Supplemental Vitamin C Alleviates the Negative Effect of High Sulfur on Meat Quality

A.S. Leaflet R2766

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Summary and Implications

The data support the conclusion that vitamin C (**VC**) supplemented to feedlot cattle consuming high sulfur (**S**) diets may increase tenderness of the beef longissimus dorsi by protecting the protease µ-calpain. Additionally, VC supplementation improved the fatty acid profile of meat products by increasing omega 3 and omega 6 fatty acids (**FA**) and decreasing saturated fatty acids (**SFA**).

Introduction

Distillers grains (**DGS**) are a nutritional and inexpensive alternative to corn in feedlot diets; however, the concentration of S in DGS remains a limiting factor to inclusion in diets. In recent years, the impact of greater DGS (and S) inclusion has been well evaluated in feedlot diets. Previous research indicates high S diets are detrimental to both live and carcass-based performance, specifically decreasing average daily gain, hot carcass weight, and marbling scores. Additionally, high S may be contributing to the development of oxidative stress in the animal.

Depletion of body antioxidants hinders both ante- and postmortem performance, specifically decreasing protein degradation (i.e. tenderness) and increasing lipid oxidation. Because consumer acceptance of meat products is primarily dependent on tenderness and meat favor, antioxidants, such as VC and vitamin E, are attractive additives to live animal diets and/or during meat processing as a means to combat the development of an oxidative environment in muscle to prolong shelf-life. The objective of this study was to examine the impact of a supplemental rumen-protected VC on meat quality, specifically protein degradation, protein oxidation, and fatty acid profile of cattle fed varying concentrations of dietary S.

Materials and Methods

Angus-cross calf-fed steers (n = 120) were blocked by initial BW (781 ± 50 lbs) and randomly assigned to the following treatments: 1) low S (**LS**, 0.22% S), 2) LS + VC, 3) medium S (**MS**, 0.34% S), 4) MS + VC, 5) high S (**HS**, 0.56% S); MS + sodium sulfate, and 6) HS + VC (**Table 1**). Vitamin C consumption averaged 10.3 g VC/steer/d, while dietary S intake across the finishing period averaged 22.2 g, 34.1g, and 55.3g S/steer/d for LS, MS, and HS cattle, respectively.

Steers were harvested at a commercial facility in Denison, IA when greater than 60% of the steers in a pen were estimated to have 0.5 in of back-fat. Cattle were graded according to USDA standards and a rib-facing was collected from each carcass (n = 116). Rib-facings were homogenized in a blender using liquid nitrogen. Proximate analyses (percent moisture, fat, and protein) were determined from each steak (n = 116). Troponin T (n = 114) and µ-calpain autolysis (n = 84) were determined via western blotting, and FA profiles were determined from 5 steers/treatment (n = 30).

Data were analyzed as a complete randomized block design using the MIXED procedure of SAS. Single degree of freedom contrast statements were designed to compare: A) VC vs. no VC, B) linear effect of S, C) VC within low S, D) VC within medium and high S, and E) VC within high S.

Results and Discussion

µ-Calpain Autolysis (Figure 1)

The calpain family of proteases are essential for the meat tenderization process. Calpains degrade a variety of muscle proteins that contribute to muscle and meat integrity. Because calpain undergoes autolysis, three distinct bands are detected via western blot: 80 kDa (the intact protein), 78 kDa (the intermediate product), and 76 kDa (fully autolyzed). The autolysis process is the hallmark of the activation of calpain, thus a greater proportion present as the 80 kDa subunit indicates that µ-calpain has not been active. There are several factors within muscle that can influence the activity of calpain, including: Ca availability (required for activation), rate and extent of pH decline, and an oxidative environment in the muscle. Previous research indicates an oxidative environment may interfere with the enzyme's ability to complete autolysis and its ability to exert proteolytic activity.

In the present study, increasing dietary S increased (P = 0.03) the quantity of the catalytic subunit of the protease µ-calpain present as the intact 80 kDa band. However, the addition of VC to the high S diet (0.56% S) tended (P = 0.09) to decrease the 80 kDa subunit, indicating the protease was able to continue the autolysis process. Interestingly, presence of the 76 kDa subunit was decreased (P = 0.03) in MS and HS diets, relative to MS+VC and HS+VC. Within the high S treatments the addition of VC increased (P = 0.05) the proportion of the 76 kDa subunit of µ-calpain from 38.1% (HS) to 55.6% (HS+VC). It is possible that this decrease in the presence of the 76 kDa subunit in the medium and high S treatments may be due to the difference in the inclusion of DGS to the diet, as the medium and high S treatments received a diet of 40% DDGS compared to the low S treatment receiving only 18% DDGS. The

concentration of fat in DDGS makes it an attractive feed ingredient, providing energy to feedlot rations, and the present study utilized full-fat DDGS (10% fat); however, the combination of dietary S and fat may be a contributing factor to the decrease in the 76 kDa subunit in the medium and high S diets. The inclusion of VC, regardless of dietary S, tended to increase ($P = 0.09$) the portion of the catalytic subunit of μ-calpain present as the fully autolyzed 76 kDa subunit. These data suggest VC may have a protective effect on calpains, supporting autolysis of the protease.

