Effects of duration of vitamin C supplementation during the finishing period on postmortem protein degradation, tenderness, and meat color of the longissimus muscle of calf-fed steers consuming a 0.31 or 0.59% sulfur diet

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ABSTRACT: High-S (HS) diets have been identified as a causative agent in the development of oxidative stress in cattle, which in postmortem muscle can negatively alter meat quality. Vitamin C (VC) is a potent antioxidant produced endogenously by cattle; however, exogenous supplementation of VC may be useful when HS diets are fed to cattle. The objective of this study was to examine the impact of duration of VC supplementation, for the first 56, 90, or 127 d, during the finishing period on meat color and tenderness of the longissimus thoracis (LT) collected from calf-fed steers consuming a 0.31 or 0.59% S diet. Angus steers (n = 42) were stratified to pens by initial BW (304 ± 13 kg) and GeneMax marbling score (4.3 ± 0.12), and each pen was randomly assigned to 1 of 7 treatments (6 steers/pen, 1 pen/treatment), including HS (0.59% S, a combination of dried distillers grains plus solubles and sodium sulfate) control (HS CON), HS CON + 10 g VC·steer−1·d−1 for the first 56 d (HS VC56), 90 d (HS VC90), or 127 d (HS VC127), low S (LS; 0.31% S) + 10 g VC·steer−1·d−1 for the first 56 d (LS VC56), 90 d (LS VC90), or 127 d (LS VC127). Steers were harvested (n = 40) and, after a 24-h chill, rib sections (LT) were collected. pH was determined on each rib section before division into 3 sections for determination of 1) 7-d retail display and color and Warner–Bratzler shear force (WBSF), 2) 14-d WBSF determination, and 3) protein degradation and collagen content (2 d postmortem). Data were analyzed by ANOVA as a completely randomized design, with the fixed effect of treatment. Individual feed intake was recorded, and steer was the experimental unit. The HS steers had a greater and lesser percent of the 80- and 76-kDa subunits of calpain-1 (P ≤ 0.05), respectively, and tended to have less (P = 0.08) troponin T degradation (d2), and more (P = 0.02) collagen than LS steers. Increasing days of VC supplementation decreased (P = 0.05) the percentage of the 80 kDa subunit of calpain-1 in HS steers but actually increased it in LS steers (P = 0.003). Supplementing VC, regardless of dietary S, did not affect meat collagen, WBSF, or color (P ≥ 0.12). a* and b* values were greater (P ≤ 0.05) in the LS treatments compared to the HS treatments. Increasing the days of VC supplementation to steers fed a HS diet appears to alleviate the negative effects of the HS diet on calpain-1 but has no effect on muscle tenderness or meat color.

Key words: calpain-1, shear force, sulfur, vitamin C

INTRODUCTION

Decreased growth and carcass traits are common in feedlot cattle consuming excessive quantities of S (Spears et al., 2011; Uwituze et al., 2011), which may partly be attributed to the role of S, as hydrogen sulfide (H₂S), in the development of oxidative stress (Truong et al., 2006; Pogge and Hansen, 2013a). In large quantities, H₂S can impede protein function and enzyme activity by interacting with the associated trace mineral cofactors (Pietri et al., 2011).

Protein and lipid oxidation in postmortem muscle can negatively alter meat quality (Morrissey et al., 1994; Rowe et al., 2004b). Previous use of exogenous antioxidants, such as vitamin C (VC), during meat processing has prolonged the reducing environment of meat and limited protein oxidation (Nam and Ahn, 2003; Realini et al., 2004). Pogge et al. (2014b) report-
ed that finishing steers fed a high-S (HS) diet showed a lesser proportion of the 76-kDa subunit of calpain-1, a historical marker of proteolytic activity in the longissimus thoracis (LT), but VC added to the HS diet increased the 76-kDa subunit to values similar to the low-S treatments.

Cattle may be at the greatest risk of developing S-induced oxidative stress within the first 10 to 35 d of consuming a HS diet, as during this phase peak H2S production is occurring (Loneragan et al., 2001). Previous studies have supplemented VC throughout the finishing period; but, given the initial 10- to 35-d risk period for the development of S-induced oxidative stress, it was hypothesized that the addition of VC to cattle diets for at least the first 56 d of consuming a HS diet would lessen the risk of steers developing S-induced oxidative stress and the associated negative effects on meat quality attributes. The objective of this study was to examine the impact of duration of VC supplementation for the first 56, 90, or 127 d during the finishing period on meat color and tenderness of the LT collected from calf-fed steers consuming a 0.31 or 0.59% S diet.

MATERIALS AND METHODS

Procedures and protocols for this experiment were approved by the Iowa State University Institutional Animal Care and Use Committee, protocol number 3-13-7225-B.

