102, p. 99-105, 2007.

ZINN, R.A.; GULATI, S.K.; PLASCENCIA, A.; SALINAS, J. Influence of ruminal biohydrogenation on the feeding value of fat in finishing diets for feedlot cattle. **Journal of Animal Science**, Albany, v. 78, p. 1738–1746, 2000.

3 THE MECHANISM BY WHICH DIETARY FAT SOURCE MAY AFFECT BEEF TENDERNESS

Abstract

The objective of this study was to understand the effects of the dietary fat source on the beef tenderization mechanism. Steers ($n=256$) were fed for 134 d on either corn, or a diet containing 40% full-fat modified distillers grains plus solubles (MDGS), 40% de-oiled MDGS, or 38% de-oiled MDGS plus 2% corn oil. Twenty-four USDA Choice carcasses (3 head/pen) were randomly selected within each dietary treatment and strip loins were collected and aged for 2, 9, 16, or 23 d. Steaks from each aging period were placed under retail display (RD) conditions for 0 and 7 d and shear force was evaluated. Feeding MDGS decreased ($P < 0.05$) concentrations of 18:1V, increased ($P < 0.05$) concentrations of linoleic acid (18:2) and tended to increase ($P = 0.06$) total PUFA in the sarcoplasmic reticulum (SR) membrane. Steaks from cattle fed MDGS had greater free calcium concentration than steaks from cattle fed corn at d 2 postmortem ($P = 0.05$). Steaks from steers fed de-oiled MDGS and de-oiled MDGS plus corn oil tended to have lower Warner-Bratzler shear force values ($P = 0.08$) than steaks from cattle fed corn at 2 d of aging with 0 day of retail display. No difference were found among dietary treatments for slice shear force values. There were no differences among dietary treatments for sarcomere length ($P = 0.92$) and troponin-T degradation at 2 d postmortem ($P = 0.60$). Results suggest that feeding MDGS may increase early-postmortem release of free calcium due increased 18:2 concentration in the SR membrane, which could result in increased beef tenderness early-postmortem.

Keywords: Beef quality; Calcium flux; Distillers grains; Sarcoplasmic reticulum

3.1 Introduction

Among the palatability traits that govern eating quality of beef, tenderness has been ranked as the most important trait for both eating quality and consumer purchasing decisions (MILLER et al., 2001; PLATTER et al., 2005).

Outcomes of several studies have provided conflicting results regarding the effects of feeding distillers grains on beef tenderness. Although several studies have shown no differences on beef tenderness from feeding distillers grains to cattle (MELLO et al., 2012a; KOGER et al., 2010; ROEBER et al., 2005), some recent studies conducted at the University of Nebraska revealed that beef from steers fed wet distillers grains plus solubles (WDGS) was more tender than beef from steers fed corn-only (SENARATNE, 2012; CHAO, 2015). Likewise, in a feeding trial including 15%, 30% or 45% de-oiled modified distillers grains plus solubles (MDGS) with either dry-rolled corn or corn oil, steaks from cattle receiving 15% de-oiled MDGS with corn oil had higher shear force values at day 3 postmortem ($P < 0.01$) than steaks

from cattle finished with 15%, 30% and 45% de-oiled MDGS with no corn oil addition (NELSON, 2016).

Although distillers grains are considered a corn by-product, they have more energy per kg than corn on a dry matter basis. Ethanol production utilizes the starch fractions of grains, concentrating the levels of other nutrients. Hence, distillers grains have up to three times more fat and protein than corn (KLOPFENSTEIN et al., 2008). Research has shown that feeding distillers grains to cattle could not only increase the concentration of polyunsaturated fatty acids (PUFA) in meat (RIBEIRO et al., 2017; MELLO et al., 2012a, b), but also increase PUFA content of the sarcoplasmic reticulum (SR) membrane (CHAO, 2017a).

Chao et al., (2017a) hypothesized that increased PUFA content of the SR membrane due feeding distillers grains would predispose the SR membrane to release calcium earlier postmortem than normal as a result of rapid membrane oxidation, causing an early activation of calcium dependent proteases (the calpain system) and thereby enhance beef tenderness early-postmortem.

The mechanism of meat tenderization is a well understood subject. However, the way animal diets with high concentration of distillers grains might affect the basic mechanism of meat tenderization requires further exploration. Therefore, the objective of this study was to evaluate the effects of dietary fat source with MDGS on the beef tenderization mechanism through examination of tenderness, sarcomere length, and proteolysis early postmortem, as well as changes in SR membrane fatty acid composition.

3.2 Materials and Methods

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

3.2.1 Cattle and Dietary Treatments

A total of 256 Angus crossbreed steers were fed for 134 d on either a corn, 40% full-fat MDGS, 40% de-oiled MDGS, or 38% de-oiled MDGS plus 2% corn oil diet. Cattle were blocked by body weight and randomly assigned 8 per pen for a total of 32 pens (8 pens/treatment). The dietary treatments are presented in Table 1 (see chapter 2, page 20).

3.2.2 Sample Collection and Fabrication

Steers were harvested at a commercial plant (Greater Omaha Packing Co., Omaha NE). After 48 h postmortem chilling, 96 carcasses were selected (24 per dietary treatment) based on quality grade. At the time of grading, 3 USDA Choice carcasses were selected per pen. Both strip loins (*Longissimus lumborum*) from all selected carcasses were collected, vacuum-packaged, and transported to the University of Nebraska Meat Laboratory.

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. Then, both left and right strip loins were divided in half, and each of the four sections per animal were randomly assigned to one of the four aging periods: 2, 9, 16, or 23 d.

Loins sections assigned for 2 d of aging were immediately trimmed of subcutaneous fat, and fabricated into three steaks (2.54 cm thickness; 1 steak for SSF and WBSF for 0 d of retail display (RD), 1 steak for SSF and WBSF for 7 d RD, and 1 steak for sarcomere length, sarcoplasmic reticulum (SR) membrane fatty acid, free calcium concentration, and troponin-T degradation for 0 d of RD.

After fabrication, steaks assigned to 0 d of RD were vacuum packaged and frozen at -80°C , except for the shear force steaks that were frozen at -20°C . Steaks assigned to 7 d of RD were placed on foam trays (21.6 x 15.9 x 2.1 cm, Styro-Tech, Denver, CO), overwrapped with oxygen permeable film (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO; oxygen transmission rate = 2.25 ml/cm²/24 hr at 23° C and 0% relative humidity; water vapor transfer rate = 496 g/m²/24 hr at 37.8° C and 90% relative humidity) and placed under RD conditions for 7 d (under continuous white fluorescence lighting at 1000 to 1800 lux) at 3°C. After 7 d of RD, steaks were stored at -20°C until use.

Steaks used for sarcomere length, SR membrane fatty acid, free calcium concentration, and troponin-T degradation were removed from the freezer, cut by hand into small cubes, and frozen in liquid nitrogen, powdered in a metal cup blender (Model 51BL32, Waring Commercial, Torrington, CT) and stored at -80°C until further analysis.

The same fabrication scheme was used at 9, 16 and 23 d postmortem for SSF and WBSF analysis. Sarcomere length, SR membrane fatty acid, free calcium concentration, and troponin-T degradation were analyzed only at d 2 postmortem.

3.2.3 Sarcoplasmic reticulum (SR) membrane extraction

The SR membrane was extracted using the procedure described by Hemmings (2001). Ten g of powdered samples were suspended in ice-cold 35 mL homogenization buffer (10 mM NaHCO₃, 2 mM sodium azide, 10 mM Tris-Cl, and 1 mM dithiothreitol) at pH 7.5 and homogenized using a Polytron homogenizer (Kinematica, Luzern, Switzerland) for 15 s. Homogenate was transferred into a 50 mL plastic centrifuge tube and centrifuged at 2,000 × *g* for 10 min at 4°C. The supernatant was collected and centrifuged at 10,000 × *g* (Sorvall RC5B Superspeed Centrifuge; Thermo Scientific, Rockford, IL) for 30 min at 4°C. Four and a half percent of KCl (w/v) was added to the supernatant and the samples were placed on a shaker platform for 30 min on ice. The supernatant was centrifuged at 100,000 × *g* for 60 min at 4°C (Beckman L7-65 Ultracentrifuge with a SW28 rotor; Beckman Coulter). The final supernatant was discarded, and the pellet was suspended in 1 mL of 10 mM tris buffer and stored (-80°C) until further analysis.

3.2.4 Sarcoplasmic reticulum membrane lipid extraction

The SR membrane lipid was extracted following the procedure described by Bligh and Dyer (1959) with modifications. Initially, 3.75 mL of 1:2 chloroform: methanol was added to the SR membrane and tris buffer mixture from the previous extraction. The tube was vortexed for 5 s, mixed on a shaker for 20 min and stored overnight at 4°C. The following morning, 1.25 mL of chloroform was added, and the sample was mixed on a shaker platform for 1 min. After shaking, 1.25 mL of ddH₂O was added, and the sample was mixed again on a shaker platform for 1 min. The homogenate was filtered through a Whatman #2 filter paper (Whatman, Clifton, NJ) into 13 x 100 mm screw cap tube. Samples were centrifuged at 1,000 × *g* for 5 min. Following centrifugation, the aqueous phase (top layer) was removed with a Pasteur pipet. The remaining was evaporated to dryness under nitrogen at 60°C.

