The use of vitamin D3 to improve beef tenderness.
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The use of vitamin D₃ to improve beef tenderness


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ABSTRACT: An experiment was designed to test the hypothesis that short-term oral administration of dietary vitamin D₃ to beef cattle before slaughter would increase beef tenderness through greater calcium-activated calpain activity in postmortem aged skeletal muscle. Thirty continental crossbred steers were allotted randomly to three treatment groups housed in one pen. One group served as a control; two other groups were administered boluses with either $5 \times 10^6$ or $7.5 \times 10^6$ IU of vitamin D₃ daily for 9 d. Cattle were slaughtered 1 d later. The longissimus lumborum was excised from each carcass 72 h postmortem and steaks removed at 3, 7, 14, and 21 d postmortem. The semimembranosus muscle (top round) was excised from each carcass 72 h postmortem and steaks removed at 7, 14, and 21 d postmortem. Blood plasma calcium concentration of cattle treated with 5 or $7.5 \times 10^6$ IU of vitamin D₃ was higher ($P < .05$) than that of controls. Strip loin and top loin steaks from cattle fed supplemental doses of vitamin D₃ had lower ($P < .05$) Warner-Bratzler (W-B) shear values at 14 d postmortem but were not significantly different from controls at 3, 7, or 21 d (strip loins) or 7 or 21 d (top rounds). No significant difference in strip loin steak tenderness was observed by sensory panel at 14 d postmortem ($P < .17$) between steaks from control and vitamin D₃-treated steers. At 14 d postmortem, strip loin and top round steaks from cattle fed $5 \times 10^6$ IU of vitamin D₃, but not from those given $7.5 \times 10^6$ IU, showed more proteolysis ($P < .05$) than did steaks from control cattle, based on Western blotting analysis. Therefore, the use of supplemental dietary vitamin D₃ given daily for 9 d before slaughter did improve tenderness (lower W-B shear values) of 14-d postmortem aged beef. Increased proteolysis seems to be the mechanism of tenderization.

Key Words: Beef, Calcium, Proteolysis, Tenderness, Vitamin D

Introduction

Tenderness has been identified as the single most important factor affecting consumers' satisfaction and perception of taste (Morgan et al., 1991; Savell et al., 1991). Injecting CaCl₂ solution into postrigor and prerigor beef carcasses and cuts improves tenderness (Wheeler et al., 1993; Kerth et al., 1995). Exogenous CaCl₂ evidently activates the intracellular calcium-dependent proteases, μ-calpain and m-calpain (Goll et al., 1992; Kooohmarai, 1992a,b), that are responsible for tenderization. An indicator of postmortem proteolysis and tenderization is the appearance of the 30-kDa component (MacBride and Parrish, 1977; Olson et al., 1977; Huff-Lonergan et al., 1996a). The 30-kDa component is a degradation product of troponin-T and can be produced by calpain digestion of troponin-T (Olson et al., 1977; Huff-Lonergan et al., 1996a).

Supplemental dietary vitamin D₃ increases blood calcium markedly via actions of additional 1,25-dihydroxyvitamin D (Horst and Littledike, 1979). Skeletal muscle is an important target organ for vitamin D₃ (de Boland and Nemere, 1992; Boland et al., 1995). Touroy et al. (1999) showed that vitamin D supplementation to rats increased bound calcium at the Z-line and increased cytosolic skeletal muscle calcium. Indeed, Swanek et al. (1999) found higher calcium concentrations in plasma and in longissimus muscle from steers fed diets containing vitamin D. Also, loin steaks from steers fed vitamin D were more tender.

We hypothesized that short-term oral administration of vitamin D₃ to steers would increase blood calcium and would cause increased beef steak tenderness be-
cause the increased blood calcium would cause greater calpain activity during postmortem aging of beef. This hypothesis was tested by feeding 5 or $7.5 \times 10^6$ IU of vitamin D$_3$ to market-weight beef steers for nine consecutive days before slaughter and evaluating measures of tenderness and postmortem proteolysis.

Materials and Methods

Preliminary Experiment

A preliminary experiment was conducted with four market-weight steers to establish the response of plasma calcium to oral administration of vitamin D$_3$ over time. The steers were administered a gelatin capsule bolus containing $7.5 \times 10^6$ IU of vitamin D$_3$. Ground corn was used as a carrier in the bolus. Boluses were administered once daily before the morning feeding for nine consecutive days. On the morning of d 10, the steers were transported 385 km to a commercial beef packing plant and slaughtered that afternoon. An additional blood sample was obtained from each steer at the slaughter facility immediately after exsanguination.