Troponin T degradation (Figure 2)

Troponin T is a component of the troponin complex, which aids in exposing the active site of actin to facilitate muscle contraction. The troponin complex is especially susceptible to degradation by calpains, making it an excellent marker for protein degradation within the muscle. In the present study, increasing dietary S tended ($P = 0.07$) to decrease the 30 kDa degradation product of troponin T, specifically noted in the high S treatments. Less troponin T degradation in steaks collected from cattle receiving the high S diets may be partially explained by a greater percent of the intact subunit of calpain (80 kDa), rendering it unable to exert proteolytic activity.

While shear force was not determined in the present study, previous research indicates a positive relationship between the extent of troponin T degradation and tenderness scores. In the present study, degradation of troponin T was negatively associated (-0.53; $P = 0.003$) with the percentage of the 80 kDa subunit of μ-calpain and positively associated (0.63; $P = 0.001$) with the percentage of the 76 kDa subunit of μ-calpain. These results suggest diets exceeding 0.34% dietary S may result in negative impacts on the tenderness and eating quality of the final beef products.

Proximate Analysis (Table 2)

Increasing dietary S decreased ($P = 0.001$) the percent fat in the steaks, primarily being driven by the high S treatments. Addition of VC to the high S treatment tended ($P = 0.07$) to decrease the percent protein of the steaks, corresponding to a numerically greater percent lipid (4.29% HS+VC and 3.56% HS).

Fatty acid percentages and ratios (Table 3)

Distillers grain products are variable in fat content, ranging from 4-12% fat. The FA profile of corn indicates a greater percentage of PUFA, specifically the omega 6 (at 42%) and omega 3 (at 73%) FA. Fatty acids entering the rumen are subject to biohydrogenation (addition of hydrogen to unsaturated fatty acids) to yield SFA; however, that at least some of the fat associated with DGS bypasses rumen alteration, resulting in increased presence of PUFA in the meat.

In the present study, as dietary S increased the total SFA decreased ($P < 0.05$); while an increase ($P < 0.05$) in total PUFA, PUFA:SFA, and omega 3 and 6 FA. Because corn oil is high in PUFA, the differences in FA profiles may be partially explained by differences in DGS inclusion, as low S diets contained 18% DDGS compared 40% inclusion in the medium and high S treatments. The inclusion of VC, regardless of dietary S concentration, magnified the decrease ($P = 0.02$) in SFA and the increase ($P < 0.03$) of PUFA, PUFA:SFA, and omega 3 and 6 FA.

Omega 3 and 6 FA are positively associated with health benefits related to heart disease, normal brain and eye development, and immunity. Benefits of VC supplementation were specifically noted in the low S treatment, as the total omega 3 FA increased from 0.30% (LS) to 0.50% (LS+VC); while the percentages remained similar within the medium (0.53%) and high S (0.50%) treatments. These results suggest the addition of VC to a lower inclusion of DDGS (18%) may result in a beef steak with omega 3 FA concentration comparable to cattle consuming 40% DDGS. These data suggest vitamin C may potentially be a way for producers to improve the lipid profile of steak to provide health benefits for consumers.

Acknowledgements

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Table 1. Ingredient composition and chemical analysis of finishing diets (% DM basis).

