Effects of the β-Adrenergic Agonist $L_{644,969}$ on Muscle Protein Turnover, Endogenous Proteinase Activities, and Meat Tenderness in Steers[1,2]

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ABSTRACT: Eight MARC III composite (1/4 Hereford, 1/4 Angus, 1/4 Pinzgauer, and 1/4 Red Poll) steers weighing approximately 350 kg were fed 0 or 3 ppm of the β-adrenergic agonist $L_{644,969}$ (Merck Sharp and Dohme Laboratories, Rahway, NJ) for 6 wk in a high-concentrate diet. Feed efficiency was higher ($P < .05$) in β-adrenergic agonist-fed steers at 1, 3, 5, and 6 wk on trial. Average daily gain was greater ($P < .05$) in β-adrenergic agonist-fed steers at 3, 5, and 6 wk on treatment. Fractional degradation rate (percentage/day) of skeletal muscle myofibrillar protein was 27.1% lower ($P < .05$) in β-adrenergic agonist-fed steers at 3 wk on trial. Fractional accretion rate (percentage/day) of skeletal muscle myofibrillar protein in β-adrenergic agonist-fed steers was higher ($P < .05$) at 1, 3, 5, and 6 wk on trial. The β-adrenergic agonist-fed steers had heavier ($P < .05$) carcasses (9.6%), larger ($P < .05$) longissimus muscle areas (24.3%), and lower ($P < .05$) USDA yield grades (43.8%). Marbling degree, USDA quality grade, kidney, pelvic, and heart fat percentage, and 12th rib fat thickness were not different ($P > .05$). Calpastatin activity was higher ($P < .05$) in muscle from the β-adrenergic agonist-fed steers at 0 and 7 d postmortem. There were no differences ($P > .05$) in μ- or m-calpain or in cathepsins B or B+L or cystatin(s) between β-adrenergic agonist-fed and control steers. The myofibril fragmentation index was greater ($P < .05$) in control steers after 1, 3, 7, and 14 d postmortem and the Warner-Bratzler shear force was lower ($P < .05$) in control steers after 7 and 14 d postmortem. These data indicate that the β-adrenergic agonist-induced muscle hypertrophy may have resulted from reduced proteolytic capacity due to increased calpastatin activity.

Key Words: Beef, Beta-Adrenergic Agonists, Proteinases, Protein Turnover, Tenderness

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Introduction

It is generally accepted that muscle proteins undergo continual degradation (Reeds, 1989). It has been estimated that 15 to 22% of the total energy expenditure of growing animals is for muscle protein turnover (Reeds et al., 1985). Regulation of the rate of skeletal muscle protein degradation could cause dramatic changes in rate of muscle growth (Goll et al., 1989). Despite its importance in muscle growth, the mechanisms and control of skeletal muscle myofibrillar protein degradation remain elusive. It has been hypothesized that several proteolytic systems are involved at different stages of degradation and that the calpain proteolytic system may initiate myofibrillar protein degradation by releasing myofilaments from the surface of the myofibril (reviewed by Goll et al., 1989).

It has been demonstrated that β-adrenergic agonists (BAA) increase muscle accretion through hypertrophy (Beermann et al., 1987; Koohmaraie et al., 1991a). Although the cause of this hypertrophy is still controversial, it results at least in part from decreased protein degradation (reviewed by Yang

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[2]Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Table 1. Composition of experimental diet

Ingredient	As fed, %
Cracked corn	65.9
Alfalfa	17.0
Corn gluten meal	8.7
Soybean meal	4.0
Cane molasses	3.0
Limestone	1.3
Salt	.05
Rumensin 60	.025
Vitamins A, D, and E	.015
TDN[a]	81.95
CP[a]	17.3
ME, Mcal/kg[a]	2.98

[a]Calculated values.

and McElligott, 1989). Consistent with the role of calpains in postmortem tenderization (reviewed by Koohmaraie, 1988, 1992), postmortem muscle proteolysis and meat tenderness were decreased with BAA feeding (Kretchmar et al., 1990; Koohmaraie et al., 1991a). These data indicate a reduction in the proteolytic capacity of the muscle largely due to increased calpastatin and are consistent with a potential role for the calpain proteolytic system in muscle protein degradation in vivo of BAA-fed animals.