Animals and Experimental Design

Angus steers (n = 42) from Iowa State University’s McNay Research Farm (Chariton, Iowa) were transported to the Iowa State University Beef Nutrition Research Center (Ames, IA) in April 2013. Steers were stratified to treatments by the average of 2 consecutive d of BW collected on d −5 and −4 (304 ± 13 kg BW) and GeneMax marbling score (4.3 ± 0.12; only steers with scores 3, 4, or 5 were used), and randomly assigned to 1 of 7 treatments arranged in an augmented factorial design (6 steers/pen, 1 pen/treatment). Treatments included 1) HS (0.59% S) control (HS CON), 2) HS CON + 10 g VC∙steer−1∙d−1 for the first 56 d of the finishing period (HS VC56), 3) HS CON + 10 g VC∙steer−1∙d−1 for the first 90 d of the finishing period (HS VC90), 4) HS diet + 10 g VC∙steer−1∙d−1 for the entire 127-d finishing period (HS VC127), 5) low S (LS; 0.31% S) + 10 g VC∙steer−1∙d−1 for the first 56 d of the finishing period (LS VC56), 6) LS diet + 10 g VC∙steer−1∙d−1 for the first 90 d of the finishing period (LS VC90), and 7) LS diet + 10 g VC∙steer−1∙d−1 for the entire 127-d finishing period (LS VC127). Diets are shown in Table 1. Two premixes, each containing dried distillers grains plus solubles (DDGS) as a carrier, were used to deliver 1) VC (containing Vitashure C50, a rumen protected ascorbate, 50% VC product; Balchem Corp., New Hampton, NY) and 2) sodium sulfate to diets to achieve the desired VC and S concentrations, respectively.

Cattle were limit fed their respective study diet (without VC) for 5 d before the start of the study (d −5 to −1) to decrease the risk of S toxicity in steers switched to the 0.59% S diet. Following the limit feeding period, steers were weighed on 2 consecutive days (d −1 and 0; 311 ± 12.4 kg BW) and on d 0 the VC premix containing Vitashure C50 (Balchem Corp.) was used to introduce VC at a dose of 10 g VC∙steer−1∙d−1 to the appropriate diets. Individual intakes were recorded using the Iowa State University Feed Intake Management System (Dahlke et al., 2008) via the unique electric identification tag assigned to each steer. Over the course of the study, dietary intake was 57.1, 55.6, 60.7, 60.9, 30.3, 31.1, and 31.3 ± 2.17 g S∙steer−1∙d−1 for HS CON, HS VC56, HS VC90, HS VC127, LS VC56, LS VC90, and LS VC127, respectively, and calculated daily supplemental VC consumption was 0, 9.8, 10.5, 10.2, 10.6, 10.6, and 10.3 ± 0.32 g VC∙steer−1∙d−1 for HS CON, HS VC56, HS VC90, HS VC127, LS VC56, LS VC90, and LS VC127, respectively. Further information regarding growth performance and carcass traits has been reported by Pogge et al. (2015).

Steers were slaughtered on d 128 (n = 40 steers; 459 ± 6.0 kg BW) at a commercial packing facility in Denison, IA (Tyson Fresh Meats) when greater than 60% of steers in a pen were estimated by visual appraisal, by trained personnel, to have approximately 1.27 cm of back fat (BF), which was confirmed with ultrasonography (1.15 ± 0.128 cm BF). Individual identification was maintained with each carcass following harvest. Following a 24-h chill period, carcasses (n = 40) were ribbed and graded according to USDA standards by trained representatives of the Tri-County Carcass Futurity (Iowa State University Beef Extension, Lewis, IA). Carcass data collected included HCW, marbling score, BF, KPH, LM area, and quality and yield grades and have been previously reported (Pogge et al., 2015).

Sample Collection

Following the 24-h chill and grading, a rib-section, approximately 7.6 cm thick, across the length and width of the LT and the 12th rib, was removed from the right side of each carcass (n = 40). Rib-sections collected from each steer were sealed in a plastic bag with an identification tag and immediately transported on ice back to the Iowa State University Meats Laboratory.
Vitamin C, sulfur, and protein degradation values were averaged and used for statistical analysis.

2.5-cm steak from each carcass was used for a 7-d trial. Upon arrival in the Meats Laboratory (2 d postmortem), Inc., Kolding, Denmark) at 2 separate locations on the retail display, analysis of meat color, and shear force measures of muscle pH were determined using a glass penetration pH electrode (pH-Star; SFK Technology, Inc., Kolding, Denmark) at 2 separate locations on the rib section. Two buffers (pH 4.0 and 7.0, at the meat temperature) were used to calibrate the probe. The 2 pH values were averaged and used for statistical analysis.

