Meat Tenderness and the Calpain Proteolytic System in Longissimus Muscle of Young Bulls and Steers[1]

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ABSTRACT: The objectives of this study were to examine the effects of castration on the calpain proteinase system (μ-calpain, m-calpain, and calpastatin) activities and meat tenderness. Six each, MARC III bulls and steers were slaughtered at approximately 12 mo of age. Longissimus muscle samples were obtained for determining myofibril fragmentation index, Warner-Bratzler shear force, and sensory panel evaluation at 1, 7, and 14 d postmortem, and μ- and m-calpain and calpastatin activities at 24 h postmortem. Bulls produced leaner carcasses with lower ($P < .05$) quality grades than did steers. Meat from bulls had higher ($P < .05$) shear force values than meat from steers; however, sensory panelists were unable ($P > .05$) to detect differences in tenderness or other sensory traits between bulls and steers. Activities of μ- and m-calpain were not affected ($P > .05$) by castration; however, calpastatin was higher ($P < .05$) in muscles from the bull carcasses. Lower ($P < .05$) myofibril fragmentation index values indicate that less proteolysis occurred in muscle from bulls than in muscle from steers during the first 7 d postmortem. Greater calpastatin 24-h activity may be associated with the increased shear force of meat from bulls.

Key Words: Beef, Calpains, Calpastatin, Castration, Meat, Tenderness

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Introduction

Numerous research studies have been conducted to assess growth and meat characteristic differences between bulls and steers. In general, results have indicated that bulls grow more rapidly (15 to 17%), utilize feed more efficiently (10 to 13%) to an age or weight end point, and produce higher yielding carcasses with less fat and more muscle than steers (Field, 1971; Seideman et al., 1982). However, in most cases, meat from young bulls was more variable in tenderness than meat from steers (Burson et al., 1986; Dikeman et al., 1986). The mechanism causing tenderness differences between bulls and steers is not well defined. Numerous studies have attempted to link bull meat toughness to higher amounts of connective tissue than that of steers (Riley et al., 1983; Crouse et al., 1985; Vanderwert et al., 1986). However, a strong relationship between 24-h postmortem calpastatin activity, myofibrillar proteolysis, and meat tenderization has been reported in steers and heifers (Whipple et al., 1990; Koohmaraie and Shackelford, 1991; Shackelford et al., 1992). The possible contribution of the calpain proteinase system to differences in postmortem tenderization of muscle from bulls and steers has not been reported. This study was conducted to examine the effect of castration on palatability traits and 24-h postmortem activities of μ-calpain, m-calpain, and calpastatin of longissimus muscle in cattle.

Materials and Methods

Animals. The Roman L. Hruska U.S. Meat Animal Research Center Animal Care and Use Committee approved the use of animals in this study. Animals to remain bulls or to be castrated were randomly

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assigned and castration was performed within 1 wk of birth. Six each, MARC III composite (1/4 Red Poll, 1/4 Pinzgauer, 1/4 Hereford, and 1/4 Angus) bulls and steers weighing approximately 180 kg at approximately 60 mo of age were given access to a diet formulated to meet NRC requirements for growing beef cattle (NRC, 1984). The diet consisted of 36% corn silage, 60% wet corn, and 4% supplement (supplement composition: 54% soybean meal, 17.9% corn, 21.7% limestone, 2% dicalcium phosphate, 3% urea, .6% vitamin A, .2% trace minerals, .5% Rumensin [containing 132 g of monensin/kg], and .1% sulfur) with a TDN of 84.16% and 10.93% CP. Animals were weighed weekly. The experiment was conducted from November through May. Animals were housed in an insulated barn (temperature maintained at approximately 13°C) in individual stalls. Lights in the building were on 9 h and off 15 h during each 24-h period. Animals were removed from the stalls and allowed to exercise outside for 3 h, twice weekly. Animals were fed for 168 d and slaughtered according to standard humane procedures at approximately 12 mo of age. Carcasses were chilled for 24 h at 1°C.

