Use of Vitamin D_3 and its Metabolites to Improve Beef Tenderness

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Summary

Our previous work has shown that feeding 5 million international units (IU) of vitamin D_3 to beef steers can produce tender strip loin and top round steaks. Our current experiment was designed to determine whether feeding two metabolites of vitamin D_3, 25-hydroxyvitamin D_3, and 1,25-dihysroxyvitamin D_3, produces tender strip loin, top round, and top blade steaks more effectively than does supplemental vitamin D_3 without leaving a substantial amount of residual vitamin D_3 in muscle. Thirty-three continental crossbred steers were randomly allotted to one of four treatment groups. The first group was fed a placebo. The second group received 5 million IU of vitamin D_3 each day for nine days and was slaughtered two days later. The third group received one dose of 125 mg of 25-hydroxyvitamin D_3 four days before harvest, and the fourth group received one dose of 500 μg of 1,25-dihydroxyvitamin D_3 three days before harvest. Blood samples were collected before treatment and at the time of slaughter for subsequent analysis of calcium, vitamin D_3, 25-hydroxyvitamin D_3, and 1,25-dihydroxyvitamin D_3 concentrations in plasma. Steaks from the longissimus lumborum (strip loin) and semimembranous (top round) muscles were collected from each animal and aged for 8, 14, and 21 days, and steaks from the infraspinatus were collected and aged for 14 and 21 days. All steaks were analyzed for tenderness by Warner-Bratzler shear force determination. Concentrations of vitamin D_3 in plasma were higher in vitamin D_3-treated cattle ($P < 0.0001$). Concentrations of plasma 25-hydroxyvitamin D_3 were increased in 25-hydroxyvitamin D_3-treated cattle, but not as high as vitamin D_3-treated cattle ($P < 0.0001$). 1,25-Dihydroxyvitamin D_3 concentrations were higher in 1,25-dihydroxyvitamin D_3-treated animals compared with all treatments ($P < 0.0001$). Supplementing steers with vitamin D_3 increased the concentration of vitamin D_3 and 25-hydroxyvitamin D_3 in the meat of all muscles sampled ($P < 0.0001$). Supplementing steers with 25-hydroxyvitamin D_3 increased the concentration of 25-hydroxyvitamin D_3 in meat, but to an amount less than half that of cattle treated with vitamin D_3. Warner-Bratzler shear force analysis showed that feeding 1,25-dihydroxyvitamin D_3 did not significantly lower shear force values, but supplemental vitamin D_3 and 25-hydroxyvitamin D_3 produced longissimus lumborum and semimembranous steaks with lower shear force values ($P < 0.06$). Analysis of Western blots showed that longissimus lumborum and semimembranous steaks from cattle fed supplemental vitamin D_3 and 25-hydroxyvitamin D_3 (but not steaks from cattle fed 1,25-dihydroxyvitamin D_3), had greater proteolysis of troponin T to a 30 kDa component.

Introduction

Tenderness has been identified as the single most important palatability factor affecting consumer satisfaction of beef. Several antemortem and postmortem procedures have been investigated to improve the tenderness of beef. Our previous work has shown that feeding supplemental vitamin D_3 to beef steers increased beef tenderness. It is hypothesized that this effect occurs because of the increase in calcium concentrations found in blood and muscle when steers were fed supplemental vitamin D_3. This increase in calcium may activate calcium-dependent proteases (calpains). Unfortunately, feeding supplemental vitamin D_3 to steers causes a substantial amount of vitamin D_3 and 25-hydroxyvitamin D_3 residue in meat. Because vitamin D_3 is hydroxylated first to 25-hydroxyvitamin D_3 and eventually to the hormonally active form of 1,25-dihydroxyvitamin D_3, we hypothesized that feeding supplemental 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 would enhance beef tenderness to a greater extent than would supplemental vitamin D_3 without generating a large concentration of vitamin D_3 residue in beef.

Materials and Methods

Study 1: Effect of dosage of 1,25-dihydroxyvitamin D_3 on plasma calcium concentrations.

Twelve market-weight, cross-bred steers were allotted randomly to four treatment groups: 0 μg, 125

Figure 1. Plasma calcium concentration as a function of time. Steers were orally administered 0 µg (+), 125 µg (♦), 250 µg ( ), or 500 µg ( ) of 1,25-dihydroxyvitamin D $_3$ on day 0.

