Use of 25-Hydroxyvitamin D_3 and Dietary Calcium Manipulations to Improve Tenderness of Beef

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Summary and Implications

Improving tenderness of beef from cows is desirable because it could increase marketability of these carcasses and, thereby, increase profits to producers. In this study, two dietary treatments, supplemental 25-hydroxyvitamin D_3 (25-OH D_3) and manipulations of dietary calcium, were examined as methods of increasing plasma and muscle calcium concentrations as a means for improving tenderness of beef. Our results indicate that the environment created in the muscle by supplementing 25-OH D_3, increasing dietary calcium, or both is conducive to increased activity of calpain, a calcium-dependent enzyme responsible for post-mortem tenderization of beef. Increased calpain activity could lead to improved beef tenderness.

Background

Tenderness is one of the most important quality characteristics of beef to both consumers and producers. To date, no practical method of producing consistently tender beef has been adopted by the beef industry. Researchers have demonstrated that feeding a supernatural dosage (0.5 to 7.5 million IU) of vitamin D_3 to beef cattle for 7 to 10 days before slaughter will result in more tender carcasses. Feeding this amount of vitamin D_3 results in elevated plasma and muscle calcium concentrations. The assumed mechanism for this tenderization effect is that the elevated muscle calcium concentration enhances the action of the calcium-dependent protease system of myofibrillar (troponin-T) protein degradation postmortem. Enhanced myofibrillar protein degradation results in more tender beef that is more desirable to consumers. For producers, being able to produce a "guaranteed tender" product even may warrant a price premium.

Some research already has been conducted to observe the effectiveness of feeding 25-OH D_3 to elicit the desired increase in plasma calcium concentrations. In these studies, minimal increases in plasma calcium and tenderness were observed. Results indicate that the necessary dosage and time of 25-OH D_3 administration was not obtained; therefore, additional research to determine the optimal dosage and time of administration was conducted. We found that feeding 500 mg of 25-OH D_3 seven days before harvest significantly increased plasma calcium concentrations and tended to increase muscle calcium in the *longissimus* muscle of beef heifers without leaving unacceptable concentrations of vitamin D_3 or 25-OH D_3 in muscle.

Because calpain is presumably responsible for increased tenderness and calcium is required for calpain activity, a second method of increasing plasma calcium concentration was considered. Decreasing dietary calcium in order to "prime" calcium homeostatic mechanisms first arose as a method for preventing milk fever in dairy cattle. Markedly decreasing calcium from the diets of dairy cattle two weeks prior to parturition and then providing calcium-sufficient feed after parturition was found to prevent milk fever in cows via transient hypercalcemia. More recently, removing supplemental calcium from the diet for 14, 21, or 28 days before harvest and then replenishing the supplemental calcium for one feeding 16 hours before harvest was found to increase plasma calcium concentrations of cattle by nearly 30%. The same study showed improved tenderness of the *semimembranosus* muscle in cattle that had received a low-calcium diet and then a full-calcium diet 16 hours before harvest. Interestingly, this study did not show an effect on tenderness of the *longissimus dorsi,* suggesting that inherent differences between muscles may influence the response to dietary calcium manipulations. The improvement in tenderness of the *semimembranosus* muscle is of particular interest because it is an underutilized muscle from the round section.