Carcass Evaluation. At 24 h postmortem, the left side of each carcass was ribbed between the 12th and 13th ribs for determination of USDA quality and yield grade factors (USDA, 1989). Hot carcass weight, dressing percentage, adjusted fat thickness, marbling degree, longissimus muscle area, and percentage of kidney, pelvic, and heart fat were recorded. Dark, coarse band formation (heat ring), lean color, lean firmness, and lean texture were scored on a 7-point scale (7 = severe, black, very soft, or very coarse, and 1 = none, very light cherry red, very firm, or very fine).

At 24 h postmortem, the longissimus muscle (right side) from the 7th thoracic to the 5th lumbar vertebra was cut into steaks 2.5-cm thick and vacuum-packaged. Two steaks each were assigned to 1, 7, or 14 d postmortem aging (2°C) by stratifying storage time along the length of the longissimus muscle. One steak was used for Warner-Bratzler shear force and one for sensory panel evaluation. The steaks were stored at −10°C for 2 to 4 wk until they were thawed and cooked.

Calpain and Calpastatin Determination. At 24 h postmortem, a 5-g sample of longissimus muscle was taken from one of the shear force steaks from the 12th rib region of the right side of each carcass for determinations of μ-calpain, m-calpain, and calpastatin activities according to the procedure described by Wheeler and Koohmaraie (1991) using 50 m*M* Tris-HCl, pH 8.3, instead of 50 m*M* sodium acetate, pH 5.8, as the extraction solution. Briefly, after homogenization, centrifugation, dialysis, and clarification, the muscle extracts were loaded onto DEAE-Sephacel (Pharmacia LKB, Piscataway, NJ) columns and washed with 10 column volumes of elution buffer to remove unbound proteins. After washing, the bound proteins were eluted with a continuous NaCl gradient from 25 to 350 m*M* NaCl. Activities were expressed as the amount of calpain caseinolytic activity per gram of muscle. One unit of μ- and m-calpain activity was defined as the amount of enzyme that catalyzed an increase of 1.0 absorbance unit at 278 nm in 1 h at 25°C. One unit of calpastatin activity was defined as the amount that inhibited 1.0 unit of DEAE-Sephacel purified m-calpain activity.

Shear Force and Sensory Evaluation. Steaks were tempered at 2°C for 24 h before cooking. The steaks were broiled to an internal temperature of 40°C, turned, and broiled to an internal temperature of 70°C on Farberware electric broilers (Farberware, Bronx, NY) for determination of shear force and sensory panel analysis at 1, 7, and 14 d postmortem. The internal temperature was monitored by a copper constantan thermocouple probe attached to a Honeywell recording thermometer (Honeywell, Scarborough, ON, Canada). Weights were recorded before and after cooking for determination of percentage of cooking loss. Steaks were allowed to cool for 2 h at 23°C, and 10 1.3-cm cores were removed from each steak parallel to the longitudinal orientation of the muscle fibers. Each core was sheared once with a Warner-Bratzler shear machine. Shear force for each cut was recorded as the mean of the 10 cores.

Steaks for sensory evaluation were cooked as described above. Warm 1-cm^3 samples were served to an eight-member sensory panel trained according to methods described by Cross et al. (1978). The panel evaluated each sample for juiciness, myofibrillar and overall tenderness, connective tissue amount, and flavor intensity based on 8-point scales (8 = extremely juicy, tender, none, and intense; 1 = extremely dry, tough, abundant, and bland).

Myofibril Fragmentation Index. Samples were excised from the longissimus muscle at 24 h postmortem and were vacuum-packaged and stored for an additional 6 or 13 d at 4°C. Myofibril fragmentation index (**MFI**) was determined on fresh muscle at 1, 7, and 14 d postmortem according to the procedures of Culler et al. (1978). The biuret procedure was used to determine protein concentrations (Gornall et al., 1949).

Statistical Analysis. Data were analyzed by analysis of variance with the GLM procedure of SAS (1985) for a split-plot design. The whole plot was animal sex class (bull vs steer) and the split plot was postmortem aging time (Warner-Bratzler shear, MFI, sensory traits, and cooking loss). The whole-plot error term in the model was replication × sex class with animal representing replication. The split-plot error term in the model was the residual error. Tukey's test was used for mean separation when needed.

Results and Discussion

Live performance traits of animals in this study were presented by Morgan et al. (1993). Bulls had heavier ($P < .05$) carcasses than steers (Table 1).

