Antioxidant status affects color stability and tenderness of calcium chloride-injected beef.

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Abstract
The objectives of this study were to determine whether vitamin E supplementation influences color and tenderness of beef injected with calcium chloride. Market heifers (n = 12) were fed a standard finishing diet with minimal levels of vitamin E (NE group). Another 12 market heifers were fed the NE diet with the inclusion of 1,000 IU/d of DL-alpha-tocopherol per animal for the last 125 d on feed (E group). Animals were slaughtered after 125 d on the diets and upon reaching an ultrasound backfat thickness > 10 mm. Half of the longissimus muscles from each treatment group (NE and E) were pumped to 10% over the original weight with 250 mM CaCl₂ (Ca) at 24 h postmortem. Remaining muscles (NE and E) were pumped to 10% over the original weight with water (NC) at 24 h postmortem. After equilibrating overnight, steaks (2.54 cm) were overwrapped with O₂-permeable film and stored for 7 d after injection. Hunter "L," "a," and "b" values were obtained each day of storage. Trained panelists evaluated color on d 1, 4, and 7 after injection. 2-Thiobarbituric acid-reactive substances (TBARS) values were measured on d 1 and 7 after injection. Warner-Bratzler (W-B) shear force values and trained sensory panel evaluations at 1, 3, and 7 d after injection were obtained. Immunoblotting techniques were used to monitor the 30-kDa degradation product of troponin-T at 1, 3, and 7 d after injection. At 4 d after injection, E/Ca steaks were the least discolored (P < 0.05). The E/Ca steak TBARS values were not significantly different from values for NE/NC steaks at 7 d after injection, whereas NE/Ca steaks had greater (P < 0.05) TBARS values after 7 d following injection compared with all other groups. Treatment with Ca resulted in higher off-flavor scores (P < 0.05). The E/Ca samples had the most rapid tenderization and proteolysis of all treatment groups. Warner-Bratzler shear values were lower in the E/Ca samples than in the E/NC samples at 1, 3, and 7 d after injection (P < 0.05). No difference in shear force was noted between NE/Ca and NE/NC samples at any time point. No difference in sensory tenderness was noted between NE/Ca and NE/NC samples at 1 d after injection. However, Ca-injected samples (NE/Ca and E/Ca) were rated as being significantly more tender than their uninjected counterparts (NE/NC and E/NC) at 3 and 7 d after injection. Injection of CaCl₂ may result in more rapid and immediate tenderization if beef from animals supplemented with vitamin E is used. Vitamin E incorporation into muscle tissue may potentiate the action of exogenously added calcium by protecting the calpains from oxidation.

Disciplines
Agriculture | Animal Sciences | Meat Science

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Antioxidant status affects color stability and tenderness of calcium chloride-injected beef¹,²

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ABSTRACT: The objectives of this study were to determine whether vitamin E supplementation influences color and tenderness of beef injected with calcium chloride. Market heifers (n = 12) were fed a standard finishing diet with minimal levels of vitamin E (NE group). Another 12 market heifers were fed the NE diet with the inclusion of 1,000 IU/d of dl-α-tocopherol per animal for the last 125 d on feed (E group). Animals were slaughtered after 125 d on the diets and upon reaching an ultrasound backfat thickness > 10 mm. Half of the longissimus muscles from each treatment group (NE and E) were pumped to 10% over the original weight with 250 mM CaCl₂ (Ca) at 24 h postmortem. Remaining muscles (NE and E) were pumped to 10% over the original weight with water (NC) at 24 h postmortem. After equilibrating overnight, steaks (2.54 cm) were overwrapped with O₂-permeable film and stored for 7 d after injection. Hunter “L,” “a,” and “b” values were obtained each day of storage. Trained panelists evaluated color on d 1, 4, and 7 after injection. 2-Thiobarbituric acid-reactive substances (TBARS) values were measured on d 1 and 7 after injection. Warner-Bratzler shear force values and trained sensory panel evaluations at 1, 3, and 7 d after injection were obtained. Immunoblotting techniques were used to monitor the 30-kDa degradation product of troponin-T at 1, 3, and 7 d after injection. At 4 d after injection, E/Ca steaks were the least discolored (P < 0.05). The E/Ca steak TBARS values were not significantly different from values for NE/NC steaks at 7 d after injection, whereas NE/Ca steaks had greater (P < 0.05) TBARS values after 7 d following injection compared with all other groups. Treatment with Ca resulted in higher off-flavor scores (P < 0.05). The E/Ca samples had the most rapid tenderization and proteolysis of all treatment groups. Warner-Bratzler shear values were lower in the E/Ca samples than in the E/NC samples at 1, 3, and 7 d after injection (P < 0.05). No difference in shear force was noted between NE/Ca and NE/NC samples at any time point. No difference in sensory tenderness was noted between NE/Ca and NE/NC samples at 1 d after injection. However, Ca-injected samples (NE/Ca and E/Ca) were rated as being significantly more tender than their uninjected counterparts (NE/NC and E/NC) at 3 and 7 d after injection. Injection of CaCl₂ may result in more rapid and immediate tenderization if beef from animals supplemented with vitamin E is used. Vitamin E incorporation into muscle tissue may potentiate the action of exogenously added calcium by protecting the calpains from oxidation.