The deboned samples were divided into 3 separate steaks (across the length and width of the LT). One, 2.54-cm steak from each carcass was used for a 7-d retail display, analysis of meat color, and shear force determination. Another, 2.54-cm steak from each carcass was immediately vacuum packaged, aged, for 14 d at 2°C, and shear force was determined. The remaining steak sample (approximately 1.27 cm) from each rib-section was trimmed of excess fat and divided into 2 portions and stored at −20°C until further analysis. Frozen samples were powdered in a Waring blender (Waring Products, Torrington, CT) before analysis using liquid N₂ to ensure a homogenized sample.

### Color Analysis, Retail Display, Warner–Bratzler Shear Force, and Collagen Content

Steaks (n = 40) designated for retail simulated display were placed on a styrofoam tray with an identification tag and wrapped with an oxygen permeable overwrap (oxygen rate of transmission 21,700 cm²·m⁻²·24 h⁻¹ at 23°C; water transmission rate 496 g·m⁻²·24 h⁻¹ at 37.8°C and 90% relative humidity; Western Plastics, Calhoun, GA). Steaks were refrigerated for 7 d at 2°C under retail simulated lighting (Sylvania F40N, 3,600 K, color rendering index = 86; 2,150 ± 50 lux of Deluxe Cool White fluorescent light; constant lighting 24 h/d; Osram Sylvania, Danveres, MS). Instrumental surface color analysis (Hunter LabScan XE; Spectrophotometer; Hunter Associates Laboratory, Inc., Reston, VA) was conducted initially on d 1 (approximately 30 h postmortem, after allowing steaks to bloom for 2 h at 2°C under the retail lighting), d 2, 5, and 7 (at the end of display), according to methods described by Pogge et al. (2014a). Hue angle and saturation index of each steak was determined according to the equations previously reported by Gardner et al. (2006).

Following the completion of the 7- and 14-d retail/aging period, samples were stored at −20°C until Warner–Bratzler shear force (WBSF) analysis. Samples were thawed at 2°C for 24 h before cooking. Steaks were cooked and analyzed according to the methods described by Pogge et al. (2014a). Total, soluble, and insoluble collagen was determined (n = 40) according to the methods described by Hill (1966) and Cross et al. (1973). Hydroxyproline was converted to collagen (μg collagen/g of sample) by multiplying the hydroxyproline concentration of supernatants by 7.52 and residuals by 7.25 (Cross et al., 1973).

### Western Blot Analysis

The protein concentrations of homogenized tissue solubilized in whole muscle buffer were determined using a modified Lowry assay (Lowry et al., 1951) with premixed reagents (DC protein assay; Bio-Rad Laboratories, Hercules, CA). The final SDS-PAGE sample (4 mg protein/mL) was prepared as described by Lonergan et al. (2001). Whole muscle protein extraction, gel composition, and Western blotting for d 2 and 7 troponin T (TT; 30-kDa subunit) and d 2 calpain-1 (n = 40; 80-, 78-, and 76-kDa subunits) were conducted according to a modified method previously reported.

### Table 1. Ingredient composition of finishing diets (% DM basis)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Low-S diet1,2</th>
<th>High-S diet1,2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry-rolled corn</td>
<td>42.92</td>
<td>42.92</td>
</tr>
<tr>
<td>Corn dried distillers grains3</td>
<td>39.73</td>
<td>38.36</td>
</tr>
<tr>
<td>Corn silage</td>
<td>15.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.62</td>
<td>1.62</td>
</tr>
<tr>
<td>Salt</td>
<td>0.31</td>
<td>0.31</td>
</tr>
<tr>
<td>Vitamin A premix4</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Trace mineral premix5</td>
<td>0.035</td>
<td>0.035</td>
</tr>
<tr>
<td>Rumensin906</td>
<td>0.014</td>
<td>0.014</td>
</tr>
<tr>
<td>Sodium sulfate7</td>
<td>0.275</td>
<td>1.64</td>
</tr>
<tr>
<td>Analyzed composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S6, %</td>
<td>0.31</td>
<td>0.59</td>
</tr>
<tr>
<td>Calculated composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid8, %</td>
<td>6.23</td>
<td>6.18</td>
</tr>
<tr>
<td>Vitamin E10, IU/kg∙1 diet DM</td>
<td>34.30</td>
<td>34.35</td>
</tr>
</tbody>
</table>