The effects of BAA feeding on both muscle proteinase activities and skeletal muscle protein degradation in cattle have not been reported. Thus, the objective of this study was to determine the effects of the BAA $L_{644,969}$ on muscle protein degradation, muscle proteinase activity, and meat tenderness of growing steers.

Materials and Methods

Animals. Eight MARC III composite (1/4 Hereford, 1/4 Angus, 1/4 Pinzgauer, and 1/4 Red Poll) steers weighing approximately 350 kg were randomly assigned to control or BAA-fed treatment groups. Animals were allowed ad libitum access to a diet (Table 1) with or without 3 ppm of $L_{644,969}$, 6-amino-alpha-([(1-methyl-3-phenylpropyl)-aminolmethyl)-3-pyridine methanol dichloride (Merck, Sharp, and Dohme Laboratories, Rahway, NJ), for 6 wk. In anticipation of a marked increase in muscle protein deposition, dietary CP was increased to 17.3% to avoid constraint of protein deposition, because exact protein requirements for steers fed a BAA are not known. All steers were fed the control diet for 5 wk before initiation of the experiment to acclimate them to the diet. The steers were individually penned in 3-m × 1-m pipe stalls with front feeding gates and rubber floor mats on concrete floors inside an enclosed, temperature-controlled (approximately 13° C) barn. Animals were removed from the stalls twice weekly and allowed 3 h of exercise in a pen outside the barn. Feed consumption was measured daily and steers were weighed weekly (after an overnight fast).

Urine Collection. Two consecutive 24-h urine collections were taken immediately before and at 1, 3, 5, and 6 wk after $L_{644,969}$ treatment began. A urine collection bag strapped to the steer was connected to a vacuum pump via 9.5-mm i.d. plastic tubing through a 20-L plastic jug. Two hundred milliliters of 6 *N* HCl was added to the collection jug as a preservative at the beginning of the collection period. 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 the total volume of urine excreted during a 24-h period. The remaining urine sample was frozen at –20° C until it was analyzed.

N^{τ}-Methylhistidine Determination. Urinary concentration of N^{τ}-methylhistidine ($N^{\tau}MH$) was determined by a modification of the procedures described by Wassner et al. (1980). Urine samples were thawed for 24 h at 1° C and centrifuged at 5,000 × *g* for 15 min, and 2 mL of the supernatant was filtered through a .45-μm filter disk. One milliliter of the filtrate was added to 1.5 mL of 3 *N* HCl and 200 μL of 1 m*M* aqueous L-histidinol (internal standard). Two milliliters of this solution was loaded onto an anion exchange chromatography column (AG 50W-8X, H+ form; Bio-Rad Laboratories, Richmond, CA) equilibrated with .6 *N* HCl. The column was washed with .6 *N* HCl and then eluted with 2 *N* NaOH. Fluorescamine derivatization was performed by adding 100 μL of sample (or standards) to 500 μL of .4 *M* sodium borate (pH 9.0) and adjusting the pH to exactly 9.0 with 3 *N* HCl. While mixing, 250 μL of fluorescamine (16 mg/10 mL of methanol) was added, then after 5 s, 250 μL of 3 *N* HCl was added. The mixture was heated at 80° C for 45 min and centrifuged at 16,000 × *g* for 2 min, and the supernatant was filtered through a .45-μm filter disk.

N^{τ}-methylhistidine separation was accomplished with a Waters model 510 HPLC with a 10 μm reverse phase (octadecylsilane) μBondapak C:18 column and a C:18 guard column (Millipore Corp., Milford, MA). Injection volume was 50 μL and the flow rate was 1.5 mL/min. Elution was performed using a 0 to 80% methanol gradient in 5 m*M* ammonium acetate (pH 5.0), over 20 min at 1.5 mL/min using Waters curve No. 4. Standard curves for $N^{\tau}MH$ (external standard) and L-histidinol (internal standard) were developed. A Shimadzu model RF-530 fluorometric HPLC monitor (Shimadzu, Kyoto, Japan) was used to detect

fluorescamine derivatives of $N^{\tau}MH$ and L-histidinol (excitation λ = 370 nm and emission λ = 475 nm).

