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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 x 10(6) or 7.5 x 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 x 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 x 10(6) IU of vitamin D3, but not from those given 7.5 x 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.

Keywords

Beef, Calcium, Proteolysis, Tenderness, Vitamin D

Disciplines

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Comments

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The use of vitamin D₃ to improve beef tenderness¹

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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 5×10^6 or 7.5×10^6 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 7.5×10^6 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 5×10^6 IU of vitamin D₃, but not from those given 7.5×10^6 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

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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; Koohmaraie, 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). Tourey et al. (1990) 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-

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cause the increased blood calcium would cause greater calpain activity during postmortem aging of beef. This hypothesis was tested by feeding 5 or 7.5×10^6 IU of vitamin D₃ 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 D₃ over time. The steers were administered a gelatin capsule bolus containing 7.5×10^6 IU of vitamin D₃. Ground corn was used as a carrier in the bolus. Boluses were administered once daily before the morning feeding for nine consecutive days. The steers were bled by jugular venipuncture once daily in the morning beginning 2 d before dosing with vitamin D₃ and continuing for 19 d after the last dose of vitamin D₃.

Design of Main Experiment

Thirty large-framed, Continental \times British, crossbred steers (approximately 23 mo of age) were allotted randomly to three treatment groups: a placebo (0 IU of vitamin D₃), 5×10^6 IU of vitamin D₃, and 7.5×10^6 IU of vitamin D₃. Each steer was implanted with Ralgru (Iowa Veterinary Supply Co., Iowa Falls, IA). In June, they were placed in drylot and fed Berseem clover as green chop. All 30 steers were fed a high-concentrate finishing diet beginning in October, at which time the steers were implanted with Revalor (Hoechst Roussel Vet, Clinton, IA). The finishing diet consisted, on a dry weight basis, of 78.2% whole shelled corn, 14.2% chopped alfalfa hay, 4.1% soybean meal, and 3.5% of a 40% crude protein liquid supplement.

In February, steers were moved to an open lot at the Iowa State University Veterinary Resource Farm, where they were fed a concentrate mix diet consisting of 85% cracked corn and 15% oats, along with .45 kg of commercial supplement (42% crude protein, 5% Ca, 1.1% P, 1.6% NaCl, and added vitamins A, D, and E) per steer per day. The supplement provided 225 mg of monensin per steer per day. The steers had free access to water and large bales of hay consisting of predominantly alfalfa with some mixed grasses. The steers were grouped together in one pen and had access to an open-front shed with straw bedding for shelter.

After 2 wk, steers were allotted to the three treatment groups. Starting 10 d before slaughter, steers were bled by venous puncture from the jugular into heparinized tubes, and plasma was stored at -18°C for subsequent analysis. Blood samples were collected each morning before bolusing. Blood collection was done at 24-h intervals and at slaughter. Also starting 10 d before slaughter, steers were given a gelatin capsule bolus containing

ground corn (control) or ground corn containing the 5 or 7.5×10^6 IU of vitamin D₃ 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 CaCl₂. One hundred microliters of plasma was added to 5 mL of .1% lanthium oxide solution.

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

Vitamin D₃, 25-hydroxyvitamin D₃, and 1,25-dihydroxyvitamin D₃ 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- \times 100-mm glass centrifuge tube. Approximately 1,000 cpm each of ³H-vitamin D₃, ³H-25-hydroxyvitamin D₃, and ³H-1,25-dihydroxyvitamin D₃ (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 D₃ 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 D₃ 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 D₃ 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 D₃ 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 7.5×10^6 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 7.5×10^6 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 7.5×10^6 IU doses increased plasma calcium concentrations (Figure 2). The concentration of plasma calcium tended to reach

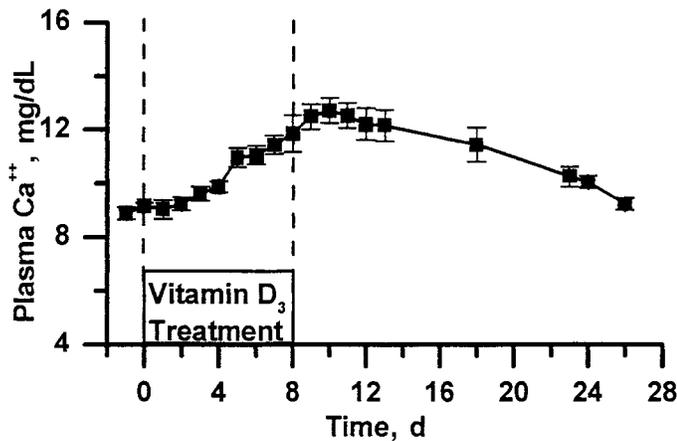


Figure 1. Influence of orally administering 7.5×10^6 IU of supplemental vitamin D₃ daily for 9 d on plasma calcium concentration. Data are means \pm SE for four steers.

