Feeding distillers grains to cattle may affect beef tenderness early postmortem¹

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ABSTRACT: The objective of this study was to evaluate the effects of dietary fat source with modified distillers grains plus solubles (MDGS) on beef tenderness through examination of sarcoplasmic reticulum (SR) membrane fatty acid composition, changes in sarcoplasmic calcium concentration, Warner–Bratzler shear force (WBSF), sarcomere length, and proteolysis early postmortem. Steers ($n = 256$) were fed for 134 d on either corn, or a diet containing 40% full-fat MDGS, 40% de-oiled MDGS, or 38% de-oiled MDGS plus 2% corn oil. Twenty-four USDA Choice carcasses (three carcasses per pen; eight pens per treatment) were selected within each dietary treatment, and strip loins were collected and aged for 2, 9, 16, or 23 d postmortem and subject to retail display (RD) for an additional 7 d. Feeding MDGS increased ($P < 0.05$)

concentrations of linoleic acid (18:2) and tended to increase ($P = 0.06$) total polyunsaturated fatty acids (PUFA) in the SR membrane. Steaks from cattle fed MDGS had greater sarcoplasmic calcium concentration than steaks from cattle fed corn at day 2 postmortem ($P = 0.05$). Steaks from steers fed de-oiled MDGS and de-oiled MDGS plus corn oil tended to have lower WBSF values ($P = 0.08$) than steaks from cattle fed corn at 2 d of aging with 0 d of RD. 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 calcium into the sarcoplasm due to increased 18:2 and PUFA concentration in the SR membrane, which could result in increased beef tenderness early postmortem.

Key words: beef quality, calcium flux, distillers grains, fatty acids, sarcoplasmic reticulum

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INTRODUCTION

Among the palatability traits that govern eating quality of beef, tenderness has been ranked as one of the most important traits for both consumer satisfaction and purchasing decisions (Huffman et al., 1996; Shackelford et al., 2001; Goodson et al., 2002), and consumers are willing to pay more for tender steaks (Lusk et al., 2001; Miller et al., 2001; Platter et al., 2005).

Conflicting results have been published regarding the effects of feeding distillers grains on beef tenderness. Although several studies have shown no effect on beef tenderness from feeding distillers grains to cattle (Roeber et al., 2005; Koger et al., 2010; Mello et al., 2012a), some recent studies have reported 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).

Distillers grains are considered as a corn by-product; they have more energy per kilogram than corn on a DM basis. Ethanol production utilizes the starch fractions of grains, concentrating the levels of other nutrients. Hence, distillers grains have up to three

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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 (Mello et al., 2012b; Domenech-Pérez et al., 2017; de Mello et al., 2018; Ribeiro et al., 2018) but also increase PUFA content of the sarcoplasmic reticulum (SR) membrane (Chao, 2017a). The mechanism of meat tenderization is a well-understood subject. However, the way animal diets with high concentration of distillers grains might affect beef tenderness requires further exploration. Therefore, the objective of this study was to evaluate the effects of dietary fat source with modified distillers grains plus solubles (MDGS) on beef tenderness by examining Warner-Bratzler shear force (WBSF), sarcomere length, and proteolysis early postmortem, as well as changes in sarcoplasmic calcium concentration and SR membrane fatty acid composition.

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 beef tenderization mechanisms by examining WBSF, sarcomere length, and proteolysis early postmortem, as well as changes in sarcoplasmic calcium concentration and SR membrane fatty acid composition.

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.

Cattle and Dietary Treatments

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

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, three USDA Choice carcasses were selected per pen. Both strip loins [Institutional Meat Purchase Specifications number 180] from all selected carcasses were collected, vacuum-packaged, and transported to the University of Nebraska Loeffel 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 postmortem and subject to retail display (RD) for an additional 7 d.

Loin sections assigned for 2 d of aging were immediately trimmed of subcutaneous fat, and

Table 1. Diet composition (% of DM basis) fed to finishing steers receiving 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% full-fat MDGS	40% de-oiled MDGS	38% de-oiled 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
Fat content	3.89	7.10	5.96	7.78

¹DRC = dry rolled corn; HMC = high moisture corn; MDGS = modified distillers grains plus solubles; FGC = fine ground corn.

²Formulated to contain 383 mg/hd per day of ruminant and 90 mg/hd per day of tylenol.

fabricated into three steaks (2.54 cm thickness; one steak for WBSF with 0 d RD, one steak for WBSF with 7 d RD, and one steak for sarcomere length, SR membrane fatty acid, sarcoplasmic calcium concentration, and troponin-T degradation with 0 d RD).

