A HYPOTHESIS ON BEEF TENDERIZATION

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Abstract - Wet distillers grains plus solubles (WDGS) in feedlot diets 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 and improving tenderness. Ninety-six cross-bred steers were finished on either corn or 50% WDGS. Fifteen striploins from each treatment (n=30) were collected and aged for 2, 7, 14, or 21 d and placed under retail display condition for 0, 4, and 7 d. Steaks were used to measure tenderness, proteolysis, free calcium concentrations, lipid oxidation, SR fatty acid, lipid and phospholipid profile. Steaks from steers fed WDGS were more tender and had higher free calcium concentration at 2 d aging. Feeding WDGS decreased C15:1, C16:1, C17:1, C18:1, C18:1V and total monounsaturated fatty acids concentrations, but increased C18:0, C18:2 and other unidentified PUFA and tended to increase total PUFA in SR membrane. Feeding WDGS also tended to decrease phospholipid concentration and increase triacylglyceride and neutral lipid concentration in SR membranes. Furthermore, feeding WDGS increased phosphatidylcholine, but decreased phosphatidylethanolamine percentages in SR phospholipids. This study confirmed that feeding WDGS tended to increase tenderness and early post-rigor calcium release. However, the true mechanism that contributes to SR membrane instability is still unclear.

I. INTRODUCTION

Tenderness has repeatedly been cited as the most important element for both eating quality and consumer purchasing decisions [1, 2] and is a high priority for research in the meat industry [3-6]. The mechanism of meat tenderization is well understood. However, meat scientists have not focused on how feed might affect the basic mechanism of meat tenderization. Our lab is now proposing a hypothesis on how feed, especially feed with high content of polyunsaturated fatty acids (PUFA), could affect meat tenderness.

Muscle is an elegant biological system with mechanisms in place to control calcium. After rigor, calcium ions slowly diffuse from the sarcoplasmic reticulum (SR) to the sarcoplasm where they activate the calcium-dependent calpain system and enhance tenderness. It is well-known that feeding cattle with feed containing high levels of PUFA [like wet distillers grains plus solubles (WDGS)] increases PUFA levels in beef. Research results from our lab have shown that beef from cattle fed WDGS tended to be more tender than beef from cattle fed corn or WDGS with dietary antioxidants [7]. Our hypothesis is that WDGS in feedlot diets increases PUFA concentration in the SR membrane, making the membrane more prone to oxidation. An unstable SR membrane occur because of altered membrane integrity, resulting in more rapid calcium leakage post-rigor and thus improve tenderness through improved activation of calcium-dependent proteolytic enzymes (the calpain system).

WDGS are the major by-product of ethanol production. When compared to corn, WDGS are not only less expensive, but also contain a higher level of protein, fat (especially PUFA) and fiber. Hence, WDGS have been used widely in feedlot diets with level varying from 10 to 80% dry matter basis. Mello, Calkins [8] showed that steers fed levels up to 30% of WDGS increased proportions of PUFA, linoleic, linoleic/linolenic, and trans fatty acids when compared to steers fed corn, but did not alter carcass characteristics. Therefore, WDGS cattle provide an excellent model to generate samples with varying degrees of oxidation capacity.

II. MATERIALS AND METHODS

This trial was designed to provide samples with differing levels of oxidations capacity to allow examination of the mechanisms by which SR membrane oxidation influences beef tenderization postmortem. Steers were fed one of two treatments (corn or 50% WDGS) with eight per pen and six replications for a total of 96 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)] 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 free calcium concentrations (via inductively coupled plasma spectroscopy) and lipid oxidation [via thiobarbituric acid reactive substances assay (TBARS)] were obtained on day 0, 4 and 7 of retail display of 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. SR were separated using the method described by Hemmings [9]. Data were analyzed by GLIMMIX procedure of SAS (version 9.2, Cary, NC, 2009) as a split-split-plot design with dietary treatments as the whole-plot, aging period as the sub-plot and retail display time as the repeated measures. Separation of means was conducted using LSMEANS procedure with PDIFF or SLICEDIFF options at $P \leq 0.05$.

