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


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


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†National Animal Disease Center, USDA/ARS, Ames 50010

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. Supplemen-tal 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 < 0.05) troponin-T degradation. 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 (Rajasee 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 myofibrill 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. Plasma 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 aged at 1°C. Steaks from LD and SMP 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 (PerkinElmer 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.
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 using the treatment and time interaction and were pooled within treatment.

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 Ca²⁺ concentrations were not increased with 25-OH D₃ supplementation.
Vitamin D metabolites and beef tenderness

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 $5.0 \times 10^{6}$ 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 $5 \times 10^{6}$ 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 $5 \times 10^{6}$ 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 $5 \times 10^{6}$ and $7.5 \times 10^{5}$ 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 × 10^6 IU or 7.5 × 10^6 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 steak 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

![Figure 3](image-url)
Table 2. Effect of supplemental vitamin D$_3$, 25-hydroxyvitamin D$_3$ (25-OH D$_3$), and 1,25-dihydroxyvitamin D$_3$ (1,25-(OH)$_2$ D$_3$) on the concentration of Ca$^{2+}$ (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 ± 0.004$^a$</td>
<td>0.120 ± 0.003</td>
<td>0.123 ± 0.007</td>
</tr>
<tr>
<td>Vitamin D$_3$</td>
<td>0.106 ± 0.003</td>
<td>0.121 ± 0.003</td>
<td>0.117 ± 0.006</td>
</tr>
<tr>
<td>25-OH D$_3$</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)$_2$ D$_3$</td>
<td>0.107 ± 0.003</td>
<td>0.128 ± 0.003</td>
<td>0.130 ± 0.006</td>
</tr>
</tbody>
</table>

$^a$Least squares means ± SE.

supplemental 25-OH D$_3$ 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 D$_3$-supplemented cattle also had more troponin-T degradation as indicated by greater accumulation of the 30-kDa band (Montgomery et al., 2000). Swanek et al. (1999) found that longissimus muscles from steers fed supplemental vitamin D$_3$ had lower $\mu$-calpain and calpastatin activities 24 h postmortem compared with controls, indicating that the vitamin D$_3$-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 D$_3$, 25-Hydroxyvitamin D$_3$, and 1,25-Dihydroxyvitamin D$_3$ on Warner-Bratzler Shear Force

There was a trend for vitamin D$_3$ 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)$_2$ D$_3$ at the same aging period. After 8 d of aging, LD steaks from steers treated with 25-OH D$_3$ had lower ($P < 0.10$) shear force values than did LD steaks from steers treated with 1,25-(OH)$_2$ D$_3$. Additionally, 25-OH D$_3$ 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)$_2$ D$_3$-supplemented ($P < 0.01$) cattle. Supplemental 1,25-(OH)$_2$ D$_3$ was ineffective ($P > 0.10$) in changing shear values.

Table 3. Effect of supplemental vitamin D$_3$, 25-hydroxyvitamin D$_3$ (25-OH D$_3$), and 1,25-dihydroxyvitamin D$_3$ (1,25-(OH)$_2$ D$_3$) on amount of the 30-kDa component in longissimus dorsi (LD), semimembranosus (SM), and infraspinatus (IF) steaks at different postmortem ages$^a$

<table>
<thead>
<tr>
<th>Treatments</th>
<th>LD</th>
<th>SM</th>
<th>IF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle/Aging</td>
<td>Control</td>
<td>Vitamin D$_3$</td>
<td>25-OH D$_3$</td>
</tr>
<tr>
<td>LD</td>
<td>1.90 ± 0.68$^b$</td>
<td>3.88 ± 0.60$^c$</td>
<td>2.96 ± 0.67$^{bc}$</td>
</tr>
<tr>
<td>Aging time, d</td>
<td>8</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>SM</td>
<td>7.95 ± 9.54</td>
<td>6.54 ± 7.79</td>
<td>8.61 ± 8.26</td>
</tr>
<tr>
<td>Aging time, d</td>
<td>8</td>
<td>14</td>
<td>20.40 ± 8.83$^c$</td>
</tr>
<tr>
<td>IF</td>
<td>1.01 ± 0.43</td>
<td>0.91 ± 0.35</td>
<td>0.59 ± 0.37</td>
</tr>
<tr>
<td>Aging time, d</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

$^a$Values 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).