Key Words: Beef Quality, Calcium Chloride, Meat Characteristics, Vitamin E

Introduction

Consumers perceive meat that is discolored to be no longer fresh and (or) wholesome. Incorporating supra-nutritional levels of vitamin E (α-tocopherol) into the diets of feedlot cattle for the final 100 to 125 d the cattle are on feed can enhance beef color stability (Faustman et al., 1989a,b; Liu et al., 1996a). Vitamin E is a potent antioxidant and improves oxidative stability of oxymyoglobin by slowing the rate of its conversion to metmyoglobin. Vitamin E, located in the cell membrane, may function by delaying formation of prooxidants from lipid oxidation during postmortem storage (Liu et al., 1994). These prooxidants have the ability to propagate lipid oxidation. Furthermore, these prooxidants also cata-lyze oxidation of many proteins, including myoglobin and other sarcoplasmic and myofibrillar proteins. Oxidation of proteins decreases protein solubility and functionality by polymerizing proteins and can cause a loss...
of enzyme activity by modifying side chains essential for activity.

Meat tenderness also has a large impact on consumers’ satisfaction with beef. Injection of calcium chloride (CaCl₂) solutions into postmortem beef has been used to improve beef tenderness (Wheeler et al., 1993, 1997). This approach was developed to enhance tenderization by stimulating proteolysis of muscle proteins by calpain enzymes (Koochmarie et al., 1988, 1989). The calpains are cysteine proteases that require calcium (Goll et al., 1999) and reducing conditions to be fully active (Guttmann et al., 1997; Gutmann and Johnson, 1998). Although evidence has shown injection of beef with CaCl₂ solutions enhances postmortem muscle protein proteolysis and subsequent tenderization, beef injected with CaCl₂ discolors more rapidly than that not injected with CaCl₂ (Wheeler et al., 1996). Because CaCl₂ seems to discolor beef by increasing the rate of oxidation (St. Angelo et al., 1991), it could also have a negative effect on calpain activity by modifying the highly susceptible cysteine residue in the active site. We hypothesized that incorporation of vitamin E in beef slows down oxidative processes in CaCl₂-injected beef and thereby limits the rate of discoloration and further enhances postmortem proteolysis and tenderization.

Materials and Methods

Animals

Angus × Gelbvieh heifers (n = 12, average starting weight = 289 kg, average age = 11 mo) were fed a finishing diet composed of corn, cottonseed hulls, and a premix protein supplement (Tend-R-Leen Beef Finisher, Gold Kist, Atlanta, GA). This diet also contained 1,000 IU of DL-α-tocopheryl acetate (ADM Animal Health & Nutrition, Des Moines, IA) (vitamin E) per heifer per day for the last 125 d on feed (E group). The vitamin E was in granulated form and mixed directly into the feed. At the same time, an additional group of Angus × Gelbvieh heifers (n = 12, average starting weight = 299 kg, average age = 11 mo) was fed the same diet without added vitamin E (NE group). The cattle used in this study originated from the same herd and were randomly assigned to the dietary treatment groups. Cattle were slaughtered after 125 d on their respective diets and upon reaching an ultrasound backfat thickness greater than 10 mm. A minimum of two animals from each diet were in all slaughter groups. Animals were slaughtered according to humane practices at the Auburn University Lamberts Meats Laboratory in groups of four or five. At 24 h postmortem, carcass data were collected including hot carcass weight, 12th rib fat depth, longissimus muscle area, percentage of kidney, pelvic, and heart fat (KPH), skeletal and lean maturity, and USDA marbling score. All carcass data were collected by the same two individuals, who were experienced in evaluating USDA quality and yield grades.

Processing

After carcass data were collected, both strip loins (IMPS # 180) were removed from every carcass. Strip loins (longissimus muscle) were chosen randomly from either the right or left side of each carcass and then injected using an Inject-Junior (Globus, Austria) to 10% over the original weight of the loin with a 250 mM calcium chloride solution (Ca, n = 24). The companion loins (those from the side of the carcass opposite the Ca treatment loins) were injected to 10% of the original weight of the loin with water (NC, n = 24). Each animal served as its own control for the injection treatment. Strip loins were then individually vacuum-packaged and stored at 4°C overnight to allow them to equilibrate. At 48 h postmortem (24 h after injection) 2.54-cm-thick steaks were removed from each strip loin for color shelf-life evaluation, Warner-Bratzler (W-B) shear force measurements, and sensory panel evaluation. Samples (0.5 cm thick) also were taken from each strip loin for measurement of vitamin E (α-tocopherol) concentration, 2-thiobarbituric acid-reactive substances (TBARS), and for whole muscle protein extractions for SDS-PAGE and immunoblotting. Samples for color shelf-life were placed on styrofoam trays, overwrapped with oxygen-permeable film, and used immediately for sensory panel color evaluation and instrumental color analysis (Hunterlab D25 DP9000, Hunter Associates Laboratory, Reston, VA). Samples for W-B shear force, sensory panel, and whole muscle protein extracts were removed from the longissimus muscle, vacuum-packaged, and stored for 1, 3, or 7 d after injection at 4°C. Samples were then frozen immediately at the end of their respective storage periods (1, 3, or 7 d after injection) at −20°C until analysis could be performed. Samples for α-tocopherol concentration and for TBARS analysis 1 d after injection were removed from the longissimus muscle 24 h after the loins were injected with their respective solutions, vacuum-packaged, and frozen (−20°C) immediately. Samples for d-7 TBARS analysis were taken from those steaks that had been displayed and used for color analysis. These samples were vacuum-packaged and frozen at the end of the 7-d display period.

