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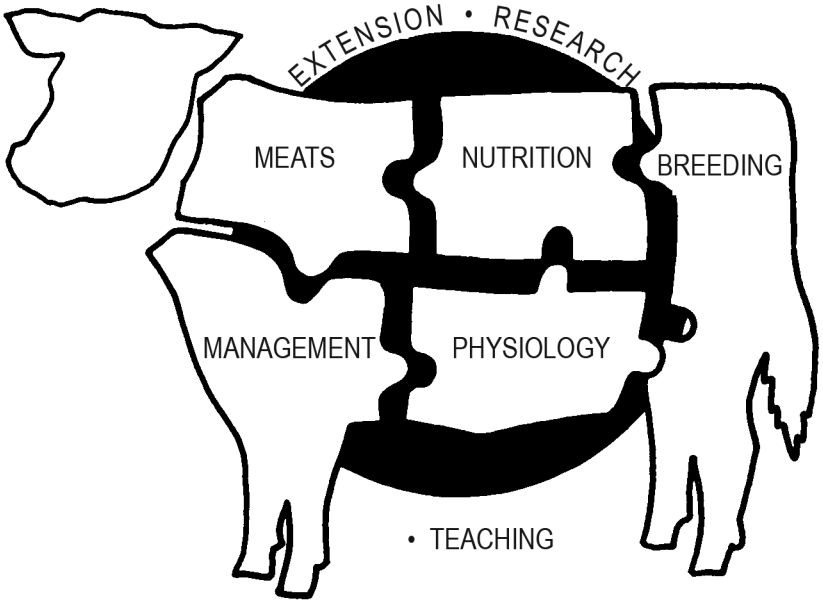

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IANR

A Basic Mechanism of Beef Tenderization: Feeding Wet Distillers Grains Plus Solubles Contributes to Sarcoplasmic Reticulum Membrane Instability

Michael D. Chao
Katherine I. Domenech
Chris R. Calkins[1,2]

Summary

Feeding wet distillers grains plus solubles (WDGS) could increase polyunsaturated fatty acid (PUFA) concentration in the sarcoplasmic reticulum (SR) membrane, thereby altering membrane integrity, resulting in more rapid post-rigor calcium leakage, greater enzyme activity and improved tenderness. Steers were finished on either 0% WDGS or 50% WDGS. Steaks from steers fed WDGS were more tender and had greater free calcium concentrations. Feeding WDGS also increased proportions of PUFA in SR membrane and altered SR lipid and phospholipid profiles. These findings suggest that feeding increased concentrations of WDGS in the finishing diet can possibly increase meat tenderness through the proposed mechanism.

Introduction

Muscle is an elegant biological system with mechanisms in place to control calcium for contraction and relaxation. After rigor, calcium ions slowly diffuse from the sarcoplasmic reticulum (SR) to the sarcoplasm where the ions activate the calcium-dependent proteolytic enzymes (the calpain system) and enhance tenderness. It is well-known that feeding cattle with feed containing greater concentrations of polyunsaturated fatty acid (PUFA) such as wet distillers grains plus solubles (WDGS) increases PUFA concentrations in beef (2011 *Nebraska Beef Cattle Report*, pp. 96-99). Research results from our lab have reported that beef from cattle fed 30% WDGS tended to be more tender than beef from cattle not fed WDGS or WDGS with dietary antioxidants (2012 *Nebraska Beef Cattle Report*, pp. 124-126). Our hypothesis is that including WDGS in feedlot diets increases PUFA concentration in the SR membrane, making the membrane more prone to oxidation. An unstable SR membrane occurs because of altered membrane integrity, resulting in more rapid calcium leakage post-rigor and, thus, improves tenderness through greater activation of the calpain system.

Table 1. Diet composition on a DM basis.

	50% WDGS	0% WDGS
Ingredients, % of DM		
Dry-rolled corn	16.5	41.5
High-moisture corn	16.5	41.5
Wet distillers grains plus solubles	50	0
Corn silage	12	12
Supplement[1]	5	5

[1]Formulated to contain 380 mg/head/day of Rumensin® and 90mg/head/day of Tylan®.