3.2.5 Sarcoplasmic reticulum membrane fatty acids

After extraction, lipids were converted to fatty acid methyl esters according to the procedures by Morrison and Smith (1964) and Metcalfe et al. (1966). Initially, 0.5 mL of 0.5 M NaOH in methanol was added to the extracted SR membrane lipid, and the solution was vortexed for 5 sec and heated for 5 min at 100°C. After heating, 0.5 mL of boron trifluoride in

14% methanol was added into the solution, and the solution was vortexed for 5 s and heated for 5 min at 100°C. One mL of a saturated NaCl solution and 1 mL of hexane were added to the solution, and the solution was centrifuged at 1,000 x g for 5 min. Following centrifugation, the hexane top layer was removed and placed in a gas chromatography (GC) vial. The prepared fatty acid methyl esters were further concentrated by drying at 60°C under constant nitrogen gas purging and mixed with 150 µL of hexane. The fatty acid methyl esters were transferred to 200 µL spring bottom vial inserts and inserted into the GC vials. The fatty acids were analyzed using gas chromatography (Hewlett-Packard 6890 FID GC System; Agilent Technologies, Santa Clara, CA) with a capillary column (Chrompack CP-Sil 88 (0.25 mm x 100 m)). Oven temperature was programmed from 140 to 220°C at 2°C/min and held at 220°C for 20 min. Injector and detector temperature was maintained at 270 and 300°C, respectively. The carrier gas Helium had a flow rate of 30 mL/min. Fatty acid were identified by comparison of retention times with known standards.

3.2.6 Free calcium concentration

Free calcium was quantified according to the procedure described by Parrish et al. (1981) with modifications. Three g of powdered strip loin was transferred to an ultracentrifuge tube (13 × 55 mm; Beckman Coluter, Brea, CA) and centrifuged at 196,000 x g (Beckman L7-65 Ultracentrifuge with a SW55Ti rotor; Beckman Coluter) at 4°C for 30 min. After centrifugation, 700 µL of the supernatant were collected and transferred to an 2 mL Eppendorf tube, treated with 0.1 mL of 27.5% trichloroacetic acid (TCA) and vortexed for 10 s. Samples were centrifuged at 6,000 x g (Eppendorf model 5430; Eppendorf, Hamburg, Germany) for 10 min. After centrifugation, 400 µL of supernatant were transferred to a plastic syringe, and the volume was brought to 4 mL with deionized distilled water. The diluted calcium sample was filtered through 13 mm diameter Millex-LG 0.20 µm syringe filters (Millipore, Bedford, MA). Calcium concentration was quantified at Ward Laboratories (Kearney, NE) using an inductively-coupled plasma emission spectrometer (iCAP 6500 Radial; Thermo Electron, Cambridge, UK) with calcium concentration standards of 0, 25, 50, 150 mg/L.

3.2.7 Tenderness determination

Steaks (2.54 cm thick) were removed from the freezer, thawed at 3°C for 24 hours, and then steak internal temperature and weight were recorded prior to cooking. Temperature was recorded for each steak using an insulated T thermocouple (5SC-TTT-30-120, OMEGA Engineering, Inc., Stamford, CT) connected to a handheld thermometer (OMEGA 450-ATT, Engineering, Inc., Stamford, CT). The thermocouples were inserted into the geometric center of each steak with large needles.

All steaks were cooked to a target temperature of 71°C on a Belt Grill (TBG60-V3 MagiGril, MagiKitch'n Inc., Quakertown, PA). Belt grill specifications were as follows: preheat = 149°C, top heat = 163°C, bottom heat = 163°C, height of gap = 2.16 cm, and cook time was approximately 6 min. After cooking, internal temperature and weight were recorded, and slice shear force evaluation was conducted using a Food Texture Analyzer (TMS-Pro, Food Technology Corp., Sterling, VA.) with a Slice Shear force blade. The remainder of the steak was individually bagged and stored overnight at 2°C for further WBSF analysis. At the following d, six (1.27 cm diameters) cores were removed with a drill press parallel to the orientation of the muscle fibers. Cores were measured using a Food Texture Analyzer (TMS-Pro, Food Technology Corp., Sterling, VA.) with a Warner-Bratzler blade. Peak WBSF values from each steak were averaged for statistical purposes.

3.2.8 Sarcomere length

Sarcomere length determination was made using the helium-neon laser diffraction method as described by Cross et al. (1981) and Dolazza and Lorenzen (2014). A few specks of powdered samples were placed on a clear glass microscope slide. Then, a drop of 0.25 M sucrose was added to the glass slide, and a coverslip was used to cover the samples. The distance between the top of the slide to the base of laser stand was set at 100 mm. A paper sheet was placed underneath the stand. The samples were moved under the laser light until a diffraction pattern was observed. The two diffraction bands were recorded on the white paper. Six sarcomeres per sample were determined, and sarcomere length (μm) was determined by the equation provided by Cross et al. (1981):

Sarcomere length (μm) =

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

Where:

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

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

T = spacing between diffraction bands (mm)

3.2.9 Myofibrillar protein isolation

Myofibrillar proteins were isolated according to Pietrzak et al. (1997) with modifications. Three g of powdered meat were placed into a 50 mL conical tube and suspended in ice-cold 15 mL rigor buffer (0.1 M KCl, 2 mM MgCl₂, 1 mM EGTA, and 10 mM K₂HPO₄) and homogenized using a Polytron (model CH-6010; Kinematica) for 5 s bursts (at setting 6) until thoroughly mixed. Homogenate was filtered through double-layered cheese cloth into a 50 mL conical tube. Homogenate (1.4 mL) was pipetted into an Eppendorf tube (2 mL safe-lock tubes; 02236352, Eppendorf AG, Hamburg, Germany) and centrifuged at 4,000 $\times g$ for 5 min. The supernatant was decanted and the pellet was resuspended in 1 mL of ice-cold rigor buffer. Suspended pellet was vortexed for 10 s and centrifuged for 5 min at 4000 $\times g$. The pellet washing step was repeated three times. One mL of extraction buffer (0.1 M Tris-HCl, 1.25 mM EDTA, 5% SDS) was added to the washed pellet and vortexed for 30 s. Then, samples were centrifuged at 4,000 $\times g$ for 5 min and the supernatant was placed into a new Eppendorf tube for determination of protein concentration. Samples were kept at -80°C overnight and protein concentration was determined on the following day.

3.2.10 Protein concentration

One hundred μL of myofibrillar protein samples were transferred to an Eppendorf tube. The samples were diluted with 900 μL of extraction buffer and vortexed for 10 s. Protein concentration was determined using a Pierce bicinchoninic acid (BCA) protein assay kit (Pierce Biotechnology, Rockford, IL). A concentration series (20 – 2,000 $\mu\text{g}/\text{mL}$) of bovine serum albumin (BSA) standards were prepared using the extraction buffer as the diluent. Twenty-five μL of BSA standards and diluted myofibrillar protein samples were deposited into their

respective well on a 96 wells microplate (Microtest III sterile 96-well flat-bottomed microplate; Becton Dickinson & Company). Two hundred μL BCA working reagents (50:1 of Reagent A: Reagent B; Pierce Biotechnology) were added to each respective well on the microplate and incubated at 37°C for 30 min. After cooling to the room temperature (23°C) for 10 min, absorbance was read at 562 nm, and protein concentrations were expressed as $\mu\text{g}/\text{mL}$. All myofibrillar protein samples were diluted to 2 mg/mL with ddH₂O.

3.2.11 Gel electrophoresis

Degree of proteolysis was measured by troponin-T degradation. All of the following procedures were conducted at room temperature. Twenty-five μL of the 2 mg/mL myofibrillar protein samples were mixed with 2x Laemmli sample buffer (65.8 mM Tris-HCl, 2.1% SDS, 26.3% glycerol, 0.01% bromophenol blue) with 5% β -mercaptoethanol at 1:1 ratio. All samples were heated at 95°C for 5 min. Ten μL Kaleidoscope Pre-stained Protein Standard and prepared myofibrillar protein samples (5 μg) were loaded on 4-20% Mini-PROTEAN TGX™ precast polyacrylamide gels (Bio Rad Laboratories, Hercules, CA) using a Bio-Rad Mini-PROTEIN 2 Cells system (Bio-Rad Laboratories). The system was run at constant voltage of 200 V for 60 min with a running buffer consisted of 25 mM Tris-base, 192 mM glycine and 0.1% SDS (pH 8.3).

3.2.12 Western Blotting

Proteins in the gels were blotted to polyvinylidene difluoride (PVDF) membranes (0.45 μm ; Immobilon-FL transfer membrane; Millipore) using a Bio-Rad Mini-Trans-Blot Electrophoretic transfer cell (Bio-Rad Laboratories) for 60 min at a constant amperage of 180 mA with ice-cold transfer buffer (25 mM Tris-base, 192 mM glycine, 20% methanol, 0.1% SDS at pH 9.2). Membranes were placed in a container with 15 mL of Odyssey Blocking Buffer (LI-COR, Lincoln, NE) for 2 h on a rocking platform and incubated in primary antitroponin-T (JLT-12; Sigma-Aldrich, St. Louis, MO) antibody at a dilution of 1:10,000 in Odyssey blocking buffer containing 0.2% Tween-20 for 1 h. Membranes were washed 3 times on a rocking platform with 15 mL of Tris Buffered Saline containing 0.2% Tween-20 for 10 min and incubated in IRDye 680LT Conjugated Goat Anti-Mouse IgG1 (LI-COR) secondary antibody at a dilution of 1:10,000 in Odyssey blocking buffer containing 0.2% Tween-20 for 1 h. Membranes were washed 3 times on a rocking platform and scanned using Odyssey Infrared

Imaging system (LI-COR) at 700 nm. All intact troponin-T and degraded troponin-T products were measured by quantifying band intensities (k. pixels) using Odyssey application software version 1.1. Bands 1 and 2 (38 and 35 kD, respectively) corresponded to intact troponin- T while bands 3 and 4 (30 and 28 kD, respectively) correspond to degraded troponin-T. Percent troponin-T degraded was measured by band intensities of degraded bands divided by band intensities of all bands in a specific lane.