Muscle α-Tocopherol Concentration

Concentrations of muscle α-tocopherol were assessed for all animals. Samples were removed, vacuum-packaged at 1 d after injection, and frozen. These samples were then packed on Dry Ice and shipped to the University of Wisconsin Soil and Plant Analysis Lab (Madison, WI) for analysis. Procedures were performed according to Liu et al. (1996b).

Color Sensory Panel

At 24 h after injection, steaks (2.54 cm thick) were removed from each strip loin, placed on styrofoam meat trays, and covered with an oxygen-permeable film. Steaks were stored at refrigerated temperatures for 7
Table 1. Carcass characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 12)</th>
<th>Vitamin E (n = 12)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean maturity</td>
<td>73.76</td>
<td>64.67</td>
<td>4.99</td>
</tr>
<tr>
<td>Skeletal maturity</td>
<td>56.00</td>
<td>59.33</td>
<td>4.06</td>
</tr>
<tr>
<td>Marbling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hot carcass weight, kg</td>
<td>304.4</td>
<td>296.6</td>
<td>8.0</td>
</tr>
<tr>
<td>Longissimus muscle area, cm²</td>
<td>83.22</td>
<td>87.43</td>
<td>1.14</td>
</tr>
<tr>
<td>12th Rib fat, cm</td>
<td>1.17</td>
<td>1.12</td>
<td>0.05</td>
</tr>
<tr>
<td>% Kidney, pelvic, and heart fat</td>
<td>2.20</td>
<td>2.23</td>
<td>0.09</td>
</tr>
</tbody>
</table>

\[a = \text{A}^2, 100 = \text{A}^{100}.\]

\[b = \text{A}^0, 100 = \text{A}^{100}.\]

\[c = \text{Traces}^0, \text{slight}^0 = 200, \text{small}^0 = 300, \text{modest}^0 = 400.\]

Cooked in a convection oven (Blodget, Model GZL-10, Burlington, VT) that had been preheated to a temperature of 176°C. Steaks were cooked to an internal temperature of 68°C. Cooking-loss data were collected on all samples by weighing the steak immediately prior to cooking and again immediately out of the oven. Cooked steaks were chilled for 24 h at approximately 4°C. To ensure all steaks were the same temperature when cored, the steaks were allowed to come to room temperature. Six 1.27-cm cores were removed from each steak perpendicular to the cut surface of the steak and sheared using a W-B shear device (1955 model, G-R Electric Manufacturing Co., Manhattan, KS). All samples were handled in the same manner. All cores were sheared perpendicular to the long axis of the core and values for each steak were averaged (AMSA, 1978; Huff and Parrish, 1993).
Steaks were cut into 1-× 2.54-cm cubes, placed monitored at the geometric center of the steak. Steaks were cooked to an internal temperature of 65 °C, and extreme [for off-flavor] to 150 (extremely tough [for tenderness], extremely dry [for juiciness], and none [for off-flavor]) to 150 (extremely tough [for tenderness], extremely dry [for juiciness], and none [for off-flavor]). Panelists were asked to refresh their palates between samples by eating an unsalted soda cracker and drinking apple juice.

**TBARS Analysis**

Analysis of TBARS was determined in duplicate for all samples. One sample from each loin was vacuum-packaged and frozen at 1 d after injection. The TBARS were also determined on steaks that had been displayed under retail conditions for 7 d. These steaks were vacuum-packaged and frozen after the completion of the 7-d display period. For samples at 1 and 7 d after injection, the entire product was ground (minus the external fat) and samples for TBARS assay were removed from the ground product. A distillation method was used to determine TBARS/1,000 g of tissue (Koniecko, 1985).

**Whole Muscle Sample Preparation for SDS-PAGE**

Whole muscle samples were prepared according to the procedures of Huff-Lonergan et al. (1996a). Steaks (0.5 cm thick) that had been aged for 1, 3, or 7 d after injection (2, 4, or 8 d postmortem) at 4°C were used. A 0.4-g sample of longissimus muscle tissue was knife-minced, added to 10 mL of a solution containing 2% (wt/vol) SDS, 10 mM sodium phosphate buffer, pH 7.0, and homogenized with a motor-driven Dounce homoge-

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**Sensory Panel**

Steaks were aged for 1, 3, or 7 d after injection (2, 4, or 8 d postmortem) at 4°C. These steaks were frozen upon completion of the appropriate aging period and stored until sensory analysis was conducted. A 10-member panel was trained using steaks prepared as standards to demonstrate distinct differences in tenderness, juiciness, and off-flavor. Steaks were thawed at 4°C for 24 h prior to sensory panel evaluation. After thawing, steaks were removed from the vacuum bags and trimmed to less than 0.5 cm external fat. Steaks were cooked in a convection oven (Blodget, Model GZL-10, Burlington, VT) that had been preheated to 176°C. Steaks were cooked to an internal temperature of 65°C as monitored at the geometric center of the steak. Steaks were cut into 1-× 1-× 2.54-cm cubes, placed in aluminum pans, and held in a warming oven for a minimal amount of time until sampling. Each panelist received two cubes randomly selected from each steak. Panelists were seated in individual booths during evaluation of the samples. Red lighting was used to eliminate personal bias due to the samples’ color. Panelists used a 15-cm line scale to evaluate the samples by placing a vertical mark on the horizontal line to indicate their score for tenderness, juiciness, and off-flavor (any flavor not normally associated with beef). Scores were recorded in millimeters and had a possible range from 0 (extremely tough [for tenderness], extremely dry [for juiciness], and extreme [for off-flavor]) to 150 (extremely tender [for tenderness], extremely juicy [for juiciness], and none [for off-flavor]). Panelists were asked to refresh their palates between samples by eating an unsalted soda cracker and drinking apple juice.