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 D₃ administration. Those steers fed no supplemental D₃ showed no change in calcium concentration throughout the preslaughter period. Also, the plasma calcium concentration of cattle fed both amounts of vitamin D₃ were similar throughout the preslaughter period, except for d 8, when the 7.5×10^6 IU dose increased plasma calcium more than did the 5×10^6 IU dose. The two groups fed supplemental vitamin D₃ 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.

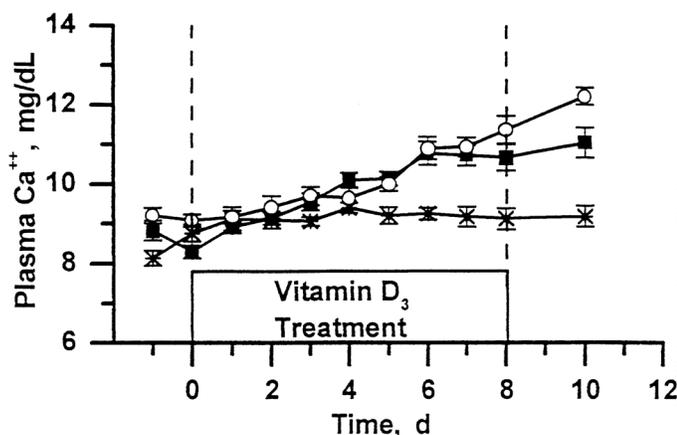


Figure 2. Influence of orally administering 0, 5.0, or 7.5×10^6 IU of supplemental vitamin D₃ daily for 9 d on plasma calcium concentration. Steers were slaughtered on d 10. Data are mean \pm SE for 10 steers. X, Control; ■, 5×10^6 IU/d; ○, 7.5×10^6 IU/d.

Oral administration of 5 or 7.5×10^6 IU of vitamin D₃ increased the concentration of vitamin D₃ in top round and strip sirloin steaks an average of 24-fold (average of 84 ng/g) above that of the control steaks (Table 1). Concentrations of vitamin D₃ in liver were increased 71- and 114-fold by the two doses; those in kidney were increased 24- and 27-fold by the two doses. Plasma from cattle given 5 and 7.5×10^6 IU dosages contained 150 and 170 times more vitamin D₃, respectively, than did plasma from controls.

The 25-hydroxyvitamin D₃ concentration in steaks was increased approximately 14-fold when cattle were fed the two doses of vitamin D₃ (Table 1). Concentration of 25-hydroxyvitamin D₃ in liver increased approximately sixfold, but that in kidney was unaffected by dietary vitamin D₃ supplementation. Supplemental vitamin D₃ increased 25-hydroxyvitamin D₃ in plasma approximately 12-fold. Supplemental vitamin D₃ caused only slight increases in 1,25-dihydroxyvitamin D₃ (up to twofold) in top round steaks, liver, kidney, and plasma. An unexplained decrease in 1,25 hydroxyvitamin D₃ was observed for strip loin steaks.

Warner-Bratzler Shear Force Values

Steaks from steers orally administered vitamin D₃ preceding slaughter had numerically lower Warner-Bratzler shear values (Table 2). Oral supplemental vitamin D₃ 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 D₃ 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 5×10^6 IU dose/d was as effective as the 7.5×10^6 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 D₃ 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)