Protein Turnover Calculations. Urinary creatinine concentration was determined with a kit (Sigma Diagnostics, St. Louis, MO). The skeletal muscle protein (**SMP**) mass was estimated from urinary creatinine concentrations according to the method described by Schroeder (1990). The total $N^{\tau}MH$ pool was calculated by multiplying the SMP by the $N^{\tau}MH$ content of skeletal muscle protein (3.5106 μmol of $N^{\tau}MH$/g of muscle protein; Nishizawa et al., 1979). The fractional degradation rate (**FDR**, percentage/day) of skeletal muscle proteins 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 skeletal muscle proteins was calculated by dividing the daily gain in SMP since the last sample time by the total SMP pool at the current sample time. The calculation was as follows: FAR = $[[(SMP_1 - SMP_0) \div t]/(SMP_1)] \times 100$. The term SMP_1 was the SMP at the time of urine collection, SMP_0 was the SMP at the time of the previous urine collection, and t was the number of days between collections. The numerator of the FAR equation was the absolute rate of skeletal muscle protein accretion (**MPA**, grams/day).

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

Carcass Measurements. After 6 wk, the steers were slaughtered according to standard procedures. Hot carcass weight was determined and the carcasses were chilled at 0°C for 24 h. At 24 h postmortem, each carcass was ribbed between the 12th and 13th ribs and USDA yield and quality grade factors were evaluated.

Semitendinosus Muscle Measurements. Within 30 min postmortem, the semitendinosus muscle from the left side was dissected, trimmed of all external fat, and weighed and a 200-g subsample was frozen in liquid nitrogen. The frozen muscle was pulverized in a blender to form a frozen powder. Aliquots of the frozen powder were analyzed for protein concentration using the biuret method (Gornall et al., 1949).

Longissimus Muscle Measurements. Within 30 min postmortem, longissimus muscle samples were taken from the left sides between the 13th rib and the 3rd lumbar vertebra for measuring calpain, calpastatin, and cathepsin activities and protein concentration. The muscle sample for the evaluation of cathepsins and protein was immediately frozen in liquid nitrogen and stored at −70°C until it was analyzed. The muscle sample for quantifying the calpain proteolytic system was immediately processed. At 24 h postmortem, the longissimus muscle from the 13th rib to the 3rd lumbar vertebra was removed from the right carcass sides, cut into five 2.54-cm-thick steaks, and vacuum-packaged. One steak each was assigned to 1, 3, 7, and 14 d of postmortem aging at 2°C by stratifying storage time along the length of the muscle.

Calpains and Calpastatin Quantification. The activities of μ-calpain, m-calpain, and calpastatin were determined on 50 g of longissimus muscle within 30 min postmortem and after 7 d of postmortem storage at 2°C. Activities were determined on fresh samples according to procedures described by Koohmaraie (1990). Activity was expressed as units/gram of muscle.

Cathepsins and Cystatin-like Quantification. Muscle extracts were prepared and cathepsin and cystatin-like activities were determined as described by Koohmaraie and Kretchmar (1990) for method D. Cystatin-like activity was calculated as the ratio of cathepsins B+L activity after affinity chromatography to B+L activity before affinity chromatography. Activities of the cathepsins were expressed as nanomoles·minute^{-1}·gram of muscle^{-1}.

Myofibril Fragmentation Index Measurement. Five grams of longissimus muscle was obtained from each shear force steak (before cooking) for determination of the myofibril fragmentation index (**MFI**). At 1, 3, 7, and 14 d postmortem, MFI was determined on fresh (not frozen) muscle samples according to the method described by Culler et al. (1978).

Muscle Fiber Histochemistry. Longissimus muscle was obtained at 24 h postmortem. Several .7-cm^3 samples were frozen on cork in liquid nitrogen and stored at −70°C. Transverse cryostat sections, 10 μm thick, were cut and allowed to air dry. Sections were stained according to the procedures for simultaneous staining of bovine muscle fiber types described by Solomon and Dunn (1988). A minimum of 200 fibers per animal were classified as βR, αR, or αW according to the classification of Ashmore and Doerr (1971). Fiber areas were measured by Microcomp PM (Southern Micro Instruments, Atlanta, GA) interactive image analysis for planar morphometry.

