Effect of Depleting and Repleting Dietary Calcium on Plasma and Tissue Calcium Concentrations and Beef Tenderness

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Story in Brief

To test effects of depletion and repletion of calcium level within the diet on beef tenderness, 35 steers (initial BW = 379 + 42 kg) were used in a complete randomized design. Steers were assigned randomly to two diets, a control diet and a calcium depletion-repletion sequence. All steers were fed a typical finishing diet (0.54% calcium) for 118 d prior to the study. On d 1 limestone was removed from the diet of those cattle assigned to the depletion-repletion sequence, with the depletion diet containing 0.30% calcium. On d 21, just 24 h before harvest, feed was removed from bunks of the depleted steers and replaced with the original diet (0.54% calcium). Efficiency of gain, DMI, and ADG did not differ among treatments. Carcass characteristics, including HCW, REA, and USDA quality grade, also were not affected by manipulation of dietary calcium concentrations. Plasma calcium concentrations of depleted steers were decreased numerically (9.45 to 8.98 mg/dL) when limestone was removed from the diet but increased numerically (to 9.70 mg/dL) with repletion. Calcium concentrations in muscle samples taken shortly post mortem did not differ among treatments (0.05 + 0.005 mg/g). The *Semimembranosus* was collected from each carcass at harvest. Warner-Bratzler shear force was determined on three steaks from each muscle on d 7, 14, and 21 post mortem. Shear force was less ($P<0.05$) for cattle on the depletion-repletion sequence than for control cattle (4.4 vs. 4.7 kg). Length of post mortem aging had no effect ($P=0.96$) on mean shear force values. Results indicate that a depletion-repletion sequence for dietary calcium can improve tenderness of beef fed high-concentrate finishing diets.

Key Words: Beef, Calcium, Tenderness

Introduction

Three National Beef Quality Audits (Lorenzen et al., 1993; Boleman et al., 1998; McKenna et al., 2002), two National Beef Tenderness Surveys (Morgan et al., 1991; Brooks et al., 2000), and research by Boleman et al. (1997) indicate that meat tenderness is important for consumers who purchase beef. With the discovery of the calpain system, the proteolytic system responsible for postmortem tenderness, research has been conducted to manipulate the system and capture premiums for consistently tender beef. Most of this research has been based on the concept that elevating calcium concentrations of postmortem cells will increase activity of the calpain system and result in improved and more consistent beef tenderness. Thus the objective of this study was to measure the response to a dietary calcium depletion-repletion sequence on calcium concentration in blood plasma and muscle tissue and on Warner-Bratzler shear force of the *Semimembranosus* muscle.

Material and Methods

Animals. Thirty-five steers (379 $\pm$ 42 kg BW) were received at the Willard Sparks Beef Research Center in July 2003. In a randomized complete block design, the steers were blocked

by weight and allotted to one of two dietary treatments: control and calcium depletion-repletion with 3 pens of steers being designated to be fed each diet at a later time. On arrival, all cattle were vaccinated, treated for internal and external parasites, and implanted with Revalor-S ™ (20 mg trenbolone acetate, 4 mg estradiol; Intervet, Inc.). Individual weights were also recorded.

Diet. For 118 d prior to the initiation of calcium depletion, all steers were fed a finishing diet composed of 77.5% rolled corn, 10% ground alfalfa, 4% molasses, 2% fat, 6.5% pelleted supplement, and 4.25% water. The diet contained .54% calcium. Nutrient composition and analyses of the diet and the pelleted supplement are shown in Table 1. On d 1 of depletion, limestone was removed from the diet of those cattle assigned to the depletion-repletion sequence, with the depletion diet containing 0.30% calcium. After three weeks (21 d) of being fed the 0.30% calcium diet, the depleted steers were replenished by replacing the limestone in the supplement (0.54% calcium ration). Cattle were harvested 24 h after they were first given access to the control supplement (d 22).

Table 1. Calculated and analyzed diet and supplement composition (DM basis)

Calculated diet composition	Control	Depletion/Repletion
NE_m, Mcal/cwt	96.58	97.20
NE_g, Mcal/cwt	62.71	63.05
Crude Protein, %	13.60	13.60
Calcium, %	.66	.31
Phosphorus, %	.39	.38
Potassium, %	.76	.77
Magnesium, %	.17	.17
Analyzed diet composition		
Calcium, %	.54	.30
Phosphorus, %	.36	.28
Potassium, %	.91	.79
Magnesium, %	.13	.12

Analyzed supplement composition		
Calcium, %	8.42	2.34
Phosphorus, %	1.88	1.88
Potassium, %	1.34	1.39
Magnesium, %	.26	.26

Weights. Cattle were individually weighed without withdrawal of feed or water on d –119, 1, 17 and 22. A 4% pencil shrink was applied to weights for calculation of ADG and efficiency of gain. On d 1 and 17, blood samples obtained by jugular venipuncture from each steer were collected into a sterile 10 mL BD Vacutainer® tube (Becton Dickinson, Rutherford, NJ) containing sodium heparin. Samples were centrifuged and plasma was collected and frozen at –20°C for later analysis of calcium and magnesium concentrations at the USDA-ARS National Animal Disease Center.