Dietary group	Top round steak	Strep loin steak	Liver	Kidney	Plasma
Vitamin D ₃ , ng/g					
Control	2.8 ± 1.0 ^a	4.1 ± 0.8 ^a	8.6 ± 2.4 ^a	7.4 ± 1.9 ^a	3.1 ± 0.6 ^a
5 × 10 ⁶ IU	78.5 ± 10.5 ^b	80.8 ± 9.4 ^b	610.1 ± 94.2 ^b	178.9 ± 12.5 ^b	464.3 ± 15.9 ^b
7.5 × 10 ⁶ IU	86.6 ± 5.5 ^b	91.1 ± 3.7 ^b	978.9 ± 165.5 ^c	200.3 ± 16.3 ^b	529.7 ± 78.0 ^b
25-Hydroxyvitamin D ₃ , ng/g					
Control	1.3 ± 0.2 ^a	1.4 ± 0.5 ^a	7.7 ± 0.9 ^a	23.3 ± 4.7	48.1 ± 5.3 ^a
5 × 10 ⁶ IU	16.1 ± 1.4 ^b	25.7 ± 1.7 ^b	50.6 ± 2.8 ^c	24.3 ± 1.5	578.4 ± 15.9 ^b
7.5 × 10 ⁶ IU	86.6 ± 1.5 ^b	20.2 ± 3.0 ^b	43.9 ± 2.8 ^b	20.7 ± 1.0	610.4 ± 31.4 ^b
1,25-Dihydroxyvitamin D ₃ , pg/g					
Control	11.8 ± 1.3 ^a	43.9 ± 4.9	103.3 ± 27.9	59.9 ± 9.6	20.8 ± 2.8
5 × 10 ⁶ IU	18.8 ± 3.4 ^{ab}	26.2 ± 10.2	117.8 ± 18.7	95.1 ± 13.0	27.2 ± 2.3
7.5 × 10 ⁶ IU	24.2 ± 3.6 ^b	20.1 ± 3.2	135.6 ± 25.5	86.6 ± 12.3	28.4 ± 2.5

^{a,b,c}Means within steak, liver, kidney, and plasma with different subscripts differ (*P* < .05) for each metabolite.

(MacBride and Parrish, 1977; Olson et al., 1977). Strip loin steaks postmortem aged 14 d from the 5 × 10⁶ IU-treated cattle had significantly (*P* < .03) more proteolytic degradation, as evidenced by higher content of the 30-kDa component, than did steaks from the control group as observed by using Western blots (Table 4 and Figure 3). Samples from strip loin steaks from cattle fed vitamin D₃ also had less intact troponin-T than did the samples from cattle fed the control diet (Figure 3), indicating greater proteolysis had occurred in these samples. No significant difference in proteolysis was observed, however, between control and 7.5 × 10⁶ IU/d samples.

Discussion

Giving supplemental daily doses of 5 or 7.5 × 10⁶ IU of vitamin D₃ to feedlot cattle decreased Warner-

Table 2. Warner-Bratzler shear force values of strip loin and top round steaks from cattle fed two levels of supplemental dietary vitamin D₃ (means ± SE)

Steak/aging	Vitamin D ₃ treatment			<i>P</i> > <i>F</i>
	Control	5 × 10 ⁶ IU/d	7.5 × 10 ⁶ IU/d	
Shear force, kg				
Strip loin steak				
Aging time, d				
3	3.58 ± .17 [*]	3.11 ± .18	3.17 ± .15	.1638
7	3.32 ± .09	3.20 ± .19	2.89 ± .16	.1873
14	3.25 ± .09 ^a	2.80 ± .08 ^b	2.78 ± .14 ^b	.0015
21	3.38 ± .11	2.90 ± .17	3.02 ± .13	.1071
Top round steak				
Aging time, d				
7	3.97 ± .23	3.56 ± .20	3.32 ± .17	.0685
14	3.91 ± .15 ^a	3.37 ± .14 ^b	3.37 ± .15 ^b	.0366
21	3.74 ± .10	3.32 ± .17	3.56 ± .16	.1973

^{a,b}Means in the same row with a common superscript or no superscript are similar (*P* > .05).