Three days after slaughter, carcasses were transported to a beef fabrication plant. Longissimus lumborum (strip loins, IMPS 180) and semimembranosus muscle (top round, IMPS 168) were placed in Cryovac B620 (Cryovac, Duncan, SC) anaerobic vacuum bags and transported to the Iowa State University Meats Laboratory. Strip loin and top round steaks were cut 2.54 cm thick, placed in Cryovac B160 beef bags, and wet-aged at 1°C. Strip loin steaks were aged for 3, 7, 14, or 21 d, and top round steaks were aged 7, 14, or 21 d. After postmortem aging, steaks were frozen at −20°C until subsequent analysis.

Plasma Calcium

Concentrations of plasma calcium were determined in duplicate by atomic absorption spectrometry (Perkin-Elmer Corp., 1965) and calculated from a standard curve consisting of 0, 5, 10, and 15 mg/dL of CaCl$_2$. One hundred microliters of plasma was added to 5 mL of .1% lanthium oxide solution.

Vitamin D$_3$, 25-Hydroxyvitamin D$_3$, and 1,25-Dihydroxyvitamin D$_3$ in Beef, Liver, Kidney, and Plasma

Vitamin D$_3$, 25-hydroxyvitamin vitamin D$_3$, and 1,25-dihydroxyvitamin D$_3$ were quantified by a modification of the method of Horst et al. (1981). Briefly, 4 mL of phosphate-buffered saline was placed in a tube containing approximately 1 g of thinly sliced tissue or of plasma and homogenized with a polytron homogenizer. A 2-mL aliquot of the homogenate (about .4 g of tissue) was transferred to a 25-× 100-mm glass centrifuge tube. Approximately 1,000 cpm each of $^3$H-vitamin D$_3$, $^3$H-25-hydroxyvitamin D$_3$, and $^3$H-1,25-dihydroxyvitamin D$_3$ (Amersham Life Sciences, Arlington, Heights, IL) were added to 2 mL aliquot for recovery estimates. Two milliliters of methanol was added to each tube and vortexed. Vitamin D$_3$ was extracted by adding 6 mL of hexane and shaking on a horizontal shaker at 120 oscillations/min for 10 min. Samples were centrifuged, and the hexane (upper) layer was removed and saved. The rest of the vitamin D$_3$ metabolites were extracted by adding 2.6 mL of chloroform and 1.4 mL of methanol to the 2-mL aliquot. The mixture was shaken for 10 min and centrifuged. The supernate was removed and placed in a separate tube containing 2 mL of chloroform. Samples were again shaken for 10 min, and phase separation was accomplished by centrifugation. The lower (chloroform) phase was removed, combined with...
the saved hexane layer, and dried under vacuum. The residue was suspended in 1 mL of hexane and applied to a Varian LRC 500 mg silica cartridge (Varian, Harbor City, CA). The cartridge was washed with 8 mL of hexane followed by 8 mL of hexane/isopropanol (99/1; vol/vol). Vitamin D₂ then was eluted with 6 mL of hexane/isopropanol (99/1), followed by 25-hydroxyvitamin D₃ that was eluted with 8 mL of hexane/isopropanol (95/5), and finally 1,25-dihydroxyvitamin D₃ was eluted with 8 mL of hexane/isopropanol (86/14). The vitamin D₂ fraction was dried and placed on a DuPont Zorbax Sil HPLC column (.46 × 25 cm) (Mac-Mod Analytical, Chads Ford, PA) developed in hexane/isopropanol (99/1). The vitamin D₃ fraction was collected and placed onto an Alltech Econosphere ODS HPLC column (.46 × 15 cm; Alltech Assoc., Deerfield, IL) developed in methanol/water (94/6). Vitamin D₃ was quantified by comparing peak areas of unknowns with those of standards described in Horst et al. (1981). The 25-hydroxyvitamin D₃ fraction was placed on a Zorbax NH₂ column (.46 × 25 cm; Mac-Mod Analytical) developed in hexane/methylene chloride/isopropanol (88/10/2). Samples were collected, and 25-hydroxyvitamin D₃ was quantified by UV peak heights (Horst et al., 1981) or by radioimmunoassay (Hollis et al., 1993). The 1,25-dihydroxyvitamin D₃ fraction was purified on a Zorbax AIL HPLC column (.46 × 25 cm; Mac-Mod Analytical) developed in hexane/isopropanol (90/10). The 1,25-dihydroxyvitamin D₃ was collected and quantified by radioimmunoassay (Hollis et al., 1996).