Warner-Bratzler Shear Force Determination. At the end of their respective postmortem aging times (1, 3, 7, or 14 d at 2°C), the steaks were broiled on Farberware Open Hearth Electric broilers (Farberware, Bronx, NY) to a 70°C internal temperature.

Table 2. Effects of 3 ppm of $L_{644,969}$ on performance and muscle protein turnover of growing steers

Item	0 wk		1 wk		3 wk		5 wk		6 wk			Probability		
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	SE	Treatment	Time	Interaction
Live wt, kg	348.2	343.8	355.7	350.0	378.0	383.8	393.5	409.0	401.0	422.3	10.2	.34	.01	.62
ADG, kg	—	—	1.43	1.38	.97	1.29	1.25	1.54	1.25	2.21	.30	.10	.28	.46
Gain/feed[a], g/kg	—	—	172.4	454.5*	112.4	153.8*	138.9	166.7*	122.0	227.3*	28.3	.01	.12	.76
SMP[b], kg	29.7	29.5	30.6	30.5	32.4	32.8	33.6	35.0	33.9	35.7	.7	.14	.01	.46
MPD[c], g/d	320.0	333.8	376.9	360.8	726.5	581.2*	605.8	617.6	475.1	557.5	41.4	.69	.01	.12
MPA[d], g/d	—	—	147.6	197.1*	103.9	164.7*	90.9	155.9*	39.2	127.5*	23.4	.01	.02	.88
MPS[e], g/d	—	—	524.5	508.6	830.4	745.9	696.7	773.5	514.2	685.0	58.0	.39	.01	.17
FDR[f], %/d	1.13	1.14	1.24	1.09	2.25	1.77*	1.81	1.77	1.41	1.56	.13	.04	.01	.15
FAR[g], %/d	—	—	.48	.63*	.32	.50*	.27	.45*	.12	.36*	.07	.01	.01	.92
FSR[h], %/d	—	—	1.72	1.72	2.57	2.27	2.08	2.22	1.53	1.92	.18	.76	.01	.25

[a]Grams of gain/kilogram of feed (as fed).
[b]Skeletal muscle protein. Estimated from urinary creatinine excretion according to Schroeder (1980).
[c]Skeletal muscle protein degradation.
[d]Skeletal muscle protein accretion.
[e]Skeletal muscle protein synthesis. MPS = MPD + MPA.
[f]Fractional degradation rate.
[g]Fractional accretion rate.
[h]Fractional synthesis rate. FSR = FDR + FAR.
*Treatment differences within week were significant ($P < .05$).

The steaks were turned after reaching 40°C. Temperature was monitored with iron constantan thermocouple wires inserted into the geometric center of a steak and attached to a Honeywell potentiometer multipoint recorder (Honeywell, Scarborough, ON, Canada). The steaks were chilled for 24 h at 3°C, then six 1.27-cm-diameter cores were removed parallel to the muscle fiber orientation and sheared once each on an Instron model 1132/Microcon II instrument (Instron, Canton, MA) with a Warner-Bratzler shear attachment. The crosshead speed was 5 cm/min.

Statistical Analyses. Data for carcass traits, muscle fiber types, and protein concentration were analyzed using an analysis of variance with the GLM procedure of SAS (1985) for a completely randomized design. Data for proteinase activities, shear force, and MFI were analyzed using analysis of variance for a split-plot design. The whole plot was BAA treatment and the split plot was postmortem time. The whole-plot error term was replication × BAA treatment and the split-plot error term was the residual error. Data for the growth and protein turnover measurements were analyzed using analysis of variance for a sequential, split-plot (repeated measures) design. The whole plot was BAA treatment and the split plot was treatment time. The whole-plot error term was replication × BAA treatment and the split-plot error term was the residual error. Tukey's procedure was used to compare treatment means.

Results

Crossbred steers fed 3 ppm of $L_{644,969}$ were not ($P > .05$) heavier, even after 6 wk on treatment (Table 2). Although the main effect of treatment for ADG was not significant ($P = .10$), ADG tended ($P < .05$) to be higher at 3, 5, and 6 wk and gain:feed ratios were higher ($P < .05$) throughout the trial for treated steers. The BAA-fed steers had heavier ($P < .05$) carcasses, larger ($P < .05$) longissimus muscle areas, and lower ($P < .05$) USDA yield grades than did control steers (Table 3). However, various measures of fat deposition (adjusted fat thickness, kidney, pelvic, and heart fat, marbling degree, and USDA quality grade) were not affected ($P > .05$) by BAA treatment. These data are in general agreement with the results of Ricks et al. (1984), Moloney et al. (1990), and Koohmaraie et al. (1991a), with the exception that fatness measures were not decreased in our study.