**Whole Muscle Sample Preparation for SDS-PAGE**

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---

**Table 3. Hunter “L” values of top loin steaks displayed under retail conditions**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Display time, d</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE/NC</td>
<td></td>
<td>32.44 (0.52)</td>
<td>34.42 (0.41)</td>
<td>34.49 (0.62)</td>
<td>33.80 (0.34)</td>
<td>34.47 (0.40)</td>
<td>33.16 (0.33)</td>
<td>33.02 (0.39)</td>
</tr>
<tr>
<td>NE/Ca</td>
<td></td>
<td>33.50 (0.52)</td>
<td>34.91 (0.41)</td>
<td>34.29 (0.62)</td>
<td>33.96 (0.34)</td>
<td>35.46 (0.40)</td>
<td>34.28 (0.33)</td>
<td>33.62 (0.39)</td>
</tr>
<tr>
<td>E/NC</td>
<td></td>
<td>34.21 (0.50)</td>
<td>35.65 (0.39)</td>
<td>34.71 (0.59)</td>
<td>33.21 (0.33)</td>
<td>34.22 (0.38)</td>
<td>32.94 (0.31)</td>
<td>32.32 (0.37)</td>
</tr>
<tr>
<td>E/Ca</td>
<td></td>
<td>33.81 (0.50)</td>
<td>35.97 (0.39)</td>
<td>35.67 (0.59)</td>
<td>33.67 (0.33)</td>
<td>35.40 (0.38)</td>
<td>34.33 (0.31)</td>
<td>34.14 (0.37)</td>
</tr>
</tbody>
</table>

Means within columns lacking a common letter differ P < 0.05 (SEM).

**Table 4. Hunter “a” values of top loin steaks displayed under retail conditions**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Display time, d</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE/NC</td>
<td></td>
<td>13.13 (0.36)</td>
<td>14.00 (0.37)</td>
<td>12.45 (0.38)</td>
<td>9.43 (0.44)</td>
<td>6.30 (0.30)</td>
<td>6.44 (0.29)</td>
<td>7.16 (0.20)</td>
</tr>
<tr>
<td>NE/Ca</td>
<td></td>
<td>15.04 (0.36)</td>
<td>14.62 (0.37)</td>
<td>13.87 (0.38)</td>
<td>10.69 (0.44)</td>
<td>6.39 (0.30)</td>
<td>6.14 (0.29)</td>
<td>6.50 (0.20)</td>
</tr>
<tr>
<td>E/NC</td>
<td></td>
<td>13.39 (0.35)</td>
<td>14.56 (0.35)</td>
<td>13.44 (0.37)</td>
<td>11.00 (0.42)</td>
<td>7.57 (0.29)</td>
<td>6.85 (0.28)</td>
<td>7.51 (0.19)</td>
</tr>
<tr>
<td>E/Ca</td>
<td></td>
<td>15.28 (0.35)</td>
<td>15.39 (0.35)</td>
<td>14.83 (0.37)</td>
<td>13.63 (0.42)</td>
<td>8.25 (0.29)</td>
<td>7.15 (0.28)</td>
<td>6.23 (0.19)</td>
</tr>
</tbody>
</table>

Means within columns lacking a common letter differ P < 0.05 (SEM).
nizer. The sample was then centrifuged at 1,500 x g for 15 min at 25°C to remove traces of insoluble components. Protein concentrations of the supernatants were determined using the Bio-Rad Detergent Compatible (DC) Protein Assay (Bio-Rad Laboratories, Hercules, CA). This procedure is a modification of the procedure by Lowry et al. (1951). Samples were diluted with water to 6.4 mg/mL and then 1 vol of each diluted sample was combined with 0.5 vol of sample buffer/stacking dye solution (3 mM EDTA, 3% [wt/vol] SDS, 30% [vol/vol] glycerol, 0.003% [wt/vol] pyronin Y, and 30 mM Tris-HCl, pH 8.0) (Wang, 1982) and 0.1 vol of 2-mercaptoethanol for a final protein concentration of 4 mg/mL. Samples were heated to 50°C before loading on SDS-PAGE gels.

**SDS-PAGE**

The SDS-PAGE was performed according to the procedures of Huff-Lonergan et al. (1996b) with minor modifications. A 15% polyacrylamide gel system was used to monitor postmortem proteolysis. These gels consisted of acrylamide (acrylamide: N,N′-bis-methylene acrylamide = 100:1 [wt/wt]), 0.1% (wt/vol) SDS, 0.67% N,N,N′N′-tetramethylethylenediamine (TEMED), 0.1% (wt/vol) ammonium persulfate, and 0.375 M Tris-HCl, pH 8.8. A 5% stacking gel was used over the 15% gel and contained acrylamide (acrylamide: N,N′-bis-methylene acrylamide = 100:1 [wt/wt]), 0.1% (wt/vol) SDS, 0.67% N,N,N′N′-TEMED, 0.1% (wt/vol) ammonium persulfate, and 0.375 M Tris-HCl, pH 6.8. Gels (8 cm wide x 9 cm tall x 1.5 mm thick) were run on Hoefer SE260 Mighty Small II units (Hoefer Scientific Instruments, San Francisco, CA). The running buffer used in both the upper and lower chambers of the slab gel unit contained 25 mM Tris, 192 mM glycine, and 15% (vol/vol) methanol. The temperature of the buffer was maintained between 4°C and 10°C using a refrigerated circulating water bath.