1. Treatments: Low-S diet: low S (0.25% S) + 10 g vitamin C∙steer⁻¹∙d⁻¹ control and high-S diet: high S (0.55% S) control, 0 VC, and a high S (0.55% S) + 10 g vitamin C∙steer⁻¹∙d⁻¹.
2. Vitashure C (donated by Balchem Corp., New Hampton, NY) replaced dried distillers grains plus solubles (DDGS), by 0.16 to 0.21% diet DM, to achieve targeted S content (low [0.31%] or high [0.59%] S, respectively) in the diet.
3. Two loads of DDGS (Lincoln Way Energy, Ames, IA) were used during the trial. S concentrations were 0.33 and 0.32% and fat content was 9.59 and 9.74%.
4. Vitamin A premix contained 4,400,000 IU∙kg⁻¹ diet DM 34.30 34.35
5. Provided at 27 g/907.18 kg diet (donated by Elanco Animal Health, Greenfield, IN).
6. Provided at 77 g/907.18 kg diet (donated by Elanco Animal Health, Greenfield, IN).
7. Sodium sulfate was included at 0.275 or 1.64% diet DM, at the expense of DDGS, to achieve targeted S content (low [0.31%] or high [0.59%] S, respectively) in the diet.
8. S content for treatments are averaged across treatment based on least squares mean averages throughout the entire study.
9. Lipid content was calculated from the analyzed lipid content of DDGS and corn silage, and the NRC (1996) value of corn was used.
10. Vitamin E concentrations were calculated based on NRC (1996) values for each ingredient.
The amount of the TT degradation product was used to calculate a sum of calpain immunoreactive bands. A common secondary antibody, goat anti-mouse IgG peroxidase conjugate (A-2554; Sigma-Aldrich), was diluted 1:10,000 and 1:5,000 for TT and calpain-1 analysis, respectively. Western blots were imaged and analyzed using ChemiImager 5500 (Alpha Innotech, San Leandro, CA) and Alpha Ease FC software (version 2.03, Alpha Innotech). Density of immunoreactive TT degradation product (30 kDa) was determined. A percentage of the total for each immunoreactive band was calculated (Cruzen et al., 2015).

### Statistical Analysis

Data were analyzed by ANOVA as a completely randomized design using the Mixed Procedure of SAS (SAS Inst. Inc., Cary, NC). The model for the analysis of single time point data of collagen content, ultimate pH, shear force, TT, and calpain-1 included the fixed effect of treatment. Meat color data were analyzed as repeated measures and included the fixed effects of treatment, day of sampling, and the interaction. Day was the repeated effect. Autoregressive 1 was selected as the covariance structure for all repeated measures analysis based on the lowest Akaike Information Corrected Criterion. Initial (d 1) color values were used as a covariate for color measures. Individual steer was the experimental unit for all data analysis (n = 6/treatment); however, the sample size was 4 for the HS VC56 steers, as 2 steers were removed from the test late in the study due to spinal cord injury (d 104) and death (d 122). Six single df contrast statements were constructed before analysis: 1) HS diets vs. LS diets; 2) HS CON vs. VC supplemented treatments within the HS treatment; 3) linear effect of days of VC supplementation within the HS diets; 4) quadratic effect of days of VC supplementation within the HS diets; 5) linear effect of days of VC supplementation within the low-S diets; F) quadratic effect of days of VC supplementation in low-S diets.

### RESULTS

#### Shear Force, Ultimate pH, and Color Analysis

Shear force, ultimate pH, and color analysis data are reported in Tables 2 and 3. On d 7, WBSF was not different (P ≥ 0.20) due to treatment; however, on d 14 a quadratic effect of VC supplementation (P = 0.07) in the HS diet was noted. This effect was likely due to a greater force required by the HS VC90 treatment compared with the HS CON, HS VC56, and HS VC127 treatments. Additionally, on d 14, a tendency (P = 0.09) for a linear effect of days of VC supplementation in the LS diet was primarily being driven by the LS VC127 treatment, which required approximately 5.1 to 6.5 N more force than LS VC56 and LS VC90 treatments. The ultimate pH of the steaks did not differ (P ≥ 0.12) by treatment.
Vitamin C, sulfur, and protein degradation

A treatment by day interaction ($P = 0.03$) was noted in L* values (Table 3). This interaction can be explained by the increase ($P \leq 0.02$) in L* values between d 1 and 2 of the HS CON, HS VC127, LS VC56, and LS VC90 treatments, and a tendency for an increase ($P = 0.06$) in the HS VC56 treatment. A further increase ($P \leq 0.01$) in L* was observed between d 2 and 5 within the LS treatments supplemented with VC for 56 or 127 d, while no difference ($P \geq 0.23$) was observed within any of the HS treatments. The HS CON and LS VC90 L* values between d 5 and 7 increased ($P \leq 0.01$), while no change in L* was noted between the remaining treatments. The HS VC90 treatment had a lesser L* value on d 2 than HS VC127 ($P = 0.08$) and LS VC90 ($P = 0.05$), but this difference was not evident by d 5 ($P \geq 0.20$).

