The use of vitamin D3 and its metabolites to improve beef tenderness

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ABSTRACT: Three experiments were conducted to determine whether feeding 25-hydroxyvitamin D3 (25-OH D3) or 1,25-dihydroxyvitamin D3 (1,25-(OH)2 D3) improves the tenderness of longissimus dorsi (LD), semimembranosus (SM), and infraspinatus (IF) muscles similar to supplemental vitamin D3 without leaving residual vitamin D3 and its metabolites in muscle. In the first two experiments, 24 crossbred steers were used to determine the effects of different oral amounts of 1,25-(OH)2 D3 (Exp. 1; n = 12) and 25-OH D3 (Exp. 2; n = 12) on plasma Ca2+ concentrations. In the third experiment, crossbred steers were allotted randomly to one of four treatments: 1) control placebo (n = 7); 2) 5 × 10⁶ IU of vitamin D3/d (n = 9) for 9 d and harvested 2 d after last treatment; 3) single, 125-mg dose of 25-OH D3 (n = 8) 4 d before harvest; or 4) single, 500-μg dose of 1,25-(OH)2 D3 (n = 9) 3 d before harvest. The LD and SM steaks from each animal were aged for 8, 14, or 21 d, whereas steaks from the IF were aged for 14 or 21 d. All steaks were analyzed for tenderness by Warner-Bratzler shear force and for troponin-T degradation by Western blot analysis. Supplementing steers with vitamin D3 increased (P < 0.01) the concentration of vitamin D3 and 25-OH D3 in all muscles sampled. Feeding steers 25-OH D3 increased (P < 0.05) the concentration of 25-OH D3 in meat, but to an amount less than half that of cattle treated with vitamin D3. Supplemental 1,25-(OH)2 D3 did not affect (P < 0.10) shear force values; however, there was a trend (P < 0.10) for supplemental vitamin D3 and 25-OH D3 to produce LD steaks with lower shear values after 8 and 14 d of aging, and lower (P < 0.10) shear force values for the SM aged for 21 d. Analysis of Western blots indicated that LD steaks from cattle supplemented with vitamin D3 and 25-OH D3 had greater (P < 0.10) shear force values for the SM aged for 21 d. Antemortem supplementation of 25-OH D3 seems to increase postmortem proteolysis and tenderness in the LD and SM without depositing large concentrations of residual vitamin D3 and its metabolite 25-OH D3.

Key Words: Beef, Calcium, Tenderness, Troponin-T, Vitamin D

Introduction

Tenderness has been identified as the single most important palatability factor affecting consumer satisfaction of beef (Savell et al., 1987, 1989; Morgan et al., 1991), and consumers are willing to pay a premium for guaranteed tenderness (Boleman et al., 1997). A recent method proposed to activate calpain-induced tenderization has been the oral supplementation of vitamin D3 (Boleman et al., 1997). A recent method proposed to activate calpain-induced tenderization has been the oral supplementation of vitamin D3 (Swanek et al., 1999; Montgomery et al., 2000). Feeding these doses of supplemental vitamin D3 to beef steers, however, results in substantial vitamin D3 and 25-hydroxyvitamin D3 (25-OH D3) residues in muscle and plasma (Montgomery et al., 2000). The 24-fold increase in raw steak meant that 125 g of steak per day from vitamin D3-supplemented cattle would meet the recommended dietary allowance for vitamin D3 for the adult human. Yet, excess vitamin D in the diet is known to cause soft tissue calcification and has recently been shown to induce arterial calcification through increased Ca²⁺ uptake in smooth muscle cells and arteries (Rajasree et al., 2002). Therefore, hypervitaminosis D may be a concern when feeding supplemental vitamin D3 to beef steers to improve tenderness.

For the current study, we hypothesized that feeding two metabolites of vitamin D3 (25-OH D3 and 1,25-dihydroxyvitamin D3 [1,25-(OH)2 D3]) to cattle would increase the Ca²⁺ concentration of blood and muscle and would thereby, increase beef tenderness via increased myofibril proteolysis to the same extent of supplemental vitamin D3 without leaving substantial residues of vita-
min D₃ and 25-OH D₃ in muscle and liver. These hypotheses were tested by feeding vitamin D₃, 25-OH D₃, and 1,25-(OH)₂ D₃ to beef steers before harvest, and evaluating measures of tenderness, postmortem proteolysis, and vitamin D metabolite residues.