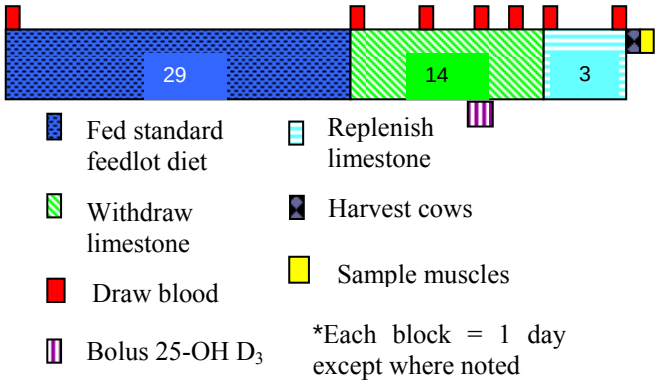
The fact that beef from cows is not as tender as that from younger steers and heifers is widely accepted. Part of the reason for this difference is attributable to the age of the animal; however, biochemical differences in the muscle might exist also. If these differences are related to calcium homeostasis or protein degradation, it is likely that they would affect tenderness of beef. Most of the steers and heifers that have been used for vitamin D_3 and 25-OH D_3 experiments produced beef that was tender even without treatment; so, any effect of treatment could be masked by the innate characteristics of the young, primarily Angus animals. By conducting a similar experiment using older animals, which are expected to be less tender, we expect to better elucidate the effect that 25-OH D_3 has on tenderness of beef. Also, because muscles are biochemically different from each other, it is possible that some muscles will react more favorably to treatment with 25-OH D_3 than will other muscles. Improving tenderness of beef from cows, especially beef from the round section, could increase marketability of the carcasses and, therefore, increase profits to producers.

We hypothesized that feeding 25-OH D_3 and manipulating dietary calcium concentration would increase plasma and meat calcium by increasing intestinal absorption of calcium and thus lead to improved tenderness of beef from cows. Therefore, our objective was to determine the effect of feeding 25-OH D_3 and manipulating dietary calcium on predictors of beef tenderness as well as on beef tenderness itself. This research is ongoing, and only completed assays are presented in this report.

Materials and Methods

We obtained 27 culled Angus beef cows from Iowa State University's Rhodes Beef Farm. All cows were between the ages of three and seven years old and 484-740 kg body weight. Cows were housed at the Iowa State University Beef Nutrition Research farm. Cows were allotted to nine pens (three cows per pen) so that the mean weight of each pen was similar. The outline of the study is shown in Figure 1. Briefly, cows were fed a corn-based finishing diet that met NRC recommendations for all vitamins and minerals for 29 days. On day 30, limestone was removed from the diet so that calcium was deficient. After calcium was withdrawn for 10 days, 0, 250, or 500 mg of 25-OH D_3 was administered in a one-time bolus. Four days after 25-OH D_3 was administered, calcium was added back to the diet at 0.25, 0.375 or 0.5% of dry matter (dry matter was 0.5, 0.75, or 1.0% limestone, respectively). Blood samples were obtained by jugular venipuncture on the first day of the study, the day that calcium was withdrawn from the diet, on day seven of calcium withdraw, on the day 25-OH D_3 was administered, two days after 25-OH D_3 was administered, on the day calcium was added back to the diet, and on the day before cows were harvested (Table 1). Cows were harvested either at a commercial abattoir 45 miles from Ames or at the Iowa State University Meat Laboratory.

Figure 1. Timeline of experiment.



Twenty-four hours after harvest, the round section of each cow was dissected, and seven muscles, *adductor, gracillus, pectineus, sartorius, semimembranosus, vastus intermedius,* and *vastus lateralis,* were collected. Each muscle was cut into three-2.54 cm slices for tenderness analysis, three small pieces for biochemical analysis, and one small piece for immediate calpastatin analysis. For each muscle, one 2.54-cm slice and one small piece was immediately frozen at -20º C for a 24-hour sample, aged for 3 days at 4º C, or aged for 7 days at 4º C. After aging, samples were frozen at -20º C until analysis.

To date, muscle samples have been analyzed for calpastatin activity, troponin-T degradation, and calcium concentration. Plasma samples have been analyzed for calcium, magnesium, and parathyroid hormone (PTH) concentration. Tests that remain to be completed on muscle samples include Warner-Bratzler shear force to test for tenderness, 25-OH D_3 analysis, and 1,25 dihydroxyvitamin D_3 (1,25-$(OH)_2$ D_3) analysis. Tests that remain to be completed on plasma samples include 25-OH D_3 and 1,25-$(OH)_2$ D_3 analysis.

Table 1. Blood sampling schedule.