Color values ($a^* [P = 0.03]$ and $b^* [P = 0.05]$) were greater in the LS treatments than in the HS treatments (Table 3). Regardless of S content, there was no effect ($P \geq 0.11$) of days of VC supplementation for $a^*$ or $b^*$. Saturation values were greater ($P = 0.02$) in the steaks collected from steers consuming the LS diet than those consuming the HS diet; however, VC supplementation did not alter the saturation values ($P \geq 0.14$). Hue angle values were not different ($P \geq 0.14$) due to S or VC content in the diet.

### Collagen Content

Soluble, insoluble, and total collagen contents of the LT are reported in Fig. 1. Total and soluble collagen (mg/g tissue) were greater ($P = 0.02$) and insoluble collagen tended to be greater ($P = 0.06$) in the steaks from steers fed HS diets compared with those fed LS diets, while no difference ($P \geq 0.12$) in the percent soluble and insoluble collagen was noted among the 2 concentrations of S. There was no effect of VC supplementation on soluble, insoluble, or total collagen content (mg/g tissue or percent; $P \geq 0.18$).

### Western Blot Analysis

Calpain-1 (2 d postmortem) and TT degradation (2 and 7 d postmortem) data are presented in Fig. 2 and 3, respectively. Steaks from steers consuming the HS diet, regardless of VC inclusion, showed a greater ($P = 0.05$) percentage of the calpain-1 catalytic subunit present as the intact 80-kDa subunit and a lesser ($P = 0.04$) percentage of the 76-kDa (fully autolyzed) subunit of calpain-1 than steers fed the LS diet. Within the HS diet, increasing the days of VC consumption linearly decreased ($P = 0.05$) the percentage of the 80-kDa subunit of calpain-1; however, the percentages of the 78- and 76-kDa subunits were not different ($P \geq 0.13$) between the HS treatments. Alternately, within the LS diet, in-

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**Table 3.** The effect of timing of supplementation of 10 g VC∙steer⁻¹∙d⁻¹ for 0, 56, 90, or 127 d of finishing on instrumental meat color of steaks collected from steers fed a 0.31 or 0.59% S diet

<table>
<thead>
<tr>
<th>Treatment¹</th>
<th>n (steers)</th>
<th>HS CON</th>
<th>HS VC56</th>
<th>HS VC90</th>
<th>HS VC127</th>
<th>LS VC56</th>
<th>LS VC90</th>
<th>LS VC127</th>
<th>SEM</th>
<th>P-value²,³</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>6</td>
<td>34.3</td>
<td>34.7</td>
<td>33.0</td>
<td>34.7</td>
<td>34.1</td>
<td>34.8</td>
<td>34.4</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>d 1</td>
<td>6</td>
<td>33.1</td>
<td>33.6</td>
<td>32.4</td>
<td>33.5</td>
<td>32.4</td>
<td>33.6</td>
<td>33.3</td>
<td>1.04</td>
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</tr>
<tr>
<td>d 2</td>
<td>6</td>
<td>34.6</td>
<td>34.8</td>
<td>32.7</td>
<td>34.8</td>
<td>33.6</td>
<td>35.2</td>
<td>33.5</td>
<td>1.03</td>
<td></td>
</tr>
<tr>
<td>d 5</td>
<td>6</td>
<td>34.0</td>
<td>35.1</td>
<td>33.2</td>
<td>34.9</td>
<td>35.0</td>
<td>34.6</td>
<td>35.3</td>
<td>1.21</td>
<td></td>
</tr>
<tr>
<td>d 7</td>
<td>6</td>
<td>35.3</td>
<td>35.2</td>
<td>33.6</td>
<td>35.5</td>
<td>35.5</td>
<td>35.8</td>
<td>35.4</td>
<td>1.12</td>
<td></td>
</tr>
<tr>
<td>a*</td>
<td>5,6</td>
<td>14.5</td>
<td>14.1</td>
<td>14.6</td>
<td>14.8</td>
<td>15.1</td>
<td>15.0</td>
<td>16.1</td>
<td>0.58</td>
<td>A</td>
</tr>
<tr>
<td>b*</td>
<td>5,7</td>
<td>9.9</td>
<td>9.6</td>
<td>9.4</td>
<td>10.1</td>
<td>9.9</td>
<td>10.2</td>
<td>10.3</td>
<td>0.33</td>
<td>A</td>
</tr>
<tr>
<td>Saturation index⁵,₆,⁸</td>
<td>17.5</td>
<td>17.0</td>
<td>17.4</td>
<td>17.9</td>
<td>18.0</td>
<td>18.1</td>
<td>19.1</td>
<td>0.62</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>Hue angle⁵,₆,⁹</td>
<td>1.23</td>
<td>1.23</td>
<td>1.33</td>
<td>1.23</td>
<td>1.30</td>
<td>1.24</td>
<td>1.34</td>
<td>1.56</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Treatments: HS CON, high-S control; HS VC56, high S + 10 g VC∙steer⁻¹∙d⁻¹ for the first 56 d of study; HS VC90, high S + 10 g VC∙steer⁻¹∙d⁻¹ for the first 90 d of the study; HS VC127, high S + 10 g VC∙steer⁻¹∙d⁻¹ for the entire finishing period (127 d); LS VC56, low S + 10 g VC∙steer⁻¹∙d⁻¹ for the first 56 d of study; LS VC90, low S + 10 g VC∙steer⁻¹∙d⁻¹ for the first 90 d of the study; LS VC127, low S + 10 g VC∙steer⁻¹∙d⁻¹ for the entire finishing period (127 d).