**Immunoblotting**

Posttransfer membranes were incubated for 1 h at room temperature in a blocking solution (80 mM disodium hydrogen orthophosphate, 20 mM sodium dihydrogen orthophosphate, 100 mM sodium chloride, 0.1% [vol/vol] polyoxyethylene sorbitan monolaurate [Tween-20], and 5% [wt/vol] nonfat dry milk). The primary antibody used was monoclonal anti-tropinin-T (JLT-12, Sigma Chemical Co., St. Louis, MO) diluted 1:20,000 in PBS-Tween (same solution as used for the blocking solution minus the non-fat dry milk). Membranes were incubated in primary antibody solution for 1 h at room temperature then washed three times, 10 min per wash, in PBS-Tween. Bound primary antibodies were labeled with goat-antimouse secondary antibody (A2554, Sigma Chemical Co.) diluted 1:5,000 in PBS-Tween. Blots were incubated for 1 h at room temperature with the secondary antibody solution. Blots were again washed in PBS-Tween three times, 10 min per wash, before detection. A chemiluminescent system was used to detect labeled protein bands (ECL, Amersham Pharmacia Biotech, Piscataway, NJ). The labeled bands were detected by exposure to Kodak Biomax ML film. Film was developed with Kodak brand GBX developer and fixer (Eastman Kodak Company, Rochester, NY).

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**Table 5. Hunter “b” values of top loin steaks displayed under retail conditions**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Display time, d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>NE/NC^a</td>
<td>7.92 (0.24)f</td>
</tr>
<tr>
<td>NE/Ca^b</td>
<td>8.75 (0.24)f</td>
</tr>
<tr>
<td>E/NC^c</td>
<td>7.99 (0.23)f</td>
</tr>
<tr>
<td>E/Ca^d</td>
<td>8.78 (0.23f)</td>
</tr>
</tbody>
</table>

^aSteaks from animals not supplemented with vitamin E and injected with water; n = 12.  
^bSteaks from animals not supplemented with vitamin E and injected with CaCl₂; n = 12.  
^cSteaks from animals supplemented with vitamin E and injected with water; n = 12.  
^dSteaks from animals supplemented with vitamin E and injected with CaCl₂; n = 12.  
*Means within columns lacking a common letter differ P < 0.05 (SEM).
Statistical Analysis

Data were analyzed using the general linear models procedure of SAS (SAS Inst. Inc., Cary, NC) using a split-split plot design. Diet was used as the whole plot, treatment as the split plot, and day of display or aging as the split-split plot. Main effects were separated using the PDIF option of SAS when that effect was determined as significant (P < 0.05) in the analysis of variance table.

Results and Discussion

Carcass Data and α-Tocopherol Concentration

Vitamin E supplementation had no effect on carcass characteristics (Table 1). Vitamin E supplementation did significantly increase the concentration of α-tocopherol in the longissimus muscle (P < 0.0001) (Table 2).

Other researchers have found similar results (Arnold et al., 1992; Sanders et al., 1997).

TBARS

Among CaCl₂-injected samples, steaks from animals supplemented with high levels of Vitamin E had significantly lower TBARS values after 1 d of aging after injection (P < 0.05) (Figure 1, NE/Ca vs E/Ca). After 7 d of aging after injection, E/NC steaks exhibited lower TBARS values than all other combinations of diet and treatment (P < 0.05). Calcium chloride increased TBARS within each diet after 7 d following injection (P < 0.05). The NE/Ca steaks exhibited higher TBARS values than any other combination of diet and treatment (P < 0.05) after 7 d of storage, but the E/Ca treatment resulted in TBARS values that were comparable (P > 0.05) to NE/NC samples after 7 d of aging after injection.

These data indicate that injection of CaCl₂ results in greater lipid oxidation in beef longissimus muscle. Other investigators have also reported increased lipid oxidation in meat products that have been injected with CaCl₂ (St. Angelo et al., 1991). This oxidation may lead to production of free radicals that could initiate further oxidation of the heme pigment as well as other proteins within meat (including the cysteine proteases, which include the calpains). Supplementation of the finishing diets of market cattle with vitamin E results in beef that is less susceptible to lipid oxidation during storage as measured by TBARS. α-Tocopherol may scavenge free radicals that can initiate lipid oxidation. Injection of CaCl₂ into beef from animals that have been supplemented with vitamin E allows the advantages of the CaCl₂ injection to be retained while curtailing lipid oxidation as measured by TBARS. This combination results in oxidation values in the product 7 d after injection with CaCl₂ that are comparable to those from meat that has not been supplemented with vitamin E or injected with CaCl₂.

Hunter Color Analysis

Vitamin E supplementation as a main effect had no influence on Hunter “L” values on any day of retail display (P > 0.1). Calcium chloride injection resulted in significantly higher “L” values among samples from animals fed the vitamin E diet on d 2, 5, 6, and 7 as well as among samples from animals fed the control diet on d 6 (P < 0.05) (Table 3). Among the samples not injected with CaCl₂, vitamin E supplementation resulted in higher “L” values on d 1 (P < 0.05). Faustman et al. (1989a) showed similar results. Additionally, on d 2, 5, 6, and 7, CaCl₂-injected samples from animals fed vitamin E had higher “L” values than did their counterparts not injected with CaCl₂. Although vitamin E supplementation had little effect on the lightness of beef products overall, these data show that CaCl₂ injection may result in beef that is lighter in color.
Harris et al.672