Procedure

This trial was designed to provide samples with differing levels of oxidation capacity to allow examination of the mechanisms by which SR membrane oxidation influences beef tenderization postmortem. Ninety-six steers were randomly assigned to one of two treatments: 0% WDGS or 50% WDGS (Table 1). For both treatments, there were six pens (replicates) with each pen having eight steers. Fifteen strip loins (*Longissimus lumborum*) from each treatment (n = 30; 2-3 per pen) were collected and aged for 2, 7, 14, or 21 days. Steaks were removed at each aging period and placed under retail display conditions for 0, 4, and 7 days.

Steak samples for tenderness assessment (via Warner Bratzler Shear Force [WBSF]), free calcium concentrations (via inductively coupled plasma spectroscopy) and proteolysis (via immunoblotting to quantify troponin-T degradation) were obtained on day 0 and 7 of retail display for each aging period. Steak samples for lipid oxidation (via t*hiobarbituric acid reactive substances assay [TBARS])* were obtained on day 0, 4 and 7 of retail display for each aging period. Steak samples for SR membrane fatty acid (via gas chromatography), lipid, and phospholipid (via thin-layer chromatography) profiles were obtained at day 0 of retail display after 14 days of aging.

Data were analyzed by GLIMMIX procedure of SAS (version 9.2; SAS Institute, Inc., Cary, N.C.) as a split-split-plot design with dietary treatments as the whole plot, aging period as the subplot and retail display time as the repeated measures. Separation of means was conducted using LSMEANS procedure with PDIFF or SLICEDIFF options at $P \leq 0.05$.

Results

Compared to steaks from steers fed 0% WDGS, steaks from steers fed 50% WDGS were more tender ($P < 0.01$; Figure 1) at two days of aging with 0 day of retail display. Meat from WDGS fed steers also had increased ($P < 0.01$) free calcium concentration (Figure 2) at two days aging after seven days of retail display. However, there were no differences in tenderness or free calcium

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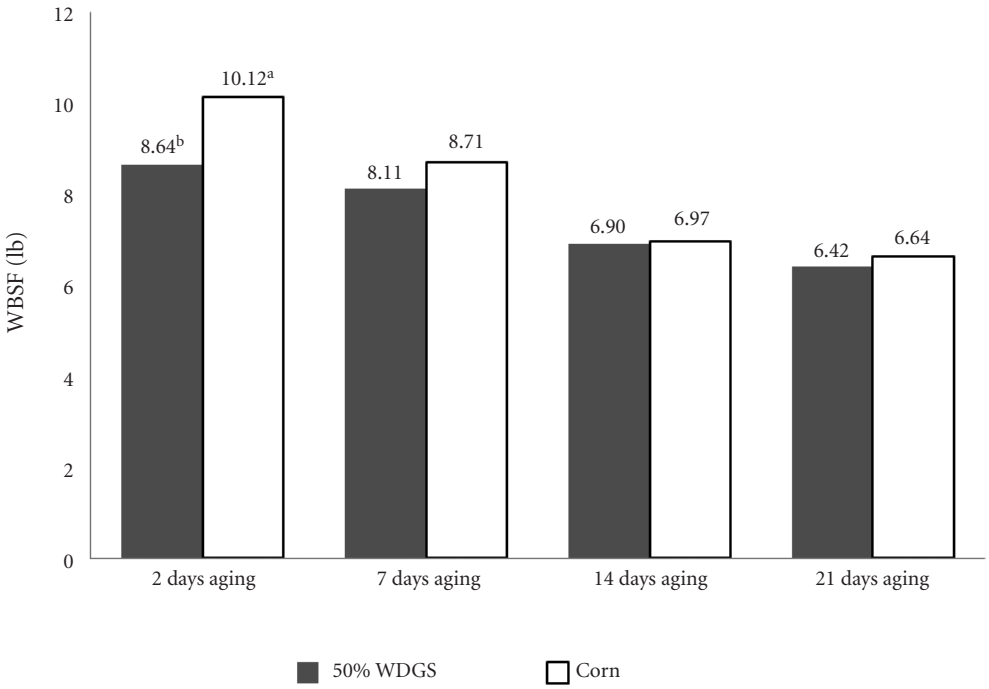

Figure 1. Warner-Bratzler shear force (WBSF) of strip loins (*m. longissimus lumborum*) from steers fed with or without wet distillers grains plus solubles (WDGS) in finishing diets without retail display.

