EFFECT OF THE β-ADRENERGIC AGONIST $L_{644,969}$ ON MUSCLE GROWTH, ENDOGENOUS PROTEINASE ACTIVITIES, AND POSTMORTEM PROTEOLYSIS IN WETHER LAMBS[1]

M. Koohmaraie[2] S. D. Shackelford[3], N. E. Muggli-Cockett[4] and R. T. Stone[2]

U.S. Department of Agriculture[5], Clay Center, NE 68933
and University of Georgia, Athens 30602

ABSTRACT

To examine the effect of a β-adrenergic agonist (BAA) on muscle growth, proteinase activities, and postmortem proteolysis, 16 wether lambs were randomly assigned to receive 0 or 4 ppm of $L_{644,969}$ in a completely mixed high-concentrate diet for 6 wk. Weight of the biceps femoris was 18.6% heavier in treated lambs. At 0 h after slaughter, treated lambs had higher cathepsin B (35.6%), cathepsins B + L (19.1%), calpastatin (62.8%), and m-calpain (24.6%) than control lambs, but both groups had similar μ-calpain activities. In both longissimus and biceps femoris muscles, treated lambs had higher protein and RNA and lower DNA concentrations. However, total DNA was not affected, indicating that the increase in muscle mass was probably due to muscle hypertrophy rather than to hyperplasia. The pattern of postmortem proteolysis was significantly altered by BAA feeding. In treated lambs, postmortem storage had no effect on the myofibril fragmentation index and degradation of desmin and troponin-T. These results indicate that the ability of the muscle to undergo postmortem proteolysis has been dramatically reduced with BAA feeding. Similar proteolytic systems are thought to be involved in antemortem and postmortem degradation of myofibrillar proteins, so BAA-mediated protein accretion is probably due, at least in part, to reduced protein degradation. To examine whether protein synthesis was altered with BAA feeding, the level of skeletal muscle α-actin mRNA was quantified. Longissimus muscle α-actin mRNA abundance was 30% greater in BAA-fed lambs. Collectively, these results indicate that dietary administration of BAA increases muscle mass through hypertrophy and that the increase in muscle protein accretion is due to reduced degradation and possibly to increased synthesis of muscle proteins.
Key Words: Beta-Adrenergic Agonist, Proteinases, Muscles, Growth, Protein Synthesis, Protein Degradation

J. Anim. Sci. 1991. 69:4823–4835

Introduction

Recently, it has been shown that β-adrenergic agonists (clenbuterol, cimaterol, fenoterol, $L_{644,969}$, and others) improve animal growth performance and carcass characteristics in laboratory and meat-producing animals (for review, see Yang and McElligott, 1989). In general these compounds increase muscle growth and decrease adipose tissue accretion. The β-adrenergic agonist (BAA)-induced mus-

[1]The authors express their gratitude to Merck Sharp and Dohme Research Laboratories for providing $L_{644,969}$. We are also grateful to Sue Hauver, Kay Theer, and Pat Tammen for their assistance in execution of these experiments and to Carol Grummert for her secretarial assistance.

[2] USDA, ARS, Roman L. Hruska U.S. Meat Anim. Res. Center, P.O. Box 166, Clay Center, NE 68933.

[3]Present address: Texas A&M Univ., Dept. of Anim. Sci., 348 Kleberg Center, College Station 77843.

[4]Present address: Utah State Univ., U.M.C. 4700, Biotech. Center, Logan 84322-4700.

[5]Mention of trade names, proprietary products, or specific equipment does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

Received April 1, 1991.
Accepted July 9, 1991.

cle growth involves a proportional increase in cell size, RNA, and protein content without an increase in DNA content in lambs (Beermann et al., 1987; Kim et al., 1987). Because muscle growth does not involve a preceding or parallel increase in DNA content, it has been concluded that BAA-induced muscle hypertrophy is not due to proliferation and incorporation of satellite cells into existing muscle fibers (Beermann et al., 1987; Kim et al., 1987). Although opinions are not unanimous, because BAA feeding does not alter fractional rate of protein synthesis in rats (Reeds et al., 1986) or lambs (Bohorov et al., 1987), it has been concluded that BAA-induced muscle hypertrophy is due to reduced protein degradation. In addition, feeding BAA decreases meat tenderness in lambs (Hamby et al., 1986; Kretchmar et al., 1990; Koohmaraie and Shackelford, 1991), cattle (Miller et al., 1988), and broilers (Morgan et al., 1989), and tenderness is usually the result of postmortem proteolysis. In summary, these results demonstrate that BAA probably exert their effect by reducing the proteolytic capacity of the muscle, which can result in increased muscle growth and decreased meat tenderness. The objectives of this study were to determine the effects of a BAA ($L_{644,969}$[6]) on growth performance, carcass characteristics, endogenous proteinases, postmortem proteolysis, and α-actin mRNA abundance in lambs.