3.2.13 Statistical Analysis

Tenderness data were analyzed as a split-plot design with dietary fat source as the whole-plot and aging period as the split-plot. Sarcomere length, SR membrane fatty acid, free calcium concentration, and troponin-T degradation were analyzed as a completely randomized design. In this experiment, pen was considered the experimental unit. Data were analyzed using the PROC GLIMMIX procedure of SAS. All means were separated with the LS MEANS and DIFF functions with $\alpha = 0.05$. Alpha values between 0.05 and 0.10 were considered as trends.

3.3 Results and Discussion

3.3.1 Sarcoplasmic reticulum (SR) membrane fatty acid profile

Results for the SR membrane fatty acid profile analyses are presented in Table 1. Feeding MDGS decreased ($P < 0.05$) concentrations of 18:1V, increased ($P < 0.05$) concentrations of linoleic acid (18:2) and tended to increase ($P = 0.06$) total PUFA in the SR membrane. Long chain PUFA were not affected by the dietary treatment. These results followed the same pattern observed for beef muscle tissue fatty acid profile, which also had increased 18:2 and PUFA content for meat from cattle finished on MDGS in comparison to corn finishing diet (Table 2; chapter 2). Interestingly, finishing diet did not alter marbling scores ($P = 0.78$) or lipid content ($P = 0.30$) in this experiment (data not shown). Similar results were reported by Mello et al. (2012b), who found no differences in marbling scores and fat content among diets when different levels of MDGS (0, 10, 20, 30, 40, or 50%) were fed to cattle.

The concentration of individual fatty acids is in the range proposed Chao et al. (2017a). They suggested that the PUFA concentration for SR membrane is four times more than the PUFA content in muscle tissue, which is in agreement with our results. Furthermore, results

from both studies follow the same pattern with increased 18:2 and PUFA concentration in SR membrane when high concentration of distillers grains were included in the finishing diet.

The levels of linoleic acid and PUFA in beef have been recognized to be affected by diet. Our results suggest that the fatty acid composition of beef and SR membrane is dependent on the dietary fat source. Although unprotected from the environment of the rumen, where PUFA are biohydrogenated, 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).

Atkinson et al. (2006) indicate that greater proportions of grains in cattle diets lead to greater unsaturated fatty acid due to less ruminal biohydrogenation. Furthermore, Vander Pol et al. (2009) associate the inclusion of WDGS in cattle diets with a protection effect, which could protect unsaturated fatty acids from biohydrogenation thus allowing greater amounts of fatty acids being available for absorption in the small intestine. In the same study, these authors also reported greater amounts of 18:2 and PUFA reaching the duodenum when cattle were fed 40% WDGS in relation to cattle fed corn control diet. These findings support the idea that fatty acids in distillers grains byproducts appear to have an added protection against ruminal biohydrogenation. Although the protection mechanism is still unclear, results from the present study support this idea, given that MDGS dietary treatments had greater 18:2 and PUFA content in comparison to the corn diet.

Table 1. Fatty acids profile of sarcoplasmic reticulum membrane from *L. lumbrorum* muscle of steers fed either a corn diet, 40% Full-fat MDGS, 40% De-oiled MDGS or 38% De-oiled MDGS plus corn oil. ^{a-b} Different superscripts within the same row indicate differences ($P < 0.10$).

Fatty Acids, %	Dietary Treatment				SEM	P-value
	Corn	Full-fat MDGS	De-oiled MDGS	De-oiled MDGS + oil		
C14:0	0.64	0.59	0.55	0.71	0.24	0.92
C15:0	0.52	0.54	0.29	0.22	0.17	0.30
C15:1	2.15	1.78	2.02	1.68	0.20	0.33
C16:0	17.73	17.84	17.23	17.89	1.01	0.97
C16:1	1.11	1.04	1.10	1.09	0.13	0.93
C17:0	0.85	0.65	0.63	0.62	0.10	0.16
C17:1	0.70	0.72	0.44	0.52	0.12	0.39
C18:0	14.87	12.97	13.22	13.84	0.61	0.20
C18:1	22.70	21.40	21.31	21.91	1.14	0.80
C18:1V	2.43 ^a	1.91 ^b	2.02 ^b	2.10 ^{ab}	0.10	< 0.01
C18:2	17.63 ^b	24.51 ^a	23.37 ^a	23.03 ^a	1.27	< 0.01
C18:3	0.67	0.41	0.66	0.69	0.16	0.61
C20:3	2.14	1.68	1.85	1.77	0.18	0.28
C20:4	8.37	8.04	8.20	7.35	0.60	0.71
C22:4	0.92	0.71	0.94	0.85	0.10	0.24
C22:5	1.81	1.55	1.59	1.62	0.13	0.77
SFA	35.18	32.09	31.95	33.48	1.45	0.62
MUFA	28.97	27.45	27.57	28.28	1.19	0.41
PUFA	31.26 ^b	36.85 ^a	36.46 ^a	37.25 ^a	1.70	0.06

SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids.

3.3.2 Free calcium concentration

Free calcium values of dietary treatments are presented in Figure 1. Dietary fat source influenced free calcium concentration of beef. Steaks from cattle fed MDGS had greater free calcium concentrations than steaks from cattle fed corn at d 2 postmortem ($P = 0.05$).

The SR is a membranous cellular organelle responsible for regulating the amount of calcium ions in the sarcoplasm of the muscle fiber. For a muscle to remain in the relaxed state, calcium must be sequestered by the SR to be at 100 nM and at 5 μ M for contraction of skeletal

muscle (ERTBJERG; PUOLANNE, 2017). After animal harvest, the calcium concentration in the sarcoplasm increases due to loss of the calcium-accumulating ability of the SR (GREASER et al., 1969) accompanied by a gradual leakage of calcium ions into the sarcoplasm.

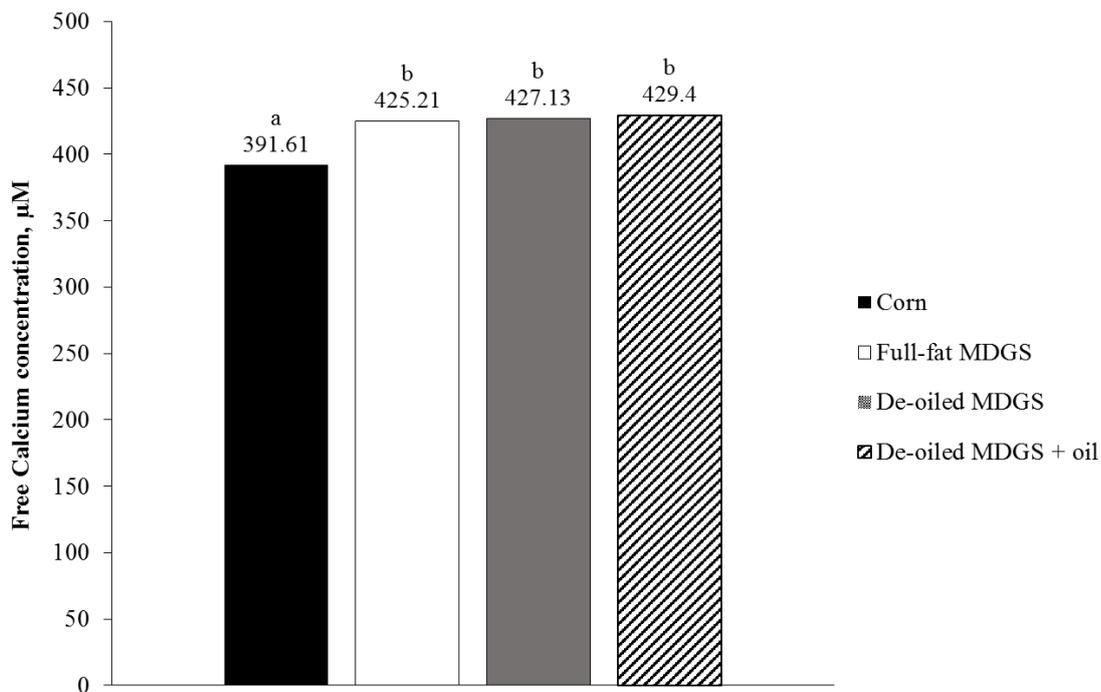


Figure 1. Free calcium concentration of strip loins (*Longissimus lumborum*) aged for 2 d from steers fed either a corn diet, 40% Full-fat MDGS, 40% De-oiled MDGS or 38% De-oiled MDGS plus corn oil. ^{a-b} Different superscripts indicate differences ($P = 0.05$).

A clear trend for changes in postmortem sarcoplasmic free calcium concentration has not been well established. Ji and Takahashi (2006) showed that calcium concentration at 40 min post-mortem increased from 16 μM to reach a maximum of 210 μM at 4 days postmortem in beef. They suggested that once the ultimate concentration is achieved, free calcium concentration in the sarcoplasm remains constant even after prolonged aging of meat. Conversely, Parrish et al. (1981) reported that concentrations of free calcium vary from 640 to 970 μM depending on maturity of beef aged for 10–14 days post-mortem. Similar results were found by Senaratne (2012), who reported that free calcium increased from 791 to 947 μM from 8 d to 28 d of aging in beef. One possible explanation for such inconsistency could be the use of different methods to quantify free calcium in these studies. Although both methods, inductively-coupled plasma emission spectrometer (ICP) and atomic absorption spectrophotometer (AA), are well documented to measure calcium ions in a solution, non-

invasive methods should be considered for future free calcium research in order to increase precision and repeatability of results.