Figure 3. Percentage cooking loss of top loin steaks. Values were calculated as \[100 - (\text{cooked weight/ raw weight})\] \times 100. Numbers on the bars represent the least squares means for the product from a particular treatment group at either 1, 3, or 7 d postmortem. NE/NC = means for steaks from heifers whose diets were not supplemented with vitamin E. These steaks were injected to 10% over their original weight with water. NE/Ca = means for steaks from heifers whose diets were not supplemented with vitamin E. These steaks were injected to 10% over their original weight with a 250 mM solution of calcium chloride. E/NC = means for steaks from heifers whose diets were supplemented with 1,000 IU of vitamin E per heifer per day for 125 d. These steaks were injected to 10% over their original weight with water. E/Ca = means for steaks from heifers whose diets were supplemented with 1,000 IU of vitamin E per heifer per day for 125 d. These steaks were injected to 10% over their original weight with a 250 mM solution of calcium chloride. a,bMeans of samples at 1 d after injection lacking a common superscript differ (\(P < 0.05\)). x,yMeans of samples at 3 d after injection lacking a common superscript differ (\(P < 0.05\)). Absence of superscripts for 7 d after injection means indicates that means were not significantly different (\(P > 0.05\)).

Hunter “a” values (Table 4) were affected by CaCl\(_2\) injection (main effects), resulting in higher “a” values on d 1 and 3 and lower “a” values on d 7 of retail display (\(P < 0.05\)). The main effects of vitamin E supplementation resulted in higher “a” values on d 4 and 5 of retail display (\(P < 0.05\)). Therefore, it seems that injection of CaCl\(_2\) results in beef that is more red initially but deteriorates more rapidly beyond 5 d of display. Vitamin E-supplemented beef holds its red color longer (from 4 to 5 d of display). Between the two groups of steaks that were injected with CaCl\(_2\), those from cattle fed supplemental vitamin E had higher (\(P < 0.05\)) Hunter “a” values on d 6 of display. The E/Ca steaks exhibited significantly greater Hunter “a” values on d 1 through 5 of retail display compared with steaks from cattle not fed vitamin E and not injected with CaCl\(_2\) (NE/NC) (\(P < 0.05\)).

Vitamin E supplementation as a main effect had no effect on Hunter “b” values (\(P > 0.05\)). Those steaks in the E/Ca group exhibited the highest Hunter “b” values on d 4 of retail display vs all other combinations of diet and treatment (\(P < 0.05\)) (Table 5). Regardless of the diet fed, calcium chloride injection resulted in higher “b” values on d 1 and 5 of display. The CaCl\(_2\) injection resulted in higher “b” values among samples from animals fed supplemental vitamin E on d 1, 4, 5, 6, and 7 of display (\(P < 0.05\)).

Color Sensory Panel

Analysis of the panelists’ scores for percentage surface discoloration showed that panelists observed no differences (\(P > 0.05\)) among any combination of diet and treatment on the 1st d of retail display (Figure 2). On d 4 of retail display, however, panelists’ scores indicated that the most discolored steaks were those from the NE/NC group (\(P < 0.05\)). Steaks from cattle not fed vitamin E and injected with CaCl\(_2\) (NE/Ca), as well as those from cattle fed vitamin E and not injected with CaCl\(_2\) (E/NC), were intermediate and not different from each other (\(P > 0.05\)). Those steaks from the E/Ca group were observed by panelists to show the least surface discoloration compared with those from any other combination of diet and treatment (\(P < 0.05\)) on d 4 of display (4 d after injection). These observations by the panel coincided with the “a”-values recorded on d 4 of display (4 d after injection). At 4 d of storage, steaks from the E/Ca group had the highest “a” values compared to steaks in the other treatment groups. Within each diet, injection of CaCl\(_2\) lowered panelists’ scores for discoloration on d 4 of retail display (\(P < 0.05\)). Additionally, within each injection treatment, supplementation of vitamin E reduced panelists’ scores for discoloration on d 4 (\(P < 0.05\)). On d 7, panelists scored all samples as having a high degree of discolor-
Vitamin E and CaCl₂ effect on beef tenderness

Figure 4. Least squares means of sensory analysis scores. (a) Tenderness scores. Panelists were asked to place a vertical mark on a 15-cm horizontal line to indicate their score for tenderness. Values were measured and are expressed as mm (0 mm = extremely tough, 150 mm = extremely tender). (b) Juiciness scores. Panelists were asked to place a vertical mark on a 15-cm horizontal line to indicate their score for juiciness. Values were measured and are expressed as mm (0 mm = extremely dry, 150 mm = extremely juicy). (c) Off-flavor scores. Panelists were asked to place a vertical mark on a 15-cm horizontal line to indicate their score for off-flavor. Values were measured and are expressed as mm (0 mm = extreme off-flavor, 150 mm = no off-flavor). NE/NC = means for steaks from heifers whose diets were not supplemented with vitamin E. These steaks were injected to 10% over their original weight with water. NE/Ca = means for steaks from heifers whose diets were not supplemented with vitamin E. These steaks were injected to 10% over their original weight with a 250 mM solution of calcium chloride. E/NC = means for steaks from heifers whose diets were supplemented with 1,000 IU of vitamin E per heifer per day for 125 d. These steaks were injected to 10% over their original weight with water. E/Ca = means for steaks from heifers whose diets were supplemented with 1,000 IU of vitamin E per heifer per day for 125 d. These steaks were injected to 10% over their original weight with a 250 mM solution of calcium chloride. * Means of samples at 1 d after injection lacking a common superscript differ (P < 0.05). ** Means of samples at 3 d after injection lacking a common superscript differ (P < 0.05). *** Means of samples at 7 d after injection lacking a common superscript differ (P < 0.05).

