# Effect of Castration on Myofibrillar Protein Turnover, Endogenous Proteinase Activities, and Muscle Growth in Bovine Skeletal Muscle[1,2]

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**ABSTRACT:** The effect of castration on endogenous proteinase activity and myofibrillar protein turnover was investigated in cattle. Six each of MARC III composite bulls and steers weighing approximately 210 kg were given ad libitum access to a typical growing diet. At 0, 42, 84, 126, and 168 d, two consecutive 24-h urine samples were obtained. Urine was analyzed for $N^{\tau}$-methylhistidine ($N^{\tau}MH$) and creatinine. Following slaughter after 170 d on feed, a longissimus muscle sample was removed immediately from each carcass for quantification of $\mu$-calpain, m-calpain, calpastatin, cystatin(s), cathepsin B, and cathepsin B + L activities. Bulls were heavier ($P < .05$) at 126 and 168 d and more efficient ($P < .05$) in conversion of feed to gain at 84 and 168 d than were steers. Compared with steers, bulls excreted less ($P < .05$) $N^{\tau}MH$ at 84, 126, and 168 d and displayed lower ($P < .05$) fractional degradation rates (FDR) at all sample times. No differences ($P > .05$) in calpain or cathepsin activities were observed between bulls and steers. However, muscle from bulls had greater ($P < .05$) activities of calpastatin and cystatin(s) than that from steers. A negative relationship existed between d-168 FDR and calpastatin ($r = -.72$; $P < .05$) and cystatin ($r = -.62$; $P < .05$) activities. These results indicate that decreased FDR of skeletal muscle from growing bulls contributes to their greater efficiency of growth and could be related partially to cystatin-mediated cathepsin activity and(or) calpastatin-mediated calpain activity.

Key Words: Beef, Calpain, Calpastatin, Castration, Growth Rate, Protein Degradation

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## Introduction

It is well established that proteins are continually synthesized and degraded in both developing and mature muscle cells (Reeds, 1989), but the proteolytic enzymes involved in protein degradation remain unknown. It is hypothesized that the calpain proteolytic system, which is known to be important in postmortem protein degradation and, thus, meat tenderization (Koohmaraie, 1992), also could be involved in or even possibly initiate muscle protein degradation in the living animal (Goll et al., 1989).

Many studies have reported that bulls gain more rapidly and are more efficient in producing leaner carcasses than steers (Hedrick, 1968; Field, 1971; Seideman et al., 1982). However, the underlying mechanisms for these advantages have not been determined. The objective of this study was to determine the effect of castration (bull vs steer) on the relationship among muscle proteinase activity, muscle fiber distribution, and myofibrillar protein turnover in growing 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. Six MARC

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III composite (1/4 Red Poll, 1/4 Pinzgauer, 1/4 Hereford, and 1/4 Angus) bulls and six MARC III steers, age 7 mo, weighing approximately 210 kg, were given ad libitum access to a diet formulated to meet NRC requirements for growing beef cattle (NRC, 1984). All animals had different sires. Animals to remain bulls or to be castrated were randomly assigned and castration was performed within 1 wk of birth. 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% Monensin premix, and .1% sulfur) with 84.16% TDN and 10.93% CP. All animals were fed the experimental diet for 5 wk before the initiation of the experiment to acclimate them to the diet. Animals were weighed weekly (after an overnight period of food deprivation) and individual feed consumption was measured daily. 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 from 0700 to 1600 daily. Animals were removed from the stalls and allowed to exercise outside for 3 h twice weekly.

*Urine Collection and Analyses.* Two consecutive 24-h urine collections were obtained immediately before (0 d) and at 42, 84, 126, and 168 d after the initiation of the study. A urine collection bag strapped to the animal was connected to a vacuum pump via 9.5 mm i.d. plastic tubing through a 20-L plastic container. At the beginning of each collection period, 200 mL of 6 *N* HCl was added to the collection container as a preservative. At the end of each 24-h collection period, the urine was weighed and a 50-mL sample was obtained. Specific gravity of a subsample was measured to calculate total volume of urine excreted in a 24-h period. The remaining urine sample was frozen at −20°C until it was analyzed. Urinary creatinine concentration was determined using a kit (Sigma Chemical, St. Louis, MO) following a commercial method (Sigma Chemical, 1983). Urinary concentration of N$^{\tau}$-methylhistidine (**N$^{\tau}$MH**) was determined by the HPLC procedures described by Wassner et al. (1980) as modified by Wheeler and Koohmaraie (1992).