Slaughter. All cattle were harvested at Excel Corporation in Dodge City, KS. Oklahoma State University personnel accompanied steers to the plant to collect carcass data (HCW, REA, marbling score, fat thickness, KPH estimates, and USDA quality and yield grade) for each steer. Blood samples collected during exsanguination were centrifuged and plasma was collected and frozen at –20°C for analysis as previously described. *Semimembranosus* (inside round) from the right side of each carcass was collected for tenderness measurement. *Semimembranosus* muscles were frozen at –16.2°C upon return to Stillwater, approximately 48 h after harvest, for later analysis. After 35 d of freezing, four steaks (2.54 cm thick) were cut from each frozen *Semimembranosus* muscle. Three steaks were vacuum packed, thawed and randomly assigned to aging times of 7, 14, or 21 d at 3.3°C. The fourth steak was packaged and frozen for later calcium and magnesium concentration analysis at the USDA-ARS National Animal Disease Center.

Following each allotted aging time, steaks were again frozen at –40°C. After all steaks had been aged, they were thawed for 24 h and cooked on an impingement oven to an internal temperature of 71°C (medium degree of doneness). Steaks were allowed to cool before six 1.27-cm cores were removed parallel to orientation of muscle fibers from each steak. Each core was then sheared once perpendicular to the orientation of muscle fibers with a Warner-Bratzler shear device attached to a texture analyzer (Kinston model 4502, Canton, MA) equipped with a 1-kiloNewton load cell and a crosshead speed of 200.0 mm/min (penetration speed of 3.3 mm/s). Warner-Bratzler shear values are presented and statistically analyzed as the mean of six cores per steak.

Statistical Analysis. All data were analyzed as a randomized complete block design using the PROC MIXED procedures of SAS. For feedlot performance and carcass measurements, pen served as the experimental unit with three pens per diet. Individual animal was considered the experimental unit for Warner-Bratzler shear force measurements and for tissue and plasma concentrations of calcium and magnesium. Means and standard errors of means for Warner-

Bratzler shear force values were determined using the AR1 repeated measures analysis. For calculation of means and standard errors for tissue and plasma concentrations, calcium treatment was considered the main effect and initial concentrations of calcium and magnesium were used as a covariate for analyzing subsequent plasma samples.

Results and Discussion

Similar to findings of Walsh et al. (2003), feedlot performance and carcass merit (Tables 2 and 3), except USDA Yield Grade, were not significantly affected by calcium manipulation. The difference in USDA Yield Grade (3.4 for control vs 3.1 for low Ca) would be commercially meaningless, as USDA Yield Grade is not accurate to a tenth of a yield grade. Yield grades calculated from physical measurements taken by OSU personnel did not differ (P=0.23) among diets.

Table 2. Effect of supplemental calcium depletion-repletion on feedlot performance

Item	Control	Depletion/Repletion	SEM
Initial weight, kg	376	379	1.96
Weight on d 22, kg	623	611	12.56
ADG, d 1 – 22, kg/d	1.43	1.19	.21
DMI, d 1 – 22, kg/d	11.70	10.80	.59
Gain:Feed, d 1 – 22, kg/kg	.12	.11	.01

Table 3. Effect of supplemental calcium depletion-repletion on carcass characteristics

Item	Control	Depletion/Repletion	SEM
Hot carcass weight, kg	391	392	6.95
12^{th} rib fat thickness, cm	1.75	1.47	.08
Longissimus muscle area, cm^2	87.87	92.00	1.84
Kidney, pelvic, and heart fat, %	2.7	3.0	.21
Marbling[a]	392	395	21.91
USDA yield grade	3.4[a]	3.1[b]	.04
Measured yield grade	3.7	3.3	.16

USDA quality grade[c]	2.43	2.60	.14

[a]Marbling score: 300 = slight00, 400 = small00

[b]USDA quality grade: 3 = select, 2 = choice

[cd]Means with different subscripts differ (P<0.05)

The blood plasma calcium concentrations were not significantly altered by time or diet when initial calcium concentration was used as a covariate (Table 4). However, mean plasma calcium concentration 16 d after limestone withdrawal began was 0.47 mg/dL less than concentrations measured on d 1 for deplete-replete cattle, while concentrations of calcium for control cattle decreased by only 0.06 mg/100 mL over this time period. Blood samples taken at exsanguination had calcium concentrations greater than those taken on d 16, being 0.72 mg/100 mL greater for replenished steers and 0.69 mg/100 mL greater for control cattle. Walsh et al. (2003) reported that serum calcium concentrations at exsanguination were greater ($P<0.01$) for calcium replenished cattle than control cattle (11.9 vs. 9.3 mg/100 mL).