Calcium has a major role in meat tenderization. Koomaraie and Geesink (2006) identified μ -calpain as the primary source of muscle tenderness. The calpain system requires the presence of calcium to be activated. In general, μ -calpains need between 3-50 μ M of calcium for its half-maximal activity (CONG et al., 1989; GOLL et al., 2003). The faster calcium release observed at d 2 postmortem in this project for all MDGS treatments compared to the corn diet supports our hypothesis that feeding high concentrations of distillers grains to cattle could increase free calcium available early-postmortem, which could result in improved tenderness.

3.3.3 Shear force

A two-way interaction between aging and retail display for shear force was found ($P < 0.0001$). As expected, decreased WBSF and SSF values were seen as aging and retail display progressed.

Dietary treatment tended to affect WBSF at d 2 postmortem ($P = 0.08$). Compared to steaks from steers fed corn, steaks from steers fed de-oiled MDGS and de-oiled MDGS plus corn oil had lower Warner-Bratzler shear force ($P = 0.03$) at 2 d of aging with 0 day of retail display (Figure 2). However, no differences in WBSF values among dietary treatments were found within any aging period when the retail display time was extended to 7 d (Figure 3). Even though SSF values for corn treatment followed the same pattern, being numerically higher than MDGS treatments at d 2 of aging, no significant differences were found in SSF values in any of the aging periods or retail display times ($P = 0.41$; Figures 4-5).

The effects of dietary treatments on tenderness at d 2 postmortem also were evident in the distribution of WBSF values of strip loin steaks (Figure 6). When cattle were fed MDGS, 44.4% of strip loin steaks had WBSF values lower than 4.4 kg, which has been reported as the threshold to claim for a certified tender label (ASTM STANDARD, 2011). In contrast, 37.5% of steaks from steers fed corn had WBSF values lower than 4.4 kg. These frequency distributions suggests that feeding MDGS to cattle increases the proportion of strip loin steaks ranked as tender early postmortem.

With regard to the distribution of SSF values, similar distributions of shear force values among dietary treatments were observed (Figure 7). When MDGS was fed to cattle, 51.4% of strip loin steaks had SSF values lower than 20 kg, compared with 50% of strip loin steaks having SSF lower than 20 kg when cattle were fed the corn diet. Although SSF frequency

distribution disagree with our WBSF distribution, these data matches our tenderness results once no significant differences among dietary treatments were found for SSF values after aging.

A moderate to strong relationship between Warner–Bratzler and slice shear force ($r = 0.64 - 0.80$) has been reported (SHACKELFORD et al., 1999; DERINGTON et al., 2011). However, the WBSF results found at day 2 of aging in this study are not in agreement with the SSF values for the same period ($r = 0.53$). One explanation for having conflicting results could be the higher variation in SSF values when compared to WBSF (Table 2). Specifically, WBSF values are grouped closer to their respective means than SSF values. Seventy-four percent of WBSF values were within $\pm 30\%$ of the mean WBSF value, whereas 68% of SSF values were within $\pm 30\%$ of the mean SSF value (Figure 8). The tendency of SSF values to diverge from the overall mean SSF value likely limits the power of the statistical test.

Table 2. Simple statistics for Warner-Bratzler shear force (WBSF), and slice shear force (SSF) from strip loin steaks aged for 2 d with 0 d retail display.

Trait	Dietary Treatment	Mean	SD	CV	Minimum	Maximum
WBSF, kg	Corn	5.30	1.54	28.98	3.05	8.60
	De-oiled MDGS	4.52	1.12	24.71	2.92	7.05
	De-oiled MDGS + oil	4.55	1.01	22.17	2.63	6.49
	Full-fat MDGS	5.03	1.27	25.21	2.90	7.89
SSF, kg	Corn	22.34	6.86	30.72	12.72	37.45
	De-oiled MDGS	19.80	5.31	26.83	12.22	33.7
	De-oiled MDGS + oil	21.29	5.30	27.86	10.83	33.03
	Full-fat MDGS	22.00	6.14	27.92	10.93	37.33

The tenderness data from several studies are in agreement with our results and support our hypothesis that feeding MDGS at high rates could increase beef tenderness early postmortem. Increased sensory overall tenderness ratings was reported as dietary level of distillers grains increased (DEPENBUSCH et al., 2009). Senaratne (2012) observed a trend ($P = 0.06$) that feeding 30% WDGS increased beef tenderness compared to feeding corn. Furthermore, Chao et al. (2017a) reported that steaks from steers finished on wet distillers grains were more tender than steaks from steers finished on corn at 2 d of aging. As expected, these differences in tenderness did not persist with increased aging time.

The results from this study suggest that feeding de-oiled MDGS and de-oiled MDGS plus corn oil to cattle has the potential to increase early postmortem tenderness, which likely was the result of increased total PUFA in the SR membrane and earlier free calcium release.

Extended aging beyond 2 d mitigated the tenderness effects, as there were no significant differences in tenderness among dietary treatments on samples aged for 9 ($P = 0.38$), 16 ($P = 0.73$) or 23 d ($P = 0.96$).

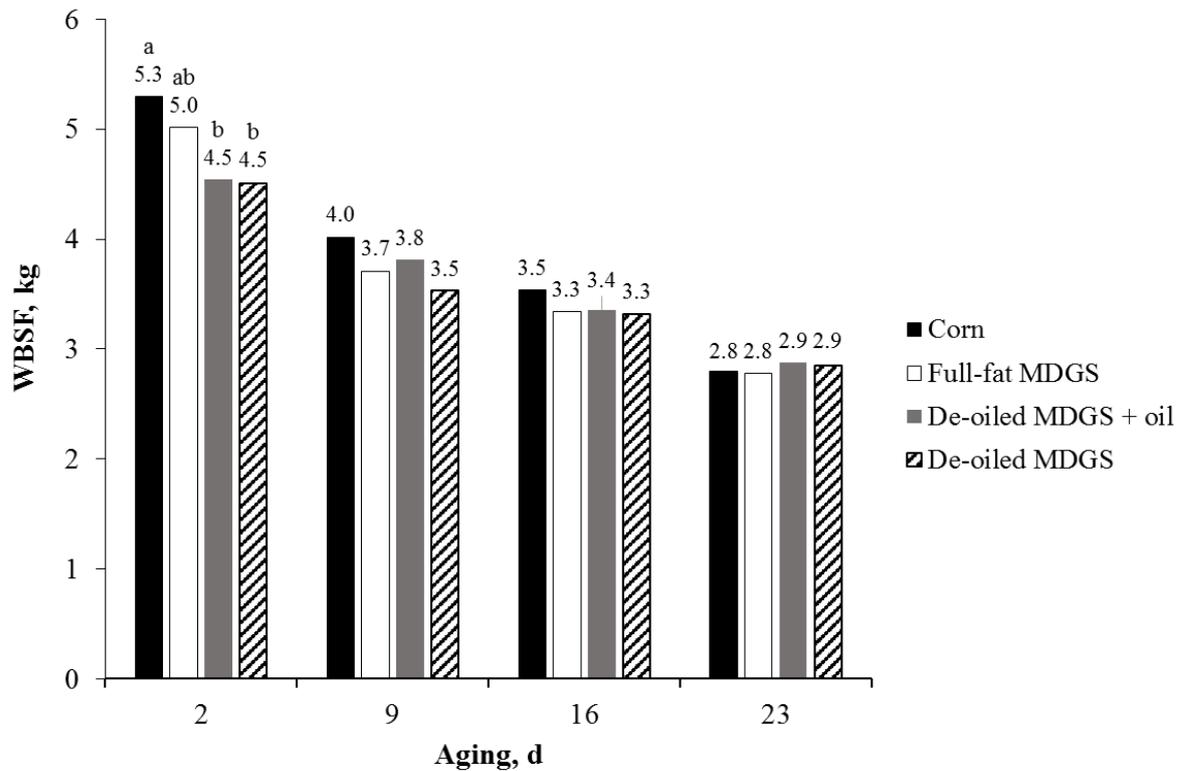


Figure 2. Warner-Bratzler shear force (WBSF) of strip loins steaks (*Longissimus lumborum*) from steers fed either a corn diet, 40% Full-fat MDGS, 40% De-oiled MDGS or 38% De-oiled MDGS plus corn oil with 0 d retail display. ^{a-b} Different superscripts indicate differences ($P < 0.05$).