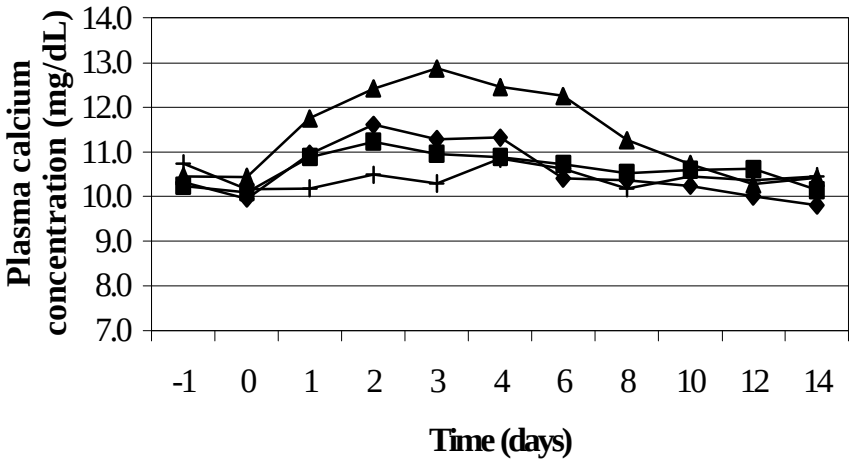

Figure 2. Plasma calcium concentration as a function Steers were orally administered 0 mg (■), 50 mg (▲), 87.5 mg (X), or 125 mg (♦) of 25-hydroxyvitamin D_3 on day 0. of time.

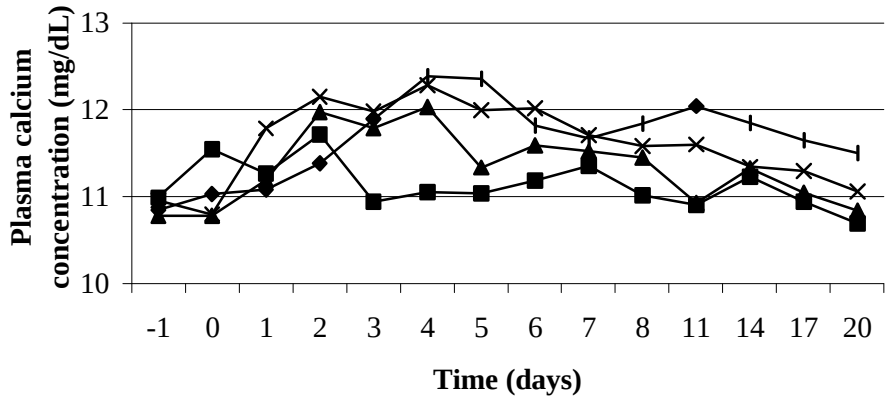

Figure 3. Plasma calcium concentration before experiment and at slaughter. Steers were orally administered placebo, 25-hydroxyvitamin D_3, 1,25-dihydroxyvitamin D_3, or vitamin D_3.

μg, 250 μg, or 500 μg of 1,25-dihydroxyvitamin D_3. Treatments were administered once to each individual animal via a bolus. Blood samples were collected two days before treatment and for four consecutive days at 24-hour intervals and every other day at 48-hour intervals up to 14 days after treatment. Blood was collected by jugular venipuncture by using sodium heparanized Vacutainer™ tubes.

Study 2: Effect of dosage of 25-hydroxyvitamin D_3 on plasma calcium concentrations.

Twelve market-weight cross-bred steers were randomly allotted to four treatment groups: 0 mg, 50 mg, 87.5 mg, or 125 mg of 25-hydoxyvitamin D_3. Treatments were individually administered orally once to each animal via a bolus. Blood samples were collected two days before treatment and for eight consecutive days at 24-hour intervals and every third day at 72-hour intervals up to 20 days after treatment.

Study 3: Effects of supplementing vitamin D_3, 25-hydroxyvitamin D_3, or 1,25-dihydroxyvitamin D_3, to beef steers on meat tenderness and meat residues.