Tenderness by Warner-Bratzler Shear Force Determination

Steaks (2.54 cm thick) were thawed at 2°C for 24 h and then broiled in a General Electric (Chicago Heights, IL) Model CNO2 industrial broiler set at a temperature of 288°C. The surfaces of the steaks were approximately 10 cm from the heating element. Steaks were turned when they reached an internal temperature of 38°C and were removed from the broiler at an internal temperature of 71°C. After cooling to 2°C, six cores 1.27 cm in diameter were removed parallel to the muscle fiber direction (two each from the central, medial, and lateral portions) of each of the steaks. Cores were sheared perpendicular to the fiber direction through the center of the core by using a shear head attached to an Instron Universal Testing Device (Model 4502) controlled with a Model 4500 computer-assisted module (Instron, Canton, MA). Peak shear force values were recorded as kilograms per 1.27-cm per 1.3-cm-diameter core. The six shear values per steak were averaged, and the means for treatments were analyzed for statistical significance.

Sensory Evaluation

Sensory evaluation of strip loin steaks was carried out by a 10-member panel trained according to the AMSA (1995) guidelines. Steaks were broiled as described above for Warner-Bratzler shear. Each panelist was served two 1.27-× 1.27-× 2.54-cm samples of steak. Water was available to rinse the palate between each sample. Samples were served to each panelist from each steak in a random sequence. An 8-point descriptive scale (1 = extremely tough, extremely dry, extremely bland, and extremely unpalatable, respectively, and 8 = extremely tender, extremely juicy, extremely flavorful, and extremely palatable, respectively) was used by the panelists to evaluate tenderness, juiciness, flavor, and overall palatability.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE) and Western Blots

Strip loin and top round steaks aged for 14 d postmortem were used for whole muscle preparations. The 14-d postmortem aging period was used because this time point gave the greatest decrease in shear force. Samples were prepared as described by Huff-Lonergan et al. (1996b). A standard (14-d-aged sample from the control group) was loaded on each gel to serve as an internal standard. Western blots were performed according to the method of Huff-Lonergan et al. (1996a) to detect the 30-kDa component, a proteolytic degradation product of troponin-T. The 30-kDa band was quantified by using a storage phosphor imaging device (model 6s525 Molecular Imager, Bio Rad, Hercules, CA) to detect the chemiluminescent signal. Analysis was accomplished using the software Molecular Analyst (Bio Rad).

Statistical Analysis

Data were analyzed as a completely randomized design with individual steers serving as the experiment unit. The GLM procedure (SAS, 1994) was used to determine means and standard errors of means. A probability of less than .05 was considered significant.

Results

Plasma and Muscle Calcium Concentration

In the preliminary experiment, daily oral doses of 7.5 × 10⁶ IU of vitamin D₃ for nine consecutive days increased plasma calcium concentrations, peaking 2 d after the last administration (Figure 1). Plasma calcium concentration decreased steadily over the next 26 d, attaining the control (d 0) concentration by 16 d. This preliminary experiment suggested that the 7.5 × 10⁶ IU dose caused a 30 to 35% increase in plasma calcium concentration and that the maximal concentration was maintained for approximately 3 d.

As expected, because of the biological effects of vitamin D₃ through its dihydroxylated hormonal form (de Boland and Nemere, 1992), both the 5 and 7.5 × 10⁶ IU doses increased plasma calcium concentrations (Figure 2). The concentration of plasma calcium tended to reach
Influence of orally administering 7.5 \( \times \) \( 10^6 \) IU of supplemental vitamin D\(_3\) daily for 9 d on plasma calcium concentration. Data are means \( \pm \) SE for four steers. A plateau after 6 d of administration of both doses at approximately 3 mg/100 mL plasma greater than the pretreatment level. The maximal calcium concentration was attained at 10 d after the initiation of vitamin D\(_3\) administration. Those steers fed no supplemental D\(_3\) showed no change in calcium concentration throughout the preslaughter period. Also, the plasma calcium concentration of cattle fed both amounts of vitamin D\(_3\) were similar throughout the preslaughter period, except for d 8, when the 7.5 \( \times \) \( 10^6 \) IU dose increased plasma calcium more than did the 5 \( \times \) \( 10^6 \) IU dose. The two groups fed supplemental vitamin D\(_3\) had higher concentrations \( (P < .05) \) of plasma calcium from d 5 through 8. On d 10 (day of slaughter), both of the treatment groups and the control groups had different \( (P < .05) \) plasma calcium concentrations.