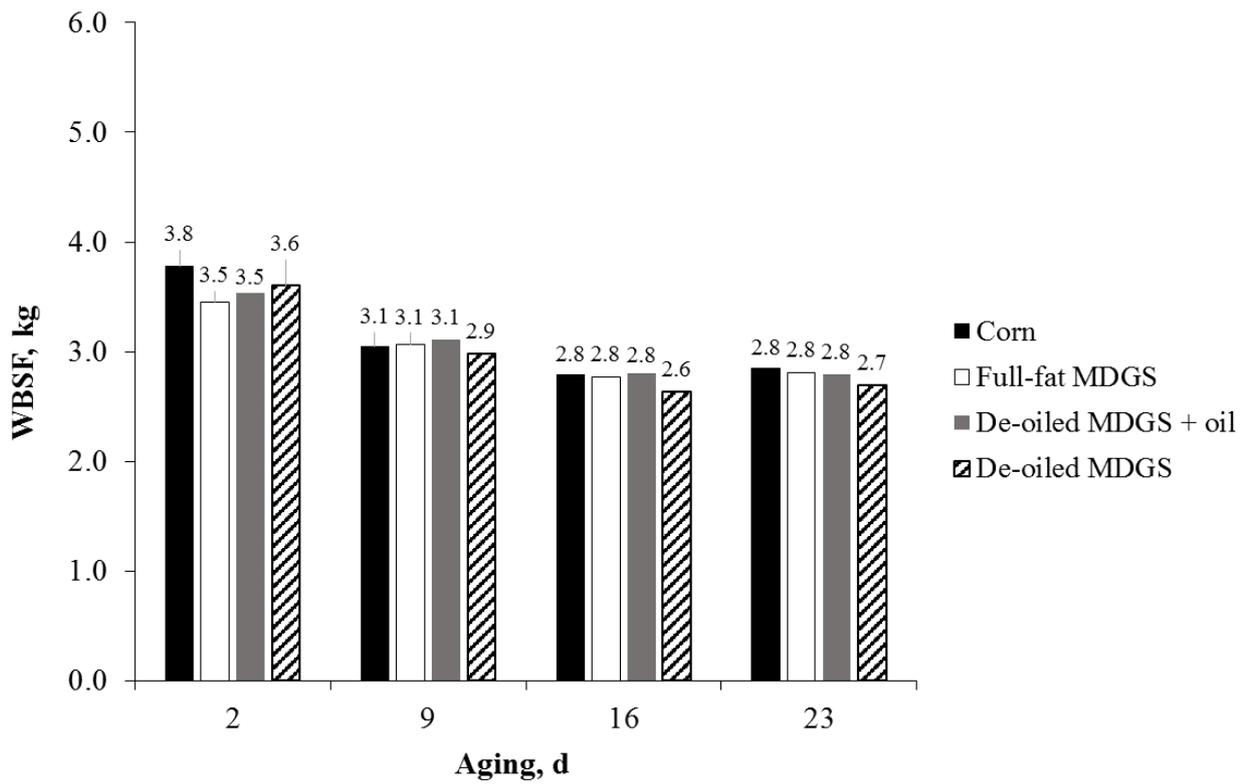


Figure 3. Warner-Bratzler shear force (WBSF) of strip loins steaks (*Longissimus lumborum*) from steers fed either a corn diet, 40% Full-fat MDGS, 40% De-oiled MDGS or 38% De-oiled MDGS plus corn oil with 7 d retail display.

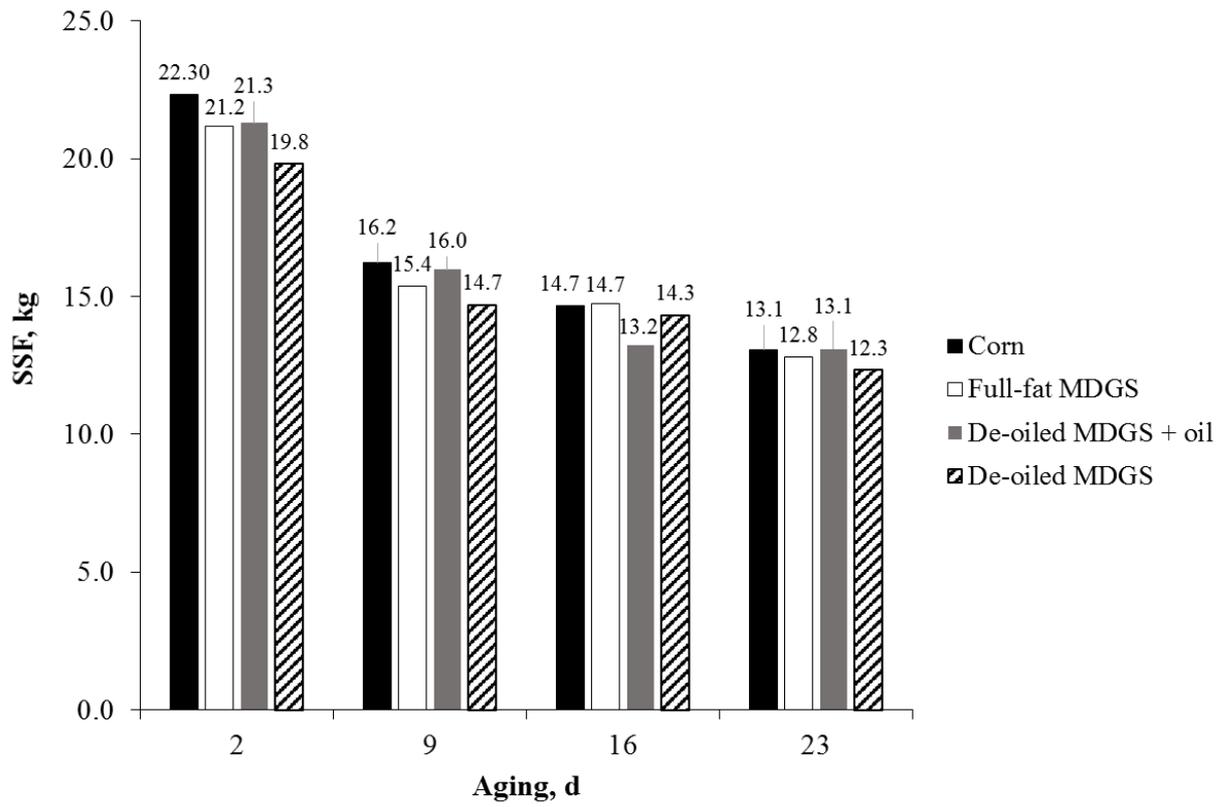


Figure 4. Slice shear force (SSF) of strip loins steaks (*Longissimus lumborum*) from steers fed either a corn diet, 40% Full-fat MDGS, 40% De-oiled MDGS or 38% De-oiled MDGS plus corn oil with 0 d retail display.

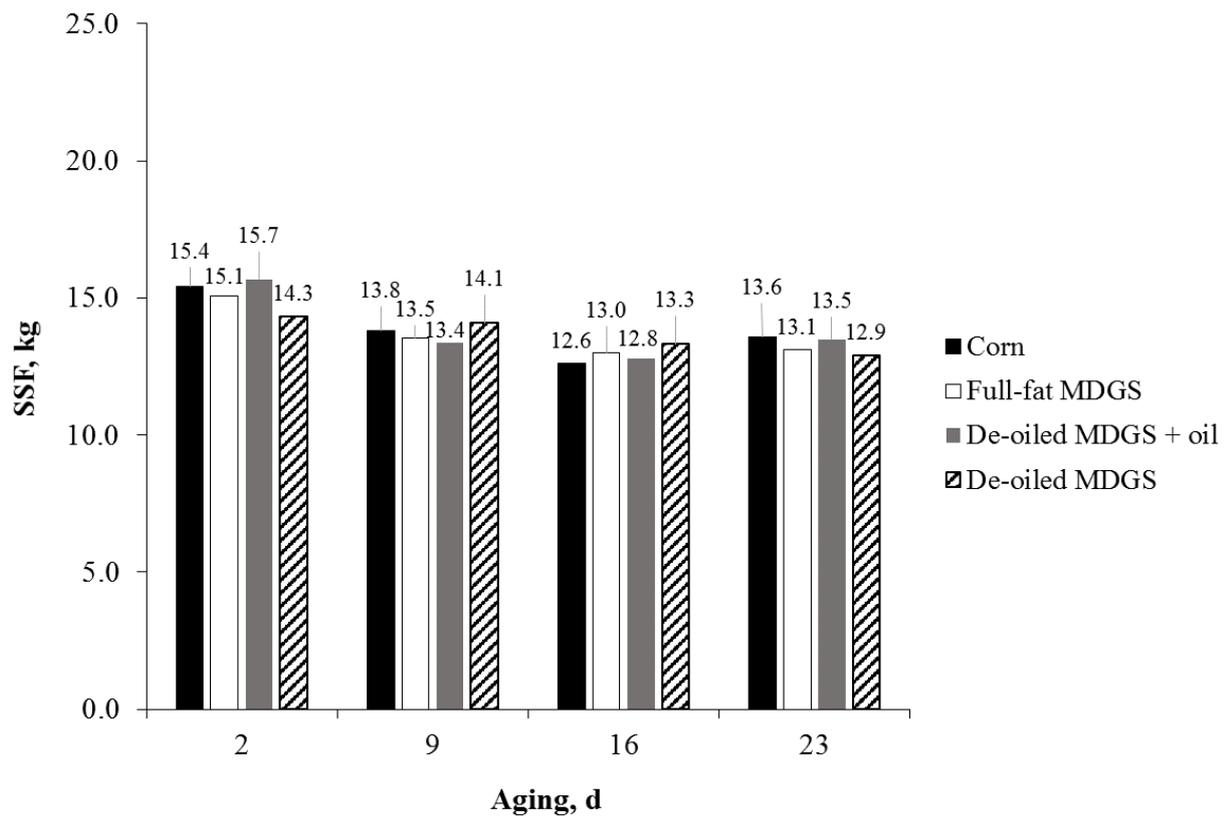


Figure 5. Slice shear force (SSF) of strip loins steaks (*Longissimus lumborum*) from steers fed either a corn diet, 40% Full-fat MDGS, 40% De-oiled MDGS or 38% De-oiled MDGS plus corn oil with 7 d retail display.