**Materials and Methods**

**Experiment 1: Effect of 1,25-Dihydroxyvitamin D₃ Dose on Plasma Ca²⁺ Concentrations**

All three experiments were approved by the Animal Care Committee at Iowa State University. Twelve market-weight crossbred steers were allotted randomly to four treatment groups: 0, 125, 250, or 500 µg of 1,25-(OH)₂ D₃. Treatments were administered once orally via boluses to each animal. Blood samples were collected 2 d before treatment and at 24-h intervals for the first 4 d after treatment, and then at 48-h intervals for the remaining 10 d of the experiment. Blood was collected in sodium-heparinized Vacutainer tubes (Beckton Dickinson, Franklin Lakes, NJ) by jugular venipuncture.

**Experiment 2: Effect of 25-Hydroxyvitamin D₃ Dose on Plasma Ca²⁺ Concentrations**

Twelve market-weight crossbred steers were allotted randomly to four treatment groups: 0, 50, 87.5, or 125 mg of 25-hydroxyvitamin D₃. Three steers on each treatment were housed in a pen with a windbreak and roof. All steers were fed a diet consisting of (DM basis) dry-rolled corn (79%) alfalfa brome chopped hay (15%), and vitamin, mineral, and rumensin supplements (6%). The diet had 0.43% calcium and no supplemental vitamin D. Treatments were administered once orally via boluses to each animal. Blood samples were collected before treatment, and at 24-h intervals for the first 8 d after treatment, and then at 72-h intervals for the remaining 12 d of the experiment. Again, blood was collected in sodium-heparinized Vacutainer tubes (Beckton Dickinson, Franklin Lakes, NJ) by jugular venipuncture.

**Experiment 3: Effects of Supplementing Vitamin D₃, 25-Hydroxyvitamin D₃, or 1,25-Dihydroxyvitamin D₃ to Beef Steers on Meat Tenderness and Meat Residues**

Thirty-three market-weight, crossbred steers of predominantly Continental breeding were housed at the Iowa State University Beef Nutrition and Management Research Center and fed a high-energy finishing diet, as described for Exp. 2. Cattle were allotted randomly to one of four treatments: 1) untreated controls (n = 7 receiving a single placebo bolus); 2) treated with 5 × 10⁶ IU of vitamin D₃ (n = 9) via a bolus at 24-h intervals for 9 d and harvested 2 d after the last bolus; 3) administered one bolus of 125 mg of 25-OH D₃ (n = 8) and harvested 4 d later; and 4) administered one bolus of 500 µg of 1,25-(OH)₂ D₃ (n = 9) and harvested 3 d later. All boluses consisted of the appropriate metabolite of vitamin D₃ mixed with dried brewer’s grain in gelatin capsules, whereas the placebo was made with only brewer’s grain in the gelatin capsules. Treatments were synchronized so that all cattle were harvested simultaneously.

Blood was collected from all steers before treatment and at time of harvest in sodium-heparinized Vacutainer tubes (Beckton Dickinson, Franklin Lakes, NJ) by jugular venipuncture. Plasma was separated and stored at −20°C for later analysis. All steers were transported to a commercial beef-processing plant (Windom, MN) and harvested that afternoon. Liver and kidney samples were collected at harvest, frozen immediately in liquid nitrogen, and stored at −80°C until later analysis. The carcasses were conventionally air-chilled at 3°C with occasional spraying to control shrink in a commercial facility, and were ribbed 3 d after harvest, following normal operating procedures. Wholesale loins, shoulder clods, and rounds from one side of each carcass were transported to the Iowa State University Meats Laboratory 7 d postmortem for subsequent measurements. Subprimals were cut at the plant according to Institutional Meat Purchase Specifications (IMPS) guidelines (USDA, 1996). Longissimus dorsi (LD) steaks from the strip loin (IMPS #180), semimembranosus (SMP) steaks from the inside round (IMPS #169A), and infraspinatus (IF) steaks from the top blade (IMPS #114D) were sliced by hand perpendicular to the length of the muscle. Steaks were sliced to be 2.54 cm thick at 8 d postmortem and assigned randomly to a specific aging treatment. Steaks were vacuum-packaged and wet-aged at 1°C. Steaks from LD and SM were aged for 8, 14, and 21 d postmortem, whereas IF steaks were aged for 14 and 21 d postmortem. Two additional 0.635-cm-thick steaks of the same muscles were cut as described above 8 d postmortem, vacuum-packaged, and immediately frozen at −20°C for determination of concentrations of Ca²⁺ and the vitamin D metabolites. After aging, steaks were frozen at −20°C until Warner-Bratzler shear force analyses. At the end of each aging period, a 0.635-cm-thick slice was removed from each steak, vacuum-packaged, and frozen at −20°C until proteolysis determination by Western blotting.