Fractional degradation rate of myofibrillar proteins was lower ($P < .05$) at 3 wk on trial and FAR was greater ($P < .05$) at 1, 3, 5, and 6 wk on trial in BAA-fed than in control steers (Table 2). However, FSR was not different ($P > .05$) between treatment

Table 3. Effects of $L_{644,969}$ feeding on carcass traits of growing steers[a]

Trait	Control	Treated	SE	Probability
Hot carcass wt, kg	241.5	264.8	6.7	.04
Adjusted fat thickness, mm	4.8	3.8	1.1	.56
Longissimus muscle area, cm^2	60.0	74.6	2.5	.01
Kidney, pelvic, and heart fat, %	1.5	1.3	.3	.58
USDA yield grade	2.3	1.6	.2	.04
Marbling[b]	353	315	20.8	.25
USDA quality grade[c]	4.2	3.7	.4	.23

[a]3 ppm of $L_{644,969}$ for 6 wk.
[b]300–399 = Slight.
[c]3 = Standard $^+$, 4 = Select −, 5 = Select $^+$.

groups. Several researchers have shown a decrease in FDR as a result of feeding BAA (Li and Jefferson, 1977; Williams et al., 1987), whereas others have inferred a reduction in FDR from a failure to find a change in FSR when accretion increased (Reeds et al., 1986; Bohorov et al., 1987). The unexpected increase in FDR of both groups over time in this study may have resulted from decreased muscular activity after steers were placed in small, individual pens, despite the twice-weekly exercise periods. Increased FDR has been reported to result from muscular disuse (Goldberg, 1969; Millward, 1980). The absolute amount of MPD in BAA-fed steers was lower ($P < .05$) at 3 wk on trial. Consistent with FAR, the absolute amount of MPA was greater ($P < .05$) in BAA-fed steers throughout the experiment, whereas absolute MPS was not altered ($P > .05$) with the feeding of BAA. Protein concentrations were not different ($P > .05$) between control and BAA-fed steers in either muscle (Table 4). The semitendinosus muscle from BAA-fed steers was 21.5% larger ($P < .05$) and total protein was increased by 23.5% ($P < .05$).

A convincing body of literature indicates that the calpain proteolytic system has a major role in postmortem proteolysis and tenderization of meat. It also has been hypothesized that the calpains initiate muscle protein turnover by releasing myofilaments from the myofibril in living muscle. It has been shown that BAA increase muscle accretion and reduce postmortem proteolysis and tenderization; thus, it was of interest to determine the effects of feeding BAA on the calpain proteolytic system. At 0 h, μ-calpain and m-calpain activities were not different ($P > .05$), but calpastatin activity was 60.0% greater ($P < .05$) in longissimus muscle from BAA-fed steers (Table 5). After 7 d of storage at 2° C, μ-calpain and calpastatin activities had decreased ($P < .05$) relative to 0 h, but, again, only calpastatin activity changed ($P < .05$) due to feeding BAA (348% increase). It also should be noted that the actual decline from 0 to d 7 in calpastatin activity was the same in BAA-treated and control muscle (approximately 3 units/g), resulting in similar activity for d-7 BAA-treated and d-0 control muscle. These data on μ-calpain and calpastatin are consistent with previous findings; however, in contrast to our data, other researchers have reported that m-calpain is increased with BAA feeding (Higgins et al., 1988; Kretchmar et al., 1989, 1990; Koohmaraie et al., 1991a).

Although they probably are not involved in postmortem tenderization, lysosomal cathepsins likely are involved in protein turnover. However, there were no significant differences ($P > .05$) in

Table 4. Effects of $L_{644,969}$ on protein in semitendinosus and longissimus muscles[a]

Muscle	Control	Treated	% Change	SE	Probability
Semitendinosus					
Muscle wt, g	1,878.1	2,282.7	+21.5	73.6	.01
Protein concentration, mg/g	179.3	181.5	+1.2	6.0	.80
Protein content, g	335.8	414.8	+23.5	17.3	.02
Longissimus					
Protein concentration, mg/g	171.4	169.3	−1.2	6.2	.82

[a]3 ppm of $L_{644,969}$ for 6 wk.

