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Effect of Castration on Skeletal Muscle Protein Turnover and Muscle Enzyme Activities in Cattle

Tommy L. Wheeler, J. Brad Morgan, Mohammad Koohmaraie, John D. Crouse, and Jeff W. Savell[1,2,3]

Introduction

It is well established that proteins are continually synthesized and degraded in skeletal muscle, but the proteolytic enzymes involved in muscle protein degradation remain unknown. It is hypothesized that the calpain proteolytic system, which is known to be important in postmortem protein degradation and thus in meat tenderization, could also be involved in or even possibly initiate muscle protein degradation in the living animal.

It is well documented that intact males grow more rapidly (15 to 17%), utilize feed more efficiently (10 to 13%) and produce higher yielding carcasses with less fat and more lean meat than castrates. However, the underlying mechanisms for these advantages have not been determined. The objective of this study was to determine the effect of gender (bull vs steer) on the relationship between muscle enzyme activity and muscle protein turnover in growing cattle.

Procedure

Six each MARC III composite (1/4 Red Poll, 1/4 Pinzgauer, 1/4 Hereford and 1/4 Angus) bulls and steers weighing approximately 397 lb were given unrestricted access to a growing/finishing diet. All animals were fed the experimental diet 5 wk before the initiation of the experiment to acclimate them to the diet. Two consecutive 24-hr urine collections were taken immediately before initiation of the experiment and at 42, 84, 126 and 168 days on feed. Urinary concentration of N^{τ}-methylhistidine ($N^{\tau}MH$) and creatinine were measured. The skeletal muscle protein mass of the steers was estimated from urinary creatinine concentrations. $N^{\tau}MH$ is a modified amino acid found only in muscle (more than 90% in skeletal muscle), so it can be used to measure the rate and amount of skeletal muscle protein degradation. At the end of the 168-day feeding period, the steers were slaughtered according to standard humane procedures.

Within 30 min postmortem, loin muscle samples were taken from the left sides for measuring the calpain enzymes and their inhibitor, calpastatin, and the lysosomal enzymes cathepsins B and B+L and cystatin (cathepsin inhibitor). Muscle for cathepsins was immediately frozen in liquid nitrogen and stored at -158°F until analyzed. Muscle for quantifying the calpain proteolytic system was immediately processed.

Results

Live animal performance data obtained in this study (Table 1) were similar to data reported previously for bull-steer comparisons. Bulls gained more rapidly than steers throughout the study. This advantage was statistically significant at both 84 and 168 days on test. Between the last two sampling periods (126 to 168 days), bulls grew more efficiently and to a heavier final body weight compared to steers (Table 1).

Fractional degradation rates (FDR) of skeletal muscle protein were lower in bulls than in steers at all sample times; however, those differences were statistically significant only at 168 days (Table 2). Fractional accretion rates (FAR) of skeletal muscle protein were not affected by gender, although bulls had numerically higher FAR at 84, 126, and 168 days. Steers were synthesizing more skeletal muscle protein per day than bulls at all times, although the differences were significant only at 168 days. The advantage in growth resulted because bulls were degrading approximately 30% less protein per day compared to steers.

No differences were detected in either μ- or m-calpain 0-hr activities between bulls and steers (Table 3). However, muscle calpastatin activity was greater for bulls than for steers. Another proteolytic system that may be involved in *in vivo* degradation of muscle proteins is the lysosomal enzyme system (cathepsins). Cathepsin B and cathepsins B+L activities were not affected by gender (Table 3). Results indicated that bull muscle contained more cystatin (cathepsin inhibitor) activity than muscle from steers.

Male cattle traditionally are castrated in the U.S., 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 when compared with steers at the same age or time on feed. 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. In our study, improvements in muscle growth observed in bulls appeared to be related to decreased FDR. These results are in agreement with others who concluded that treating rats and lambs with testosterone increased muscle growth by suppressing muscle protein degradation. Additionally, female rats injected with a synthetic androgen, trenbolone, increased muscle gain primarily by reducing protein degradation. In addition, several reports have concluded that feeding β-agonists to growing animals increased muscle mass and improved whole body composition due to reductions in FDR. These results have been observed in lambs, rats, veal calves, chickens, rabbits and cattle.

