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Feeding Vitamin E May Reverse Sarcoplasmic Reticulum Membrane Instability Caused by Feeding Wet Distillers Grains Plus Solubles to Cattle

Michael D. Chao, Katherine I. Domenech and Chris R. Calkins

Summary

Steers were finished on either 0% or 30% wet distillers grains plus solubles with four antioxidant treatments (control; vitamin E; Agrado; vitamin E + Agrado). Feeding wet distillers grains plus solubles increased polyunsaturated fatty acids contents in SR membrane and altered sarcoplasmic reticulum phospholipid profiles. Steaks from steers fed wet distillers grains plus solubles also had more protein degradation. Supplementing vitamin E reversed the alteration of sarcoplasmic reticulum phospholipid profiles and prevented the accelerated protein degradation resulted from feeding wet distillers grains plus solubles. These findings suggest that feeding wet distillers grains plus solubles in the finishing diet may accelerate beef tenderization, while supplementing vitamin E may inhibit this distillers grains-induced beef tenderization effect.

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 cytoplasm where the ions activate the calcium-dependent proteolytic enzymes (the calpain system) and enhance tenderness. Research results from our lab (2012 *Nebraska Beef Cattle Report*, pp. 124–126; 2015 *Nebraska Beef Cattle Report*, pp. 117–119) showed that beef from cattle fed high concentration of WDGS is more tender than beef from cattle not fed WDGS or WDGS with dietary antioxidants. Our hypothesis is that including WDGS in feedlot diets increases PUFA concentration in the SR membrane, making the membrane more prone to oxidation. Such membrane integrity alterations may result in more rapid calcium leakage post-rigor and thus improve tenderness through greater activation of the calcium-dependent proteolytic enzyme. Antioxidants may preserve membrane integrity and inhibit this WDGS-accelerated tenderization process. Feeding WDGS and different antioxidants provides an excellent model to generate samples with varying degrees of SR membrane oxidation capacity to explore this proposed mechanism of beef tenderization.

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Procedure

One hundred and sixty Continental x British steers were blocked by BW, stratified by BW within each block, and assigned randomly to pens within block. Pens were randomly assigned to one of the eight treatments with two pens/treatment and 10 heads/pen. Cattle were fed for 106 d on a corn-based diet with 0% WDGS or 30% WDGS (DM basis) with four dietary antioxidant treatments (control; E at 1,000 IU/hd/d; AG at 215 ppm of feed; a combination of E at 500 IU/hd/d and AG at 215 ppm of feed). It is important to note that although 0% WDGS-control, 0% WDGS+AG, 30% WDGS-control and 30% WDGS+AG treatments were not supplemented with additional E, 50 IU hd/d of E was included in the mineral premix for all dietary treatments. For ease of comprehension, 0% WDGS-control, 0% WDGS+AG, 30% WDGS-control and 30% WDGS+AG refer to dietary treatments without additional E supplementation throughout this paper.

Ten strip loins (*Longissimus lumborum*) from each treatment (n=80) were collected and aged for 2, 7, or 14 d. Steaks were removed from each loin at each aging period and placed under retail display conditions (36 ± 4°F, and exposed to continuous 1,000–1,800 lux warm white fluorescence lighting). On d 0 and 7 of retail display for each aging period, steak samples were obtained 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). Steak samples for SR membrane fatty acid (via gas chromatography), total lipid, neutral lipid and phospholipid profiles (via thin-layer chromatography) were obtained at d 0 of retail display after 14 d of aging.

Data were analyzed using the GLIMMIX procedure of SAS (version 9.2, Cary, NC, 2009). Data for WBSF, free calcium concentration and proteolysis were analyzed as a split-split plot design with dietary treatments as the whole-plot, aging period as the split-plot and retail display time as the split-split plot. Sarcomere length, SR fatty acid, total lipid, neutral lipid and phospholipid profiles were analyzed as a completely randomized design. Separation of means was conducted using LSMEANS procedure with PDIFF or SLICEDIFF options at $P \leq 0.05$.

Results

Fatty acid analysis revealed distinct differences among fatty acid profiles of SR membrane from cattle fed different diets. Feeding 30% WDGS decreased ($P \leq 0.05$) the proportions of the fatty acids 16:0, 16:1, 18:0, 18:1V and total saturated fatty acids. There was also a tendency ($P \leq 0.10$) to decrease 18:1 and total monounsaturated fatty acids. However, feeding 30% WDGS increased ($P \leq 0.05$) 15:0, 17:0, 18:1 trans, 18:2 and total PUFA in the SR membrane (Table 1). The increase in PUFA content of the SR membrane supports the hypothesis that feeding WDGS may impair SR membrane integrity and thus accelerate free calcium release.