After fabrication, steaks assigned to 0 d of RD were vacuum packaged and frozen at -80°C , except for the WBSF steaks that were frozen at -20°C . Steaks assigned to 7 d of RD were placed on foam trays ($21.6 \times 15.9 \times 2.1$ cm, Styro-Tech, Denver, CO), overwrapped with oxygen permeable film (Prime Source PSM 18 #75003815, Bunsz Processors Division, North Kansas City, MO; oxygen transmission rate = $2.25\text{ mL/cm}^2/24\text{ h}$ at 23°C and 0% relative humidity; water vapor transfer rate = $496\text{ g/m}^2/24\text{ h}$ at 37.8°C and 90% relative humidity) and placed under RD conditions for 7 d (under continuous white fluorescence lighting at 1,000 to 1,800 lux; F32T8/TL741, 700 series, 32 W, Philips) 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 profile, sarcoplasmic calcium concentration, and troponin-T degradation were removed from the freezer, cut by hand into small cubes, 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 WBSF analysis. Sarcomere length, SR membrane fatty acid, free calcium concentration, and troponin-T degradation were analyzed only at day 2 postmortem.

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 method 920.39 (AOAC, 1990). Protein content was calculated by difference.

SR Membrane Extraction

The SR membrane was extracted using the procedure described by Hemmings (2001). Ten grams of powdered samples were suspended in ice-cold 35 mL homogenization buffer (10 mM NaHCO_3 , 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 $2,000 \times g$ for 10 min at 4°C . The supernatant was collected and centrifuged at $10,000 \times g$ (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 $100,000 \times g$ 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.

SR Membrane Lipid Extraction

The SR membrane lipids were 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 ($\sim 35\text{ mL}$) 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 double-distilled water (ddH_2O) 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 a 13×100 mm screw cap tube. Samples were centrifuged at $1,000 \times g$ for 5 min. Following centrifugation, the aqueous phase (top layer) was removed with a Pasteur pipet. The remaining fraction was evaporated to dryness under nitrogen at 60°C .

SR Membrane Fatty Acid Profile

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 lipids, and the solution was vortexed for 5 s and heated for 5 min at 100°C . After heating, 0.5 mL of boron trifluoride in 14% methanol was added and the solution was vortexed for 5 s and heated for 5 min at 100°C . One milliliter of a saturated NaCl solution and 1 mL of hexane were added to the solution, and the solution was centrifuged at $1,000 \times g$ 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 GC (Hewlett-Packard 6890 FID GC System; Agilent Technologies, Santa Clara, CA) with a capillary column (Chrompack CP-Sil 88 (0.25 mm × 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 was identified by comparison of retention times with known standards.

Sarcoplasmic Calcium Concentration

Sarcoplasmic calcium was quantified according to the procedure described by [Parrish et al. \(1981\)](#) with modifications. Three grams of powdered strip loin were transferred to an ultracentrifuge tube (13 × 55 mm; Beckman Coluter, Brea, CA) and centrifuged at 196,000 × *g* (Beckman L7-65 Ultracentrifuge with a SW55Ti rotor; Beckman Coluter) at 4 °C for 30 min. After centrifugation, 700 µL of the supernatant was 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 × *g* (Eppendorf model 5430; Eppendorf, Hamburg, Germany) for 10 min. After centrifugation, 400 µL of supernatant was 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, and 150 mg/L.

Tenderness Determination

Steaks (2.54 cm thick) were removed from the freezer, thawed at 3 °C for 24 h, steak internal temperatures and weights 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 (450-ATT, OMEGA Engineering, Inc.). 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 MagiGrill, 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 ~6 min. After cooking, internal temperature and weight were recorded. Then, steaks were individually bagged and stored overnight at 2 °C for further WBSF analysis. The following day, 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 Corporation, Sterling, VA.) with a Warner–Bratzler blade. Peak WBSF values from each steak were averaged for statistical purposes.

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, and 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. \(1981\)](#):

$$\text{Sarcomere length } (\mu\text{m}) = \frac{0.6328 \times D \sqrt{(T/D)^2 + 1}}{T}$$

where 0.6328 denotes 632.8 (the wavelength of the laser) × 10^{−3}, *D* is the distance from specimen to diffraction pattern screen (100 mm), and *T* is the spacing between diffraction bands (mm)

Myofibrillar Protein Isolation

Myofibrillar proteins were isolated according to [Pietrzak et al. \(1997\)](#) with modifications. Three grams of powdered meat were placed into a 50-mL

conical tube and suspended in 15 mL ice-cold 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 re-suspended in 1 mL of ice-cold rigor buffer. The suspended pellet was vortexed for 10 s and centrifuged for 5 min at $4,000 \times g$. The pellet washing step was repeated three times. One milliliter 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.