²Contrast statements: A) high-S diets vs. low-S diets; B) control (0 VC) vs. VC-supplemented treatments within the high-S treatment; C) linear effect of days of VC supplementation within high-S diets; D) quadratic effect of days of VC supplementation within high-S diets; E) linear effect of days of VC supplementation within the low-S diets; F) quadratic effect of days of VC supplementation in low-S diets.

³P ≤ 0.05 for contrast statement A.

⁴Treatment P = 0.75; day P < 0.001; treatment by day P = 0.03.

⁵Means are pooled across all sampling days based on repeated measures analysis.

⁶Treatment P ≥ 0.19; day P < 0.001; treatment by day P ≥ 0.16.

⁷Treatment P = 0.23; day P < 0.001; treatment by day P = 0.06.

⁸Saturation index (vividness of color) can be calculated as $(a^{*2} + b^{*2})^{1/2}$.

⁹Hue angle is calculated as $\tan^{-1}(b^{*}/a^{*})$, representing the color of the sample.
creasing the days of VC supplementation increased ($P = 0.003$) the proportion of the catalytic subunit present as the intact 80-kDa subunit of calpain-1 and decreased ($P = 0.05$) the proportion of the catalytic subunit present as the 76-kDa autolysis product.

Troponin T degradation (2 d postmortem) tended to be lesser ($P = 0.08$) in steers fed HS diets than in those fed LS diets. A quadratic effect of VC supplementation ($P = 0.02$) for TT degradation (2 d postmortem) was noted within the LS diet. The quadratic effect is explained by more extensive degradation of the LS VC90 than the LS VC127, while no difference was noted between the LS VC56 and LS VC90 or LS VC56 and LS VC127. However, within the HS treatments, the duration of VC supplementation did not affect ($P \geq 0.21$) TT degradation 2 d postmortem. Dietary S ($P = 0.98$) or duration of VC supplementation ($P \geq 0.55$) had no effect on d 7 TT degradation.

**DISCUSSION**

Much of the research concerning supplementation of VC in finishing cattle diets has been focused on enhancement of carcass traits, specifically marbling, with little investigation on the subsequent influence on meat quality (Oohashi et al., 1999; Yano, 2001; Pogge and Hansen, 2013a,b). This study was designed to expound further on research conducted by Pogge et al. (2014a,b) supplementing VC to cattle fed HS diets and the impact on postmortem protein degradation and meat color. Cattle growth performance and carcass characteristics of the steers used in the present study were previously discussed by Pogge et al. (2015). Similar to previous work by Pogge and Hansen (2013a,b), the HS steers had lesser plasma ascorbate concentrations than the LS steers. As hypothesized, the steers fed the HS diet experienced some oxidative stress, evidenced by greater liver oxidized-to-reduced glutathione (GSH) ratios, a marker of oxidative stress, greater than the 10% threshold established by Ithayaraja (2011). Specifically within the HS diet, the HS CON steers had a greater liver oxidized-to-reduced GSH ratio than the HS steers supplemented with VC.