Furthermore, steaks from steers fed 30% WDGS-control had more ($P \leq 0.05$)

Table 1. Fatty acid profiles (%) of sarcoplasmic reticulum membranes from strip loins from steers fed a corn-based diet with 0% wet distillers grains plus solubles (WDGS) or 30% WDGS supplemented with or without vitamin E (E) or Agrado (AG) or a combination of E and AG

Fatty Acids	Dietary treatments								*P*-value
	0% WDGS	0% WDGS+E	0% WDGS+AG	0% WDGS+E+AG	30% WDGS	30% WDGS+E	30% WDGS+AG	30% WDGS+E+AG	
14:0	0.66^{ab}	0.69^{ab}	1.25^{a}	1.30^{a}	0.89^{ab}	1.05^{ab}	0.59^{ab}	0.45^{b}	≤ 0.01
15:0	0.30^{ab}	0.25^{b}	0.39^{ab}	0.42^{ab}	0.47^{a}	0.43^{ab}	0.41^{ab}	0.33^{ab}	≤ 0.01
16:0	20.52^{abc}	18.71^{bc}	21.98^{ab}	22.14^{a}	20.65^{abc}	19.83^{abc}	19.26^{abc}	17.84^{c}	≤ 0.01
16:1	2.23^{abc}	2.10^{bc}	3.03^{a}	2.81^{ab}	1.76^{c}	2.01^{bc}	1.81^{c}	1.70^{c}	≤ 0.01
17:0	0.89^{b}	0.83^{b}	1.03^{ab}	0.98^{ab}	1.36^{a}	1.24^{ab}	1.15^{ab}	1.09^{ab}	≤ 0.01
17:1	0.92	0.84	1.09	1.08	1.20	1.21	1.12	1.06	0.14
18:0	10.22	10.79	10.62	9.41	9.86	10.13	9.43	9.30	0.09
18:1T	1.90^{bcd}	1.43^{d}	2.03^{abcd}	1.71^{cd}	2.77^{ab}	2.79^{a}	2.29^{abc}	2.42^{abc}	≤ 0.01
18:1	30.45	32.08	35.60	31.55	26.45	31.15	27.68	27.31	0.06
18:1V	2.17^{ab}	2.14^{ab}	2.38^{a}	2.27^{ab}	2.05^{ab}	2.07^{ab}	1.99b	2.03^{b}	0.01
18:2	13.82^{ab}	11.58^{b}	11.12^{b}	13.81^{ab}	19.54^{a}	15.66^{ab}	17.55^{a}	18.61^{a}	≤ 0.01
20:1	0.52	0.49	0.46	0.55	0.41	0.41	0.47	0.42	0.23
20:3	1.16	1.17	1.01	1.26	1.04	0.94	1.21	1.09	0.74
20:4	4.55	3.99	3.30	4.61	3.78	3.51	4.51	4.05	0.56
20:5	0.43	0.47	0.38	0.44	0.35	0.35	0.46	0.39	0.75
22:4	0.56	0.51	0.41	0.49	0.44	0.37	0.58	0.49	0.34
22:5	1.08	0.93	0.72	0.98	0.80	0.69	1.03	0.85	0.23
SFA	32.60^{abc}	31.15^{abc}	35.16^{a}	34.26^{ab}	33.24^{ab}	32.68^{abc}	30.78^{bc}	28.90^{c}	≤ 0.01
MUFA	38.31	39.16	44.69	40.28	34.85	39.84	35.39	34.99	0.09
PUFA	21.60^{ab}	18.56^{ab}	16.78^{b}	21.59^{ab}	25.91^{a}	21.45^{ab}	25.14^{a}	25.39^{a}	0.03

Note: SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids
[a-d]Within a row, means without a common superscript differ at $P \leq 0.05$