**Plasma and Muscle Ca²⁺ Determination**

All plasma and muscle Ca²⁺ concentrations were determined by atomic absorption spectrometry (Perkin-Elmer Corp, Norwalk, CT). Plasma samples were prepared and measured in duplicate by diluting 100 µL of plasma in 5 mL of 0.1% lanthanum oxide solution. A standard curve was calculated by using 0, 5, 10, and 15 mg/dL of CaCl₂. Meat samples were measured similarly in duplicate. Approximately 5 g of wet tissue was excised from each steak and dried overnight. Samples were then ashed at 600°C in an Isotemp Muffle Furnace 550 (Fisher Scientific, Pittsburgh, PA) for 24 h. Ashed samples were suspended in 25 mL of 3 N hydrochloric
acid and measured in duplicate by diluting 1 mL of the hydrochloric acid preparation in 4 mL of 0.1% lanthanum oxide solution.

Concentration of Vitamin D₃, 25-Hydroxyvitamin D₃, and 1,25-Dihydroxyvitamin D₃ in Beef, Liver, Kidney, and Plasma

Concentrations of vitamin D₃, 25-OH D₃, and 1,25-(OH)₂ D₃ in muscle and organ tissue were measured by a modification of the method of Horst et al. (1981). Apparatus identical to that used in the method of Horst et al. (1981) was used in the present study, excluding the HPLC fraction collector. In this study, a Gilson FC204 fraction collector (Gilson, Inc., Middleton, WI) was used to collect purified fractions at specific intervals. The method of Montgomery et al. (2000) was used to extract the vitamin D₃ metabolites from muscle, whereas 25-OH D₃ and 1,25-(OH)₂ D₃ concentrations were quantified by RIA using the methods of Hollis et al. (1993) and Hollis et al. (1996), respectively.

Tenderness Determination Using Warner-Bratzler Shear Force Analysis

Frozen steaks were thawed at 2°C for 48 h, and cooked on an industrial broiler (Model CNO2; General Electric, Chicago Heights, IL) preset to a temperature of 288°C. The top surface of the steaks was approximately 10 cm from the heating element. Steaks were turned when they had reached an internal temperature of approximately 38°C (measured by a digital meat thermometer), and were removed from the broiler when they had reached an internal temperature of 71°C. Cooked steaks were then cooled at 2°C for 24 h. For each muscle, two steaks from each carcass were subjected to shear force evaluation. Four 1.27-cm cores were removed parallel to muscle fiber orientation from each steak (AMSA, 1995) and sheared once perpendicular to the muscle fiber orientation with a Warner-Bratzler shear device attached to a texture analyzer (Texture Technologies Corp., Scarsdale, NY) equipped with a 5-kg load cell and a crosshead pretest speed of 2.0 mm/s (penetration speed of 3.3 mm/s). Warner-Bratzler shear values are presented as a mean of eight cores per carcass.

SDS-PAGE and Western Blots

Proteolysis of troponin-T was determined by Western blots and quantifying the 30-kDa band (a proteolytic degradation product of troponin-T). Samples were prepared as described by Huff-Lonergan et al. (1996b). One 14-d-aged sample from the control group was loaded onto each gel to serve as an internal standard. Internal standards were of the same muscle as the experimental sample to decrease error caused by troponin-T isoforms that differed between muscles. Western blots were performed according to the method of Huff-Lonergan et al. (1996a) to detect the 30-kDa band. Images of the blots were captured (Kodak DC120 camera; Eastman Kodak, New Haven, CT) and analyzed using Kodak ID Version 3 image analysis software (Eastman Kodak, New Haven, CT). Values were expressed as ratios of the intensity of the 30-kDa band in the experimental samples to the intensity of the 30-kDa band of an internal standard.