*Calculations.* The calculations of the characteristics of muscle protein metabolism were performed according to the methods of Gopinath and Kitts (1984) and McCarthy et al. (1983). The skeletal muscle protein (**SMP**) mass was estimated from urinary creatinine concentrations according to procedures described by Schroeder (1990) and Schroeder et al. (1990). The total N$^{\tau}$MH pool in skeletal muscle was calculated by multiplying the SMP mass by the N$^{\tau}$MH content of SMP (3.5106 μmol of N$^{\tau}$MH/g of muscle protein; Nishizawa et al., 1979. The fractional degradation rate (**FDR**, percentage/day) of SMP was calculated by the following equation: FDR = [N$^{\tau}$MH urinary excretion (μmol/d)/skeletal muscle N$^{\tau}$MH pool (μmol)] × 100. The fractional accretion rate, (**FAR**, percentage/day) of SMP was calculated by dividing the daily gain in SMP since the last sample time by the total pool at the current sample time. The calculation was as follows: FAR = $[(MP_1 - MP_0/T) \div MP_1] \times 100$, where $MP_1$ is the measure of total muscle protein at the current urine collection period, $MP_0$ is the measure of muscle protein from the previous urine collection period, and T is the number of days between collection periods. The numerator of the FAR equation is equal to the absolute rate of muscle protein accretion (**MPA**, grams/day).

The fractional synthesis rate (**FSR**, percentage/day) of the mixed protein pool was calculated as the sum of FDR and FAR. Myofibrillar protein degradation (**MPD**, grams/day) was calculated by dividing daily N$^{\tau}$MH excretion by the concentration of N$^{\tau}$MH in muscle. The rate of muscle protein synthesis (**MPS**, grams/day) was calculated as the sum of MPD and MPA.

Urinary excretion of N$^{\tau}$MH is often used to estimate myofibrillar protein turnover and has been validated for use in cattle (Harris and Milne, 1981; McCarthy et al., 1983). In cattle, the degradation of actin and myosin releases N$^{\tau}$MH that is not reutilized or modified but is rapidly and quantitatively excreted in the urine (Young et al., 1972). Because N$^{\tau}$MH is present primarily (> 93%) in skeletal muscle (Nishizawa et al., 1979), this procedure provides a good comparison of skeletal muscle myofibrillar protein turnover in bulls and steers, assuming that castration does not alter the relative rates of myofibrillar to sarcoplasmic protein turnover (based on the findings of Bates and Millward, 1983) in skeletal muscle or change the turnover of smooth muscle N$^{\tau}$MH.

*Calpain and Calpastatin Determination.* A longissimus muscle sample was obtained within 30 min postmortem and activities of μ-calpain, m-calpain, and calpastatin were determined on a fresh, unfrozen 5-g longissimus muscle sample 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. After washing, the bound proteins were eluted with a continuous NaCl gradient (total volume = 6.5 column volumes) from 25 to 350 m*M* NaCl. Activities were expressed as the amount of 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 inhibits 1.0 unit of DEAE-Sephacel purified m-calpain activity.

*Cathepsin B, Cathepsins B + L, and Cystatin Determination.* Five grams of longissimus muscle that had been frozen within 30 min postmortem and stored at −70°C was extracted to determine the activities of cathepsins B and B + L according to Method D of Koohmaraie and Kretchmar (1990). Cystatin-like activity was defined as the ratio of cathepsins B + L activity after affinity chromatography:cathepsins B + L activity before affinity chromatography. Activities were determined using synthetic fluorometric substrates assayed at 37°C and expressed as nanomoles·minute$^{-1}$·gram of muscle$^{-1}$.