Magnesium concentrations within the blood (Table 5) were not significantly altered by time or diet when analyzed with initial level as the covariate, except at harvest when the magnesium concentration of control cattle was greater ($P<0.04$) than that of calcium replenished cattle (2.56 vs. 2.38 mg/100 mL).

Calcium and magnesium muscle concentrations did not differ ($P=0.72$ and 0.26 respectively) between control and calcium replenished cattle. In agreement with Walsh et al. (2003), muscle calcium concentrations were numerically greater in calcium replenished cattle than control cattle (51.7 vs. 54.2 μg/g).

Table 4. Effect of calcium depletion-repletion on plasma and tissue concentrations

	Control	Depletion/Repletion	SEM
Plasma calcium, mg/dL			
Initial	9.16	9.45	.23
16 d after withdrawal	9.09	8.98	.16
Harvest	9.78	9.70	.10
Plasma magnesium, mg/dL			
Initial	1.83	1.87	.07
16 d after withdrawal	1.85	1.82	.05

Harvest	2.56[a]	2.37[b]	.08
Tissue calcium, mg/g			
Wet muscle	.052	.054	.005
Ash	4.41	4.52	.42
Tissue magnesium, mg/g			
Wet muscle	.229	.236	.005
Ash	19.3	19.7	.39
[ab] Means in a row with different subscripts differ (P<0.05)			

In the present study, overall Warner-Bratzler shear values were not altered by aging of steaks, but averaged across aging time were lower for replenished steers than for control steers (Table 5). The absence of an aging effect may be due to the aging protocol followed in the study. By freezing the whole muscle before steaks were cut and aged, the slow freezing process may have resulted in formation of ice crystals that would damage the myofibrils. Walsh et al. (2003) reported that *Semimembranosus* shear force values were lower ($P<0.05$) for calcium replenished than for control cattle after steaks had been aged for 5 d (3.6 vs. 4.2 kg), but they observed no improvement in shear values for steaks aged 10 or 15 d. Walsh et al. (2003) also reported no difference ($P<0.20$) in Warner-Bratzler shear force values between calcium replenished and control cattle for other muscles (*Longissimus dorsi, Triceps brachii)* after 5, 10, or 15 d of aging.

Table 5. Effect of calcium depletion-repletion on Warner-Bratzler shear values

Age	Control	Depletion/Repletion	Aging response
7	4.7	4.5	4.6
14	4.7	4.5	4.6
21	4.8	4.2	4.5
Diet Means	4.7[a]	4.4[b]	

[ab] Means with different subscripts differ ($P<0.05$)

Main diet effect, $P=0.04$

Main aging effect, $P=0.96$

Diet * aging interaction, $P=0.44$

SEM for aging and diet were 0.13 and 0.12, respectively

The distribution of Warner-Bratzler shear force by treatments is shown in Table 6. Control cattle had 17.2% more steaks that received a shear value corresponding to a tough tenderness score compared with replenished cattle. Moreover, depleted-repleted cattle had 5% more steaks receiving a shear value considered to be very tender compared with control cattle.

Table 6. Effect of calcium manipulation on Warner-Bratzler shear force distribution

Shear force distribution	Control	Depletion/Repletion
Under 3.9 kg (very tender)	8.9 %	13.9%
From 3.9 to 4.5 kg (tender)	37.8%	50%
Over 4.5 kg (tough)	53.3%	36.1%

"Very tender" generally corresponds to shear force values < 3.9 kg

"Tender" generally corresponds to shear force values 3.9 – 4.5 kg

"Tough" generally corresponds to shear force values > 4.5 kg

Implications

A dietary scheme of calcium depletion and repletion appears to have potential as an economical method to improve the tenderness of beef without adversely affecting feedlot performance or carcass merit. Additional research is needed to examine different degrees and durations of calcium withdrawal and repletion. Further research also is needed to examine the consistency of this method to improve tenderness not only of the *Semimembranosus*, but also of other muscles of the carcass.

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