Statistical Analysis

Data were analyzed as a completely randomized design using the mixed-model procedure (PROC MIXED) of SAS (SAS Inst. Inc., Cary, NC). Steer served as the experimental unit in the analysis of all data. Concentrations of Ca²⁺ in plasma for the three experiments were analyzed as repeated measures in time using PROC MIXED. The model included the fixed effects of treatment, time, and the treatment × time interaction, whereas steer was included in the model as the random effect. Least squares means were generated and separated using the PDIFF procedure of SAS. Standard errors were calculated by using the treatment and time interaction and were pooled within treatment.

The effects of vitamin D₃, 25-OH D₃, and 1,25-(OH)₂ D₃ treatment on concentrations of the vitamin D metabolites in plasma and tissue at the time of harvest and on Warner-Bratzler shear force value, troponin-T degradation, and concentration of Ca²⁺ in muscle were also analyzed using PROC MIXED. Dietary treatment and steer were included in the model as fixed and random effects, respectively. Least squares means were generated and separated using the PDIFF option.

Results and Discussion

Effects of Supplemental Vitamin D₃, 25-Hydroxyvitamin D₃, and 1,25-Dihydroxyvitamin D₃ on Ca²⁺ Concentrations in Plasma and Muscle

Results of the first two studies indicated that feeding supplemental 1,25-(OH)₂ D₃ and 25-OH D₃ increased (P < 0.05) plasma Ca²⁺ concentration (Figures 1 and 2, respectively). Feeding 500 µg of 1,25-(OH)₂ D₃ resulted in the highest (P < 0.01) plasma Ca²⁺ concentrations, and the concentration peaked 3 d after treatment (Figure 1). All three doses of supplemental 25-OH D₃ increased (P < 0.05) plasma Ca²⁺ concentration (Figure 2), but the 125-mg treatment caused the largest numerical (P > 0.05) increase in concentration 4 d after treatment.

Concentrations of Ca²⁺ in plasma increased (P < 0.05) in steers treated with vitamin D₃ and 1,25-(OH)₂ D₃ compared with control steers. Furthermore, steers treated with 25-OH D₃ had higher (P < 0.05) circulating concentrations of 25-OH D₃ than did controls (Table 1); however, the 25-OH D₃-treatment did not (P > 0.10) elevate plasma Ca²⁺ concentrations (Figure 3). Although plasma Ca²⁺ concentrations were increased with vitamin D₃ and 1,25-(OH)₂ D₃ supplementation, total
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Figure 1. Plasma Ca$^{2+}$ concentrations as a function of time (Exp. 1). Steers were orally administered 0 (Control), 125, 250, or 500 µg of 1,25-dihydroxyvitamin D$_3$ (1,25-(OH)$_2$ D$_3$) on d 0. An asterisk (*) indicates that cattle fed 500 µg of 1,25-(OH)$_2$ D$_3$ had higher (P < 0.01) plasma Ca$^{2+}$ concentrations compared with all other treatments after 3 d of treatment. Ca$^{2+}$ concentrations in the LD, SM, and IF were not affected (P > 0.10) by any antemortem treatment (Table 2). Concentrations, however, were similar to published values (Price and Schweigert, 1987). Although Swanek et al. (1999) found higher Ca$^{2+}$ concentrations in steaks from vitamin D$_3$-supplemented steers, water-extractable Ca$^{2+}$ was measured rather than total Ca$^{2+}$ as in the current study. Measurement of water-extractable Ca$^{2+}$ provides a value that is almost equal to free Ca$^{2+}$ in muscle that may be available for the activation of the calpains (Nakamura, 1973).