*DNA, RNA, and Protein Quantification.* Longissimus muscle, frozen within 30 min postmortem, was pulverized for quantitative determinations of RNA and DNA. Procedures of Labarca and Paigen (1980) using Hoechst 33258 reagent (bisbenzimidazole) were used to determine DNA concentrations. Content of RNA was determined according to the method of Munro and Fleck (1969). Protein concentrations were determined by the biuret procedure (Gornall et al., 1949). Bovine serum albumin was used as a standard.

*Histochemical Determinations.* Longissimus muscle samples (.7 cm$^3$) removed at 24 h postmortem were placed on cork perpendicular to fiber direction, frozen in liquid nitrogen, and stored at −70°C. Transverse cryostat sections, 10 mm thick, were cut and allowed to air-dry. Sections were stained according to the procedures described by Solomon and Dunn (1988). A minimum of 200 fibers per sample were classified as βR, αR, or αW according to the scheme described by Ashmore et al. (1972).

Table 1. Effect of castration and time on feed on animal performance traits

| Trait and sample time[a] | Bulls | Steers | Sig[b] | SEM |
|---|---|---|---|---|
| Live wt, kg | | | | |
| 0 d | 213 | 210 | — | 13.1 |
| 42 d | 272 | 259 | — | — |
| 84 d | 335 | 309 | — | — |
| 126 d | 388 | 361 | * | — |
| 168 d | 455 | 403 | * | — |
| ADG, kg | | | | |
| 0 d | — | — | — | — |
| 42 d | 1.35 | 1.13 | — | .12 |
| 84 d | 1.47 | 1.18 | * | — |
| 126 d | 1.23 | 1.00 | — | — |
| 168 d | 1.56 | 1.01 | * | — |
| Gain/feed, g/kg | | | | |
| 0 d | — | — | — | — |
| 42 d | 243.9 | 212.3 | * | 2.04 |
| 84 d | 237.5 | 206.6 | * | — |
| 126 d | 196.1 | 173.6 | * | — |
| 168 d | 153.8 | 106.3 | * | — |

[a]Days from initiation of the study. The ADG and gain/feed data represent the average since the previous sample time.
[b]Sig = significance. Means in a row marked with an asterisk differ ($P < .05$).

*Statistical Analysis.* Protein turnover data were analyzed using an analysis of variance with a sequential, split-plot treatment design (Steel and Torrie, 1980). The whole plot was castration (bull vs steer) and the split plot was sampling time (42, 84, 126, and 168 d). The whole-plot error term in the model was replication × castration, with animal representing replication. The split-plot error-term in the model was the residual error. A modified $t$-statistic ($t'$) was calculated to allow whole and subplot comparisons (Steel and Torrie, 1980). Postmortem data (calpain system, cathepsin activity, nucleic acid determination, and fiber type distribution) were analyzed using the GLM procedure of SAS (1985) with animal as the experimental unit. Simple correlation coefficients between FDR and proteinase activities were generated.

## Results

Live animal performance data obtained in this study (Table 1) were similar to data reported previously (for review see Field, 1971; Seideman et al., 1982) of bull vs steer comparisons. Bulls gained more rapidly ($P < .05$) than steers at both 84 and 168 d on test. Bulls grew more efficiently ($P < .05$) throughout the experiment and were heavier ($P < .05$) than steers at 126 and 168 d (Table 1).

As time increased, a gradual increase in the amount of N$^\tau$MH excreted was observed in both groups (Table 2). The quantities of N$^\tau$MH excreted in this study were in the range similar to those reported by Nishizawa et al. (1979) and Harris and Milne (1981). At 84, 126, and 168 d, bulls excreted lower ($P < .05$) levels of N$^\tau$MH than did steers. Greater N$^\tau$MH excretion by steers (combined with equal or smaller SMP mass) indicated a higher amount of muscle protein degradation than indicated by N$^\tau$MH excretion by bulls. This was supported by N$^\tau$MH:creatinine ratios. Creatinine excretion increased linearly with time ($P < .05$) for both steers and bulls (Table 2). During the last two sampling periods, higher creatinine excretion in bulls indicated a greater ($P < .05$) muscle mass for those animals.