Timepoint	Event
1	Cows brought to feedlot
2	Calcium withdrawn from diet
3	Midpoint of calcium withdrawal
4	25-OH D_3 administered
5	Midpoint of 25-OH D_3
6	Calcium replenished
7	Day before harvest

Results and Discussion

Because calpain is the enzyme responsible for post-mortem protein degradation and its inhibitor is calpastatin, we determined calpastatin activity in the seven muscles. We used samples from the four most extreme treatments: 0 mg 25-OH D_3 and 0.25% dietary calcium, 500 mg 25-OH D_3 and 0.25% dietary calcium, 0 mg 25-OH D_3 and 0.50% dietary calcium, and 500 mg 25-OH D_3 and 0.50% dietary calcium. Calpastatin activity was measured in a protein extract that was prepared immediately following dissection of each muscle. Activity was measured by adding a known amount of calpain activity to each tube along with substrate and samples. By using spectroscopy, we were able to measure the amount of substrate that was not hydrolyzed by calpain and thus determine how much calpain was inhibited by calpastatin.

In some muscles, *gracillus*, for example, calpastatin activity was highest when neither 25-OH D_3 nor additional calcium was administered to the cows (Table 2). Our findings were expected because some studies have shown that calpain activity is increased when vitamin D_3 is added to the diet of beef steer shortly before harvest. Also, as expected, the calpastatin activity in the *gracillus* muscle was decreased when dietary calcium was increased or when 500 mg 25-OH D_3 was administered. When calpastatin activity is decreased, calpain activity is increased. So, we expect

Table 2. Effect of 25-OH D_3 and manipulations of dietary calcium on calpastatin activity in muscle of beef cows.

	Gracillus	*Adductor*	*Pectinius*	*Semimembranosus*	*Sartorius*	*Vastus Lateralis*	*Vastus Intermedius*
0 mg 25-OH D_3 & 0.5% limestone	83.28^{a}±6.98	44.71±4.68	53.89±4.75	46.23±7.98	40.07±4.04	61.76±16.14	96.47^{a}±11.91
0 mg 25-OH D_3 & 1.0% limestone	40.89^{b}±6.98	33.1±4.68	48.65±4.75	32.21±7.98	37.21±4.95	46.91±16.14	44.85^{b}±11.91
500 mg 25-OH D_3 & 0.5% limestone	44.91^{b}±6.98	36.86±4.68	43.19±4.75	44.04±7.98	41.3±4.04	55.58±16.14	83.44^{a}±11.91
500 mg 25-OH D_3 & 1.0% limestone	59.38^{a}±6.98	31.61±4.68	45.57±4.75	41.86±7.98	44.41±4.04	92.6±16.14	63.32^{ab}±11.91

*Values are mean SEM ± SEM

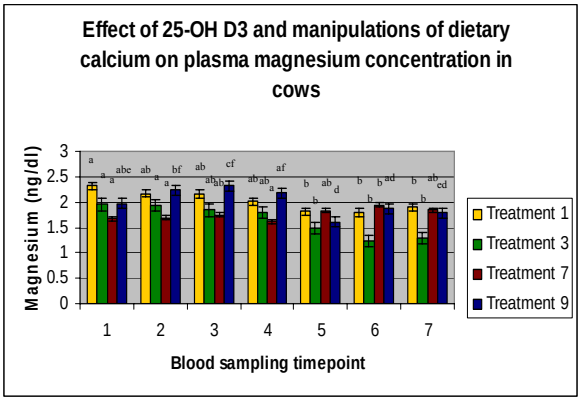
Values within a muscle with differeing subscripts are significantly different ($p \leq 0.05$)

decreased calpastatin activity to correlate negatively with improved beef tenderness.

Plasma calcium concentrations were measured by atomic absorption. As predicted, plasma calcium concentrations were similar in most treatment groups at the beginning of the study (Figure 2). After calcium was withdrawn from the diet, plasma calcium concentration decreased numerically in all treatment groups. When dietary calcium was replenished, plasma calcium concentration in all treatment groups except Treatment 1 (control) increased; however, the groups that received 500 mg of 25-OH D_3 were increased to a greater extent.