Pleasure et al. (1979) reported that vitamin D$_3$ supplementation increased the concentration of Ca$^{2+}$ in skeletal muscle mitochondria in chicks, whereas Selles and Boland (1990) reported that supplemental 1,25-(OH)$_2$ D$_3$ decreased the Ca$^{2+}$ content of muscle mitochondria in chicks. Clearly, some vitamin D metabolites have the ability to influence mitochondrial Ca$^{2+}$ content in muscle, although the true mechanism is yet unclear. Vitamin D repletion of rats caused substantial deposits of Ca$^{2+}$ to form pronounced lines within the myofilament of the I-band parallel to the Z-disk (Toury et al., 1990). Toury et al. (1990) also reported that vitamin D may influence the ratio of bound Ca$^{2+}$ to available Ca$^{2+}$ in skeletal muscle of rats. Although we did not change total Ca$^{2+}$, the ratio of bound Ca$^{2+}$ to available Ca$^{2+}$ may have been changed by 25-OH$_2$ D$_3$ treatment. Montgomery (2001) demonstrated an increase in free cytosolic Ca$^{2+}$ with increased vitamin D$_3$ supplementation (0.5 to 5.0 × 10$^6$ IU of vitamin D$_3$) and increased postmortem aging time; unfortunately, we were unable to measure the effect of vitamin D$_3$ and its metabolites on cytosolic Ca$^{2+}$ concentration.

Concentration of Vitamin D$_3$ Metabolites in Plasma, Muscle, Liver, and Kidney

Supplemental vitamin D$_3$ increased (P < 0.01) the concentration of both vitamin D$_3$ and 25-OH D$_3$ in plasma, liver, kidney, and all three muscles tested (Table 1). Furthermore, supplementing steers with 25-OH D$_3$ increased (P < 0.01) plasma, liver, kidney, and muscle 25-OH D$_3$ concentrations, but concentrations were almost 50% lower (P < 0.01) than those reported in plasma and organ tissues of vitamin D$_3$-treated steers. Even though plasma concentrations of 1,25-(OH)$_2$ D$_3$ were increased (P < 0.01) with 1,25-(OH)$_2$ D$_3$ supplementation, concentrations of 1,25-(OH)$_2$ D$_3$ in muscle remained unchanged (P > 0.05). The concentration of 1,25-(OH)$_2$ D$_3$ in the liver was lower (P < 0.05) with 25-OH D$_3$ supplementation when compared with either vitamin D$_3$ or 1,25-(OH)$_2$ D$_3$ supplementation.

The daily recommended dietary allowance of adult men and women (11 to 24 yr of age) for vitamin D$_3$ is 10 µg/d (NRC, 1989). This allowance is less in infants (7.5 µg/d) and adults over 25 yr of age (5 µg/d). Montgomery et al. (2000) reported that feeding steers 5 × 10$^6$ IU of vitamin D$_3$/d for 9 d resulted in a residue of approximately 80 ng/g of vitamin D$_3$—a 24-fold increase above that of controls. In the present study, feeding 5 × 10$^6$ IU of vitamin D$_3$/d for 9 d resulted in residues ranging from 28.6 to 58.9 ng/g or an average increase of 43-fold compared with that of controls. Additionally, Montgomery et al. (2000) reported a greater increase in vitamin D$_3$ concentrations in liver with vitamin D$_3$ supplementation (concentrations increased to 610 ng/g and 979 ng/g in cattle supplemented with 5 × 10$^6$ and 7.5 × 10$^5$ IU vitamin D$_3$, respectively) than in the
present study. Assuming the concentration of vitamin D₃ in steak was 80 ng/g, Montgomery et al. (2000) calculated that an adult human could meet their daily recommended allowance for vitamin D₃ by consuming approximately 125 g of steak from vitamin D₃-supplemented cattle. Based on results from the present study, an adult would need to consume 238 g of steak/d from vitamin D₃-supplemented cattle before meeting their daily recommended allowance, assuming an average of 42 ng vitamin D₃ per gram of steak. Because there is no recommended daily allowance specifically for 25-OH D₃, it is difficult to compare the daily intake of 25-OH D₃ consumed from steak of vitamin D₃- or 25-OH D₃-treated cattle with human needs. Cooking can decrease the concentration of vitamin D₃ in liver for untreated cattle up to 28%; however, concentrations of 25-OH D₃ actually increased slightly in cooked liver from cattle supplemented the two high doses of vitamin D₃ (Montgomery et al., 2002). The destruction of vitamin D₃ in liver as a result of cooking, however, decreases from 28% to 10% for cattle fed 5 × 10⁶ IU or 7.5 × 10⁶ IU of vitamin D₃. Because hypervitaminosis D is a concern, the fact that feeding supplemental 25-OH D₃ to beef steers did not increase concentrations of vitamin D₃ in steak, liver, and kidney compared with controls is noteworthy. Additionally, the fact that supplemental 25-OH D₃ increased concentrations of 25-OH D₃ in steaks to concentrations that were less than half that of vitamin D₃ supplementation makes the commercial adoption of feeding 25-OH D₃ to improve beef tenderness more feasible than the adoption of feeding vitamin D₃.