Beef quality traits are impacted by a host of different factors such as genetics, composition, livestock handling, nutrition, and carcass processing (reviewed by Warner et al., 2010). Many of these factors relate to the early postmortem metabolism that can have a profound effect on the degradation and/or oxidation of sarcoplasmic, myofibrillar, and intermediate filament proteins (Huff-Lonergan et al., 2010; D’Alessandro and Zolla, 2013). An oxidative environment in postmortem muscle may be the lingering result of live ani-
Vitamin C, sulfur, and protein degradation

This may be related to an improved antioxidant capacity environment impedes the ability of calpain-1 to achieve from steers fed the HS diet, regardless of the duration increasing the days of VC supplementation linearly de-

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in the meat packaging, or endogenous components of oxidants, such as irradiation, the presence of oxygen

mal conditions or may develop from exposure to pro-

oxidants, such as irradiation, the presence of oxygen

in the meat packaging, or endogenous components of muscle (transition metals or unsaturated fatty acids; Rowe et al., 2004a,b; Kim et al., 2010; Estevez, 2011).

Several authors have reported that an oxidative en-

vironment impedes the ability of calpain-1 to achieve autolysis and proteolysis (Guttmann and Johnson, 1998; Carlin et al., 2006), by specifically forming a disulfide bond in the catalytic domain of calpain-1 (Lametsch et al., 2008). Importantly, the effect of oxidation is reversible because the addition of reducing agents rescues activity (Lametsch et al., 2008). In the present study, steaks from steers fed the HS diet, regardless of the duration of VC supplementation, had a greater quantity of the intact (80 kDa) subunit and less of the fully autolyzed (76 kDa) subunit of calpain-1 than did steaks from the LS steers. These results are consistent with less activation of calpain-1 and less proteolysis. Within the HS treatments, increasing the days of VC supplementation linearly decreased the proportion of the 80-kDa subunit of calpain-1 while having minimal impact on the 76-kDa subunit. This may be related to an improved antioxidant capacity of the muscle to limit the development of an oxidative environment, protecting the protease. Similar benefits of VC supplementation to a 0.55% S diet on calpain-1 autolysis were also observed by Pogge et al. (2014b) in calf-fed steers supplemented during a 149 d finishing period compared to their unsupplemented counterparts.

However, in yearling steers fed varying doses of VC in a 0.55% S diet for 91 to 112 d, no difference in calpain-1 autolysis was noted, which may be due to the lack of a LS treatment comparison (Pogge et al., 2014a).

Interestingly, the effects of VC supplementation in the LS treatment were dissimilar, as increasing days on VC actually decreased the proportion of the 76-kDa subunit of calpain-1. This decrease in the 76-kDa subunit in the LS steers may be the result of a lesser demand for antioxidants compared to the HS steers, as plasma ascorbate concentrations in the LS steers were less than the HS steers (Pogge et al., 2015). Furthermore, with a potentially lessened oxidative demand on the LS steers, the exogenous supplementation of VC may have prompted a decrease in the endogenous production of VC from the liver, suggesting a potential dependency on the exogenously supplied VC, which is similar to findings previously reported in supplemented mice (Tsao and Young, 1989).

Differences in the response of calpain-1 autolysis to VC supplementation between the studies conducted by Pogge et al. (2014a,b, 2015) may be related to the age at which the cattle were exposed to a HS diet and the duration of that exposure. Similarities in the response of the 80-kDa subunit of calpain-1 to VC supplementation in the HS diets in the current study and Pogge et al. (2014b) may indicate that calf-fed steers have a greater susceptibility to S-induced oxidative stress, as in both studies the steers fed the HS diet had liver oxidized-to-reduced GSH ratios greater than the acceptable 10% threshold reported by Ithayaraja (2011). Additionally, the greater number of days on feed for the calf-feds from the present study and Pogge et al. (2014b) compared with the fewer days on feed of the yearling steers (Pogge et al., 2014a) may suggest that more time on a HS diet increases the risk of protein oxidation and cross-linking, which could contribute to differences in calpain-1 autolysis.

Oxidation can modify muscle protein structure and change overall protein functionality and contribute negatively to meat quality, specifically tenderness (Xiong and Decker, 1995; Stadtmann and Levine, 2000; Estevez, 2011). Rowe et al. (2004b) exposed steaks to irradiation, inducing an oxidative environment, which resulted in a lesser extent of calpain-1 autolysis and TT degradation and increased shear force values of the steaks. Because TT is easily degraded by calpain-1 and is associated with the contractile filaments of the muscle, the extent of TT degradation may be used to mark the extent of muscle protein degradation (Ho et al., 1994; Huff-Lonergan et al., 1996). In the present study, similar to Pogge et al. (2014b), TT degradation (2 d postmortem) tended to be less in the steaks from steers fed the HS diet than those fed the LS diet, which corresponds with the greater proportion of the intact subunit (80 kDa) of calpain-1 in the steaks from the HS steers. The authors are unaware of any additional literature, regardless of