Cooking Loss

Steaks in the E/Ca group had the lowest numerical percentage cooking loss on all days but differences were statistically significant (P < 0.05) only for NE/NC vs E/Ca on d 1 and 3 after injection and for NE/Ca vs E/Ca on d 3 after injection (Figure 3).

Sensory Analysis

On d 1 of postinjection aging, panelists evaluated E/Ca steaks as more tender than NE/NC steaks (P < 0.05) (Figure 4a). The NE/Ca steaks were not evaluated as significantly more tender than steaks that were injected only with water (NE/NC). By d 3 of postinjection aging, however, panelists found all steaks injected with CaCl₂ to be more tender than those not injected with CaCl₂, regardless of diet (P < 0.05). After 7 d, CaCl₂ injection increased tenderness scores within the control diet (P < 0.05) but had no effect within the vitamin E diet. As a main effect, vitamin E supplementation had no effect.
Figure 5. Warner-Bratzler shear force values of top loin steaks. Numbers on the bars represent the least squares means for the product from a particular treatment group at either 1, 3, or 7 d postmortem. NE/NC = means for steaks from heifers whose diets were not supplemented with vitamin E. These steaks were injected to 10% over their original weight with water. NE/Ca = means for steaks from heifers whose diets were not supplemented with vitamin E. These steaks were injected to 10% over their original weight with a 250 mM solution of calcium chloride. E/NC = means for steaks from heifers whose diets were supplemented with 1,000 IU of vitamin E per heifer per day for 125 d. These steaks were injected to 10% over their original weight with water. E/Ca = means for steaks from heifers whose diets were supplemented with 1,000 IU of vitamin E per heifer per day for 125 d. These steaks were injected to 10% over their original weight with a 250 mM solution of calcium chloride. a,bMeans of samples at 1 d after injection lacking a common superscript differ (P < 0.05). c,d,eMeans of samples at 3 d after injection lacking a common superscript differ (P < 0.05). x,y,zMeans of samples at 7 d after injection lacking a common superscript differ (P < 0.05).

on panelist tenderness ratings (P > 0.05), but CaCl$_2$ injection (as a main effect) did result in steaks that were perceived by panelists to be more tender. It is notable that differences in tenderness due to treatments were distinguished by panelists regardless of the fact that all samples were relatively tender even after 1 d of postinjection aging. It is possible that greater differences between treatments would have been noted if there had been a greater variation in initial tenderness.

Figure 6. Immunoblots of beef loin samples 1 d after injection with CaCl$_2$. These represent samples from all treatment groups. E/Ca = means for steaks from heifers whose diets were supplemented with 1,000 IU of vitamin E per heifer per day for 125 d. These steaks were injected to 10% over their original weight with a 250 mM solution of calcium chloride. NE/Ca = means for steaks from heifers whose diets were not supplemented with vitamin E. These steaks were injected to 10% over their original weight with a 250 mM solution of calcium chloride. E/NC = samples from steaks from heifers whose diets were supplemented with 1,000 IU of vitamin E per heifer per day for 125 d. These steaks were injected to 10% over their original weight with water. NE/NC = samples from steaks from heifers whose diets were not supplemented with vitamin E. These steaks were injected to 10% over their original weight with water.
At 1 d of postinjection aging, panelists did not detect differences in juiciness due to diet or treatment (Figure 4b). Injection of CaCl₂ increased panelists’ juiciness scores after 3 d of postinjection aging within the control diet (P < 0.05). Although not significant, there was a trend for panelists to perceive E/Ca steaks to be more juicy than NE/NC steaks after 7 d of postinjection aging (P = 0.051).

Panelists gave significantly lower scores for off-flavor to each of the CaCl₂ injection treatments at each aging period (P < 0.001) (Figure 4c), indicating that CaCl₂ injection resulted in very noticeable off-flavors regardless of diet or day of aging. Other researchers have reported similar results regarding the effect of CaCl₂ injection on flavor (Morgan et al., 1991).

**Warner-Bratzler Shear Force**

A t1d of postinjection aging, those steaks from the E/Ca group had the lowest W-B shear force values compared with any other combination of diet and treatment (P < 0.05) (Figure 5). The other three combinations of diet and treatment did not differ from each other (P > 0.05) with respect to W-B shear force values. At 3 and 7 d of PI aging, E/Ca steaks still possessed the lowest mean W-B shear force values but were not significantly different from NE/Ca steaks (P = 0.13, d 3; P = 0.08, d 7). There was a significant diet × treatment interaction on all days (P < 0.05). Among the samples from animals fed supplemental vitamin E, CaCl₂ injection significantly reduced W-B shear force values at 1, 3, and 7 d of postinjection aging (P < 0.05). Within the control diet, however, CaCl₂ injection did not significantly reduce shear force values at any time (P > 0.05). At 1 d of postinjection aging, the E/Ca treatment resulted in a 21% decrease in W-B shear force values compared with beef from E/NC treatment. Decreases of 22 and 30% within the vitamin E-supplemented group were achieved by CaCl₂ injection at 3 and 7 d of postinjection aging, respectively. In contrast, compared with water-injected beef, NE/Ca treatment resulted in no difference in shear force at 1 d and in decreases of only 6.7 and 10% after 3 and 7 d of postinjection aging, decreases that were not statistically significant. Further weight can be given to these results because within a diet (E vs NE) each animal served as its own control. These data indicate that injection of CaCl₂ into vitamin E-supplemented beef may result in accelerated tenderization, even in very tender product.