Materials and Methods

Animals. Sixteen crossbred wether lambs (1/2 Finnsheep × 1/4 Dorset × 1/4 Rambouillet), within 2 wk of age, were randomly assigned to two treatment groups: control and BAA-fed. Animals were allowed ad libitum access to a diet (Table 1) with or without 4 ppm of $L_{644,969}$, 6-amino-alpha-([1-methyl-3-phenylpropyl)amino]methyl)-3-pyridine methanol dichloride for 6 wk. The diet was formulated to contain 18% CP. After 6 wk, the animals were slaughtered according to standard procedures and hot carcass weight was determined, and then carcasses were stored in a holding cooler (–1.1°C, no forced air movement) for 24 h.

[6]Merck Sharp and Dohme Research Laboratories, Division of Merck and Co., Inc., Rahway, NJ.

TABLE 1. COMPOSITION OF DIET

Ingredient	%
Corn	59.39
Alfalfa	20.00
Soybean meal	15.00
Liquid molasses	3.00
Limestone	1.00
Ammonium chloride	.50
Steamed bone meal	.50
Salt	.50
Vitamins A, D, and E	.05
Aureomycin 50	.05
Rumensin 60	.008

Carcass Measurements. At 24 h postmortem, each carcass was ribbed between the 12th and 13th ribs to determine USDA quality and yield grade (USDA, 1982) factors. Dressing percentage, actual fat thickness, adjusted fat thickness, leg score, longissimus muscle area, and percentage of kidney, pelvic, and heart fat and marbling were recorded. Lean color and lean firmness were scored on a scale of 1 to 8 (1 = dark and soft to 8 = bleached and firm).

Biceps Femoris Muscle Collection and Analysis. Biceps femoris muscle was dissected, trimmed of any external fat, weighed, frozen in liquid nitrogen, and stored at –70°C. The frozen muscle was then pulverized in a blender to form a frozen powder. Aliquots of the frozen powder were analyzed for DNA, RNA, and protein concentration.

Longissimus Muscle Collection and Analysis. Left sides of all carcasses were used for those determinations made immediately after slaughter and the right sides were used for other measurements. Immediately after slaughter, about 100 g of longissimus muscle was removed for the following determinations: calpains and calpastatin, lysosomal proteinases, protein, DNA and RNA concentration, and SDS-PAGE analysis of myofibrillar proteins. Also, about 10 g of muscle was aseptically removed, chopped into small pieces, and frozen in liquid nitrogen for mRNA analysis. At 24 h postmortem, and after determination of USDA quality and yield grade, the entire longissimus muscle from the right side of each carcass was cut into 2.54-cm-thick chops and vacuum-packaged. Chops were assigned to 1, 7, and 14 d postmortem vacuum aging by stratifying storage time along the length of the longissimus muscle.

Calpains and Calpastatin Quantification. The activities of calpains and calpastatin were determined on 50 g of longissimus muscle immediately after slaughter and after 7 d of postmortem storage at 4°C. Activities were determined on fresh samples according to procedures described by Koohmaraie (1990b). Briefly, after homogenization, centrifugation, dialysis, and clarification, the muscle extracts were loaded onto DEAE-Sephacel columns[7]. After extensive washing (≥ 10 column volumes) to remove unbound proteins, the bound proteins were eluted with a continuous NaCl gradient (total volume = 6.5 column volumes) from 0 to 400 m*M* NaCl. Activities were expressed as the amount of calpain caseinolytic activity in 50 g of muscle. One unit of μ-calpain 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 one unit of m-calpain activity.