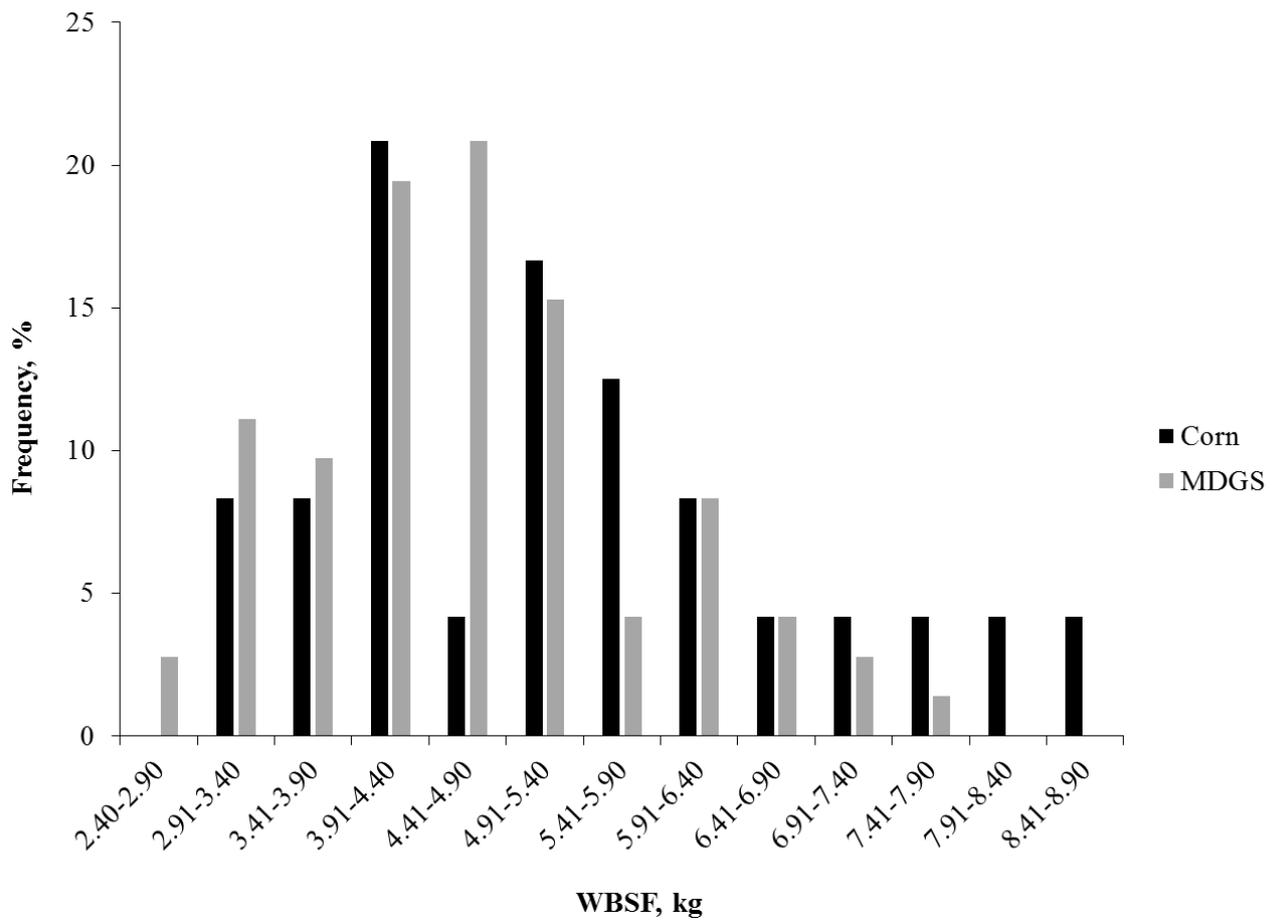


Figure 6. Frequency distributions of Warner-Bratzler shear force (WBSF) values for strip loin (*Longissimus lumborum*) steaks aged for 2 days from steers fed either a corn diet or 40% MDGS (all three MDGS dietary treatments were combined and compared to the corn diet).

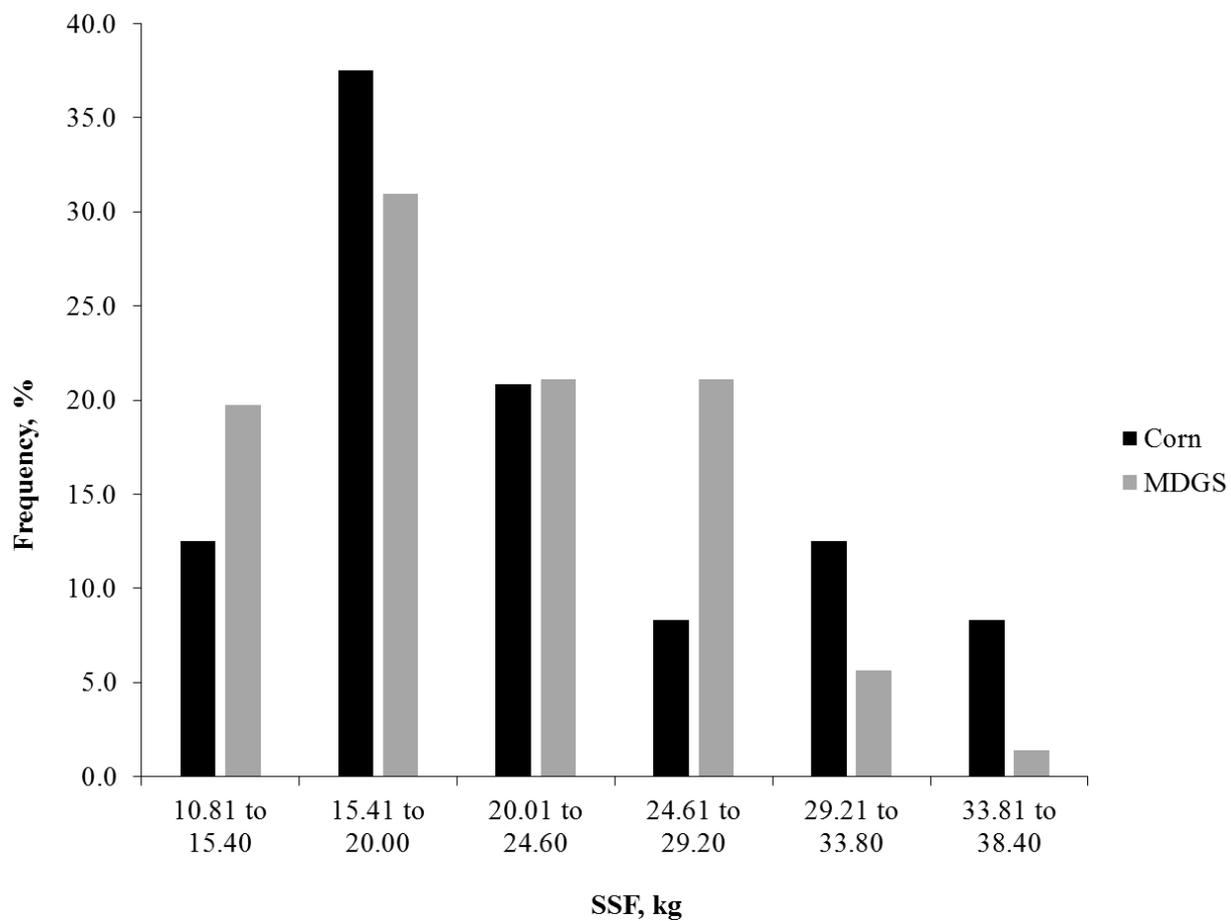


Figure 7. Frequency distributions of slice shear force (SSF) values for strip loin (*Longissimus lumborum*) steaks aged for 2 days from steers fed either a corn diet or 40% MDGS (all three MDGS dietary treatments were combined and compared to the corn diet).

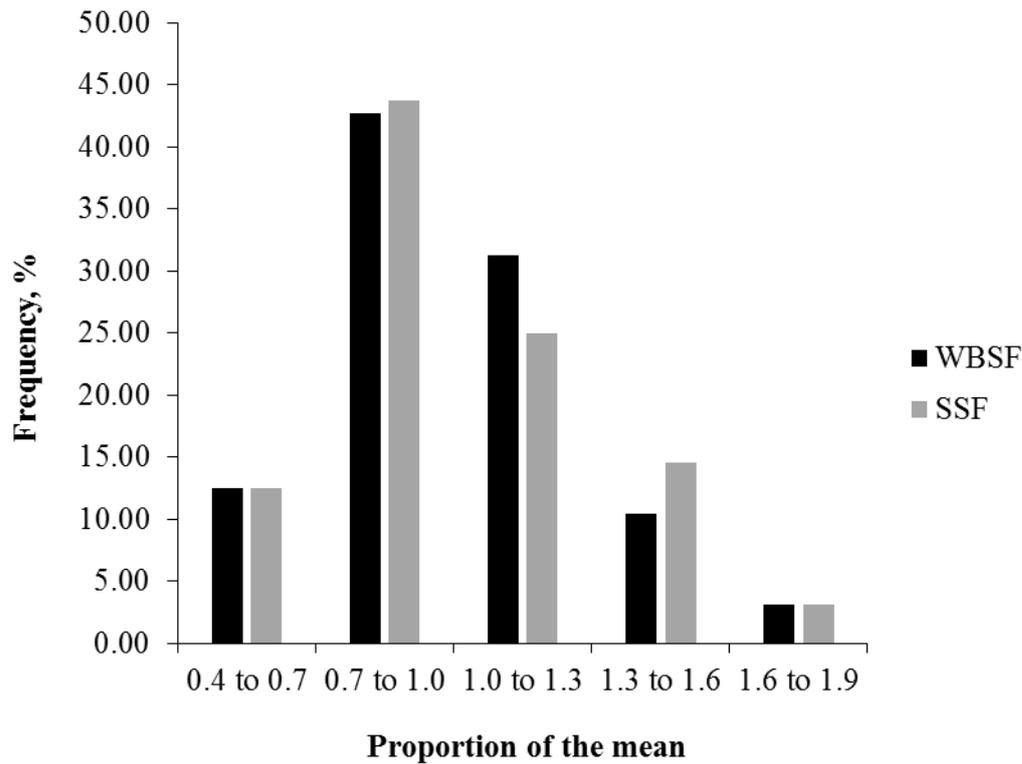


Figure 8. Relative frequency distributions of Warner-Bratzler shear force (WBSF) and slice shear force (SSF) values. Frequencies were calculated relative to the mean for each trait to normalize data for the mean difference between WBSF and SSF (e.g. 74% of WBSF values were within $\pm 30\%$ of the mean WBSF value, whereas 68% of SSF values were within $\pm 30\%$ of the mean SSF value).