Table 5. Effects of $L_{644,969}$ feeding and time postmortem on endogenous muscle proteinase activities in longissimus muscle

Item	μ-Calpain[a]	m-Calpain[a]	Calpastatin[b]	Cathepsin B[c]	Cathepsin B+L[c]	Cystatin[d]
Control						
0 d	1.05	1.44	3.72	46.0	235.5	3.9
7 d	.02	1.27	.69	33.8	193.5	4.5
Treated[e]						
0 d	1.15	1.51	5.95*	36.5	215.5	4.3
7 d	.08	1.27	3.09*	26.8	181.5	4.6
SE	.14	.14	.48	4.2	12.0	.4
Probability						
Treatment (Trt)	.44	.82	.01	.07	.21	.50
Time	.01	.06	.01	.02	.01	.30
Trt × time	.36	.80	.86	.77	.74	.68

[a]Total caseinolytic activity/gram of muscle.
[b]Inhibition of casein hydrolysis by m-calpain. Total activity/gram of muscle.
[c]Nanomoles of product released·minute^{-1}·gram of muscle^{-1}.
[d]Measured as the ratio of B+L activity after to before cystatin(s) removal by affinity chromatography.
[e]3 ppm of $L_{644,969}$ for 6 wk.
*Treatment differences within postmortem time were significant ($P < .05$).

cathepsin B, B+L, or cystatin(s) activities between control and BAA-fed steers at 0 h or 7 d postmortem (Table 5). There was a decrease ($P < .05$) in cathepsin B and B+L activities measured at 7 d compared with 0 h postmortem. Previous data indicate very inconsistent effects of BAA on cathepsin activities (Kretchmar et al., 1989, 1990; McElligott et al., 1989; Béchet et al., 1990; Koohmaraie et al., 1991a).

Consistent with the role of the calpain proteolytic system in postmortem proteolysis and tenderization, tenderness and myofibrillar proteolysis were dramatically reduced in the longissimus muscle from BAA-fed steers (Figure 1). Warner-Bratzler shear force decreased ($P < .05$) from 1 to 7 d postmortem in steak from control steers. However, shear force of steaks from BAA-fed steers did not vary ($P > .05$) from 1 to 14 d postmortem. In addition, MFI (a measure of postmortem proteolysis) did not change ($P > .05$) in muscle from BAA-fed steers from 1 to 14 d postmortem, whereas MFI increased ($P < .05$) from 1 to 7 d postmortem in control steers. The inhibition of calpain activity by the high calpastatin activity probably is responsible for the lack of normal postmortem tenderization in BAA-fed steers. These effects are consistent with previous findings (Miller et al., 1988; Kretchmar et al., 1990; Koohmaraie et al., 1991a).

The increased muscle accretion resulting from BAA feeding has been attributed to muscle hypertrophy. The percentage distribution of longissimus muscle fiber types was not ($P > .05$) altered by BAA feeding (Table 6). However, βR fiber areas decreased by 41.0% ($P < .05$), and αR and αW areas increased by 24.5 and 7.6% ($P < .05$). Kim et al. (1987) and Miller et al. (1988) found no change in the percentage distribution of muscle fiber types from BAA feeding. However, Beermann et al. (1987) reported that the percentage of Type II fibers increased and both Type I and II fiber areas were increased with BAA feeding. Kim et al. (1987) also reported that Type II fiber areas increased.

Table 6. Effects of $L_{644,969}$ on longissimus muscle fiber-type distribution and areas[a]

Fiber type	Control	Treated	% Change	SE	Probability
Percentage					
βR	19.3	21.6	+11.9	3.4	.65
αR	27.2	23.8	−14.3	2.6	.38
αW	53.5	54.6	+2.1	2.5	.79
Area, μm^2					
βR	1,936.6	1,374.0	−41.0	88.7	.01
αR	2,117.6	2,635.9	+24.5	79.4	.01
αW	3,553.0	3,821.8	+7.6	60.0	.01

[a]3 ppm of $L_{644,969}$ for 6 wk.