Warner-Bratzler Shear Force Values

Steaks from steers orally administered vitamin D\(_3\) preceding slaughter had numerically lower Warner-Bratzler shear values (Table 2). Oral supplemental vitamin D\(_3\) caused only a difference in shear force in steaks aged for 14 d \( (P < .05) \). Strip loin and top round steaks from both treatment groups postmortem aged for 14 d had shear force values of almost .5 kg lower \( (P < .05) \) than those of control steaks. As expected, shear force tended to decrease for all steaks with increasing time of postmortem aging. More specifically, strip loin steaks from all three groups postmortem aged for 14 d had lower Warner-Bratzler shear values than the steaks postmortem aged for 3 or 7 d \( (P < .05) \). Supplemental vitamin D\(_3\) decreased Warner-Bratzler shear force values at all postmortem aging times, but the maximal improvement was noted for those steaks postmortem aged for 14 d \( (P < .05) \). Moreover, the 5 \( \times \) \( 10^6 \) IU dose/d was as effective as the 7.5 \( \times \) \( 10^6 \) IU/d dose in lowering Warner-Bratzler shear force after 14 d of postmortem aging.

Sensory Evaluation

Results of sensory evaluation of strip loin steaks postmortem aged for 14 d are presented in Table 3. Only 14-d postmortem aged strip loins were evaluated by using sensory procedures, because only the 14-d postmortem strip loin steaks had lower Warner-Bratzler shear force values. Supplemental vitamin D\(_3\) did not significantly increase tenderness, juiciness, flavor, or overall palatability scores of strip loin steaks.

Western Blots

Degradation of troponin-T by proteolysis to a 30-kDa component is related positively to improved tenderness.
Table 1. Concentrations of vitamin D₃ and two of its metabolites in beef steaks, liver, kidney, and plasma of cattle given two supplemental doses of vitamin D₃ (means ± SE)

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Top round steak</th>
<th>Strip loin steak</th>
<th>Liver</th>
<th>Kidney</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.8 ± 1.0⁷</td>
<td>4.1 ± 0.8</td>
<td>8.6 ± 2.4</td>
<td>7.4 ± 1.9</td>
<td>3.1 ± 0.6</td>
</tr>
<tr>
<td>5 × 10⁶ IU</td>
<td>78.5 ± 10.5⁶</td>
<td>80.8 ± 9.4</td>
<td>610.1 ± 94.2</td>
<td>178.9 ± 12.5</td>
<td>464.3 ± 15.9</td>
</tr>
<tr>
<td>7.5 × 10⁶ IU</td>
<td>86.6 ± 5.5</td>
<td>91.1 ± 3.7</td>
<td>978.9 ± 165.5</td>
<td>200.3 ± 16.3</td>
<td>529.7 ± 78.0</td>
</tr>
<tr>
<td>Control</td>
<td>1.3 ± 0.2</td>
<td>1.4 ± 0.5</td>
<td>7.7 ± 0.9</td>
<td>23.3 ± 4.7</td>
<td>48.1 ± 5.3</td>
</tr>
<tr>
<td>5 × 10⁶ IU</td>
<td>16.1 ± 1.4</td>
<td>25.7 ± 1.7</td>
<td>50.6 ± 2.8</td>
<td>24.3 ± 1.5</td>
<td>578.4 ± 15.9</td>
</tr>
<tr>
<td>7.5 × 10⁶ IU</td>
<td>86.6 ± 1.5</td>
<td>20.2 ± 3.0</td>
<td>43.9 ± 2.8</td>
<td>20.7 ± 1.0</td>
<td>610.4 ± 31.4</td>
</tr>
<tr>
<td>Control</td>
<td>11.8 ± 1.3</td>
<td>43.9 ± 4.9</td>
<td>103.3 ± 27.9</td>
<td>59.9 ± 9.6</td>
<td>20.8 ± 2.8</td>
</tr>
<tr>
<td>5 × 10⁶ IU</td>
<td>18.8 ± 3.4</td>
<td>26.2 ± 10.2</td>
<td>117.8 ± 18.7</td>
<td>95.1 ± 13.0</td>
<td>27.2 ± 2.3</td>
</tr>
<tr>
<td>7.5 × 10⁶ IU</td>
<td>24.2 ± 3.6</td>
<td>20.1 ± 3.2</td>
<td>135.6 ± 25.5</td>
<td>86.6 ± 12.3</td>
<td>28.4 ± 2.5</td>
</tr>
</tbody>
</table>