**SDS-PAGE and Western Blots**

Analysis of the intensity of the 30-kDa band in Western blots showed that feeding supplemental 1,25-(OH)₂ D₃ to cattle was not effective (P > 0.05) in changing the proteolysis of troponin-T (Table 3). There was, however, an increase (P < 0.05) in proteolysis in LD steaks aged for 8 d and in SM steaks aged for 14 d from vitamin D₃-treated cattle compared with control steers. Feeding

Table 1. Concentrations (as-is basis) of vitamin D₃, 25-hydroxyvitamin D₃ (25-OH D₃), and 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂ D₃) in steaks from the longissimus dorsi (LD), semimembranosus (SM), and infraspinatus (IF), as well as in liver, kidney, and plasma, of cattle given supplemental doses of vitamin D₃, 25-OH D₃ or 1,25-(OH)₂ D₃.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>LD</th>
<th>SM</th>
<th>IF</th>
<th>Liver</th>
<th>Kidney</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.10 ± 0.57</td>
<td>0.76 ± 0.74</td>
<td>1.08 ± 2.46</td>
<td>1.92 ± 2.44</td>
<td>1.32 ± 1.31</td>
<td>3.34 ± 14.34</td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>38.06 ± 4.47</td>
<td>28.58 ± 3.30</td>
<td>58.86 ± 2.17</td>
<td>72.65 ± 2.15</td>
<td>34.24 ± 1.16</td>
<td>438.93 ± 12.65</td>
</tr>
<tr>
<td>25-OH D₃</td>
<td>0.65 ± 4.74</td>
<td>1.32 ± 3.50</td>
<td>0.49 ± 2.30</td>
<td>1.43 ± 2.28</td>
<td>1.01 ± 1.23</td>
<td>3.85 ± 13.42</td>
</tr>
<tr>
<td>1,25-(OH)₂ D₃</td>
<td>0.73 ± 4.47</td>
<td>0.68 ± 3.30</td>
<td>1.66 ± 2.17</td>
<td>0.42 ± 2.15</td>
<td>1.14 ± 1.16</td>
<td>4.81 ± 12.65</td>
</tr>
<tr>
<td>Control</td>
<td>1.68 ± 0.37</td>
<td>1.39 ± 0.77</td>
<td>0.90 ± 1.57</td>
<td>2.59 ± 0.73</td>
<td>3.02 ± 1.13</td>
<td>62.66 ± 16.74</td>
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<tr>
<td>Vitamin D₃</td>
<td>9.58 ± 0.33</td>
<td>10.62 ± 0.68</td>
<td>17.04 ± 1.38</td>
<td>16.81 ± 0.64</td>
<td>18.35 ± 0.99</td>
<td>412.49 ± 14.78</td>
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<tr>
<td>25-OH D₃</td>
<td>3.00 ± 0.34</td>
<td>4.65 ± 0.72b</td>
<td>5.66 ± 1.47b</td>
<td>10.92 ± 0.68b</td>
<td>13.17 ± 1.05b</td>
<td>269.24 ± 15.67b</td>
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<tr>
<td>1,25-(OH)₂ D₃</td>
<td>1.82 ± 0.33</td>
<td>1.43 ± 0.68</td>
<td>1.20 ± 1.38</td>
<td>2.41 ± 0.64</td>
<td>1.69 ± 0.99</td>
<td>60.85 ± 14.78</td>
</tr>
<tr>
<td>Control</td>
<td>54.31 ± 8.40</td>
<td>84.04 ± 16.69</td>
<td>59.13 ± 5.25</td>
<td>108.77 ± 18.64ab</td>
<td>117.22 ± 16.86</td>
<td>143.14 ± 20.08</td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>57.94 ± 7.41</td>
<td>94.57 ± 14.72</td>
<td>69.29 ± 4.63</td>
<td>151.83 ± 16.44a</td>
<td>106.00 ± 14.86</td>
<td>179.53 ± 20.08</td>
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<tr>
<td>25-OH D₃</td>
<td>57.51 ± 7.86</td>
<td>71.96 ± 15.61</td>
<td>63.84 ± 4.91</td>
<td>84.94 ± 17.44b</td>
<td>107.83 ± 15.77</td>
<td>164.39 ± 21.17</td>
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<tr>
<td>1,25-(OH)₂ D₃</td>
<td>58.07 ± 7.41</td>
<td>96.44 ± 15.61</td>
<td>61.61 ± 4.63</td>
<td>157.82 ± 16.44a</td>
<td>138.81 ± 14.86</td>
<td>320.88 ± 20.08a</td>
</tr>
</tbody>
</table>