3.3.4 Sarcomere length

No differences in sarcomere length among dietary treatments were observed ($P = 0.92$; Table 3). The average sarcomere length of strip loins from cattle fed corn-only, de-oiled MDGS, de-oiled MDGS plus corn oil and full-fat MDGS were 1.77, 1.76, 1.75 and 1.76 μm , respectively. Wheeler and Koohmaraie (1999) reported a range in sarcomere lengths from 1.36 μm (cold-shortened longissimus) to 1.69 μm (normal longissimus). Similar results were found by King et al. (2003) and England et al. (2012) who reported post-mortem sarcomere lengths of 1.78 μm and 1.82 μm in bovine longissimus, respectively. Furthermore, many studies on feeding distillers grains reported no differences in sarcomere length (OLTRA et al., 2008; CHAO et al., 2017a,b).

Given the higher calcium concentration in the sarcoplasm observed for all MDGS treatments early postmortem in this experiment, one would expect sarcomeres to shorten. However, our results showed that sarcomere length was not compromised. Sarcomere length

has long been recognized as a factor influencing meat tenderness. Our results suggest the increase in sarcoplasmic calcium occurs postmortem, when no additional shortening can occur. Relevant changes in length begin to occur early postmortem when sarcomeres contract as the muscle goes into rigor post-mortem. Wheeler and Koohmaraie (1994) reported increased shear force values as the sarcomere length decreased from 2.24 μm at death to 1.69 μm at 24 h post-mortem. Later on, Koohmaraie et al. (1996) concluded that meat toughening that occurs in lamb longissimus during the first 24 h postmortem is caused by sarcomere shortening, as shear force did not increase when sarcomere shortening was prevented.

The proteolytic process and the resultant degree of tenderization seems to be dependent on sarcomere length. Sarcomere shortening has been suggested to reduce the accessibility of proteolytic enzymes to myofibrillar proteins (IVERSEN et al., 1995). Some studies have suggested that proteolysis of troponin T and titin occur faster in muscles with longer sarcomere lengths (WEAVER et al., 2008; ENGLAND et al., 2012). This is in contrast to Koohmaraie et al. (1984) and Wheeler and Koohmaraie (1999), who reported no difference in proteolysis between cold-shortened and normal muscle.

Our results showed that sarcomere shortening was not affected by dietary fat source. Hence, sarcomere shortening cannot explain the difference in WBSF values observed at 48 h postmortem among dietary treatments in this experiment.

3.3.5 Troponin-T degradation

No differences in troponin-T degradation were found between dietary treatments at 2 d postmortem ($P = 0.60$; Table 3). In agreement with our results, Chao et al. (2017a) showed that the inclusion of 50% WDGS in finishing diets did not affect troponin-T degradation when compared with diets with no addition of WDGS in any of the aging periods studied: 2, 7, 14 or 21 ($P > 0.10$). On the other hand, Chao et al. (2017b) indicated that feeding 40% WDGS increased troponin-T degradation at 2 d of aging compared with no addition of WDGS.

Troponin-T degradation results from this study suggest that calpain activity was not different among dietary treatments; however, the WBSF results suggest the opposite. Although Lonergan et al. (2001) indicated that degradation of troponin-T at day 2 postmortem could be used as a consistent indicator of proteolysis and tenderization, some studies have also reported contradictory results between WBSF values and troponin-T degradation (WHEELER; KOOHMARAIE, 1999; CHAO et al., 2017a). It seems that the relationship between troponin-T degradation and improvement in beef tenderness is not linear and that some minimum

threshold of degradation is necessary for proteolysis to have any measurable effect on meat tenderness.

Table 3. Troponin-T (TNT) degradation and sarcomere length of strip loins (*Longissimus lumborum*) from steers fed either a corn diet, 40% Full-fat MDGS, 40% De-oiled MDGS or 38% De-oiled MDGS plus corn oil.

	Dietary Treatment				SEM	P-value
	Corn	Full-fat MDGS	De-oiled MDGS	De-oiled MDGS + oil		
TNT, % Degraded	20.44	24.72	23.85	22.24	2.49	0.60
Sarcomere Length, uM	1.77	1.76	1.76	1.75	0.02	0.92

It may be unrealistic to expect that the degradation of a single regulatory protein could be used to explain postmortem tenderization. The evidence from the work of Taylor et al. (1995) indicates that the degradation of proteins found at the periphery of the Z-disk such as desmin, titin, vinculin, and nebulin seem related to rate and extent of postmortem tenderization.

3.4 Conclusion

Feeding MDGS resulted in increased 18:2 content in the SR membrane in comparison to corn finishing diet. Results suggest that increased PUFA in the SR membrane may increase early-postmortem release of free calcium and thus, increase beef tenderness.

References

ASTM STANDARD, 2011. **Standard specification for tenderness marketing claims associated with meat cuts derived from beef.** ASTM International, West Conshohocken, PA.

ATKINSON, R.L.; SCHOLLJEGERDES, E.J.; LAKE, S.L.; NAYIGIHUGU, V.; HESS, B.W.; RULE, D.C. 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. **Journal of Animal Science**, Albany, v. 84, p. 387–396, 2006.

BLIGH, E.G.; DYER, W.J. A rapid method of total lipid extraction and purification. **Canadian Journal of Biochemistry and Physiology**, Ottawa, v. 37, p. 911–917, 1959.

CHAO, M.D. **Impact of wet distillers grains plus solubles and antioxidants on a basic mechanism of beef tenderization**. 2015. 184p. Thesis (Ph.D. in Animal Science) University of Nebraska, Lincoln, 2015.

CHAO, M.; DOMENECH-PEREZ, K.I.; SENARATNE-LENAGALA, L.S.; CALKINS, C.R. Feeding wet distillers grains plus solubles contributes to sarcoplasmic reticulum membrane instability. **Animal Production Science**, Clayton South, v. Online Early, p. A-I, 2017a.

CHAO, M.; DOMENECH-PEREZ, K.I.; CALKINS, C.R. Feeding vitamin E may reverse sarcoplasmic reticulum membrane instability caused by feeding wet distillers grains plus solubles to cattle. **Professional Animal Scientist**, Champaign, v. 33, p. 12–23, 2017b.

CONG, J.; GOLL, D.E.; PETERSON, A.M.; KAPPELL, H.P. The role of autolysis in activity of the Ca²⁺-dependent proteinases (μ -calpain and m-calpain). **Journal of Biological Chemistry**, Rockville, v. 264, p. 10096-10103, 1989.

CROSS, H.; WEST, R.; DUTSON, T. Comparison of methods for measuring sarcomere length in beef semitendinosus muscle. **Meat Science**, Barking, v. 5, p. 261-266, 1981.

DEPENBUSCH, B.E.; COLEMAN, C.M.; HIGGINS, J.J.; DROUILLARD, J.S. Effects of increasing levels of dried corn distillers grains with solubles on growth performance, carcass characteristics, and meat quality of yearling heifers. **Journal of Animal Science**, Albany, v. 87, p. 2653–2663, 2009.

DERINGTON, A.J.; BROOKS, J.C.; GARMYN, A.J.; THOMPSON, L.D.; WESTER, D.B.; MILLER, M.F. Relationships of slice shear force and Warner-Bratzler shear force of beef strip loin steaks as related to the tenderness gradient of the strip loin. **Meat Science**, Barking, v. 88, p. 203-208, 2011.

DOLAZZA, R.M.; LORENZEN, C.L. Can samples be powdered to determine sarcomere length? **Journal of Animal Science**, Albany, v. 92, p. 129–130, 2014.

ENGLAND, E.M.; FISHER, K.D.; WELLS, S.J.; MOHRHAUSER, D.A.; GERRARD, D.E.; WEAVER, A.D. Postmortem titin proteolysis is influenced by sarcomere length in bovine muscle. **Journal of Animal Science**, Albany, v. 90, p. 989–995, 2012.

ENSER, M.; HALLETT, K.; HEWITT, B.; FURSEY, G.A.J.; WOOD, J.D. Fatty acid content and composition of english beef, lamb and pork at retail. **Meat Science**, Barking, v. 42, p. 443–456, 1996.

ERTBJERG, P.; PUOLANNE, E. Muscle structure, sarcomere length and influences on meat quality: A review. **Meat Science**, Barking, v. 132, p. 139–152, 2017.

GOLL, D.E.; THOMPSON, V.F.; LI, H.Q.; WEI, W.; CONG, J.Y. The calpain system. **Physiological Reviews**, Bethesda, v. 83: p. 731–801, 2003.