Protein Concentration

One hundred microliters 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 to 2,000 $\mu\text{g/mL}$) of BSA standards were prepared using the extraction buffer as the diluent. Twenty-five microliters of BSA standards and diluted myofibrillar protein samples were deposited into their respective wells on a 96-well microplate (Microtest III sterile 96-well flat-bottomed microplate; Becton Dickinson & Company, Franklin Lakes, NJ). Two hundred microliters 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 room temperature (23°C) for 10 min, absorbance was read at 562 nm, and protein concentrations were expressed as microgram per milliliter. All myofibrillar protein samples were diluted to 2 mg/mL with ddH₂O.

Gel Electrophoresis

Degree of proteolysis was measured by troponin-T degradation. All of the following procedures were conducted at room temperature. Twenty-five microliters of the 2 mg/mL myofibrillar

protein samples were mixed with $2\times$ Laemmli sample buffer (65.8 mM Tris-HCl, 2.1% SDS, 26.3% glycerol, and 0.01% bromophenol blue) with 5% β -mercaptoethanol at 1:1 ratio. All samples were heated at 95°C for 5 min. Ten microliters Kaleidoscope Pre-stained Protein Standard and prepared myofibrillar protein samples (5 μg) were loaded on 4% to 20% Mini-PROTEAN TGX pre-cast 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 consisting of 25 mM Tris-base, 192 mM glycine, and 0.1% SDS (pH 8.3).

Western Blotting

Proteins in the gels were blotted onto polyvinylidene difluoride 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, and 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 anti-troponin-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 three 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 three times on a rocking platform and scanned using an 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 the 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.

Statistical Analysis

Tenderness data were analyzed as a split-plot design with dietary fat source as the whole plot,

aging period as the split-plot and RD as the split-split-plot. Separation of means for tenderness data was conducted using LS MEANS procedure with SLICEDIFF function at $P < 0.05$. Sarcomere length, SR membrane fatty acid, sarcoplasmic calcium concentration, and troponin-T degradation were analyzed as a completely randomized design. Pen was considered the experimental unit and the mean of the three animals collected within each pen was considered as a replication. 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.

RESULTS AND DISCUSSION

Proximate Composition and Marbling Score

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 ($P = 0.78$), for the subset of samples ($n = 96$) evaluated in this project, neither for all 256 steers finished ($P = 0.64$). The overall

averages ($n = 256$) for marbling scores were: 463 for the corn control, 458 for de-oiled MDGS, 446 for de-oiled MDGS plus corn oil, and 467 for the full-fat MDGS diet (marbling score: 400 = Small⁰⁰, 450 = Small⁵⁰, 500 = Modest⁰⁰). Overall, the USDA Quality Grade frequency distribution for all finished steers ($n = 256$) was 2.27% Prime, 74.03% Choice, and 23.37% Select. Within the USDA Choice grade, 28.25% were graded as upper 2/3 USDA Choice (modest or moderate marbling), and 45.78% were graded as USDA low Choice (small marbling). Although the relationship between intramuscular fat and tenderness is not always strong, intramuscular fat content is associated with tenderness of meat. In order to eliminate the intramuscular fat content effect on tenderness and to cover the USDA quality grade with the highest frequency, we decided to collect samples only from USDA low Choice carcass. As a result, no differences in proximate composition and marbling scores were expected as only samples from USDA low Choice carcass were collected.

Fatty Acid Profile

Results for the SR membrane fatty acid profile analyses are presented in Table 2. Cattle fed full-fat and de-oiled MDGS had lower ($P < 0.05$) cis

Table 2. Fatty acid profile of sarcoplasmic reticulum membrane from *Longissimus lumborum* muscle of steers fed a corn diet, 40% full-fat MDGS, 40% de-oiled MDGS, or 38% de-oiled MDGS plus corn oil

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

^{a,b}Different superscripts within the same row indicate differences ($P < 0.05$). Alpha values between 0.05 and 0.10 were considered as trends.

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

vaccenic acid (18:1 V) concentration when compared to cattle fed corn and de-oiled MDGS plus corn oil. Feeding MDGS 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 which contain three or more double bonds were not affected by the dietary treatment.