Lysosomal Proteinases and Cystatin-Like Quantification. Samples for determination of lysosomal enzyme activities were frozen in liquid nitrogen immediately after slaughter and after 7 d postmortem and stored at –70°C before extraction. Muscle extracts were prepared; lysosomal cysteine proteinases and cystatin-like activities were determined as described previously (Koohmaraie and Kretchmar, 1990). Briefly, after homogenization and centrifugation, an aliquot of muscle extract was subjected to S-carboxymethylated-papain-Sepharose affinity chromatography to remove cystatins. After washing to remove unbound proteins, the bound proteins were eluted. Muscle extracts before and after affinity chromatography were assayed for cathepsin B (using N-CBZ-L-arginyl-L-arginine-7-amido-4-methylcoumarin; Z-Arg-Arg-NMec[8] as the substrate) and cathepsins B + L (using Z-Phe-Arg-NMec[8] as the substrate) activities. Cystatin-like activity was calculated as cathepsins B + L activity after affinity chromatography/ cathepsins B + L activity before affinity chromatography. Activities of the cathepsins were expressed as nanomoles·minute^{-1}·gram of muscle^{-1}.

DNA, RNA, and Protein Quantification. Concentrations of RNA were determined by the method of Munro and Fleck (1969). The DNA concentrations were determined according to the procedure described by Labarca and Paigen (1980) using Hoechst 33258 reagent[9]. Protein concentrations were determined by the biuret method (Gornall et al., 1949).

Myofibril Fragmentation Index Measurements. At 1, 7, and 14 d postmortem, myofibril fragmentation indices (MFI) were determined on fresh (not frozen) muscle samples according to the procedure of Culler et al. (1978).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. Myofibrils were isolated after 0, 1, 7 and 14 d postmortem and analyzed by SDS-PAGE according to the procedure described by Koohmaraie (1990a).

Quantification of α-Actin mRNA. Total RNA was isolated from longissimus muscle using a method we adapted by combining and modifying two procedures. These procedures were developed by Chomczynski and Sacchi (1987) and Birnboim (1988). Briefly, 1 g of muscle was homogenized with 10 ml of solution D (4 *M* guanidinium thiocyanate, 25 m*M* sodium citrate, pH 7.0; .5% sarcosyl, .1 *M* mercaptoethanol [Chomczynski and Sacchi, 1987]) using a Polytron[10] set at 1/2 maximum speed. To the homogenate, 1 ml of 2 *M* sodium acetate, pH 4.0, 10 ml of water-saturated phenol[11] and 2 ml of chloroform-isoamyl alcohol mixture (49:1) were sequentially added, with thorough mixing by inversion after each addition. The final suspension was vortexed vigorously for approximately 15 s and allowed to stand on ice for 15 min followed by centrifugation at 10,000 × *g* for 20 min. The aqueous phase, which contained RNA, was carefully removed and transferred to another tube, mixed with 10 ml of cold ethanol, and stored at –20°C for 3 to 24 h to precipitate RNA. The RNA was sedimented by centrifugation as before. The pellet was dissolved in 3 ml of solution D and precipitated with 6 ml of 100% ethanol at –20°C for 1 to 2 h. After centrifugation the RNA pellet was washed with 10 ml of 80% ethanol and centrifuged. The pellet was dissolved in 4 ml of RES-1 (.5 *M* LiCl, 1 *M* urea, 1.0% SDS, .02 *M* sodium citrate, and 2.5 m*M* 1,2-cyclohexanediaminetetraacetic acid [CDTA], pH 6.8; Birnboim, 1988) and to it was added 20 μl of 2

[7]Pharmacia LKB, Piscataway, NJ.
[8]Bachem Fine Chemicals, Torrance, CA.
[9]Sigma Chemical Co., St. Louis, MO.
[10]Brinkman Instruments, Westbury, NY.
[11]Boehringer Mannheim, Indianapolis, IN.