$^b$Within a row, least squares means (± SE) that do not have a common superscript letter differ ($P < 0.05$).
Table 4. Warner-Bratzler shear force values of longissimus dorsi (LD), semimembranosus (SM), and infraspinatus (IF) steaks at different postmortem aging times from cattle administered a placebo (Control), vitamin D$_3$, 25-hydroxyvitamin D$_3$ (25-OH D$_3$), or 1,25-dihydroxyvitamin D$_3$ (1,25-(OH)$_2$ D$_3$)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Control</th>
<th>Vitamin D$_3$</th>
<th>25-OH D$_3$</th>
<th>1,25-(OH)$_2$ D$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aging time, d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3.24 ± 0.24$^{ab}$</td>
<td>3.15 ± 0.21$^{ab}$</td>
<td>3.03 ± 0.22$^{ab}$</td>
<td>3.62 ± 0.21$^a$</td>
</tr>
<tr>
<td>14</td>
<td>3.45 ± 0.24$^a$</td>
<td>2.84 ± 0.21$^{ab}$</td>
<td>3.14 ± 0.22$^{ab}$</td>
<td>3.17 ± 0.21$^{ab}$</td>
</tr>
<tr>
<td>21</td>
<td>3.03 ± 0.24</td>
<td>2.82 ± 0.21</td>
<td>2.57 ± 0.22</td>
<td>2.84 ± 0.21</td>
</tr>
<tr>
<td><strong>SM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aging time, d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3.23 ± 0.25</td>
<td>3.02 ± 0.22</td>
<td>2.80 ± 0.24</td>
<td>3.29 ± 0.22</td>
</tr>
<tr>
<td>14</td>
<td>3.71 ± 0.25</td>
<td>3.33 ± 0.22</td>
<td>3.45 ± 0.24</td>
<td>3.65 ± 0.22</td>
</tr>
<tr>
<td>21</td>
<td>4.44 ± 0.25$^a$</td>
<td>4.43 ± 0.22$^{a}$</td>
<td>3.80 ± 0.24$^b$</td>
<td>4.71 ± 0.22$^a$</td>
</tr>
<tr>
<td><strong>IF</strong></td>
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<td></td>
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<tr>
<td>Aging time, d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>2.33 ± 0.17$^{ab}$</td>
<td>2.13 ± 0.15$^{b}$</td>
<td>2.27 ± 0.15$^{ab}$</td>
<td>2.51 ± 0.15$^a$</td>
</tr>
<tr>
<td>21</td>
<td>2.64 ± 0.17</td>
<td>2.61 ± 0.15</td>
<td>2.68 ± 0.15</td>
<td>2.85 ± 0.15</td>
</tr>
</tbody>
</table>

$^{a,b}$Within a row and within a muscle, least squares means (± SE) that do not have a common superscript letter differ ($P < 0.10$).

The difference in muscle response is not understood, but could possibly be the result of fiber type differences. It is unclear why we did not observe a distinct relationship between appearance of the 30-kDa component and shear force value, but the small number of experimental units per treatment may have been a limiting factor.

These results support previous work by Montgomery et al. (2000) that feeding supplemental daily doses of $5 \times 10^6$ IU of vitamin D$_3$ for 9 d to feedlot cattle decreases Warner-Bratzler shear force values in the LD aged for 14 d. Previous work has indicated that both LD and SM steaks from cattle fed $5 \times 10^6$ or $7.5 \times 10^6$ IU of vitamin D$_3$ and aged for 14 d had lower Warner-Bratzler shear force values than did those of untreated controls (Montgomery et al., 2000). In other studies focusing on vitamin D$_3$ supplementation, $5 \times 10^6$ and $7.5 \times 10^6$ IU of vitamin D$_3$ fed to cattle for 7 or 10 d before harvest effectively decreased shear force values of LD steaks aged for 7 d, but not in steaks aged for 14 or 21 d (Swanek et al., 1999). More recently, Scanga et al. (2001) demonstrated that, even though supplementing steers with various amounts of oral vitamin D$_3$ increased Ca$^{2+}$ concentrations in serum, it failed to improve cooked LD tenderness as measured by shear force values.

The fact that we did not see any kind of tenderness effect with 1,25-(OH)$_2$ D$_3$ supplementation raises questions as to the mechanism of vitamin D$_3$-induced tenderness. We may not have seen a response because cattle supplemented with 1,25-(OH)$_2$ D$_3$ were not hypercalcemic for as long as those treated with vitamin D$_3$. Results from Exp. 1 indicated that cattle supplemented with 1,25-(OH)$_2$ D$_3$ should have been hypercalcemic for approximately 2 d at the time of exsanguinations, whereas Montgomery et al. (2000) reported that cattle supplemented with vitamin D$_3$ should have been hypercalcemic for approximately 7 d. The observation that there were no differences among treatments in total muscle Ca$^{2+}$ concentration contradicts this theory; however, it may not be the absolute increase in muscle Ca$^{2+}$ but an increase in cytosolic Ca$^{2+}$ that is associated with the tenderizing effect of vitamin D$_3$ supplementation. Although direct influence of 25-OH D$_3$ on muscle metabolism has been described (Birge and Haddad, 1975), the direct influence of 25-OH D$_3$ on meat tenderness has not. 1,25-Dihydroxyvitamin D$_3$ acts directly on the brush border region of the intestine (Fullmer et al., 1996) to increase calcium absorption from the diet (Mykkanen and Wasserman, 1982; Chandra et al., 1990), resulting in increased plasma concentrations of Ca$^{2+}$. Therefore, initially we hypothesized that increased concentrations of plasma Ca$^{2+}$ via 1,25-(OH)$_2$ D$_3$ would increase the concentration of Ca$^{2+}$ in muscle, thereby activating calpain-proteolysis of myofibrils and improving cooked meat tenderness. Given our results, however, we must consider other mechanisms that may require either extended periods of hypercalcemia in cattle or the direct effect of 25-OH D$_3$ on muscle metabolism, tenderness, or Ca$^{2+}$ compartmentalization within the myofibril. Concentrations of Ca$^{2+}$ in plasma and muscle were not increased with 25-OH D$_3$ supplementation, indicating that 25-OH D$_3$ may have a role in beef tenderization excluding the role of the calpains.

Implications

The ability to improve beef tenderness through management 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**