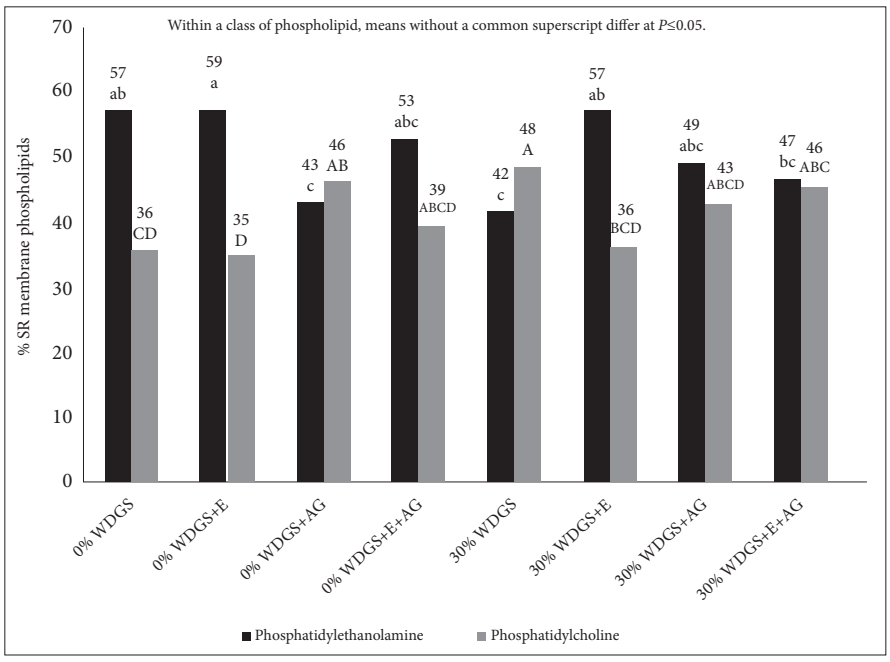

Figure 1. Phospholipid profile of sarcoplasmic reticulum membrane from strip loins from steers fed a corn-based diet with 0% wet distillers grains plus solubles (WDGS) or 30% WDGS supplemented with or without vitamin E (E) or Agrado (AG) or a combination of E and AG.

phosphatidylcholine in the SR membrane, but less ($P \leq 0.05$) phosphatidylethanolamine compared to steaks from steers fed 0% WDGS-control or 30% WDGS supplemented with E. It is interesting to note that supplementing AG in the 0% WDGS diet also created similar effects as the 30% WDGS diet for SR membrane phospholipid profile (Figure 1). Many studies have demonstrated that the conversion of phosphatidylethanolamine to phosphatidylcholine is an indicator of oxidative stress. It is possible that feeding a diet high in PUFA like WDGS might have induced oxidative stress in the SR, while supplementing E likely alleviated such stress on the SR. Furthermore, AG may have failed to act as an antioxidant, but transitioned into pro-oxidants, which stimulated the SR oxidative stress observed in this study.

At 2 d postmortem, steaks from steers fed 30% WDGS had more muscle protein degradation compared to steaks from steers fed 0% WDGS without any antioxidant

supplementation or either diet supplemented with E only. What is even more interesting is that AG in the 0% WDGS diet again created similar effects as the 30% WDGS diet, which not only failed to hold back proteolysis, but accelerated proteolysis (Figure 2). It is also worth noting that the least square means for troponin-T degradation at 2 d aging is highly correlated with the least square means for SR membrane phosphatidylcholine ($R^2 = 0.90$) and phosphatidylethanolamine ($R^2 = 0.88$) of the treatments (Figure 3). Based on these results, it is reasonable to suspect that SR oxidative stress may play a significant role in controlling rate of early postmortem protein degradation.

Finally, there were no differences in tenderness ($P > 0.10$) or free calcium concentrations ($P > 0.10$) among steaks from steer in any of the treatments, aging or display periods. It is likely that such subtle differences in tenderness can only be detected using extremely sensitive methodologies like protein degradation. These findings suggest that the WDGS-induced tenderness observed in this study may not be caused by lipid oxidation of SR membrane as proposed in our hypothesis. Evidence suggests that feeding WDGS in the finishing diet may induce SR oxidative stress, and such oxidative stress may hamper the ability of SR to effectively sequester free calcium early postmortem, leaving more free calcium behind in the cytoplasm. The free calcium interacts with the calcium-dependent proteinase and accelerates early postmortem protein degradation. Vitamin E appears to alleviate, while AG stimulates, oxidative stress.