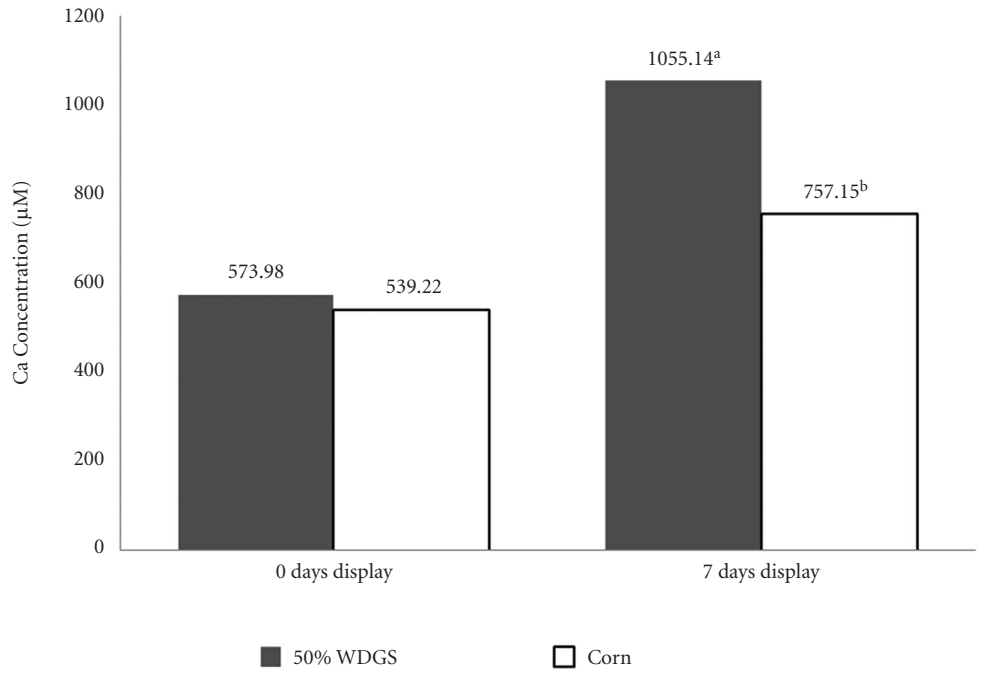

Figure 2. Free calcium concentration of strip loins (*m. longissimus lumborum*) aged for two days from steers fed with or without wet distillers grains plus solubles (WDGS) in finishing diets.

Table 2. Fatty acid profile of sarcoplasmic reticulum membrane from strip loins (*m. longissimus lumborum*) from steers fed with or without wet distillers grains plus solubles (WDGS) in finishing diets.

Fatty Acids (%)	50% WDGS	0% WDGS	*P*-value
C15:0	0.50	0.53	0.56
C15:1	1.51[b]	2.81[a]	0.04
C16:0	22.16	23.25	0.13
C16:1	2.32[b]	3.32[a]	< 0.01
C17:0	0.95	0.94	0.94
C17:1	0.97[b]	1.19[a]	< 0.01
C18:0	10.30[a]	9.06[b]	0.04
C18:1	26.48[b]	30.30[a]	0.03
C18:1V[2]	1.93[b]	2.47[a]	< 0.01
C18:2	16.81[a]	12.46[b]	0.03
C18:3	0.42	0.39	0.63
C20:3	1.30	1.39	0.59
C20:4	4.97	5.57	0.37
C20:5	0.48	0.52	0.71
C22:4	0.80	0.85	0.75
C22:5	0.22	0.19	0.08
SFA[1]	36.04	35.53	0.72
UFA[1]	63.96	64.47	0.72
SFA:UFA[1]	0.57	0.56	0.70
MUFA[1]	33.09[b]	38.52[a]	0.01
PUFA[1]	28.73	23.91	0.09

[1]SFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids.
[2]C18:1V is cis vaccinic acid.
[a-b]Within a row, means without a common superscript differ at $P \leq 0.05$.