Thirty-three market-weight steers of largely continental breeding fed a high energy finishing diet and housed at the Iowa State University Beef Nutrition and Management Research Center were divided randomly into four groups. One group (n = 7) was given a placebo bolus. The second group (n = 9) was given 5 million IUs of vitamin D_3 via a bolus at 24-hour intervals for nine days and was killed two days later. The third group (n = 8) was given one bolus of 125 mg of 25-hydroxyvitamin D_3 and was killed four days later. The fourth treatment group (n = 9) was given one bolus of 500 μg of 1,25-dihydroxyvitamin D_3 and was killed three days later. All boluses consisted of the appropriate metabolite of vitamin D_3 mixed with dried brewers grain in gelatin capsules. The placebo was made with only brewers grain in the gelatin capsules.

Blood was collected from all steers before treatment and at the time of slaughter via jugular venipuncture by using sodium heparanized Vacutainer™ collection tubes and was stored at -18°C for later analysis. All steers were transported to a commercial beef packing plant and slaughtered that afternoon. The carcasses were ribbed three days after slaughter, at which time ribeye areas and 12th rib fat measurements were taken. Wholesale loins, shoulder clods, and rounds were transported to the Iowa State University Meat Laboratory seven days after slaughter for subsequent measurements. Boneless longissimus lumborum (strip loin), semimembranous (top round), and infraspinatus (top blade) steaks were cut 2.54 cm and 0.635 cm thick eight days after slaughter and were vacuum packaged and wet-aged at 1°C. Strip loin and top round steaks were both aged for 8, 14, and 21 days, whereas top blade steaks were aged for 14 and 21 days. After aging, the 2.54 cm steaks were frozen at -20°C until subsequent Warner-Bratzler shear force analyses. The 0.635 cm steaks were frozen at -20°C until later proteolysis determination by Western blotting. Two 0.635 cm steaks of the same muscles also were cut eight days after slaughter and were vacuum packaged and immediately frozen at -20°C for later chemical analyses.

For Warner-Bratzler shear force analysis, steaks were thawed at 2°C for 48 hours, broiled to an internal temperature of 71°C, and then placed in a 2°C cooler for 24 hours. Two steaks were analyzed from each animal, and four cores for shear force analysis were removed from each steak. Cores were removed parallel to the direction of the fiber.

All blood calcium concentrations were determined in duplicate by atomic absorption spectrometry. The concentration of vitamin D_3 in plasma and meat was measured by using reverse phase high-performance liquid chromatography (HPLC). The concentrations of both 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 in plasma and meat were determined by using straight phase HPLC and radioimmunoassay. Proteolysis of troponin T, (shown to be involved with the mechanism of meat tenderization), was determined by Western blots and subsequent quantification by densitometry of the 30 kDa band, which is a proteolytic degradation product of troponin T. An increase in proteolysis, which is highly correlated with an increase in meat tenderness, is expressed by a greater intensity of the 30 kDa band.

Results and Discussion

Our results of the first two studies indicated that feeding supplemental 1,25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_3 increases blood calcium concentration (Figures 1 and 2). Figure 1 shows that feeding 500 μg of 1,25-dihydroxyvitamin D_3 gave the highest blood calcium concentration and that this concentration peaked three days after treatment. All three doses of supplemental 25-hydroxyvitamin D_3 caused an increase in blood calcium concentration (Figure 2), but the 125 mg treatment caused the largest increase in concentration four days after treatment. Analysis of the blood calcium concentrations collected from the third study gave unexpected results; although calcium concentrations in plasma from steers treated with vitamin D_3 and 1,25-hydroxyvitamin D_3 increased, those treated with 25-hydroxyvitamin D_3 did not increase (Figure 3). The reason for this is unknown, but 25-hydroxyvitamin D_3 concentrations in the plasma of these steers did increase, indicating that the cattle indeed were administered 25-hydroxyvitamin D_3 (Table 1).

Feeding supplemental vitamin D_3 increased the concentration of both vitamin D_3 and 25-hydroxyvitamin D_3 in blood and in all three steaks analyzed (Table 1). Cattle fed supplemental 25-hydroxyvitamin D_3 had increased concentrations of 25-hydroxyvitamin D_3 in blood and in all three steaks as well, but these concentrations were significantly lower than those found in vitamin D_3-treated cattle. The concentration of 1,25-dihydroxyvitamin D_3 in steak was not affected and changed only in blood of 1,25-dihydroxyvitamin D_3-treated cattle as expected.