Bull carcasses had lower ($P < .05$) adjusted fat thicknesses, kidney, pelvic, and heart fat percentages, USDA yield grades, and larger ($P < .05$) longissimus muscle areas. Steer carcasses had higher ($P < .05$) marbling scores and USDA quality grades (Table 1). Similar findings have been reported in other investigations (reviewed by Seideman et al., 1982). In our study, bull carcasses had a higher ($P < .05$) incidence of dark, coarse band formation (heat ring) than did steer carcasses (Table 1). The decreased 12th rib fat thickness could have resulted in greater chilling rate, and thus, decreased the rate of decline in calpastatin activity in meat from bulls. However, several studies have reported no effect on tenderness of removing the subcutaneous fat over the longissimus muscle before chilling (Koohmaraie et al., 1988; Johnson et al., 1989).

Many conflicting reports exist concerning differences in tenderness of meat from carcasses of bulls and steers. Many researchers have reported that meat from bull carcasses was less tender and less palatable than meat from steer carcasses (Field, 1971; Seideman et al., 1982; Burson et al., 1986; Dikeman et al., 1986), whereas others have been unable to detect significant differences in tenderness of meat from young bulls and steers slaughtered at comparable ages (Naunde et al., 1977; Riley et al., 1983; Calkins et al., 1986; Vanderwert et al., 1986). Bull longissimus muscle steaks had higher ($P < .05$) shear force values and lower ($P < .05$) MFI than steer longissimus muscle steaks (Table 2). In addition, shear force decreased ($P < .05$) and MFI increased ($P < .05$) as postmortem aging time increased. Longissimus tenderness is highly and positively correlated with MFI (Davey and Gilbert, 1969; Olson et al., 1976; Culler et al., 1978; Parrish et al., 1979) and indicates the amount of myofibrillar proteolysis that has occurred. Although the sex class × postmortem aging interaction was not significant, it seems that a greater amount of proteolysis had occurred in muscle from steers during the first 7 d postmortem.

A convincing body of literature indicates that the calpain proteolytic system plays a major role in postmortem tenderization (reviewed by Koohmaraie, 1988, 1992a; Dransfield, 1992b). The myofibrillar proteins hydrolyzed in vitro by calpain proteinases closely mimic changes in muscle observed under normal postmortem conditions (Dayton et al., 1976; Olson et al., 1977; Elgasim et al., 1985; Koohmaraie, 1988, 1992a). In addition, the enhanced and accelerated tenderization after the addition of exogenous calcium results from proteolysis due to activation of both μ- and m-calpain (Koohmaraie et al., 1988; Koohmaraie, 1990; Koohmaraie and Shackelford, 1991; Wheeler et al., 1992).

In our study, there were no differences due to sex class in 24-h m-calpain activities (Table 3). These data are consistent with previous findings indicating no differences in 24-h m-calpain activities between *Bos taurus* and *Bos indicus* breeds of cattle, although, tenderness was decreased in meat from *Bos indicus* cattle (Wheeler et al., 1990; Whipple et al., 1990; Shackelford et al., 1991). Research has indicated that

Table 1. Means and standard errors for carcass and longissimus muscle traits of bulls and steers

Trait	Bulls	Steers	SEM
Hot carcass wt, kg	273[a]	242[b]	6.0
Dressing percentage	60.0	60.0	1.7
Adjusted fat thickness, cm	.21[a]	.71[b]	.02
Longissimus muscle area, cm^2	76.7[a]	67.7[b]	3.2
KPH, %[c]	1.0[a]	3.0[b]	.1
USDA yield grade	1.3[a]	2.3[b]	.1
Marbling score[d]	3.60[a]	4.34[b]	.2
USDA quality grade[e]	4.1[a]	5.2[b]	.1
Lean color[f]	2.5	2.0	.2
Lean firmness[g]	1.5	2.0	.3
Lean texture[h]	2.3	2.2	.1
Heat ring[i]	2.8[a]	2.0[b]	.2

[a,b]Means within a row with a different superscript letter differ ($P < .05$).
[c]Kidney, pelvic, and heart fat.
[d]3.00 = Slight[00], 4.00 = Small[00].
[e]4 = USDA Select[+], 5 = USDA Choice[−].
[f]1 = very light cherry red, 7 = black.
[g]1 = very firm, 7 = very soft.
[h]1 = very fine, 7 = very coarse.
[i]1 = none, 7 = severe.