25-Hydroxyvitamin D₃, ng/g
<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Control</th>
<th>5 × 10⁶ IU</th>
<th>7.5 × 10⁶ IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>106 IU</td>
<td>106 IU</td>
<td>106 IU</td>
</tr>
<tr>
<td>5 × 10⁶ IU</td>
<td>106 IU</td>
<td>1.5b 20.2</td>
<td>3.0b 135.6</td>
</tr>
<tr>
<td>7.5 × 10⁶ IU</td>
<td>106 IU</td>
<td>1.5b 20.2</td>
<td>3.0b 135.6</td>
</tr>
</tbody>
</table>

1,25-Dihydroxyvitamin D₃, pg/g
<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Control</th>
<th>5 × 10⁶ IU</th>
<th>7.5 × 10⁶ IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>106 IU</td>
<td>106 IU</td>
<td>106 IU</td>
</tr>
<tr>
<td>5 × 10⁶ IU</td>
<td>106 IU</td>
<td>1.5b 20.2</td>
<td>3.0b 135.6</td>
</tr>
<tr>
<td>7.5 × 10⁶ IU</td>
<td>106 IU</td>
<td>1.5b 20.2</td>
<td>3.0b 135.6</td>
</tr>
</tbody>
</table>

Given supplemental daily doses of 5 or 7.5 × 10⁶ IU of vitamin D₃ to feedlot cattle can increase vitamin D₃ and improve tenderness (decrease Warner-Bratzler shear values) within 14 days of postmortem aging of loin and round muscles.

In other studies of vitamin D₃ supplementation on beef tenderness, Swanek et al. (1999) observed improvements in Warner-Bratzler shear values in 7 d postmortem, but not 14 or 21 d postmortem, longissimus muscle steaks from cattle fed 5 × 10⁶ IU of vitamin D₃ daily for 7 d before slaughter. In a second experiment, Swanek et al. (1999), showed that longissimus muscle steaks from cattle fed 7.5 × 10⁶ IU of vitamin D₃ daily for 7 d immediately before slaughter had lower Warner-Bratzler shear values at both 7 and 14 d, but not at 21 d postmortem. Therefore, on the basis of studies at two different experimental stations, 5 or 7.5 × 10⁶ IU of vitamin D₃ can improve tenderness of beef as measured by Warner-Bratzler shear force. Sensory panel evaluation of strip loin steaks at 14 d postmortem in our study could not detect any significant difference (P < .17) in tenderness. Future research studies, however, need to address the optimal doses and length of time to feed vitamin D₃ and optimal postmortem storage time for beef tenderness.
Table 4. Effect of 5 and 7.5 × 10^6 IU/d of vitamin D3 administered for 9 d to steers on amount of the 30-kDa component in 14-d postmortem aged steaks (means ± SE)\(^a\)

<table>
<thead>
<tr>
<th>Steaks</th>
<th>Control (n = 10)</th>
<th>5 × 10^6 IU/d (n = 10)</th>
<th>7.5 × 10^6 IU/d (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strip loin steaks</td>
<td>.614 ± .09(^b)</td>
<td>.889 ± .09(^c)</td>
<td>.631 ± .09(^b)</td>
</tr>
<tr>
<td>Top round steaks</td>
<td>.965 ± .16</td>
<td>1.207 ± .16</td>
<td>1.029 ± .17</td>
</tr>
</tbody>
</table>

\(^a\)Means represent relative values of the increase in appearance of the 30-kDa band in Western blot analyses. A 14-d postmortem aged sample (longissimus dorsi for loin gels and semimembranosus for top round gels) was loaded on every gel. These samples served as an internal standard to make comparisons across blots. Values were expressed as a ratio of the intensity of the 30-kDa band in the 5 and 7.5 × 10^6 IU samples to the 30-kDa band in the standard sample.

\(^b\)Means in the same row with a different superscript letter differ (P < .05).

Maximal tenderization and to elucidate why different responses seem to occur in top rounds and strip loins. Moreover, it is important to discover why the current study and that of Swanek et al. (1999) indicated that the differences in tenderness disappeared by 21 d postmortem.