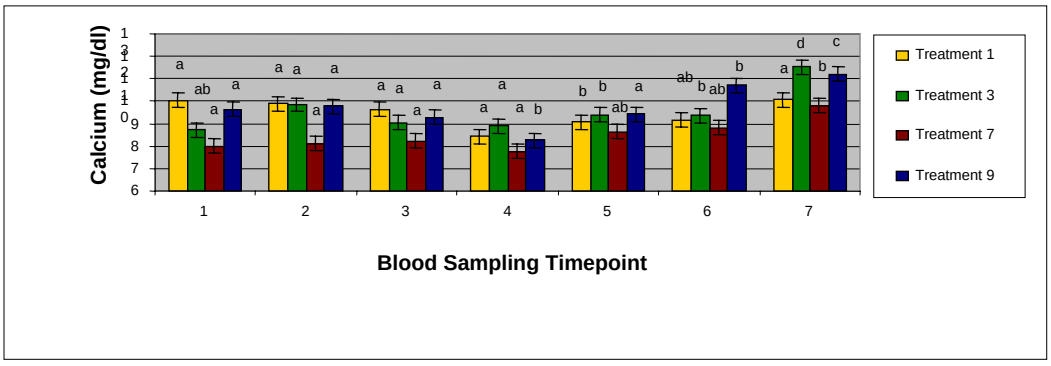
We analyzed plasma magnesium concentration to test our hypothesis that plasma calcium concentration was increased through increased intestinal absorption of calcium because of the action of 1,25-$(OH)_2$ D_3 on increasing the transcription of calcium binding protein and not because of increased bone resorption. If the increase in plasma calcium that we observed was caused by bone resorption, plasma magnesium concentrations would be increased too. As shown in Figure 3, plasma magnesium concentrations are

Figure 3.



Letters denote differences in treatment effect over time ($p \leq 0.05$).

Figure 2. Effect of dietary calcium and 25-OH D3 on plasma calcium concentration.



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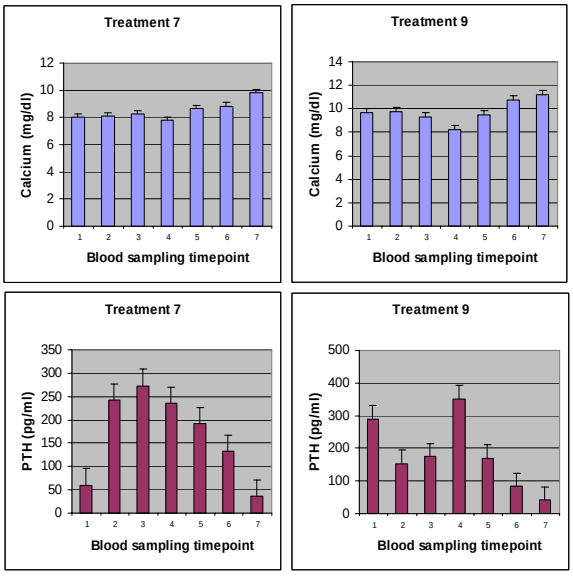
decreased when the plasma calcium concentrations are increased, signifying a decrease in bone resorption.

Plasma parathyroid hormone was determined by radioimmunoassay (Nichols Institute Diagnostics, San Clemente, CA). Because PTH is responsible for increasing bone resorption to maintain plasma calcium concentrations, we expected PTH concentrations to increase when dietary calcium was decreased. Our findings, at least numerically, were in accordance with our expectations (Figures 4a and 4b). Statistical analysis has not been completed on PTH data; however, by looking at the data, PTH concentration seems increased when plasma calcium concentrations are decreased. Because calcium was withdrawn from all treatment groups, this effect is observed, regardless of calcium or 25-OH D_3 treatment. Figures 4a and 4b show only data for the four most extreme treatments, but results are similar for all treatments.

Table 3. Key to treatments.