There was no difference ($P > .05$) between bulls and steers in creatinine:BW ratio (Table 2). This observation indicates that muscle mass per unit of BW did not differ ($P > .05$) between bulls and steers. Values reported for creatinine excretion were similar to those previously reported in cattle (Nishizawa et al., 1979; Harris and Milne, 1981; Jones et al., 1990). Bull and steer creatinine and creatinine:BW ratios were increasing through 168 d of feeding; this indicates that muscle mass was still increasing with BW.

As shown in Table 2, N$^\tau$MH per unit of BW (at 84, 126, and 168 d) and N$^\tau$MH per unit of creatinine (at

126 and 168 d) were lower in bulls. This indicates that at these times bulls degraded less muscle protein per unit of muscle mass. In support, FDR also was lower ($P < .05$) for bulls than for steers at all sample times except d 0 (Table 3). The FDR observed (Table 3) was similar to the results reported by Harris and Milne (1981). The $N^{\tau}$MH pool increased with time in both groups and was greater in bulls than in steers at 84, 126, and 168 d, indicating that bulls were accreting more myofibrillar protein, although FAR was greater ($P < .05$) in bulls only at 42 d.

At 168 d on feed, bulls had a lower ($P < .05$) FSR than did steers (Table 3). Total protein synthesis and degradation has been shown to decline during later stages of the feeding period in wether lambs administered testosterone (Lobley et al., 1987). At the 168-d sampling period, steers were synthesizing more ($P < .05$) SMP per day than were bulls (Table 4). However, muscle from bulls had greater ($P < .05$) net protein accretion at 42, 84, and 168 d than did muscle from steers. This advantage in growth resulted because bulls were degrading approximately 30% less ($P < .05$) protein per day than were steers at 168 d.

No differences ($P > .05$) in activity/gram of muscle were seen for either $\mu$- or m-calpain activities between bulls and steers (Table 5). However, muscle calpastatin activity was greater ($P < .05$) for bulls than for steers. These results conflict with those reported by Ou et al. (1991), who detected similar calpastatin levels in castrated and intact lambs.

Another proteolytic system that may be involved in in vivo degradation of muscle proteins is the lysosomal enzyme system. Cathepsin B and cathepsins B + L activities were not affected ($P > .05$) by castration (Table 5). Bull muscle contained more ($P < .05$) cystatin-like activity than did steer muscle, similar to results observed for calpastatin. Castration did not affect ($P > .05$) RNA concentration or RNA:DNA ratios in muscle (Table 6). Similar results were observed by Ou et al. (1991) in that no differences in RNA were detected between intact and castrated lambs. However, protein concentration was lower ($P < .05$) and DNA concentration higher ($P < .05$) in

Table 2. Effect of castration on $N^{\tau}$-methylhistidine and creatinine measures

| Trait and sample time[a] | Bulls | Steers | Sig[b] | SEM |
|---|---|---|---|---|
| $N^{\tau}$-methylhistidine, mmol/d | | | | |
| 0 d | .78 | .89 | — | .09 |
| 42 d | .95 | 1.09 | — | — |
| 84 d | 1.07 | 1.30 | * | — |
| 126 d | 1.44 | 1.61 | * | — |
| 168 d | 1.29 | 1.94 | * | — |
| Creatinine, g/d | | | | |
| 0 d | 5.16 | 5.06 | — | .31 |
| 42 d | 5.19 | 5.40 | — | — |
| 84 d | 8.82 | 8.57 | — | — |
| 126 d | 10.30 | 9.19 | * | — |
| 168 d | 13.69 | 12.47 | * | — |
| Creatinine excretion/BW, mg/kg | | | | |
| 0 d | 24.11 | 24.09 | — | 1.48 |
| 42 d | 19.08 | 21.09 | — | — |
| 84 d | 26.32 | 27.73 | — | — |
| 126 d | 26.54 | 25.45 | — | — |
| 168 d | 30.08 | 30.86 | — | — |
| $N^{\tau}$-methylhistidine excretion/BW, $\mu$mol/kg | | | | |
| 0 d | 3.64 | 4.24 | — | .21 |
| 42 d | 3.49 | 4.18 | — | — |
| 84 d | 3.19 | 4.21 | * | — |
| 126 d | 3.71 | 4.46 | * | — |
| 168 d | 2.83 | 4.80 | * | — |
| $N^{\tau}$-methylhistidine:creatinine ratio | | | | |
| 0 d | .151 | .176 | — | .013 |
| 42 d | .183 | .168 | — | — |
| 84 d | .121 | .152 | — | — |
| 126 d | .140 | .175 | * | — |
| 168 d | .094 | .156 | * | — |