Discussion

The general effects of BAA on muscle protein accretion, feed efficiency, and carcass composition have been well established (Yang and McElligott, 1989). However, the mechanisms responsible for the increased muscle protein accretion are still very much in question. Increased protein synthesis (Bergen et al., 1989; Claeys et al., 1989), decreased protein degradation (Reeds et al., 1986; Bohorov et al., 1987; Williams et al., 1987; MacRae et al., 1988), or both (Maltin et al., 1989; Koohmaraie et al., 1991a), have been reported as a result of BAA feeding. Our results indicate that FDR in BAA-fed steers began to decline after 1 wk, became

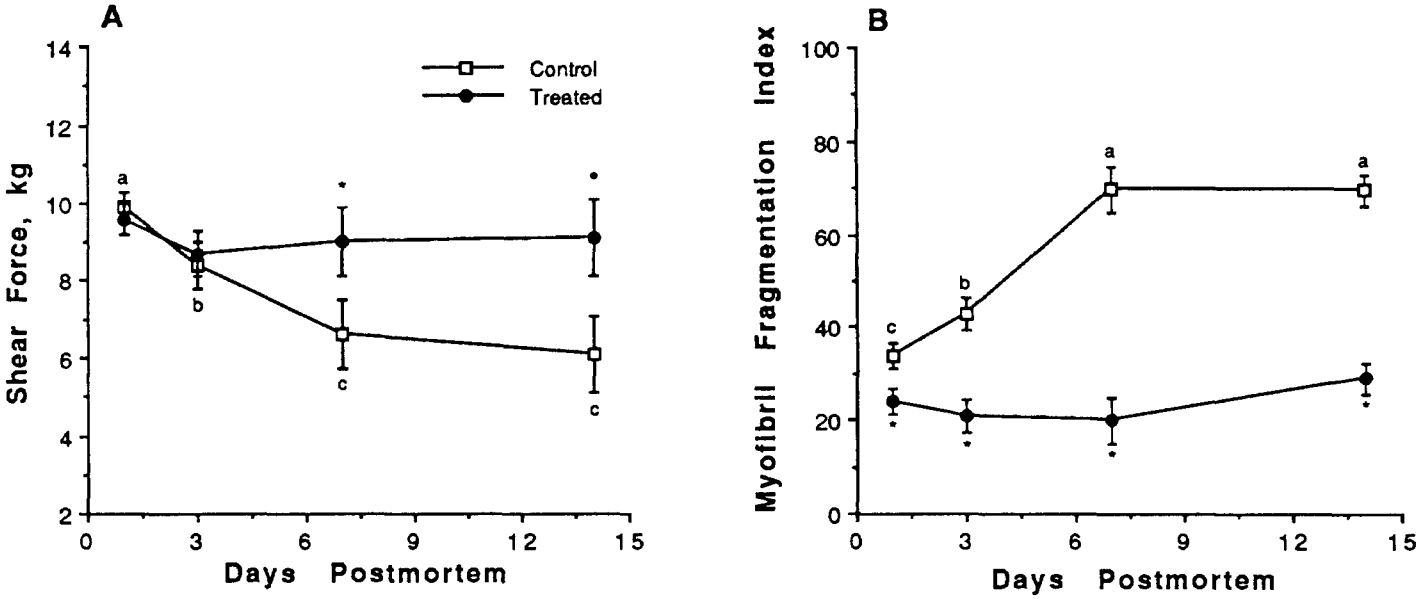

Figure 1. The effects of feeding 3 ppm of $L_{644,969}$ to growing steers for 6 wk on (A) Warner-Bratzler shear force and (B) myofibril fragmentation index during postmortem storage at 2°C. Bars represent standard errors of the means. *Treatment differences within postmortem time are significant ($P < .05$). ^{a,b,c}Means for postmortem aging times within a treatment with a common superscript are not different ($P > .05$).