Our results suggest that the fatty acid composition of the SR membrane is dependent on the dietary fat source. After the milling process, distillers grains have greater concentrations of fat when compared to corn (Klopfenstein et al., 2008). According to Díaz-Royón et al. (2012), the fat in distillers grains is mainly composed of unsaturated fat acids, and 18:2 is the most abundant fatty acid, accounting for almost 50% of the total fatty acids. Although unprotected from the environment of the rumen, where most 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) indicated that greater proportions of grains in cattle diets lead to greater unsaturated fatty acid due to less ruminal biohydrogenation. Similarly, Vander Pol et al. (2009) suggested inclusion of 40% WDGS in cattle diets translated in more 18:2 passing through the rumen (vs. corn) due to a protective effect from rumen biohydrogenation, thus allowing greater amounts of unsaturated fatty acids being available for absorption in the small intestine. In contrast, Castillo-Lopez et al. (2014) reported a volume effect in that the more WDGS were provided, the higher the fatty

acid content in the diet. Thus, an increase in 18:2 passing through the rumen was observed. However, Castillo-Lopez also noted an increase in the percentage of 18:2 that was biohydrogenated when WDGS were fed, suggesting the more fat being fed translated into more total volume of unsaturated fatty acids passing through to the duodenum.

Therefore, the higher content of C18:2 and PUFA in the SR membrane for MDGS diets relative to the corn control diet appears to be related to the higher volume of lipid in MDGS diets, or a fat protection from rumen biohydrogenation, or a combination of both.

Sarcoplasmic Calcium Concentration

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

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 obtain a sarcoplasmic concentration of 100 nM and at 5 μ M for contraction of skeletal muscle (Ertbjerg and 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 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 ($P = 0.05$).

In fact, there are contradictory results in the literature regarding calcium release postmortem. [Ji and Takahashi \(2006\)](#) showed that calcium concentration 40 min postmortem increased from 16 μM to reach a maximum of 210 μM at 4 d postmortem in beef. They suggested that once the ultimate concentration is achieved, calcium concentration in the sarcoplasm remains constant even after prolonged aging of meat. Conversely, some studies found that aging influenced sarcoplasmic calcium concentration. [Senaratne \(2012\)](#) reported that sarcoplasmic calcium concentration increased with longer aging time, while [Chao et al. \(2017b\)](#) found that sarcoplasmic calcium concentration decreased during aging.

Calcium has a major role in meat tenderization. [Koohmaraie 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 and 50 μM of calcium for its half-maximal activity ([Cong et al., 1989](#); [Goll et al., 2003](#)).

We speculate a few possible mechanisms for the difference in free calcium concentration observed among dietary treatments. First, [Cheah \(1981\)](#) reported that 18:2 fatty acid can stimulate calcium release and inhibit calcium uptake from the SR membrane. Perhaps, linoleic acid from the SR membrane released by endogenous phospholipase A2 induced the ryanodine receptor (RyR1) to release calcium. Second, the protein oxidation could be considered a possible mechanism. The RyR1 of SR membrane possesses highly reactive sulfhydryl (SH) groups that are susceptible to oxidation. [Sun et al. \(2001\)](#) indicated that extensive oxidizing conditions favor channel opening. [Abramson and Salama \(1989\)](#) reported that oxidized SH groups can trigger rapid calcium release from the SR by forming disulfide bonds, which cause the RyR1 to maintain in the open state.

Several studies have found that beef from cattle fed distillers grains was more tender than beef from steers fed corn ([Depenbusch et al., 2009](#); [Segers et al., 2011](#); [Senaratne, 2012](#); [Chao et al., 2017a](#)). Interestingly, opposite results were found when antioxidants were added in the diet. [Chao et al. \(2017b\)](#) and [Senaratne \(2012\)](#) found that beef from cattle fed antioxidants was tougher than beef from steers that were not fed antioxidants. These findings suggest a significant role for SR membrane integrity on the tenderization mechanism in beef from cattle fed a high concentration of distillers grains. The work of [Malheiros et al. \(2018\)](#) indicates that tender meat is associated with an increase of oxidative stress of antioxidant enzymes, heat shock

proteins and structure proteins, as well as being associated with a decrease of oxidative stress of metabolic enzymes. Perhaps, an increase of PUFA in the SR membrane contributed to instability of the SR, making it more susceptible to protein oxidation, which could induce early calcium release postmortem.

The higher concentration of calcium observed at day 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.