[a]Days from initiation of the study.
[b]Sig = significance. Means in a row marked with an asterisk differ ($P < .05$).

Table 3. Skeletal muscle $N^{\tau}$-methylhistidine pool, fractional degradation, accretion, and synthesis rates in growing bulls and steers

| Trait and sample time[a] | Bulls | Steers | Sig[b] | SEM |
|---|---|---|---|---|
| $N^{\tau}$-methylhistidine pool, mmol | | | | |
| 0 d | 38.79 | 38.14 | — | 1.47 |
| 42 d | 49.34 | 46.99 | — | — |
| 84 d | 57.75 | 53.30 | * | — |
| 126 d | 70.37 | 65.44 | * | — |
| 168 d | 82.57 | 73.34 | * | — |
| Fractional degradation rate, %/d | | | | |
| 0 d | 1.64 | 1.80 | — | .09 |
| 42 d | 1.45 | 1.78 | * | — |
| 84 d | 1.41 | 1.80 | * | — |
| 126 d | 1.83 | 2.26 | * | — |
| 168 d | 1.30 | 2.14 | * | — |
| Fractional accretion rate, %/d | | | | |
| 0 d | — | — | — | — |
| 42 d | .37 | .31 | * | .02 |
| 84 d | .35 | .32 | — | — |
| 126 d | .28 | .25 | — | — |
| 168 d | .29 | .22 | — | — |
| Fractional synthesis rate, %/d[c] | | | | |
| 0 d | — | — | — | — |
| 42 d | 1.82 | 2.09 | — | .21 |
| 84 d | 1.76 | 2.12 | — | — |
| 126 d | 2.11 | 2.51 | — | — |
| 168 d | 1.59 | 2.36 | * | — |

[a]Days from initiation of the study. Values represent the average since the previous sample time.
[b]Sig = significance. Means in a row marked with an asterisk differ ($P < .05$).
[c]The summation of fractional degradation rate and fractional accretion rate.

Table 4. Absolute rate of muscle protein degradation, accretion, and synthesis in growing bulls and steers

| Trait and sample time[a] | Bulls | Steers | Sig[b] | SEM |
|---|---|---|---|---|
| Muscle protein degradation, g/d | | | | |
| 0 d | 222.62 | 247.47 | — | 38.60 |
| 42 d | 268.97 | 310.48 | — | — |
| 84 d | 305.33 | 370.70 | — | — |
| 126 d | 411.32 | 458.81 | — | — |
| 168 d | 366.75 | 525.56 | * | — |
| Muscle protein accretion, g/d | | | | |
| 0 d | — | — | — | — |
| 42 d | 71.52 | 59.95 | * | 8.20 |
| 84 d | 55.66 | 42.80 | * | — |
| 126 d | 87.02 | 82.38 | — | — |
| 168 d | 82.71 | 53.59 | * | — |
| Muscle protein synthesis, g/d[c] | | | | |
| 0 d | — | — | — | — |
| 42 d | 340.49 | 370.43 | — | 30.32 |
| 84 d | 360.99 | 413.50 | — | — |
| 126 d | 498.34 | 541.19 | — | — |
| 168 d | 449.46 | 579.15 | * | — |

[a]Days from initiation of the study. Values represent the average since the previous sample time.
[b]Sig = signifigance. Means in a row marked with an asterisk differ ($P < .05$).
[c]The summation of muscle protein degradation and muscle protein synthesis.

longissimus muscle from bulls than in muscle from steers. Protein:DNA and protein:RNA ratios were higher ($P < .05$) in muscle from steers than in muscle from bulls.