Item	Low S[1]	Medium S[1]	High S[1,6]
Corn	69.7	48.0	48.0
Corn dried distiller's grains[2]	18.0	40.0	38.9
Chopped hay	9.00	9.00	9.00
Limestone	2.00	2.00	2.00
Salt	0.31	0.31	0.31
Vitamin A premix[3]	0.10	0.10	0.10
Trace mineral premix[4]	0.035	0.035	0.035
Rumensin90[5]	0.016	0.016	0.016
Sodium sulfate[6]	--	--	1.11
Urea	0.80	--	--
Analyzed composition			
CP, %	14.3	16.6	16.6
NEg, Mcal/kg DM	1.3	1.3	1.3
S[7], %	0.22	0.34	0.55

[1]Vitashure C, provided by Balchem Corp., replaced DDGS at 0.215% DM to achieve 10 g of vitamin C per steer per day; vitamin C intake averaged 10.3 g per steer per day

[2]Five loads of DDGS from Lincoln Way Energy (Nevada, IA) were used during the trial with S concentrations of 0.72%, 0.67%, 0.70%, 0.79%, and 0.55%

[3]Vitamin A premix contained 4,400,000 IU/kg

[4]Provided per kg of diet: 30 mg Zn as $ZnSO_4$; 20 mg Mn as $MnSO_4$; 0.5 mg I as $Ca(IO_3)_2(H_2O)$; 0.1 mg Se as Na_2SeO_3; 10 mg Cu as $CuSO_4$; and 0.1 mg Co as $CoCO_3$

[5]Provided at 27 g/ton diet (donated by Elanco Animal Health)

[6]Sodium sulfate added to the diet, at the expense of DDGS, to increase the percent S of the medium S diet by 0.36% S

[7]Percent S for low, medium, and high diets are based on repeated measures analysis of samples collected over the 149 d study

Table 2. Proximate analysis of rib-facings collected from cattle supplemented with vitamin C on a low, medium, or high S diet.

Diet S	Low		Medium		High			
Diet vitamin C	-	+	-	+	-	+	SEM	Sig[1-3]
Item								
Pen (steers)	5(13)	5(13)	5(15)	5(15)	5(14)	5(15)		
Moisture, %	71.9	72.2	72.1	72.4	73.2	73.0	0.29	B**
Fat, %	5.40	5.03	5.48	5.05	3.56	4.29	0.38	B**
Protein, %	22.3	22.1	21.5	22.0	22.3	21.9	0.10	E†

[1]Sig: Significance of Contrast Statement

[2]Contrast Statements: A = vitamin C vs. no vitamin C; B = linear effect of S; C = vitamin C within low S corn diet; D = vitamin C within medium and high S; E = vitamin C within high S

[3]**($P \leq 0.01$);* ($P \leq 0.05$); †($P \leq 0.10$)

Table 3. Effect of vitamin C on fatty acid percentages and ratios of rib-facings collected from cattle consuming a low, medium, or high S diet.

Diet S	Low		Medium		High			
Diet vitamin C	-	+	-	+	-	+	SEM	Sig[1-3]
Item								
Steers per treatment (n)	5	5	5	5	5	5		
SFA, %[4]	46.26	44.78	44.29	42.56	43.86	43.42	0.72	A*B*C†D†
PUFA, %[5]	5.98	6.69	8.09	8.44	8.84	10.01	0.34	A*B**D*E*
PUFA:SFA	0.14	0.15	0.18	0.20	0.21	0.23	0.01	A**B**D**E**
n3, %[6]	0.30	0.50	0.53	0.53	0.52	0.47	0.03	A*B**C**
n6, %[7]	5.65	6.19	7.58	7.92	8.31	9.53	0.33	A*B**D*E*
n3:n6	0.05	0.08	0.07	0.07	0.06	0.05	0.004	B**C**D†E*

[1]Sig: Significance of Contrast Statement
[2]Contrast Statements: A= vitamin C versus no vitamin C; B = linear effect of S; C = vitamin C within low S corn diet; D = vitamin C within medium and high S; E = vitamin C within high S
[3]**($P \leq 0.01$);* ($P \leq 0.05$); †($P \leq 0.10$)
[4]Saturated fatty acid calculation, sum of: C10:0, C12:0, C14:0, C15:0, C17:0, C18:0, and C24:0
[5]Polyunsaturated fatty acid calculation, sum of: C18:2n6, C18:3n3, C20:2n6, C20:3n3, C20:4n6, C20:5, C22:5n3, c9-t11 CLA
[6]Omega 3 fatty acid calculation, sum of: C18:3n3, C20:5, and C22:5n3
[7]Omega 6 fatty acid calculation, sum of: C18:2n6, C20:2n6, C20:3n6, C20:4n6, c9-t11 CLA

Figure 1.