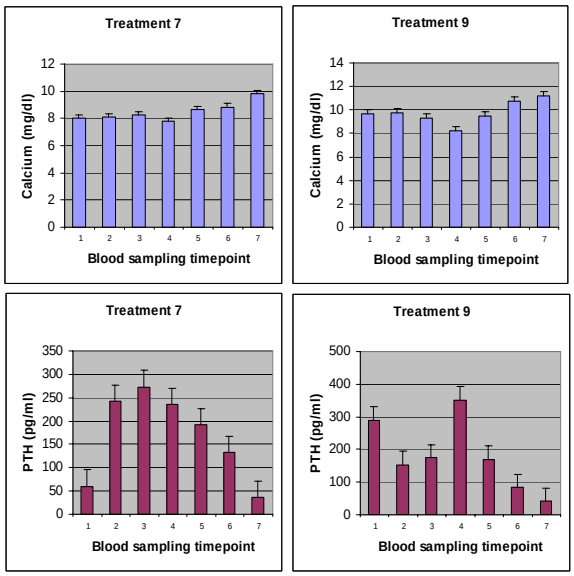
Treatment	Limestone (% DM)	25-OH D_3 (mg)
1	0.5	0
2	0.5	250
3	0.5	500
4	0.75	0
5	0.75	250
6	0.75	500
7	1.0	0
8	1.0	250
9	1.0	500

Figure 4a. Effect of 25-OH D3 and calcium on plasma.



Treatment 7 $R^2 = 0.37$
Treatment 9 $R^2 = 0.78$

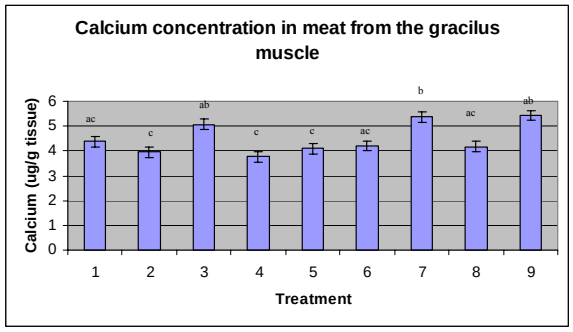
Figure 4b. Effect of 25-OH D_3 and calcium on plasma calcium and PTH concentrations: high dietary calcium.



Treatment 7 $R^2 = 0.37$
Treatment 9 $R^2 = 0.78$

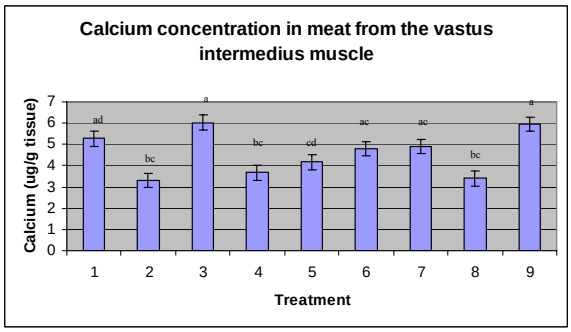
Muscle calcium concentration was determined by wet ashing followed by atomic absorption. Calcium concentrations in most muscles were higher in cows from treatment groups that received increased dietary calcium, 25-OH D_3, or both. Two muscles that show these results very clearly are the *gracillus* and the *vastus intermedius* (Figures 5 and 6). An increase in calcium concentration in muscle at the time of harvest creates an environment in which calpain could be activated to a greater extent and lead to increased meat tenderness.

Figure 5.



Letters denote differences in treatment effect over time ($p \leq 0.05$).

Figure 6.



Letters denote differences in treatment effect over time ($p \leq 0.05$).

Conclusion

These results indicate that withdrawing and then replenishing dietary calcium, administering 25-OH D_3 seven days before harvest, or both decreases calpastatin activity in some muscles and increases plasma and muscle calcium concentrations. The increase in calcium concentration we see is, in fact, caused by an increase of calcium transporters in the small intestine as a result of up-regulation of calcium homeostatic mechanisms in the absence of adequate dietary calcium or from increased 1,25-$(OH)_2$ D_3 (acquired by hydroxylation of 25-OH D_3 in the kidney) concentrations in the body. Increased calcium concentrations in muscle at the time of harvest may lead to improved tenderness of beef because of increased action of calpain, especially in muscles in which calpastatin activity is decreased.

Acknowledgements

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