Table 3. Phospholipid and lipid profile of sarcoplasmic reticulum membrane from strip loins (*m. longissimus lumborum*) from steers fed with or without wet distillers grains plus solubles (WDGS) in finishing diets.

	50% WDGS	0% WDGS	*P*- value
Phospholipids (%)			
Phosphatidylcholine	43.00[a]	36.07[b]	< 0.01
Phosphatidylethanolamine	31.89[b]	38.78[a]	0.03
Phosphatidylinositol	2.86	2.66	0.56
Phosphatidylserine	1.03	1.15	0.53
Sphingomyelin	21.89	21.71	0.93
Lipid (%)			
Phospholipid	47.90	53.74	0.10
Mono, Di & Triacylglyceride	47.55	41.06	0.08
Cholesterol	4.36	5.01	0.36
Free Fatty Acids	0.18	0.19	0.90
Total Neutral Lipid	52.10	46.26	0.10

[a-b]Within a row, means without a common superscript differ at $P \leq 0.05$.

concentration between treatments for any other aging and retail display period. Extended aging beyond two days appeared to mitigate the tenderness effects.

In addition, feeding WDGS decreased ($P \leq 0.05$) concentrations of fatty acids C15:1, C16:1, C17:1, C18:1, C18:1V and total monounsaturated fatty acid, but increased ($P \leq 0.05$) concentrations of fatty acids C18:0, C18:2 and tended to increase ($P \leq 0.1$) total PUFA in SR membrane (Table 2). The increase in PUFA content of the SR membrane supports our hypothesis that feeding WDGS may impair SR membrane integrity and, thus, accelerate free calcium release.

Feeding WDGS also tended to decrease ($P \leq 0.1$) phospholipid concentration and tended to increase ($P \leq 0.1$) mono, di and triacylglyceride and neutral lipid concentration in SR membrane (Table 3). Also, feeding WDGS increased ($P < 0.01$) phosphatidylcholine, but decreased ($P \leq 0.05$) phosphatidylethanolamine percentages in SR phospholipids (Table 3). It has been reported that the phospholipids in the SR membrane are degraded during postmortem aging and that calcium leaks through channels formed by this degradation in the SR membrane. Phosphatidylethanolamine is bound to the transmembrane helices of the membrane-bound structure that pumps calcium into the SR. When calcium is bound, the phosphatidylethanolamine is released. We hypothesize that a reduction in phosphatidylethanolamine is related to the increase in free calcium concentration. It is likely, then, that the difference in SR fatty acid profile is not the only contributor to the differences in tenderness and free calcium concentration.

There were no differences in troponin-T degradation between treatments in any of the aging and retail display periods, which indicated that the calpain activity was not different between treatments. Steaks from 0% WDGS steers had increased lipid oxidation values compared to steaks from steers fed WDGS ($P \leq 0.05$) at 21 day aging, and the reason behind it is still unclear.

Although lipid oxidation values did not agree with our hypothesis, it is likely that measuring lipid oxidation on muscle tissue is not the best way to measure SR membrane oxidative status. A sensitive, simple, and reliable method that can detect lipid oxidation in extremely small sample volume is needed for direct measurement of SR membrane oxidative status. These findings suggest that feeding WDGS in the finishing diet can possibly increase meat tenderness through the proposed mechanism.

[1]Michael D. Chao, graduate student; Katherine I. Domenech, graduate student; Chris R. Calkins, professor, University of Nebraska–Lincoln Department of Animal Science, Lincoln, Neb.

[2]This project was funded in part by The Beef Checkoff.