Even though all three cuts of meat were quite tender, supplemental vitamin D_3 and 25-hydroxyvitamin D_3 treatment resulted in decreased shear force values compared with controls (Table 2). Vitamin D_3 decreased shear force values of strip loin steaks aged for 14 days, whereas 25-hydroxyvitamin D_3 decreased shear force values of strip loin steaks aged for eight days and of top round steaks aged for 21 days. Supplemental 1,25-dihydroxyvitamin D_3 was ineffective in changing shear force values. Top blade steaks did not seem overly affected, although a trend for vitamin D_3 to cause a decrease in Warner-Bratzler shear force value in steaks aged for 14 days was evident ($P < 0.07$). Analysis of the intensity of the 30-kDa band in Western blots similarly showed that feeding supplemental 1,25-dihydroxyvitamin D_3 to cattle was not effective in changing proteolysis of troponin T (Table 3). However, there was an increase in proteolysis in strip loin steaks aged for eight days and in top round steaks aged for 14 days of vitamin D_3-treated cattle over those of control cattle ($P < 0.033$). Feeding supplemental 25-hydroxyvitamin D_3 to cattle produced more proteolysis in strip loin steaks aged for 21 days ($P < 0.033$).

Implications

Our results would suggest that feeding one dosage of 125 mg of 25-hydroxyvitamin D_3 four days before slaughter could be implemented in a commercial feedlot system more easily than feeding 5 million IU of vitamin D_3 per day for nine days before slaughter. Besides being easier, feeding supplemental 25-hydroxyvitamin D_3 produces significantly more tender strip loin and top round steaks without generating a large concentration of vitamin D_3 residue in beef. Therefore, antemortem feeding of supplemental 25-hydroxyvitamin D_3 is an effective and easy way to increase beef tenderness before slaughter and may improve overall beef palatability. Thus, this technology should improve consumer acceptance of beef.

Table 1. Warner-Bratzler shear force values of strip loin, top round, and chuck steaks at different postmortem aging times from cattle fed a placebo, vitamin D_3, 25-hydroxyvitamin D_3, or 1,25-dihydroxyvitamin D_3.

	Treatments*			
Steak/Aging	**Control**	Vitamin D_3	25-Hydroxyvitamin D_3	1,25-Dihydroxyvitamin D_3
	---------- kg ----------			
Strip loin steak				
Aging time (days)				
8	3.24 ± 0.24	3.15 ± 0.21	3.03 ± 0.22[a]	3.62 ± 0.21[c]
14	3.45 ± 0.24[c]	2.84 ± 0.21[a]	3.14 ± 0.22	3.17 ± 0.21
21	3.03 ± 0.24	2.82 ± 0.21	2.57 ± 0.22	2.84 ± 0.21
Top round steak				
Aging time (days)				
8	3.23 ± 0.25	3.02 ± 0.22	2.80 ± 0.24	3.29 ± 0.22
14	3.71 ± 0.25	3.33 ± 0.22	3.45 ± 0.24	3.65 ± 0.22
21	4.44 ± 0.25[d]	4.43 ± 0.22[d]	3.80 ± 0.24[a]	4.71 ± 0.22[b]
Chuck steak				
Aging time (days)				
14	2.33 ± 0.17	2.13 ± 0.15[a]	2.27 ± 0.15	2.51 ± 0.15[d]
21	2.64 ± 0.17	2.61 ± 0.15	2.68 ± 0.15	2.85 ± 0.15

*Mean ± SE.

[ab]Means with superscript a are different from means in the same row with a superscript b ($p < 0.01$).

[ac]Means with superscript a show a trend for difference from means in the same row with a superscript c ($p < 0.06$).

[ad]Means with superscript a show a trend for difference from means in the same row with a superscript d ($p < 0.07$).

Table 2. Concentrations of vitamin D_3, 25-hydroxyvitamin D_3, and 1,25-dihydroxyvitamin D_3, in three different cuts of beef and in plasma of cattle given supplemental doses of vitamin D_3, 25-hydroxyvitamin D_3, or 1,25-dihydroxyvitamin D_3.