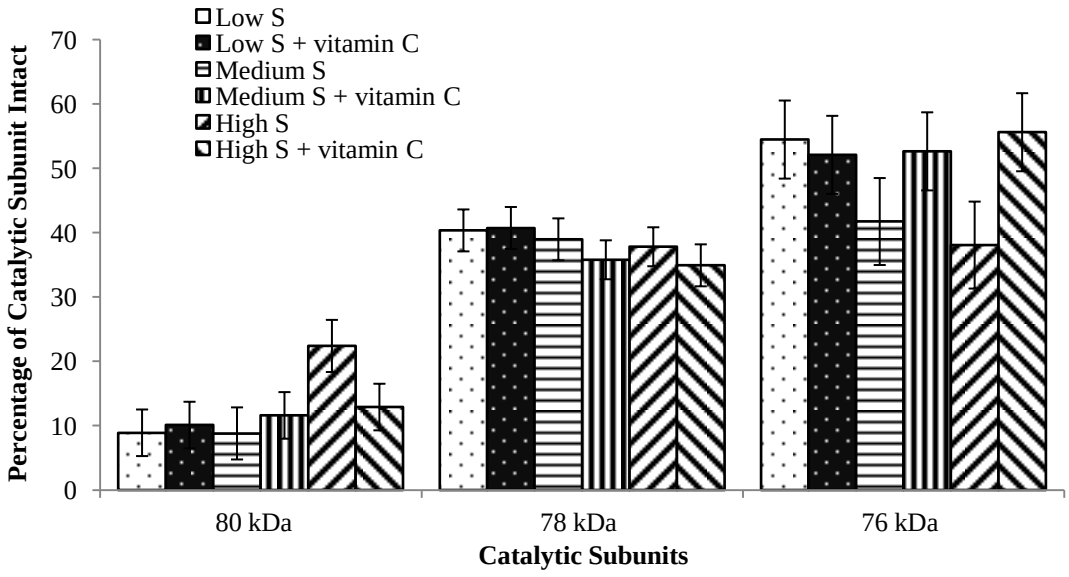

Figure 1. Impact of a rumen-protected supplemental vitamin C source on µ-calpain autolysis at 2 d postmortem in rib-facings collected from cattle consuming a low S (0.22%), medium S (0.34%), or high S (0.56%) diet. 80 kDa subunit: linear effect of S ($P = 0.03$); vitamin C within high S ($P = 0.09$); 78 kDa subunit: linear effect of S ($P = 0.108$); 76 kDa subunit: vitamin C versus no vitamin C ($P = 0.09$); vitamin C within high S ($P = 0.05$); vitamin C within medium and high S ($P = 0.03$).

Figure 2.

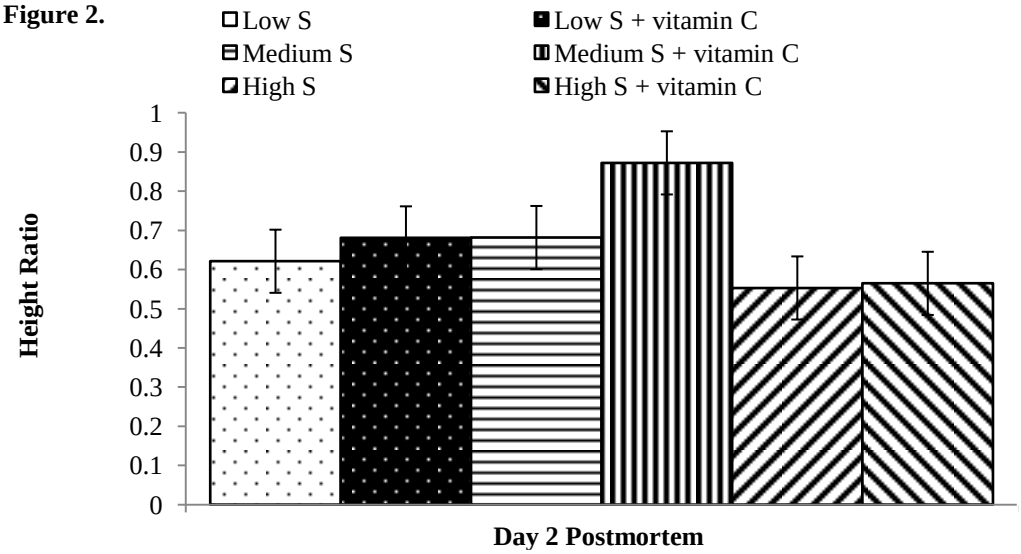

Figure 2. Effects of a rumen-protected supplemental vitamin C source on troponin-T degradation at 2 d postmortem in rib-facings collected from cattle consuming a low S (0.22%), medium S (0.34%), or high S (0.56%) diet. Tendency for a linear effect of S ($P = 0.07$). Height ratio represents the sample peak height (of the band detected) divided by the control sample peak height; greater ratio indicates a greater degradation.