Tenderness Measurements

There was a trend ($P = 0.08$) for three-way interaction among dietary treatment, aging and RD time on steak WBSF. Compared with steaks from steers fed the corn control diet, steaks from steers fed de-oiled MDGS and de-oiled MDGS plus corn oil were more tender ($P < 0.001$) at 2 d of aging with 0 d of RD ([Figure 2](#)). Steaks from steers fed full-fat MDGS had intermediate WBSF values at 2 d of aging with 0 d of RD and did not differ from the corn control group ($P = 0.21$). Lower WBSF values for the full-fat group in comparison to corn group were expected at day 2 since the full-fat group had greater 18:2 content in the SR membrane and calcium concentration in the sarcoplasm than the corn control group. The authors have no clear explanation as to why full-fat MDGS had intermediate WBSF values at day 2. Extended aging beyond 2 d mitigated tenderness effects among all dietary treatments ([Figure 2](#)), as there were no significant differences in tenderness among dietary treatments on samples aged for 9, 16, or 23 d. No differences in WBSF values among dietary treatments were found within any aging period when the RD time was extended to 7 d ([Figure 3](#)).

This is not the first time that increased tenderness due to feeding distillers grains to cattle has been reported in scientific literature. Increased sensory overall tenderness ratings were 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 WDGS were more tender than steaks from steers finished on corn at 2 d of aging. The tenderness data from these studies are in agreement with our results and support our hypothesis that

Figure 2. Warner–Bratzler shear force (WBSF) of strip loins steaks (*L. lumbarum*) from steers fed 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 ($P = 0.08$).

Figure 3. Warner–Bratzler shear force (WBSF) of strip loins steaks (*L. lumbarum*) from steers fed a corn diet, 40% full-fat MDGS, 40% de-oiled MDGS, or 38% de-oiled MDGS plus corn oil with 7 d retail display.

feeding MDGS at high rates could increase beef tenderness early postmortem.

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 post-mortem tenderness, which likely was the result of increased total PUFA in the SR membrane and earlier calcium release into the sarcoplasm.

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. In agreement with our results, no differences in sarcomere length have been reported in studies on feeding distillers grains (Oltra et al., 2008; Chao et al., 2017a, 2017b).

Relevant changes in sarcomere length begin to occur early postmortem when sarcomeres contract as the muscle goes into rigor. If calcium is released when ATP is still available, muscle contraction can occur, resulting in shorter sarcomeres and detrimental effects on meat tenderness. However, sarcomere length was not compromised in this project, indicating that the earlier release of calcium found in all MDGS treatments occurred after the depletion of ATP reserves (completion of rigor mortis).

Table 3. Troponin-T (TNT) degradation and sarcomere length of strip loins (*Longissimus lumborum*) from steers fed 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, μm	1.77	1.76	1.76	1.75	0.02	0.92

Sarcomere length has long been recognized as a factor influencing meat tenderness (Koohmaraie et al., 1996). Wheeler and Koohmaraie (1994) reported increased shear force values as the sarcomere length decreased from 2.24 μm at death to 1.69 μm 24 h postmortem. 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.

Longissimus tenderness depends on the extent of rigor shortening and postmortem proteolysis during aging (Wheeler and Koohmaraie, 1999). Iversen et al. (1995) suggested that muscle shortening reduces the accessibility of proteolytic enzymes to myofibrillar proteins, thereby reducing their degradation and subsequent improvement in tenderness. Weaver et al. (2009) reported greater extent of troponin-T proteolysis in muscles with longer sarcomeres, while England et al. (2012) reported that proteolysis of titin occurs faster in muscles with longer sarcomere lengths. This is in contrast to Koohmaraie et al. (1984) and Wheeler and Koohmaraie (1999), who reported no difference in proteolysis between cold-shortened and un-shortened 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 48 h postmortem among dietary treatments in this experiment.

Troponin-T Degradation

No differences in troponin-T degradation were found between dietary treatments 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. Conversely, Chao et al. (2017b) indicated that feeding 40% WDGS increased troponin-T degradation at 2 d of aging compared with no addition of WDGS.

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. Troponin-T degradation results from this study suggest that extent of proteolysis was not different among dietary treatments at day 2 postmortem; however, the WBSF results suggest the opposite. Similarly, Chao et al. (2017a) reported contradictory results between WBSF values and troponin-T degradation at day 2 postmortem.

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

CONCLUSIONS

Feeding MDGS to cattle resulted in increased 18:2 content in the SR membrane and calcium concentration in the sarcoplasm in comparison to corn finishing diet, which is in agreement with our hypothesis. Results suggest that feeding de-oiled and de-oiled plus oil MDGS to cattle may increase beef tenderness early postmortem. We hypothesized that early release of calcium postmortem could improve beef tenderness through increased proteolysis. However, proteolysis postmortem measured by troponin-T degradation at day 2 was not affected by MDGS inclusion on cattle diets, which did not fit our hypothesis.

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