

Project Summary

Novel Strategies to Improve Tenderness of Underutilized Round Muscles from Cows

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**Study Completed
May 2005**



Funded by The Beef Checkoff

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Background

Past research has shown that feeding a supernatural dosage (0.5 to 7.5 million IU) of vitamin D₃ to beef cattle for seven to 10 days before slaughter can improve tenderness in beef.

Supplementing vitamin D increases muscle calcium concentrations, which are thought to enhance the action of intercellular calcium-dependent proteases, or calpains. These proteases cause enhanced myofibrillar protein degradation that ultimately results in beef that is more tender and desirable to consumers.

The disadvantage of feeding a high dosage of vitamin D₃ close to harvest is that it results in a high concentration of vitamin D₃ and its metabolite, 25-hydroxyvitamin D₃ (25-OH D₃) in muscle tissues. In contrast, feeding 25-OH D₃ directly results in less accumulation of vitamin D₃ metabolites in the muscle and therefore has a greater chance of being approved by the Food and Drug Administration (FDA) for the use of improving tenderness in meat-producing animals.

A second method of increasing plasma calcium concentration to improve meat tenderness is to decrease and then replenish dietary calcium. This strategy has been used as a means of preventing milk fever in dairy cattle. The standard practice is to decrease calcium in the diets of dairy cows two weeks prior to parturition (calving) and then provide calcium-sufficient feed after parturition. Recently, researchers have found that 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 increased plasma calcium concentrations of cattle by nearly 30 percent. The same study showed a decrease in the shear force in a muscle from the round (*semimembranosus*) from cattle that had received a low-calcium diet and then 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 among muscles may influence the response to dietary calcium.

In this study, researchers from Iowa State University sought to identify biochemical differences influencing aging rate for muscles from the round. Researchers also examined the effect of various dietary treatments to increase calcium concentrations in muscle with the goal of improving tenderness.

Methodology

Twenty-seven English cows, approximately three to seven years old, were separated and randomly assigned into nine pens of three cows each. Each pen was assigned randomly to one of nine treatments:

- 0 mg 25-OH D₃/0.51 percent limestone
- 0 mg 25-OH D₃/0.75 percent limestone
- 0 mg 25-OH D₃/1 percent limestone
- 250 mg 25-OH D₃/0.51 percent limestone
- 250 mg 25-OH D₃/0.75 percent limestone

- 250 mg 25-OH D₃/1 percent limestone
- 500 mg 25-OH D₃/.51 percent limestone
- 500 mg 25-OH D₃/.75 percent limestone
- 500 mg 25- OH D₃/1 percent limestone

Three days before harvest, one group of three cows for each 25-OH D₃ treatment (0, 250, or 500 mg) was fed one of three (0.51%, 0.75%, and 1%) concentrations of limestone in the diet.

Cows were weighed and a baseline blood sample was obtained. Cows were fed a standard feedlot diet for 60 days. Seventeen days before harvest, limestone (calcium supplement) was withdrawn from the diet. Blood samples were drawn three, two, and one week before harvest. The remainder of the diet did not change and contained 0.09% calcium (60% of NRC recommendation). The three 25-OH D₃ treatments (0 mg, 250 mg, and 500 mg 25-OH D₃) were administered by gelatin capsule as a one-time bolus (ROVIMIX® Hy•D® 1.25% DSM Nutritional Products, Inc., Ames, IA) seven days before harvest. The control group received gelatin capsules containing only cornstarch. Three days before harvest, one group of three cows for each 25-OH D₃ treatment (0, 250, or 500 mg) was fed one of three (0.51 percent, 0.75 percent, and one percent) concentrations of limestone in the diet. Limestone normally is supplemented at 0.50 percent of a feedlot diet; so, these concentrations correspond to one, 1.5, and two times the normal dietary calcium concentration that is recommended for feedlot cattle. Additional blood samples were drawn two, four, and six days after bolusing 25-OH D₃. All blood samples were centrifuged to separate plasma, and plasma was stored at -20°C until analysis.

All cattle were harvested and samples from each of seven muscles, *gracillus*, *adductor*, *pectineus*, *semimembranosus*, *sartorius*, *vastus lateralis*, and *vastus intermedius*, were obtained, packaged, and aged for 24 hours, 3 days, or 7 days. Two samples from each muscle were subjected to each aging period. Samples of aged muscles were stored at -20° C until further analysis.

Analysis for calpain and calpastatin activity was completed for the 12 cows in the most extreme dietary treatments (normal calcium, 0 mg 25-OH D₃; normal calcium, 500 mg 25-OH D₃; 2 times normal calcium, 0 mg 25-OH D₃; or 2 times normal calcium, 500 mg 25-OH D₃) as soon as possible after harvest. Calcium and magnesium concentrations in plasma were analyzed by atomic absorption. Degradation of Troponin-T (protein complex that confers calcium sensitivity to muscle cells) was determined through Western blotting. 25-Hydroxyvitamin D₃ and 1,25-dihydroxyvitamin D₃ concentrations in blood plasma and in meat will also be analyzed to determine if levels are above an acceptable threshold. Beef muscle tenderness will be measured with Warner Bratzler shear force. At the time of submission of the final report, not all analyses were complete.

Findings

Results from calpastatin analysis indicate that 25-OH D₃ and manipulations of dietary calcium decreased postmortem calpastatin activity in some muscles. The *gracillus* and *vastus intermedius* muscles were affected by dietary manipulations more than other muscles. In all muscles except the *sartorius*, postmortem calpastatin activity was numerically higher for the 0 mg of 25-OH D₃ treatment and the normal calcium group than in any other treatment. A decrease in postmortem

calpastatin activity could lead to an increase in calpain activity and, therefore improve muscle tenderness.

Table 1. Calpastatin activity in beef cow muscles

Treatment	<i>Gracillus</i>	<i>Adductor</i>	<i>Pectineus</i>	<i>Semimembranosus</i>	<i>Sartorius</i>	<i>Vastus lateralis</i>	<i>Vastus intermedius</i>
0 25-OH D ₃ 0.5% limestone	83.3 ^a ±7.0*	44.7±4.7	53.9±4.8	46.2±8.0	40.1±4.0	61.8±16.1	96.5 ^a ±11.9
0 25-OH D ₃ 1.0% limestone	40.9 ^b ±7.0	33.1±4.7	48.7±4.8	32.2±8.0	37.2±5.0	46.9±16.1	44.9 ^b ±11.9
500 25-OH D ₃ .5% limestone	44.9 ^a ±7.0	36.9±4.7	43.2±4.8	44.0±8.0	41.3±4.0	55.6±16.1	83.4 ^a ±11.9
500 25-OH D ₃ 1.0% limestone	59.4 ^a ±7.0	31.6 ^a ±4.7	45.6±4.8	41.9±8.0	44.4±4.0	92.6±16.1	63.3 ^{ab} ±11.9

* Values are units calpastatin activity per gram protein extracted ± SEM

^{ab} Numbers with differing superscripts in the same column are different (p≤0.05)

Implications

The researchers anticipated that based on these results, further analysis with Warner-Bratzler shear force will demonstrate that more tender meat will be from muscles with higher calcium concentrations. The researchers also expected that some of the dietary treatments will influence the protein profile of the postmortem muscle and will aid in discovering more precise mechanisms for improving tenderness in the round muscles of older cattle.

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