The percentage distribution of longissimus muscle fiber-types was not ($P > .05$) altered by castration (Table 6). These findings are consistent with those of Seideman et al. (1986), who reported that as feeding time increased, very few differences in muscle fiber-type distribution were observed between bulls and steers. In addition, the relative percentages of fiber-types was similar to those reported by Seideman et al. (1986).

## Discussion

Male cattle traditionally are castrated in the United States, primarily to improve ease of management and palatability traits. However, young bulls have up to a 15% advantage in growth rate, feed efficiency, and carcass leanness compared with steers at the same age or time on feed (Seideman et al., 1982). Many reports link the growth advantages associated with intact males to greater amounts of androgens such as testosterone.

The direct mechanism by which castration alters protein turnover remains unclear. Lobley et al. (1987) concluded that individual tissues may respond differently to the administration of androgen; however, the overall effects on whole-body protein metabolism may mask changes in the opposite direction for specific cell types. In this study, improvements in MPA observed in bulls seemed to be related to decreased FDR. These results are in agreement with those of Santidrian et al. (1982) and Lobley et al. (1987), who concluded that treating rats and lambs with testosterone increased muscle growth by suppressing muscle protein degradation. Additionally, Heitzman (1980) injected female rats with a synthetic androgen, trenbolone acetate, which increased muscle gain primarily by the reduction of protein degradation. In addition, several reports have concluded that feeding $\beta$-adrenergic agonists to growing animals increased muscle mass and improved whole-body composition due at least in part to reductions in FDR. These results have been observed in lambs (Bohorov et al., 1987), rats (Reeds et al., 1986), veal calves (Williams et al., 1987), chickens (Morgan et al., 1989), rabbits (Forsberg et al., 1989), and cattle (Wheeler and Koohmaraie, 1992).

Koohmaraie (1992) stated that calpastatin is a powerful regulator of calpain-mediated proteolysis during postmortem aging of meat. Unlike protein degradation occurring in postmortem muscle, very little is known about the mechanisms or factors that control intracellular protein degradation in growing muscle. The ability to regulate muscle protein degradation could have a large effect on the rate of muscle growth (Goll et al., 1989). The proteolytic capacity of the calpain system may regulate muscle protein degradation during both muscle growth and postmortem storage of meat (Wheeler and Koohmaraie, 1992). The current data support this possibility.

Table 5. Effect of castration on calpain proteolytic system and lysosomal proteinase activities of longissimus muscle

| Trait | Bulls | Steers | Sig[a] | SEM |
|---|---|---|---|---|
| $\mu$-Calpain[b] | 1.32 | 1.22 | — | .09 |
| m-Calpain[c] | .81 | 1.02 | — | .13 |
| Calpastatin[d] | 3.28 | 2.24 | * | .17 |
| Cathepsin B[e] | 28.47 | 32.17 | — | 2.1 |
| Cathepsins B + L[e] | 126.59 | 108.17 | — | 10.5 |
| Cystatin(s) | 3.84 | 2.78 | * | .37 |

[a]Sig = signifigance. Means in a row marked with an asterisk differ ($P < .05$).
[b]Low $Ca^{2+}$-requiring calpain proteinase activity/gram of muscle (casienolytic activity).
[c]High $Ca^{2+}$-requiring calpain proteinase activity/gram of muscle (casienolytic activity).
[d]Units of inhibition of casein hydrolysis by m-calpain/gram of muscle.
[e]Values expressed are nanomoles amino-methylcoumarin released·minute$^{-1}$·gram of muscle$^{-1}$.