III. RESULTS AND DISCUSSION

Compared to steaks from steers fed corn only, steaks from steers fed 50% WDGS were more tender ($P < 0.01$; figure 1) and had higher sarcoplasmic free calcium concentrations ($P < 0.01$) at 2 d aging. In addition, feeding WDGS decreased C15:1 ($P < 0.05$), C16:1 ($P < 0.01$), C17:1 ($P < 0.01$), C18:1 ($P < 0.05$), C18:1V ($P < 0.01$) and total monounsaturated fatty acid ($P < 0.01$) concentrations, but increased C18:0 ($P < 0.05$), C18:2 ($P < 0.05$), and unidentified PUFA ($P < 0.01$) and tended to increase total PUFA ($P < 0.1$) in SR membrane (Table 1). The unidentified PUFA contained peaks that fell between C18:0 and C17:0; as all the SFA and MUFA in this region are defined by the standards, odd chain PUFAs are the only possible components of the unidentified fatty acids in this particular region. Feeding WDGS also tended to decrease phospholipid concentration ($P < 0.1$) and tended to increase mono, di and triacylglyceride and neutral lipid concentration ($P < 0.1$) in SR membranes (Table 2). Also, feeding WDGS increased phosphatidylcholine ($P < 0.01$), but decreased phosphatidylethanolamine ($P < 0.05$) percentages in SR phospholipids (Table 2). There were no differences in troponin-T degradation at any of the aging periods. Steaks from corn-fed steers had higher lipid oxidation values compared to steaks from steers fed WDGS ($P < 0.05$) at 21 d aging.

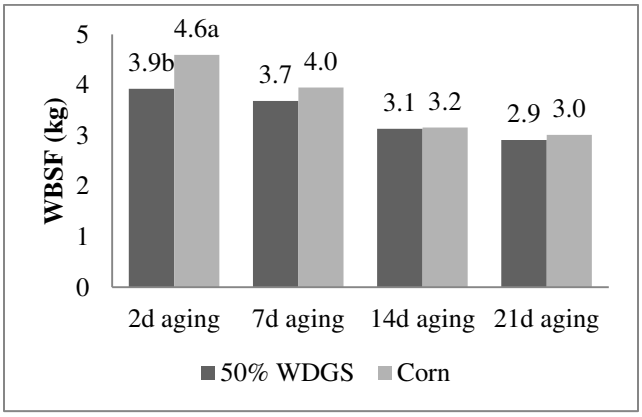

Figure 1. Warner-Bratzler shear force (WBSF) of strip loins (*m. longissimus lumborum*) from steers fed wet distillers grains plus solubles (WDGS) and corn finishing diets.

Table 1. Fatty acids profile of sarcoplasmic reticulum membrane from strip loins (*m. longissimus lumborum*) from steers fed wet distillers grains plus solubles (WDGS) and corn finishing diets.

Fatty Acids (%)	50% WDGS	Corn	P-value
C15:0	0.50	0.53	0.56
C15:1	1.51	2.81	0.04
C16:0	22.16	23.25	0.13
C16:1	2.32	3.32	0.00
C17:0	0.95	0.94	0.94
C17:1	0.97	1.19	0.00
C18:0	10.30	9.06	0.04
C18:1	26.48	30.30	0.03
C18:1V	1.93	2.47	0.00
C18:2	16.81	12.46	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
Unidentified PUFA	6.05	4.32	0.00
SFA	36.04	35.53	0.72
UFA	63.96	64.47	0.72
SFA:UFA	0.57	0.56	0.70
MUFA	33.09	38.52	0.01
PUFA	28.73	23.91	0.09

SFA = saturated fatty acids, UFA = unsaturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids

Table 2. Phospholipid and lipid profile of sarcoplasmic reticulum membrane from strip loins (*m. longissimus lumborum*) from steers fed wet distillers grains plus solubles (WDGS) and corn finishing diets.