Bratzler shear force (*P* < .05) of beef strip loin and top round steaks that were postmortem aged for 14 d. Therefore, feeding vitamin D₃ may offer an effective way to improve tenderness (decrease Warner-Bratzler shear values) within 14 d 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 experiment stations, 5 or 7.5 × 10⁶ IU daily of supplemental 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

Table 3. Sensory evaluation of strip loin steaks postmortem aged 14 d (means ± SE)

Treatment group	Tenderness ^a	Juiciness ^a	Flavor ^a	Overall ^a palatability
Control	5.07 ± .25 ^b	4.47 ± .33	4.96 ± .17	4.98 ± .29
5 × 10 ⁶ IU/d	5.31 ± .25	4.47 ± .28	4.90 ± .15	4.98 ± .28
7.5 × 10 ⁶ IU/d	5.19 ± .19	4.56 ± .26	5.23 ± .15	4.99 ± .21

^aPanel scores are based on an 8-point descriptive scale as follows: tenderness, 1 = extremely tough, 8 = extremely tender; juiciness, 1 = extremely dry, 8 = extremely juicy; flavor, 1 = extremely bland, 8 = extremely flavorful; palatability, 1 = extremely unpalatable, 8 = extremely palatable.

^bAll values within a column are similar (*P* > .05).

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 \pm SE)^a

Steaks	Vitamin D ₃ treatments		
	Control (n = 10)	5×10^6 IU/d (n = 10)	7.5×10^6 IU/d (n = 10)
Strip loin steaks	.614 \pm .09 ^b	.889 \pm .09 ^c	.631 \pm .09 ^b
Top round steaks	.965 \pm .16	1.207 \pm .16	1.029 \pm .17

^aMeans 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,c}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; Koohmariaie, 1992a; Huff-Lonergan et al., 1996a). Huff-Lonergan et al. (1996a), employing SDS-PAGE and Western blotting techniques, demonstrated that μ -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 m-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 μ 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

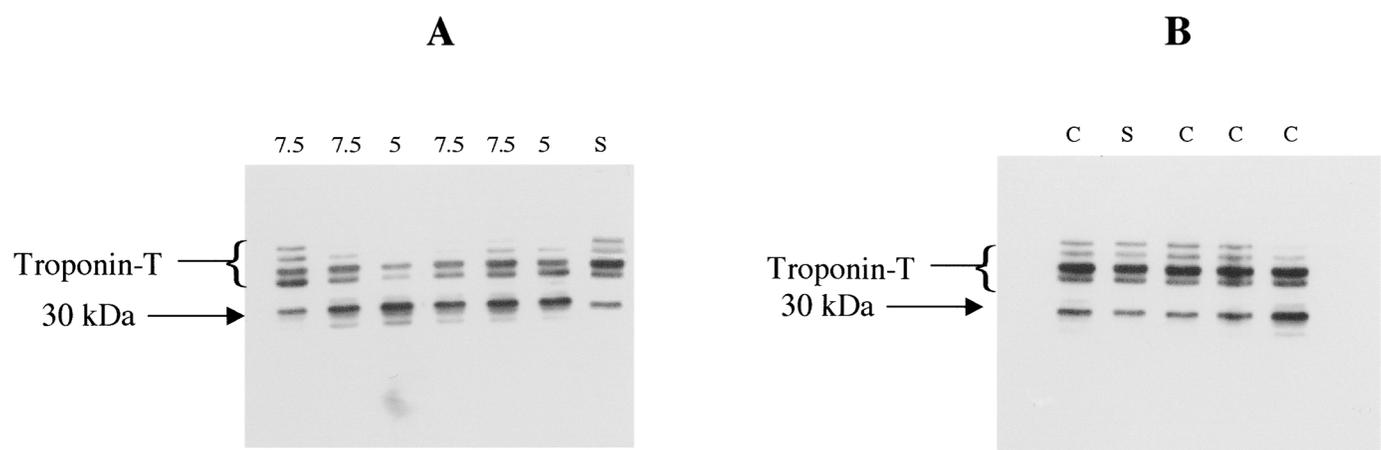


Figure 3. Western blots of 14-d postmortem whole muscle samples from longissimus dorsi (20 μ g protein/lane) run on SDS-PAGE 15% gels (acrylamide:bis ratio = 100:1) and transferred to PVDF membranes. The blots were incubated for 1 h at 25°C with monoclonal troponin-T antibody JLT-12 (Sigma Chemical Co., 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 D₃ by consuming 16.4 g of liver from the 5×10^6 IU/d-fed cattle (610 ng of vitamin D₃/g). These calculations raise a caveat with regard to the commercial adoption of feeding vitamin D₃ to improve beef tenderness, because consumption of as little as 45 µg of vitamin D₃ 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 × 10⁶ IU of vitamin D₃ 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 D₃ 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 D₃ 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 D₃-supplemented cattle that have elevated vitamin D₃ content.

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