**Postmortem Myofibrillar Protein Proteolysis**

Postmortem myofibrillar protein proteolysis was evaluated using immunoblotting techniques with whole muscle protein samples from longissimus muscle. For this study, the myofibrillar protein troponin-T was examined. The rate of postmortem degradation of this protein and the appearance of its degradation products that migrate at approximately 30 kDa have been shown to be related to the rate of tenderization (Olson et al., 1977; Huff-Lonergan et al., 1995, 1996b). Samples from the E/Ca-treated group consistently showed a more intense 30-kDa band than did samples from any other treatment group at 1 d of postinjection aging (Figure 6). Samples from the other treatment groups exhibited more variation in the appearance of the 30-kDa band. Figure 7 shows two E/Ca samples at 1 and 3 d of postinjection aging and two NE/Ca samples at 1 and 3 d of postinjection aging. From this figure it can be seen that production of the 30-kDa band is already very intense at 1 d of aging in the product from the E/Ca treatment. This is not the case in the NE/Ca samples. However, the 30-kDa band did become more intense with further aging time in the NE/Ca samples, indicating that proteolysis within E/Ca samples was mostly complete at 1 d of postinjection aging, whereas unsupplemented beef that had been injected with CaCl₂ showed further proteolysis beyond 1 d of postinjection aging. Figure 8 shows one E/Ca sample and one NE/Ca sample at 1, 3, and 7 d of postinjection aging. This figure further supports the preceding statements and shows that although proteolysis of troponin-T seems to have been complete in the E/Ca samples by approximately 1 d after injection with calcium chloride, proteolysis of troponin-T continues through 7 d of postinjection aging in the NE/Ca samples.
Harris et al. (2006) Figure 8. (a) Immunoblots of beef loin samples 1, 3, and 7 d after injection with CaCl₂. A indicates samples from a single animal from the group fed vitamin E. B represents samples from a single animal from the control diet group. Treatment group (Figure 8a). The 30-kDa component is consistently more intense in E/Ca samples at an early time postmortem (d 1) than in the other treatment groups at this aging time, indicating that proteolysis occurred earlier in E/Ca samples. This early appearance of the 30-kDa band coincided with the accelerated reduction in shear force that was seen in the E/Ca treatment group (Figure 8b).

Calpains are calcium-dependent cysteine proteases. The two most ubiquitous forms are called μ-calpain and m-calpain. These isoforms are named in reference to the amount of calcium that is required for their activity. μ-Calpain requires 30 to 50 μM calcium, and m-calpain requires 200 to 1,000 μM calcium for half-maximal activity (Goll et al., 1999). Both μ-calpain and m-calpain are able to degrade myofibrillar proteins (Kendall et al., 1993; Huff-Lonergerl et al., 1996a). One of the proteins that they degrade is troponin-T. The 30-kDa degradation products of troponin-T produced by in vitro incubation of μ- or m-calpain with myofibrils migrate the same on SDS-PAGE gels and have the same immunoreactivity as the 30-kDa product found in aged beef (Ho et al., 1994; Huff-Lonergerl et al., 1996a). The fact that calpains are cysteine proteases is another key feature of the enzymes. Hydrolysis of peptide bonds by cysteine proteases requires the transfer of electrons between specific cysteine and histidine residues in the active site (Mehdi, 1991). In order for this reaction to proceed, it is necessary that the cysteine and histidine residues be maintained in their properly charged state, a property that may be influenced by the microenvironment that is found around the enzyme, including the redox state of the cell. The cysteine residue in the active site of both μ- and m-calpain is highly susceptible to oxidation and to subsequent inactivation (Guttmann et al., 1997; Guttmann and Johnson, 1998). Therefore, one of many variables that must be controlled to ensure maximal μ- and/or m-calpain activity may be the redox state of the cell. In the current study, it was observed that CaCl₂ increased TBARS values significantly, even at 1 d of aging. Those samples that showed the fastest response in tenderization and proteolysis were CaCl₂-injected steaks from vitamin E-supplemented animals. It is hypothesized that vitamin E’s antioxidant properties may have protected calpain enzymes from oxidation while at the same time injection of CaCl₂ provided levels of calcium required for maximal μ- and m-calpain activity. Therefore, this treatment combination could allow greater calpain activity and subsequently more tenderization to occur by 1 d after injection.

Figure 8. (b) Postinjection Warner-Bratzler shear force means for all samples in the E/Ca and NE/Ca treatment groups. E/Ca = samples from steaks from heifers whose diets were supplemented with 1,000 IU of vitamin E per heifer per day for 125 d. These steaks were injected to 10% over their original weight with a 250 mM solution of calcium chloride. NE/Ca = samples from steaks from heifers whose diets were not supplemented with vitamin E. These steaks were injected to 10% over their original weight with a 250 mM solution of calcium chloride.
This study has shown that CaCl₂ injection results in more tender beef but can result in very noticeable off-flavors. When these Warner-Bratzler shear force data are evaluated with data from immunoblotting, it seems that injection of CaCl₂ into beef with elevated levels of α-tocopherol resulted in accelerated tenderization such that most tenderization and proteolysis had occurred by 1 d after injection. Injection of CaCl₂ into beef from vitamin E-supplemented animals may provide even more rapid tenderization of beef than CaCl₂ injection in beef from unsupplemented animals.

**Literature Cited**