GREASER, M.; CASSENS, R.; HOESKSTRA, W.; BRISKEY, E. The effect of pH and temperature on the calcium accumulating ability of purified sarcoplasmic reticulum. **Journal of Food Science**, Chicago, v. 34, p. 633–637, 1969.

HEMMINGS, S.J. New methods for the isolation of skeletal muscle sarcolemma and sarcoplasmic reticulum allowing a comparison between the mammalian and amphibian beta (2)-adrenergic receptors and calcium pumps. **Cell Biochemistry and Function**, Beijing, v. 19, p. 133–141, 2001.

IVERSEN, P.; HENCKEL, P.; LARSEN, L.M.; MONLLAO, S.; MOLLER, A.J. Tenderisation of pork as affected by degree of cold-induced shortening. **Meat Science**, Barking, v. 40, p. 171–181, 1995.

JI, J.R.; TAKAHASHI, K. Changes in concentration of sarcoplasmic free calcium during post-mortem ageing of meat. **Meat Science**, Barking, v. 73, p. 395–403, 2006.

KING, D.A.; DIKEMAN, M.E.; WHEELER, T.L.; KASTNER, C.L.; KOOHMARAIE, M. Chilling and cooking rate effects on some myofibrillar determinants of tenderness of beef. **Journal of Animal Science**, Albany, v. 81, p. 1473–1481, 2003.

KLOPFENSTEIN, T.J.; ERICKSON, G.E.; BREMER, V.R. Board-invited review: Use of distillers by-products in the beef cattle feeding industry. **Journal of Animal Science**, Albany, v. 86, p. 1223–1231, 2008.

KOGER, T.J.; WULF, D.M.; WEAVER, A.D.; WRIGHT, C.L.; TJARDES, K.E.; MATEO, K.S.; ENGLE, T.E.; MADDOCK, R.J.; SMART, A.J. 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. **Journal of Animal Science**, Albany, v. 88, p. 3399–3408, 2010.

KOOHMARAIE, M.; KENNICK, W.H.; ANGLEMIER, A.F.; ELGASIM, E.A.; JONES, T.K. A research note: Effect of postmortem storage on cold-shortened bovine muscle: Analysis by SDS-polyacrylamide gel electrophoresis. **Journal of Food Science**, London, v. 49, p. 290–291, 1984.

KOOHMARAIE, M.; DOUMIT, M.E.; WHEELER, T.L. Meat Toughening Does Not Occur When Rigor Shortening Is Prevented. **Journal of Animal Science**, Albany, v. 74, p. 2935–2942, 1996.

KOOHMARAIE, M.; GEESINK, G.H. Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. **Meat Science**, Barking, v. 74, p. 34–43, 2006.

LONERGAN, S.M.; HUFF-LONERGAN, E. Postmortem proteolysis and tenderization of top loin steaks from Brangus cattle. **Journal of Muscle Foods**, Trumbull, v. 12, p. 121–136, 2001.

MELLO, A.S.; CALKINS, C.R.; JENSCHKE, B.E.; CARR, T.P.; DUGAN, M.E.R.; ERICKSON, G.E. Beef quality of calf-fed steers finished on varying level of corn-based wet distillers grains plus solubles. **Journal of Animal Science**, Albany, v. 90, p. 4625–4633, 2012a.

MELLO, A.S.; JENSCHKE, B.E.; SENARATNE, L.S.; CARR, T.P.; ERICKSON, G.E.; CALKINS, C.R. Effects of feeding modified distillers grains plus solubles on marbling attributes, proximate composition, and fatty acid profile of beef. **Journal of Animal Science**, Albany, v. 90, p. 4634-4640, 2012b.

METCALFE, L.D.; SCHMITZ, A.A.; PELKA, J.R. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. **Analytical Chemistry**, Moscow, v. 38, p. 514–515, 1966.

MILLER, M.F.; CARR, M.A.; RANSEY, C.B.; CROCKETT, C.L.; HOOVER, L.C. Consumer thresholds for establishing the value of beef tenderness. **Journal of Animal Science**, Albany, v. 79, p. 3062–3068, 2001.

MORRISON, W.R.; SMITH, L.M. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. **Journal of Lipid Research**, Rockville, v. 5, p. 600–608, 1964.

NELSON, M.A. **Effects of varying levels of reduced-fat modified distillers grains with solubles in finishing diets of feedlot steers on beef quality Characteristics**. 2016. 90p. Dissertation (Master of Science) University of Minnesota, Minneapolis, 2016.

OLTRA, O.; FARMER, L.; MOSS, B.; BIRNIE, J. The effect of distiller's and brewer's grains on beef acceptability. In: Proceedings of International Congress of Meat science and Technology, 54., 2008, Cape Town.

PARRISH, F.C.; SELVIG, C.J.; CULLER, R.D.; ZECCE, M.G. CAF activity, calcium concentration, and the 30,000-dalton component of tough and tender bovine longissimus muscle. **Journal of Food Science**, Chicago, v. 46, p. 308–311, 1981.

PIETRZAK, M.; GREASER, M.L.; SOSNICKI, A.A. Effect of rapid rigor mortis processes on protein functionality in pectoralis major muscle of domestic turkeys. **Journal of Animal Science**, Albany, v. 75, p. 2106–2116, 1997.

PLATTER, W.J.; TATUM, J.D.; BELK, K.E.; KOONTZ, S.R.; CHAPMAN, P.L.; SMITH, G.C. Effects of marbling and shear force on consumers' willingness to pay for beef strip loin steaks. **Journal of Animal Science**, Albany, v. 83, p. 890–899, 2005.

RIBEIRO, F.A.; DOMENECH-PEREZ, K.I.; WILKERSON, E.K.; VOEGELE, H.; HERRERA, N.J.; HART, K.B.; CALKINS, C.R. Relationship between fat dietary source and beef display life. In: Proceedings of International Congress of Meat science and Technology, 63., 2017, Cork.

ROEBER, D.; GILL, R.; DiCOSTANZO, A. Meat quality responses to feeding distiller's grains to finishing Holstein steers. **Journal of Animal Science**, Albany, v. 83, p. 2455-2460, 2005.

SCOLLAN, N.; HOCQUETTE, J.F.; NUERNBERG, K.; DANNENBERGER, D.; RICHARSON, I.; MOLONEY, A. Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. **Meat Science**, Barking, v. 74, p. 17-33, 2006.

SENARATNE, L.S. **Mechanism and control of beef toughening during retail display in high oxygen modified atmosphere packages**. 2012. 238p. Thesis (Ph.D. in Animal Science) University of Nebraska, Lincoln, 2012.

SHACKELFORD, S.D.; WHEELER, T.L.; KOOHMARIE, M. Evaluation of slice shear force as an objective method of assessing beef longissimus tenderness. **Journal of Animal Science**, Albany, v. 77, p. 2693–2699, 1999.

TAYLOR, R.G.; GEESINK, G.; THOMPSON, V.; KOOHMARAIE, M.; GOLL, D. Is Z-disk degradation responsible for post-mortem tenderization? **Journal of Animal Science**, Albany, v. 73, p. 1351–1367, 1995.

VANDER POL, K.; LUEBBE, M.; CRAWFORD, G.I.; ERICKSON, G.E.; KLOPFENSTEIN, T.J. Performance and digestibility characteristics of finishing diets containing distillers grains, composites of corn processing coproducts, or supplemental corn oil. **Journal of Animal Science**, Albany, v. 87, p. 639–652, 2009.

WEAVER, A.D.; BOWKER, B.C.; GERRARD, D.E. Sarcomere length influences postmortem proteolysis of excised bovine semitendinosus muscle. **Journal of Animal Science**, Albany, v. 86, p. 1925–1932, 2008.

WHEELER, J.L.; KOOHMARAIE, M. Pre-rigor and post-rigor changes in tenderness of ovine longissimus muscle. **Journal of Animal Science**, Albany, v. 72, p. 1232–1238, 1994.

WHEELER, J.L.; KOOHMARAIE, M. The extent of proteolysis is independent of sarcomere length in lamb longissimus and psoas major. **Journal of Animal Science**, Albany, v. 99, p. 2444–2451, 1999.

4 FINAL CONSIDERATIONS

There are still some questions that needed to be answered for this research. A clear relationship between oxidative stress and earlier calcium release has not been well described. The calcium results from this study indicated that the SR membrane lost the ability of efficiently regulate free calcium early post-mortem, leaving more free calcium in the sarcoplasm, however the TBARS results indicated no differences among dietary treatments at 48 h postmortem. Perhaps, such subtle differences in oxidation early postmortem can only be detected using extremely sensitive methodologies capable to quantify lipid oxidation and oxidative status directly in the SR membrane.

Hence, a portion of this work was dedicated to the quantification of oxidative stress early postmortem using more sensitive methodologies. Initially, some studies conducted by our group were dedicated to the quantification of superoxide dismutase, which is an enzyme that prevents the accumulation of reactive oxygen species (ROS). Then, we evaluated tumor necrosis factor alpha (TNF alpha), which is an inflammatory protein produced during acute inflammation and is responsible for a diverse range of signaling events within cells, leading to necrosis or apoptosis. Lastly, we measured interleukin 6 (IL-6), which is a small protein produced in response to infections and tissue injuries, contributes to host defense through the stimulation of acute phase responses, hematopoiesis, and immune reactions. One possible explanation for having no success on these attempts was mainly due the quantification process of analyzing animal tissue in conjunction with the highly reactive nature of ROS.

Understanding the time course of SR membrane oxidation and ascertaining the role of SR membrane integrity on free calcium release could clarify the meat tenderization process early postmortem from a new and different perspective at the cellular level.