Table 2. Means for Warner-Bratzler shear force and myofibril fragmentation index of longissimus muscle of bulls and steers at 1, 7, and 14 days postmortem

Trait	Shear force, kg	Myofibril fragmentation index[a]
Sex class		
Bull	4.9[b]	49.4[c]
Steer	4.2[c]	56.8[b]
SEM	.2	2.0
Day postmortem		
1	5.3[b]	28.6[d]
7	4.6[c]	57.8[c]
14	3.8[d]	72.9[b]
SEM	.2	2.4
Interaction		
Bull		
Day 1	5.5	22.0
Day 7	5.0	53.5
Day 14	4.2	72.6
Steer		
Day 1	5.1	35.2
Day 7	4.2	62.0
Day 14	3.4	73.2
SEM	.2	2.5
P-value	.73	.42

[a]Absorbance at 540 nm of .5 mg of protein/mL of myofibril solution × 200.
[b,c,d]Means in a column within a main effect with a different superscript letter differ ($P < .05$).

Table 3. Means for 24-hour calpain and calpastatin activities for longissimus muscle in bulls and steers

Item	Bulls	Steers	SEM
μ-calpain[a]	.29	.21	.02
m-calpain[b]	.80	.90	.07
Calpastatin[c]	2.41[d]	1.33[e]	.28

[a]Low Ca^{2+}-requiring calpain protease total activity/gram of muscle (caseinolytic activity).
[b]High Ca^{2+}-requiring calpain proteinase total activity/gram of muscle (caseinolytic activity).
[c]Inhibition of casein hydrolysis by m-calpain/gram of muscle.
[d,e]Means within a row with a different superscript letter differ ($P < .05$).

under typical postmortem conditions, m-calpain is very stable, whereas there is a decline in the activity of μ-calpain and calpastatin (Vidalenc et al., 1983; Ducastaing et al., 1985; Koohmaraie et al., 1987). Previous data have indicated that there is a gradual increase in water-extractable calcium in beef (Arnold et al., 1956) and chicken (Nakamura, 1973) during postmortem storage. Thus, intracellular calcium concentrations in postmortem bovine muscle (Koohmaraie et al., 1990) are high enough to activate μ-calpain but not m-calpain (for review, see Koohmaraie, 1992a). In addition to myofibrillar proteolysis, calpain also goes through autolysis upon activation (Guroff, 1964; Koohmaraie, 1992b). Thus, the amount of μ-calpain activity remaining at any time postmortem reflects the extent to which the proteinase has been activated and, therefore, has an inverse relationship with the amount of proteolysis (and tenderization) that has occurred during postmortem storage (Koohmaraie et al. 1987; Dransfield, 1992a). In our study, 24-h postmortem μ-calpain activity tended to be higher ($P < .08$) in bulls than in steers (Table 3) and was consistent with the higher shear force values in bull meat.

Calpastatin activity (endogenous calpain inhibitor) was 81% greater in longissimus muscle from bulls at 24 h postmortem (Table 3). These data agree with the results of several recent experiments that indicate calpastatin is probably a primary regulator of μ-calpain in postmortem muscle. Furthermore, the postmortem activity of calpastatin is highly related to the rate of postmortem proteolysis and tenderness in meat from *Bos indicus* breeds of cattle (Whipple et al., 1990; Shackelford et al., 1991), in meat from animals fed a β-adrenergic agonist (Kretchmar et al., 1990; Koohmaraie et al., 1991a; Wheeler and Koohmaraie, 1992), and in meat from different species (Ouali and Talmant, 1990; Koohmaraie et al., 1991b). In our study, calpastatin activity of meat from bulls at 24 h postmortem (2.41 units/g of muscle) was similar to the 0-h calpastatin values for steers (2.24 units/g of muscle) reported by Morgan et al. (1993) from the same animals. The greater calpastatin activity in bull longissimus muscle likely decreased the amount of myofibrillar protein proteolysis by μ-calpain through 7 d postmortem, resulting in less tender meat.