*Within a column (i.e., tissue type) for a metabolite (i.e., vitamin D₃, 25-(OH) D₃, or 1,25-(OH)₂ D₃), least squares means (± SE) that do not have a common superscript letter differ (P < 0.05).*

Figure 3. Plasma Ca²⁺ concentrations before treatment and at harvest (Exp. 3). Steers were orally administered placebo (Control), 1,25-dihydroxyvitamin D₃, 25-hydroxyvitamin D₃, or vitamin D₃. Bars that do not have a common letter differ (P < 0.01).
Table 2. Effect of supplemental vitamin D₃, 25-hydroxyvitamin D₃ (25-OH D₃), and 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂ D₃) on the concentration of Ca²⁺ (mg/g of dry tissue) in longissimus dorsi (LD), semimembranosus (SM), and infraspinatus (IF) steaks

<table>
<thead>
<tr>
<th>Treatments</th>
<th>LD</th>
<th>SM</th>
<th>IF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.107 ± 0.004ᵃ</td>
<td>0.120 ± 0.003</td>
<td>0.123 ± 0.007</td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>0.106 ± 0.003</td>
<td>0.121 ± 0.003</td>
<td>0.117 ± 0.006</td>
</tr>
<tr>
<td>25-OH D₃</td>
<td>0.099 ± 0.004</td>
<td>0.125 ± 0.003</td>
<td>0.118 ± 0.006</td>
</tr>
<tr>
<td>1,25-(OH)₂ D₃</td>
<td>0.107 ± 0.003</td>
<td>0.128 ± 0.003</td>
<td>0.130 ± 0.006</td>
</tr>
</tbody>
</table>

ᵃLeast squares means ± SE.

supplemental 25-OH D₃ to cattle resulted in more \((P < 0.05)\) proteolysis in LD steaks aged for 21 d (Table 3).

On the basis of previous studies, postmortem tenderness is highly correlated with the proteolysis of certain myofibrillar proteins (Olson and Parrish, 1977; Goll et al., 1983; Huff-Lonergan et al., 1996a). It is generally recognized that postmortem proteolysis is caused by the calcium-dependent calpains (Huff-Lonergan et al., 1996a; Koohmaraie, 1996). Huff-Lonergan et al. (1996a) reported that myofibrils digested with \(\mu\)-calpain produced the 30-kDa degradation component of troponin-T similar to that found in myofibrils from steaks that have been aged. Previous work indicated that tender steaks from vitamin D₃-supplemented cattle also had more troponin-T degradation as indicated by greater accumulation of the 30-kDa component (Montgomery et al., 2000). Swanek et al. (1999) found that longissimus muscles from steers fed supplemental vitamin D₃ had lower \(\mu\)-calpain and calpastatin activities 24 h postmortem compared with controls, indicating that the vitamin D₃-induced tenderness was a function of the ratio of calpain to calpastatin activity; however, Montgomery et al. (2002), found no difference in longissimus \(\mu\)- and \(m\)-calpain and calpastatin activities.

Effects of Supplemental Vitamin D₃, 25-Hydroxyvitamin D₃, and 1,25-Dihydroxyvitamin D₃ on Warner-Bratzler Shear Force

There was a trend for vitamin D₃ to decrease \((P < 0.10)\) shear force values of LD steaks aged for 14 d compared with those of controls aged for 14 d (Table 4). Moreover, there was a tendency for shear force values of IF steaks aged for 14 d to be lower \((P < 0.10)\) when compared with steaks from cattle treated with 1,25-(OH)₂ D₃ at the same aging period. After 8 d of aging, LD steaks from steers treated with 25-OH D₃ had lower \((P < 0.10)\) shear force values than did LD steaks from steers treated with 1,25-(OH)₂ D₃. Additionally, 25-OH D₃ tended to decrease Warner-Bratzler shear force values of SM steaks aged for 21 d compared with those from untreated \((P < 0.10)\) and 1,25-(OH)₂ D₃-supplemented \((P < 0.01)\) cattle. Supplemental 1,25-(OH)₂ D₃ was ineffective \((P > 0.10)\) in changing shear