Acknowledgement

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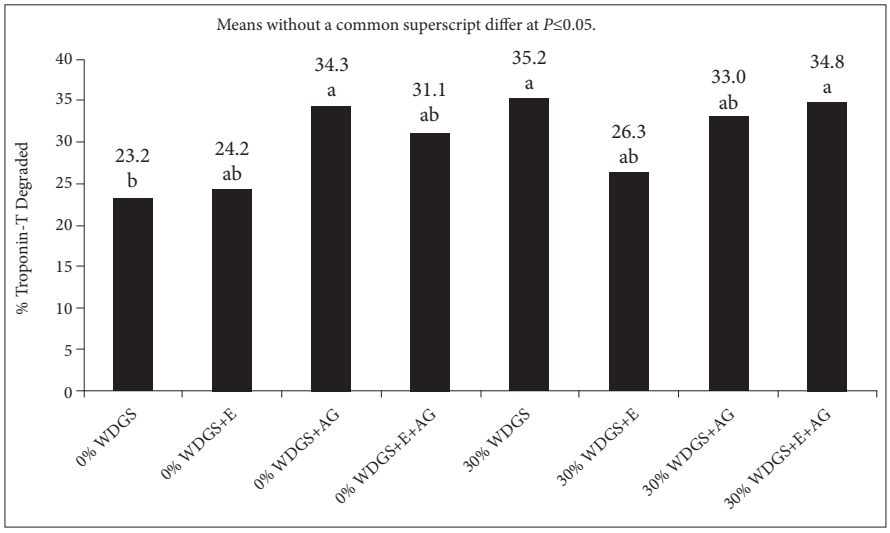

Figure 2. Protein degradation (Measured by troponin-T degradation) of strip loins (aged for 2 d) from steers fed a corn-based diet with 0% wet distillers grains plus solubles (WDGS) or 30% WDGS supplemented with or without vitamin E (E) or Agrado (AG) or a combination of E and AG.

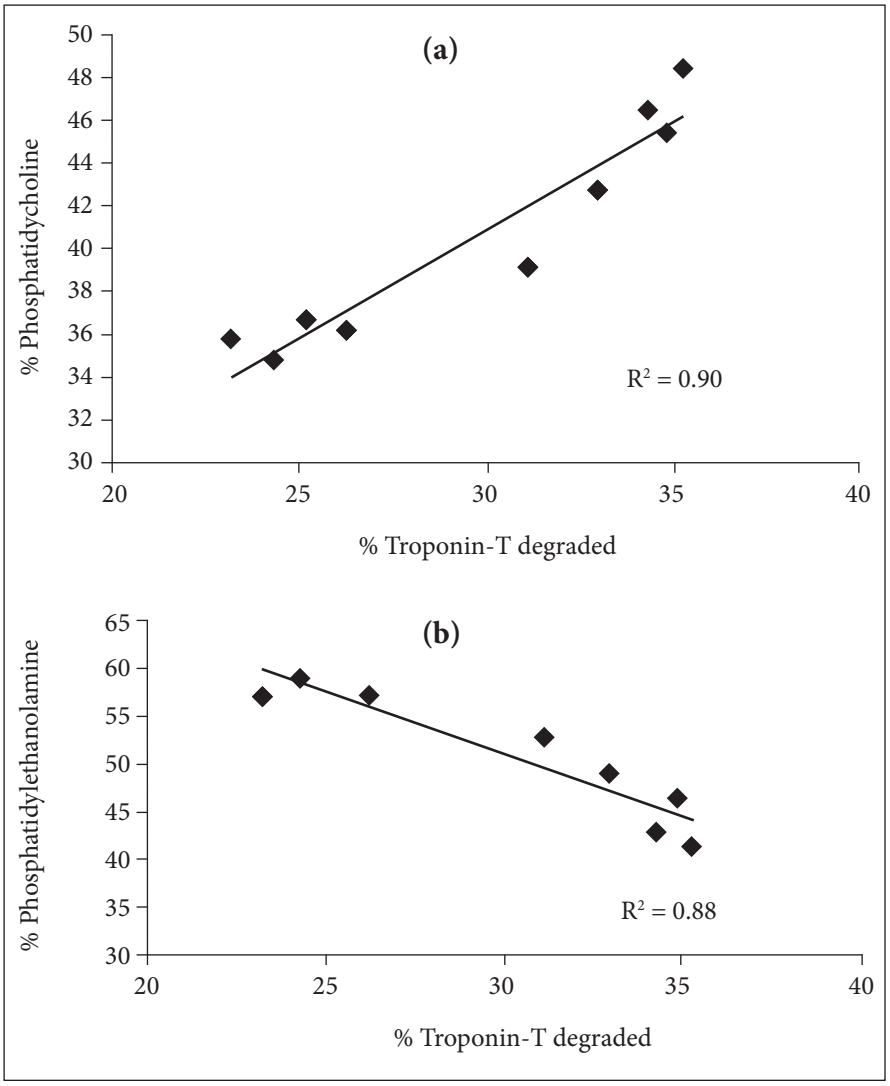

Figure 3. Relationship of the LS means of protein degradation (Measured by troponin-T degradation) of muscle aged for 2 d with a) LS means of phosphatidylcholine and b) LS means of phosphatidylethanolamine in total sarcoplasmic reticulum (SR) membrane phospholipid.