MARC scientists have demonstrated that calpastatin is a powerful regulator of calpain-mediated proteolysis during postmortem aging. In fact, differences in the rate of postmortem proteolysis and tenderization of meat, regardless of species, are negatively correlated with the inhibitor of calpains, calpastatin. Several investigations substantiate the fact that animals with higher calpastatin activity produced meat which was tougher and exhibited less postmortem proteolysis compared to muscle displaying lower calpastatin activity. Unlike protein degradation occurring in postmortem muscle, very little is known about the mechanisms or factors which control or influence intracellular protein degradation in growing muscle. It has been proposed that the proteolytic capacity of the calpain system may regulate muscle protein degradation during both muscle growth and postmortem storage of meat. The lower FDR observed in bulls may be a

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[3]The full report of this work has been submitted to the J. Anim. Sci. for publication.

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) between calpastatin and FDR (at 168 days) indicates that animals with higher calpastatin activities had lower FDR. Bulls exhibited higher calpastatin activities and decreased FDR compared to steers. We previously reported increased calpastatin activity was associated with decreased FDR in β-agonist fed steers.

Although no differences were observed in cathepsins B or B+L, greater cystatin activity was observed in bulls than in steers. Like calpastatin, a significant negative correlation (r = -.62) between cystatin and FDR was observed in our study. The relationship of cystatin to FDR may be in regulating cathepsin activity in later stages of muscle fiber disassembly.

Results suggest that the increased growth rate and efficiency of bulls compared to steers is partially due to increased protein muscle accretion resulting from reduced muscle protein degradation. Although no differences in m-calpain or m-calpain activities were observed between bulls and steers, the reduced proteolytic capacity of muscle due to increased calpastatin activity may serve as a regulator of muscle protein degradation. This information contributes to a better understanding of the complex mechanism and regulation of muscle protein metabolism that occurs in cattle.

Table 1—Effect of gender on animal performance traits

Trait and sample time[a]	Bulls	Steers
Live wt, lb		
0 day	470	463
42 day	600	571
84 day	738	681
126 day	855	796
168 day	1033	888
Avg daily gain, lb		
0 day	---	---
42 day	3.0	2.5
84 day	3.2	2.6
126 day	2.7	2.2
168 day	3.4	2.2
Feed/gain, lb/lb		
0 day	---	---
42 day	.24	.21
84 day	.28	.21
126 day	.20	.17
168 day	.15	.11

[a] Days from initiation of the study.

Table 2—Effect of gender on fractional degradation, accretion, and synthesis rates of skeletal muscle protein in growing bulls and steers

Trait and sample time[a]	Bulls	Steers
Fractional degradation rate, %/day		
0 day	1.64	1.80
42 day	1.45	1.78
84 day	1.41	1.80
126 day	1.83	2.26
168 day	1.30[c]	2.14[b]
Fractional accretion rate, %/day		
0 day	---	---
42 day	.37	.41
84 day	.35	.32
126 day	.28	.25
168 day	.29	.22
Fractional synthesis rate, %/day[d]		
0 day	---	---
42 day	1.82	2.19
84 day	1.76	2.12
126 day	2.11	2.51
168 day	1.59[c]	2.36[b]

[a] Days from initiation of the study.
[b c] Means in a row with different superscripts differ (P < .05).
[d] The summation of fractional degradation rate and fractional accretion rate.

Table 3—Effect of gender on 0 hr calpain enzyme system and cathepsin enzyme activities of loin muscle

Trait	Bulls	Steers
μ-Calpain[c]	1.32	1.22
m-Calpain[d]	.81	1.02
Calpastatin[e]	3.28[a]	2.24[b]
Cathepsin B[f]	28.47	32.17
Cathepsins B+L[f]	126.59	108.17
Cystatin[g]	3.84[a]	2.78[b]

[a b] Means in a row with different superscripts differ (P < .05).
[c] Low calcium-requiring calpain.
[d] High calcium-requiring calpain.
[e] Units of inhibition of m-calpain.
[f] Total activity.
[g] Measured as the ratio of B+L activity after to before cystatin removal.