Figure 3. The effect of timing of supplementation of 10 g vitamin C (VC)∙steer−1∙d−1 for the first 0, 56, 90, or 127 d of the finishing period on the percentage of the degradation of troponin T (TT; 30-kDa subunit), 2 and 7 d postmortem, of the longissimus thoracis of steers consuming a 0.31 or 0.59% S diet. Treatments include high-S (0.59%) control (HS CON), high S + 10 g VC∙steer−1∙d−1 for the first 56 d of study (HS VC56), high S + 10 g VC∙steer−1∙d−1 for the first 90 d of the study (HS VC90), high S + 10 g VC∙steer−1∙d−1 for the entire 127-d finishing period (HS VC127), low S (0.31%) + 10 g VC∙steer−1∙d−1 for the first 56 d of study (LS VC56), low S + 10 g VC∙steer−1∙d−1 for the first 90 d of the study (LS VC90), and low S + 10 g VC∙steer−1∙d−1 for the entire 127-d finishing period (LS VC127). Troponin T degradation (2 d postmortem): high-S diets vs. low-S diets (P = 0.08); quadratic effect of VC supplementation within the low-S diets (P = 0.02). Troponin T degradation (7 d postmortem): all contrast statements (P ≥ 0.55). Standard error of the mean: TT d 2 postmortem (±0.096) and TT d 7 postmortem (±0.131).
species, regarding the effect of VC supplementation or dietary S content on the extent of TT degradation. A relationship has been established between the extent of TT degradation and shear force values, as both measures correspond with the extent of protein degradation (Olson and Parrish, 1977; Huff-Lonergan et al., 1996).

At the retail case, meat color is one of the determining factors in consumer acceptance and selection of meat products (Grunert, 1997). It was our hypothesis that VC is capable of maintaining a reducing environment in postmortem muscle to prolong the color stability of beef. In the current study, steaks from steers fed the LS diet were redder than steaks from the HS steers; however, the supplementation of VC, regardless of dietary S concentration, had no effect on a* values. Similarly, Pogge et al. (2014a) reported no difference in a* values due to VC supplementation in a HS diet. In contrast, the jugular infusion of VC immediately prior to slaughter resulted in an accumulation of ascorbate in the muscle and extended the color shelf-life of 3 LM by 3 d compared to saline-injected cattle (Hood, 1975). The lack of difference in meat color noted by Pogge et al. (2014a) may be due to the difference in the delivery method of the VC, as jugular infusion (Hood, 1975) may have had a more direct influence on muscle that oral supplementation of VC may not provide.

As collagen is the most abundant protein in the body, the presence and maturity of collagen can contribute to variation in meat tenderness, specifically as the heat stable crosslinks of mature collagen are formed (Weston et al., 2002). The structure and characteristic AA of collagen, hydroxyproline, and hydroxylysine are formed by enzymes requiring Fe, Cu, and VC as cofactors (Kadler et al., 1996). Based on the involvement of VC and Cu in the synthesis and maintenance of collagen structure and antioxidant functions, it was hypothesized that less-mature collagen would form, thus contributing to a more tender meat product. Archile-Contreras and Purslow (2011) indicated that reactive oxygen species (ROS) might negatively affect collagen turnover, resulting in a greater presence of insoluble collagen and decreased meat tenderness. These authors observed that VC and VE added to the media resulted in synergistic actions to negate the negative effects of ROS on collagen turnover (Archile-Contreras and Purslow, 2011). In the present study, the total and insoluble collagen content was greater in steaks from steers fed the HS diet, but no effect of supplemental VC was noted. The greater quantity of total and insoluble collagen from the HS steers may be a function of S-induced oxidative stress, as Archile-Contreras and Purslow (2011) indicated the presence of ROS can decrease collagen turnover. The lack of benefit from VC supplementation in the HS treatments may indicate that the greater contribution of S from the HS diet may have been overwhelming the body’s capacity of VC to maintain a reducing environment and encourage the turnover of collagen, thus increasing the maturity of established collagen.

The current results support the conclusion that the LT from steers fed the HS diet demonstrate less activation of calpain-1 (increased proportion of the 80-kDa subunit of calpain-1) and less protein degradation (decreased TT degradation) at 7 d postmortem. While the greater S content of the diet resulted in less autolysis and protein degradation, there was no difference in WBSF values between the dietary S concentrations. Increasing the days of VC supplementation in the HS diet decreased the percentage of the 80-kDa subunit of calpain-1; however, within the LS diet, the opposite occurred when VC was included. Whereas the consumption of a HS diet appears to be negatively influencing calpain-1 degradation in early postmortem muscle, the lack of difference among TT degradation (d 7), WBSF measures, and meat color values indicates that feeding cattle a HS diet will likely have minimal impacts on meat tenderness and color.

**LITERATURE CITED**


Vitamin C, sulfur, and protein degradation