	50% WDGS	Corn	P-value
Phospholipids (%)			
Phosphatidylcholine	43	36.07	< 0.01
Phosphatidylethanolamine	31.89	38.78	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	47.9	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	52.1	46.26	0.10

Our data matched with the results from Senaratne [7] in that feeding a high percentage of WDGS increased tenderness and calcium release early postmortem. This also confirmed with our hypothesis that the integrity of SR membranes of beef muscles are likely altered by feeding WDGS. However, the true mechanism that contributes to SR membrane instability is still unclear. The increase of PUFA in SR membrane is likely not the only contributor to the accelerated calcium release. The changes in phospholipid profile as well as the decrease in total phospholipid in the membrane might also contribute to SR membrane instability. Ji and Takahashi [10] found that SR phospholipids content decreases while sarcoplasmic calcium ions increase during the aging of pork and beef. This agrees with our results that the decrease in SR phospholipid had a big effect on calcium flux. It is likely that the increase in PUFA contributed to membrane oxidation, which increases activity of phospholipases to remove esterified fatty acids, causing the liberation of phospholipids. Finally, measuring lipid oxidation on muscle tissue is likely not the best way to measure SR membrane oxidation. A sensitive, simple, and reliable method that can detect lipid oxidation in extremely small sample

volume needs to be developed for direct measurement of SR membrane oxidative status.

IV. CONCLUSION

This study confirmed that feeding WDGS tended to increase tenderness and early postmortem calcium release, likely a result of increased total PUFA and decreased total phospholipid in SR membrane. Although the true mechanism and time course of membrane oxidation and calcium release are still unclear, these results provide conceptual foundation for a new research perspective on meat tenderization.

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REFERENCES

Papers:

1. Boleman, S., Boleman, S., Morgan, W., Hale, D., Griffin, D., Savell, J., Ames, R., Smith, M., Tatum, J., & Field, T. (1998). National Beef Quality Audit-1995: survey of producer-related defects and carcass quality and quantity attributes. Journal of Animal Science, 76(1), 96-103.
2. Garcia, L., Nicholson, K., Hoffman, T., Lawrence, T., Hale, D., Griffin, D., Savell, J., VanOverbeke, D., Morgan, J., & Belk, K. (2008). National Beef Quality Audit–2005: Survey of targeted cattle and carcass characteristics related to quality, quantity, and value of fed steers and heifers. Journal of Animal Science, 86(12), 3533-3543.
3. Hemmings, S. J. (2001). 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 Biochem Function, 19(2), 133-141.
4. Ji, J. R., & Takahashi, K. (2006). Changes in concentration of sarcoplasmic free calcium during post-mortem ageing of meat. Meat Science, 73(3), 395-403.
5. McKenna, D., Roebert, D., Bates, P., Schmidt, T., Hale, D., Griffin, D., Savell, J., Brooks, J., Morgan, J., & Montgomery, T. (2002). National Beef Quality Audit-2000: survey of targeted cattle and carcass characteristics related to quality, quantity, and value of fed steers and heifers. Journal of Animal Science, 80(5), 1212-1222.
6. Mello, A. S., Calkins, C. R., Jenschke, B. E., Carr, T. P., Dugan, M. E. R., & Erickson, G. E. (2012). Beef quality of calf-fed steers finished on varying levels of corn-based wet distillers grains plus solubles. Journal of Animal Science, 90(12), 4625-4633.
7. Miller, M. F., Carr, M. A., Ramsey, C. B., Crockett, K. L., & Hoover, L. C. (2001). Consumer thresholds for establishing the value of beef tenderness. Journal of Animal Science, 79(12), 3062-3068.
8. Platter, W. J., Tatum, J. D., Belk, K. E., Koontz, S. R., Chapman, P. L., & Smith, G. C. (2005). Effects of marbling and shear force on consumers' willingness to pay for beef strip loin steaks. Journal of Animal Science, 83(4), 890-899.
9. Senaratne, L. S. (2012). Mechanism and Control of Beef Tougheningduring Retail Display in High Oxygen Modified Atmosphere Packages. Unpublished Dissertation, University of Nebraska-Lincoln, DigitalCommons.

Proposal:

10. NCBA. (2012). FY 2013 Request for Proposals-Product Enhancement Research. In. Beef Research: The National Cattlemen's Beef Association (NCBA).