Based on this and previous studies, postmortem increase in tenderness is most likely the result of the degradation of myofibrillar proteins responsible for the integrity of the myofibril (Olson et al., 1977; Koohmaraie, 1992a; Huff-Lonergan et al., 1996a). Huff-Lonergan et al. (1996a), employing SDS-PAGE and Western blotting techniques, demonstrated that \(\mu\)-calpain proteolytically degraded five key myofibrillar and cytoskeletal proteins under postmortem-like conditions similar to changes observed in postmortem muscle. Additionally, the degradation of these five proteins, titin, nebulin, filamin, desmin, and troponin-T, was related to beef steak tenderness. Degradation of troponin-T and the simultaneous appearance of polypeptides migrating at approximately 30 kDa is correlated strongly to beef tenderness (MacBride and Parrish, 1977; Olson et al., 1977; Penny and Dransfield, 1979). Olson et al. (1977) showed \(\mu\)-calpain degraded purified bovine troponin-T to produce polypeptides in the 30-kDa region. This result of the degradation of troponin-T to a 30-kDa component recently has been confirmed by using Western blotting techniques (Ho et al., 1994). Using Western blotting techniques to detect the increase in the 30-kDa component, we have demonstrated that proteolysis seems to be involved in tenderization of beef from cattle fed supplemental doses of vitamin D3. Swanek et al. (1999) observed a 43% increase in calcium content of beef longissimus by daily doses of 5 × 10^6 IU for 7 d and a 50% increase by the 7.5 × 10^6 IU dose for 7 d. Increased muscle calcium could enhance the ability of the calcium-activated proteases to degrade troponin-T to the 30-kDa component at 14 d postmortem. Proteolysis was not examined at other postmortem aging times.

The steaks from vitamin D3-fed cattle contained from 78 to 91 ng of vitamin D3 per gram, which is approximately 24-fold that found in control steaks. To relate this concentration to recommended dietary allowances of adult humans (19 to 24 yr of age) for vitamin D3 (10 \(\mu\)g/d; NRC, 1989), adult humans would need to eat about 125 g of top round or strip loin steaks from the vitamin D3-supplemented cattle per day to meet their daily needs for this nutrient, assuming 80 ng of vitamin D3 per gram. Likewise, adult humans could meet their

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**Figure 3.** Western blots of 14-d postmortem whole muscle samples from longissimus dorsi (20 \(\mu\)g protein/lane) run on SDS-PAGE 15% gels (acylamide:bis ratio = 100:1) and transferred to PVDF membranes. The blots were incubated for 1 h at 25°C with monoclonal troponin-T antibody JLT-12 (Sigma Chemical Co., St. Louis, MO) diluted 1:20,000 in PBS-Tween. Blots were detected using an enhanced chemiluminescent system (ECL, Amersham Pharmacia Biotech, Arlington Heights, IL). (A) Samples from animals fed either 7.5 × 10^6 IU (7.5) or 5 × 10^6 IU (5). (B) Samples from animals fed the control diet (C). Troponin-T = intact isoforms of troponin-T. 30 kDa = degradation product of troponin-T. S = Standard, this same sample was loaded on all gels and served as an internal standard.
daily requirement of vitamin D$_3$ by consuming 16.4 g of liver from the 5 × 10$^6$ IU/d-fed cattle (610 ng of vitamin D$_3$/g). These calculations raise a caveat with regard to the commercial adoption of feeding vitamin D$_3$ to improve beef tenderness, because consumption of as little as 45 µg of vitamin D$_3$ per day has been associated with signs of hypervitaminosis D in young children (American Academy of Pediatrics, 1963).

In summary, our results have confirmed our hypothesis that short-term oral administration of 5 and 7.5 × 10$^6$ IU of vitamin D$_3$ will improve tenderness of beef strip loin and top round steaks postmortem aged for 14 d. This increased tenderness could occur because increased intracellular calcium concentration is available to augment proteolysis during postmortem aging.

Implications

Feeding 5 × 10$^6$ IU of vitamin D$_3$ per day for 9 d before slaughter could be implemented in a commercial feedlot system to improve tenderness (based on decreased Warner-Bratzler shear force values) of strip loin and top round steaks within 14 d postmortem. Therefore, antemortem feeding of supplemental vitamin D$_3$ may hold the potential of improving beef tenderness and increasing consumer acceptance of beef. Acceptance of the technology depends on approval of marketing the beef, and especially the liver, of vitamin D$_3$-supplemented cattle that have elevated vitamin D$_3$ content.

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