Treatments	Strip sirloin steak	Top round steak	Chuck steak	Plasma
		Vitamin D_3*		
	---------- (ng/g) ----------			--- (ng/ml) ---
Control	1.10 ± 5.07	0.76 ± 3.74	1.08 ± 2.46	3.34 ± 14.34
Vitamin D_3	38.06 ± 4.47[a]	28.58 ± 3.30[a]	58.86 ± 2.17[a]	438.93 ± 12.65[a]
25-Hydroxyvitamin D_3	0.65 ± 4.74	1.32 ± 3.50	0.49 ± 2.3	3.85 ± 13.42
1,25-Dihydroxyvitamin D_3	0.73 ± 4.47	0.68 ± 3.30	1.66 ± 2.17	4.81 ± 12.65
		25-Hydroxyvitamin D_3*		
	---------- (ng/g) ----------			--- (ng/ml) ---
Control	1.68 ± 0.37	1.39 ± 0.77	0.90 ± 1.57	62.66 ± 16.74
Vitamin D_3	9.58 ± 0.33[a]	10.62 ± 0.68[a]	17.04 ± 1.38[a]	412.49 ± 14.78[a]
25-Hydroxyvitamin D_3	3.00 ± 0.34[b]	4.65 ± 0.72[b]	5.66 ± 1.47[b]	269.24 ± 15.67[c]
1,25-Dihydroxyvitamin D_3	1.82 ± 0.33	1.43 ± 0.68	1.20 ± 1.38	60.85 ± 14.78
		1,25-Dihydroxyvitamin D_3*		
	---------- (pg/g) ----------			--- (pg/ml) ---
Control	54.31 ± 8.40	84.04 ± 16.69	59.13 ± 5.25	143.14 ± 20.08
Vitamin D_3	57.94 ± 7.41	94.57 ± 14.72	69.29 ± 4.63	179.53 ± 20.08
25-Hydroxyvitamin D_3	57.51 ± 7.86	71.96 ± 15.61	63.84 ± 4.91	164.39 ± 21.17
1,25-Dihydroxyvitamin D_3	58.07 ± 7.41	96.44 ± 15.61	61.61 ± 4.63	320.88 ± 20.08[a]

* Means ± SE.
[a] Means with superscript a are different from all means within column ($p < 0.0001$).
[b] Means with superscript b are different from means with no superscript within column ($p < 0.04$).
[c] Means with superscript c are different from all means within column ($p < 0.0001$).

Table 3. Effect of supplemental vitamin D_3, 25-hydroxyvitamin D_3, and 1,25-dihydroxyvitamin D_3 on amount of the 30-kDa component in strip loin, top round, and chuck steaks at different ages.

	Treatments*			
Steak/aging	**Control**	Vitamin D_3	25-Hydroxyvitamin D_3	1,25-Dihydroxyvitamin D_3
Strip loin steak				
Aging time (days)				
8	1.90 ± 0.68[a]	3.88 ± 0.60[b]	2.96 ± 0.67	2.17 ± 0.63[c]
14	2.51 ± 0.68[c]	4.31 ± 0.60[b]	2.65 ± 0.64[d]	2.35 ± 0.60[a]
21	3.20 ± 0.68[b]	5.00 ± 0.60[c]	5.22 ± 0.64[a]	3.98 ± 0.60
Top round steak				
Aging time (days)				
8	7.95 ± 9.54	6.54 ± 7.79	8.61 ± 8.26	8.82 ± 7.79
14	20.40 ± 8.83[a]	47.42 ± 7.79[b]	15.23 ± 8.26[a]	25.81 ± 7.79[d]
Chuck steak				
Aging time (days)				
14	1.01 ± 0.43	0.91 ± 0.35	0.59 ± 0.37	0.49 ± 0.35

* Values represent means of relative values of the increase in the amount of the 30-kDa band in Western blot analysis. Values are expressed as ratios of the intensity of the 30-kDa band in the experimental samples to the 30-kDa band of an internal standard.
[ab] Values with superscript a are different from values within the same row with superscript b ($p < 0.033$).
[bc] Values with superscript b are different from values within the same row with superscript c ($p < 0.052$).
[bd] Values with superscript b show trend for difference from values within the same row with superscript d ($p < 0.06$).