In addition, zinc is a potent inhibitor of calpain proteinases (Guroff, 1964; Koohmaraie, 1990). Seideman et al. (1989) reported that longissimus muscle from bulls was tougher and contained higher concentrations of zinc than muscle from steers (45.1 vs 34.8 ppm, respectively). Thus, in addition to the elevated calpastatin activity, higher endogenous zinc concentration in bull longissimus muscle could also contribute to the decreased activation of μ-calpain and the resulting decrease in meat tenderness.

In agreement with shear force values, sensory panel ratings indicated that as postmortem aging time increased, longissimus muscle samples became more ($P < .05$) tender with less ($P < .05$) detectable connective tissue (Table 4). Sex class had little ($P > .05$) effect on sensory characteristics or cooking loss (Table 4). It was anticipated that because shear force values indicated that meat from bull carcasses was less tender than meat from steer carcasses, differences in sensory tenderness ratings also would be detected. The overall tenderness rating only tended ($P < .07$) to be higher in meat from steers. In several cases involving assessment of palatability between bull and steer longissimus samples, meat from bull carcasses was less tender as indicated by increased shear force values; however, in most cases, the differences were small and more than likely would not result in consumer objection. Additionally, juiciness, flavor intensity, and percentage of cooking loss were not affected ($P > .05$) by time postmortem (Table 4).

Collectively, available evidence indicates that meat from bulls is slightly, but consistently, less tender than meat from steers, although the difference is frequently not statistically significant. Our data indicate that tenderization was greater in steers early postmortem (0 to 7 d), implying that tenderness differences due to castration may depend on the time of measurement postmortem. Thus, this tenderness difference may not be of practical importance. But mechanistically, many previous investigations reporting that bulls were less tender than steers have implicated decreased collagen solubility as sexual development progressed. However, Boccard et al. (1979) reported that collagen solubility decreased between 12 and 16 mo of age in bulls, although Cross et al. (1984) reported no interaction between sex class and age (6 to 18 mo) for total collagen or collagen solubility. We slaughtered our animals at 12 mo to avoid the possibility of confounding collagen solubility with myofibrillar tenderness differences. Furthermore, our data are consistent with the current hypothesis regarding the involvement of the calpain proteolytic system in postmortem tenderization, such that higher calpastatin activity results in less tender meat due to decreased proteolysis by μ-calpain.

Table 4. Sensory characteristics and cooking traits of longissimus muscle of bulls and steers at 1, 7, and 14 days postmortem

Item	Juiciness[a]	Myofibrillar tenderness[b]	Connective tissue amount[c]	Overall tenderness[b]	Flavor intensity[d]	Cooking loss, %
Sex class						
Bull	5.12	5.05	6.13	5.00	5.60	22.4
Steer	5.05	5.49	6.49	5.52	5.76	23.2
SEM	.1	.4	.3	.2	.2	.7
Day postmortem						
1	5.14	4.33[f]	5.53[g]	4.28[g]	5.64	22.0
7	4.98	5.00[f]	6.19[f]	5.03[f]	5.54	21.8
14	5.14	6.47[e]	7.19[e]	6.45[e]	5.74	23.4
SEM	.1	.4	.5	.4	.1	.8
Interaction						
Bull						
Day 1	5.21	4.15	5.26	4.01	4.61	22.0
Day 7	5.05	4.68	5.81	4.72	5.41	22.1
Day 14	5.10	6.31	7.30	6.26	5.77	23.5
Steer						
Day 1	5.08	4.52	5.80	4.56	5.68	22.5
Day 7	4.90	5.32	6.58	5.36	5.68	23.4
Day 14	5.18	6.64	7.08	6.64	5.92	23.8
SEM	.2	.7	.6	.7	.1	.9
P-value	.83	.90	.30	.93	.75	.95

[a]8 = Extremely juicy, 1 = extremely bland.
[b]8 = Extremely tender, 1 = extremely tough.
[c]8 = None, 1 = abundant.
[d]8 = Extremely intense, 1 = extremely bland.
[e,f,g]Means in a column within a main effect with a different superscript letter differ ($P < .05$).

Implications

Bulls would not be expected to produce longissimus steaks that are as tender as those from steers when slaughtered at the same age. The higher calpastatin activity at 24 h postmortem in meat from bulls could be responsible for their decreased tenderness.

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