significantly lower at 3 wk, but were again similar to controls by 6 wk of BAA feeding. These results are consistent with previous reports indicating that the effects of BAA on protein degradation are rapid and subsequently attenuated (Reeds et al., 1986; Eisemann et al., 1988; Eadara et al., 1989; McElligott et al., 1989; Yang and McElligott, 1989; Bergen and Merkel, 1991). Bergen and Merkel (1991) indicated that BAA induced a short-term decrease in muscle protein degradation and that as degradation returned to normal, protein synthesis was increased. Collectively, these data support the proposed (Anderson et al., 1991) diphasic effect of BAA on protein metabolism resulting from an initial reduction in protein degradation followed by an increase in protein synthesis. Estimating fractional FSR as the sum of FDR and FAR indicated that FSR was not different between control and BAA-fed steers. However, FSR was numerically higher at 5 and 6 wk. Thus, our data tend to support the proposed diphasic effects on protein metabolism. The different BAA agents, species to which they are administered, variation in dosages, length of treatment, methodologies, and time course for measuring FSR and FDR all contribute to discrepancies in the literature. However, based on our data and a broad interpretation of previous data (to account for differences in experimental conditions), it seems likely that BAA exert their effect on muscle hypertrophy through both decreased degradation and increased synthesis of muscle proteins. This is in contrast to increased hypertrophy of normal, rapidly growing muscle, in which both synthesis and degradation are increased, although the former to a greater extent (Millward, 1980; Reeds, 1989).

The relative role of various proteinases in muscle protein turnover has not been determined. However, the calpain proteolytic system has been implicated in the initial, and possibly rate-limiting, steps of muscle protein degradation (van der Westhuyzen et al., 1981; Zeman et al., 1985; Goll et al., 1989). Furthermore, it was clearly demonstrated that the calpain proteolytic system was largely responsible for the observed variation in postmortem meat tenderness (Koohmaraie, 1988, 1992; Ouali and Talmant, 1990; Whipple et al., 1990; Koohmaraie et al., 1991b). Thus, it is believed that the proteolytic capacity of the calpain system could regulate muscle protein degradation during both muscle growth and postmortem storage of meat. In support of this hypothesis, our data indicate that BAA feeding increases muscle calpastatin activity, thereby reducing postmortem porteolysis and meat tenderness, which agrees with the conclusions of Higgins et al. (1988), Kretchmar et al. (1989, 1990), and Koohmaraie et al. (1991a). Because postmortem proteolysis by calpain is reduced, it is conceivable that calpain proteolysis in vivo also might be reduced, thereby reducing the rate of muscle protein degradation, if the calpain system were involved in the rate-limiting step. The combined observations of

decreased calpain proteolytic capacity and reduced FDR in BAA-fed steers compared to control animals supports this hypothesis. The fact that the FDR at the time calpastatin activity was determined (6 wk) was not different between treatments may indicate that the calpain system is not involved in a rate-limiting step or that short-term down-regulation of calpastatin activity occurs through a mechanism other than a reduced amount of the protein. A greater understanding of the in vivo regulation of this proteolytic system is needed to evaluate further its role in muscle protein turnover.

It has been documented that protein turnover varies in different skeletal muscles due to fiber-type composition (Goldberg, 1967; Garlick et al., 1989). There is generally a positive relationship between βR muscle fiber content and protein turnover rate. There also is variability in endogenous proteolytic capacity of different muscle types (Ouali, 1990) and in the same muscle from different species (Ouali and Talmant, 1990; Koohmaraie et al., 1991b). Red muscles (greater βR fiber composition) have lower calpastatin activity than do white muscles (Koohmaraie et al., 1988), and pork longissimus muscle has lower calpastatin activity than does beef or lamb (Ouali and Talmant, 1990; Koohmaraie et al., 1991b). Porcine longissimus muscle also has a larger percentage βR fibers than does ovine or bovine longissimus muscle (Solomon and Dunn, 1988). Our results and others (Kim et al., 1987; Miller et al., 1988) indicate that the effects of BAA feeding on muscle hypertrophy seem to occur through increased size of αR and αW fiber types. Collectively, these results could explain the increased calpastatin activity and decreased FDR in BAA-fed animals from the current study.

Implications

The previously documented effects of increased rate and efficiency of growth, muscle hypertrophy, decreased meat tenderness, and increased calpastatin activity were observed in β-adrenergic agonist-fed growing steers. In conjunction with those effects, a short-term decline in fractional degradation rate of skeletal muscle proteins occurred. Collectively, these results are consistent with a role for the calpain proteolytic system in muscle protein degradation of growing steers.

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