The calpain proteolytic system seems to be a good candidate to initiate myofibrillar protein turnover (for review see Dayton et al., 1975; Goll et al., 1989). Reports by Etlinger et al. (1976) and van der Westhuyzen et al. (1981) stated that striated muscle contains approximately 5 to 10% myofilaments referred to as "easily-releasable myofilaments." Conditions that are known to decrease proteolysis reduce the releasable myofilament pool and conditions that stimulate proteolysis increase this pool (van der Westhuyzen et al., 1981). The lower FDR observed in bulls (Table 3) may be a result of lower proteolytic capacity from calpain proteinases due to greater calpastatin activities. If calpastatin is related to protein turnover in living muscle, then an increase in calpastatin activity could possibly decrease calpain-mediated degradation and, in turn, reduce FDR. The significant negative correlation ($r = -.72$; $P < .05$) between calpastatin activity and FDR (at 168 d) indicates that animals with higher calpastatin activities had lower FDR. Bulls exhibited higher calpastatin activities and decreased FDR compared with steers. Wheeler and Koohmaraie (1992) reported increased calpastatin activity associated with decreased FDR in steers fed $\beta$-adrenergic agonists.

Although no differences ($P > .05$) were observed in cathepsins B or B + L, greater cystatin-like activity was observed in bulls than in steers (Table 5). Like calpastatin, a significant negative correlation ($r = -.62$; $P < .05$) between cystatin(s) and FDR was observed in this study. The relationship of cystatin(s) to FDR may be in regulating cathepsin activity in later stages of myofilament disassembly.

The concentration of RNA and the RNA:DNA ratios were not affected by castration, indicating that transcriptional activity was not different. However, decreased protein:RNA ratios in longissimus muscle from bulls indicated a decrease in transcriptional activity. Lower protein:DNA ratios due to increased DNA concentration and decreased protein concentration reflect a change in cell size. It is not clear whether these changes contribute to greater muscle accretion in bulls.

Table 6. Effect of castration on protein and nucleic acid concentration and fiber-type distribution of longissimus muscle

| Item | Bulls | Steers | Sig[a] | SEM |
|---|---|---|---|---|
| Protein concentration, mg/g | 188.9 | 207.4 | * | 5.1 |
| DNA concentration, $\mu$g/g | 949.2 | 899.1 | * | 12.4 |
| RNA concentration, $\mu$g/g | 399.5 | 387.2 | — | 12.3 |
| Protein:DNA ratio | 199.0 | 230.7 | * | 5.7 |
| Protein:RNA ratio | 472.8 | 535.6 | * | 14.2 |
| RNA:DNA ratio | .42 | .43 | — | .02 |
| Fiber-type percentage | | | | |
| $\beta$R | 29.1 | 28.9 | — | 1.2 |
| $\alpha$R | 22.3 | 20.5 | — | .9 |
| $\alpha$W | 48.6 | 51.6 | — | 1.2 |

[a]Sig = significance. Means in a row marked with an asterisk differ ($P < .05$).

The heterogeneous composition of most muscles with regard to fiber-type makes it difficult to associate fiber-type distribution of specific muscles with changes in overall skeletal muscle myofibrillar protein degradation, or even postmortem proteolysis and tenderization. No difference occurred in the percentage of distribution of muscle fiber-types in longissimus muscle between bulls and steers, although differences in FDR (this study) and postmortem tenderization (Morgan et al., unpublished data) did occur. It has been demonstrated that measurable amounts of postmortem proteolysis and tenderization do not occur in some muscles (e.g., psoas major) containing predominantly $\beta$R fiber types (Koohmaraie et al., 1988). Guroff (1964) reported that zinc chloride was a potent inhibitor of calpains, and Kondo et al. (1991) reported that muscles consisting primarily of $\beta$R fibers contained 4.3 times higher amounts of zinc than muscles having $\alpha$R or $\alpha$W fibers. Therefore, in addition to calpastatin, zinc concentration may also regulate calpain activity and thus have a role in muscle protein turnover. It is clear that a better understanding of the complex regulation of proteolysis in both postmortem and growing muscle is needed to optimize both the efficiency of lean muscle growth and ultimate meat quality.

## Implications

Results suggest that the increased growth rate and efficiency of bulls compared with steers is due partially to increased protein muscle accretion resulting from reduced muscle protein degradation. Although no differences in $\mu$-calpain or m-calpain activities were observed between bulls and steers, the reduced proteolytic capacity of muscle due to increased calpastatin and(or) cystatin activity may serve as a regulator of myofibrillar protein degradation.

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