Table 3. Effect of supplemental vitamin D₃, 25-hydroxyvitamin D₃ (25-OH D₃), and 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂ D₃) on amount of the 30-kDa component in longissimus dorsi (LD), semimembranosus (SM), and infraspinatus (IF) steaks at different postmortem ages

<table>
<thead>
<tr>
<th>Muscle/Aging</th>
<th>Control</th>
<th>Vitamin D₃</th>
<th>25-OH D₃</th>
<th>1,25-(OH)₂ D₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD Aging time, d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1.90 ± 0.68ᵇ</td>
<td>3.88 ± 0.60ᶜ</td>
<td>2.96 ± 0.67ᵇ</td>
<td>2.17 ± 0.63ᵇ</td>
</tr>
<tr>
<td>14</td>
<td>2.51 ± 0.68ᵇ</td>
<td>4.31 ± 0.60ᶜ</td>
<td>2.65 ± 0.64ᵇ</td>
<td>2.35 ± 0.60ᵇ</td>
</tr>
<tr>
<td>21</td>
<td>3.20 ± 0.68ᵇ</td>
<td>5.00 ± 0.60ᶜ</td>
<td>5.22 ± 0.64ᶜ</td>
<td>3.98 ± 0.60ᵇ</td>
</tr>
<tr>
<td>SM Aging time, d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>7.95 ± 9.54ᶜ</td>
<td>6.54 ± 7.79ᵇ</td>
<td>8.61 ± 8.26ᶜ</td>
<td>8.82 ± 7.79ᶜ</td>
</tr>
<tr>
<td>14</td>
<td>20.40 ± 8.83ᶜ</td>
<td>47.42 ± 7.79ᵇ</td>
<td>15.23 ± 8.26ᶜ</td>
<td>25.81 ± 7.79ᶜ</td>
</tr>
<tr>
<td>IF Aging time, d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1.01 ± 0.43</td>
<td>0.91 ± 0.35</td>
<td>0.59 ± 0.37</td>
<td>0.49 ± 0.35</td>
</tr>
</tbody>
</table>

ᵃValues represent means of relative values of the increase in the amount of the 30-kDa band in Western blot analysis (expressed as a ratio of the intensity of the 30-kDa band in the experimental samples to the 30-kDa band of an internal standard).
ᵇWithin a row, least squares means (± SE) that do not have a common superscript letter differ \((P < 0.05)\).
Steers with various amounts of oral vitamin D3 in- 
(2001) demonstrated that, even though supplementing 
(Swanek et al., 1999). More recently, Scanga et al. 
age for 7 d, but not in steaks aged for 14 or 21 d. 
effectively decreased shear force values of LD steaks 
warmer-Bratzler shear force values in the LD aged for 
Warner-Bratzler shear force values than did those of untreated controls 
Montgomery et al. (2000) reported that cattle 
approximately 2 d at the time of exsanguinations, whereas Montgomery et al. (2000) reported that cattle 
the tenderizing effect of vitamin D3 supplementation. Although direct influence of 25-OH D3 on muscle metabo- 
improving cooked meat tenderness. Given our results, however, we must consider other mechanisms that may 
require either extended periods of hypercalcemia in cat- 
beef tenderization excluding the role of the calpains. 

**Implications**

The ability to improve beef tenderness through man- 
agement can help cattle producers supply beef that
meets the demands of consumers. Feeding supplemental 25-hydroxyvitamin D₃ to beef cattle before harvest may result in more tender longissimus dorsi and semimembranosus steaks without generating a large vitamin D₃ residue as observed in muscle from vitamin D₃-treated cattle. Thus, the more consistent tenderness that may be possible through use of this technology could improve consumer acceptance of beef. To maximize the effectiveness of 25-hydroxyvitamin D₃ for improved beef tenderness, additional research is needed to discern the time of administration before harvest and the required dosage.

**Literature Cited**