M sodium acetate, pH 4.0 and 4 ml of LiCl/ethanol (3 volumes of 5 *M* LiCl + 2 volumes of 100% ethanol) and stored on ice at 4°C overnight (Birnboim, 1988). The RNA was sedimented by centrifugation at 10,000 × *g* for 10 min, washed with 15 ml of 80% ethanol, and centrifuged again. Finally, the RNA was dissolved in 300 to 500 μl of water and A_{260}: A_{280} ratio was determined, and RNA concentration was determined from the A_{260}. Relative mRNA for α-actin was quantified by slot-blot hybridization methods described by Sambrook et al., (1989). Total RNA was blotted onto nitrocellulose paper at .1, .2, .3, and .4 μg of longissimus muscle RNA per slot using a 24-well manifold[12]. After blotting, RNA was fixed onto the nitrocellulose paper using a UV-crosslinker[13] and prehybridized for 12 to 24 h at 37°C. The prehybridization solution consisted of 50% deionized formamide, 5×SSC (1× SSC is .15 *M* NaCl, .015 *M* sodium citrate, pH 7.0), 1× Denhart's solution, 100 μg/ml of salmon sperm DNA, 30 m*M* 1,4-piperazinediethanesulfonic acid (PIPES), and .1% SDS. Because the cDNA probe used contains a long poly A tail, prehybridization solution also contained 1 μg/ml of poly A to block nonspecific hybridization. After prehybridization, the solution was removed and brought to 10% dextran sulfate by adding solid dextran sulfate. To a 1-ml aliquot of this solution, nick-translated probe (1×10^6 cpm/ml of hybridization solution) was added, boiled for 4 min, then chilled on ice for 4 min and added to hybridization bags. The ratio of hybridization solution to nitrocellulose paper was .2 ml/cm^2 (Sambrook et al., 1989). Hybridization was carried out overnight at 37°C. After hybridization, the nitrocellulose papers were washed 2 × 10 min with 2× SSC and .5% SDS at room temperature and then washed 3 × 15 min with .2 × SSC and .5% SDS at 65°C. Hybridization of muscle RNA to α-actin cDNA was quantified by a densitometer[14] . The cDNA probe was obtained from L. Kedes at Stanford University (248 probe). The insert was excised by digesting the plasmid with *PstI/PvuII*.

Statistical Analysis. An analysis of variance (Steel and Torrie, 1980) for a completely randomized design was used to analyze the following traits: carcass and performance traits, nucleic acid, protein concentration and content, and α-actin mRNA abundance. A factorial arrangement (two postmortem times × two dietary treatments) of a completely randomized design was used to analyze calpains, lysosomal proteinases, MFI, and shear force. When the main effect or interaction was significant, means separation was accomplished using least squares procedures (SAS, 1985). The predetermined level of significance of $P < .05$ was used for all comparisons and will be used for the remainder of this discussion.

Results

Performance and Carcass Characteristics. The effects of BAA on the performance and carcass characteristics of lambs are summarized in Table 2. The ADG was improved (16.7%) with BAA feeding compared with control feeding ($P < .05$). Though statistically not significant ($P > .05$), final weight, hot carcass weight, and dressing percentage were all numerically higher in BAA-fed animals than in control animals. Indices of carcass fatness were mostly lowered with BAA feeding; marbling and kidney, pelvic, and heart fat were not affected by BAA, but actual and adjusted fat thicknesses were reduced in BAA-fed compared with control lambs. Traits indicating the muscling of the carcass were all higher with BAA feeding. These include muscle weight (e.g., biceps femoris weight was increased by 11.5% when expressed as a percentage of carcass weight, $P > .05$), longissimus muscle area ($P < .05$), and leg score ($P < .05$). These results are in general agreement with those reported by Kim et al. (1987) and Kretchmar et al. (1990).

Meat Tenderness and Postmortem Proteolysis. Indices of meat tenderness (i.e., shear force, MFI) were determined at different postmortem times to examine the effects of BAA feeding on meat quality of BAA-fed lambs (Figure 1). In control animals, shear force decreased and MFI increased with 14 d postmortem storage at 4°C. However, the extent of these changes was much less (especially MFI) in BAA-fed lambs. For example, the shear force value decreased by 52.6% in control animals and only by 18.3% in

[12]BRL, Gaithersburg, MD.
[13]Stratagene, La Jolla, CA.
[14]Shimadzu Scientific Instruments, Inc., Columbia, MA.

TABLE 2. LEAST SQUARES MEANS FOR THE EFFECT OF TREATMENT ON GROWTH PERFORMANCE AND CARCASS CHARACTERISTICS IN WETHER LAMBS

Item	Control	$L_{644,969}$[a]	SE
Starting wt, kg	34.2	34.2	.6
Final wt, kg	41.6	43.2	1.3
Average daily gain, kg	.18[b]	.21[c]	.01
Hot carcass wt, kg	22.2	23.7	.8
Dressing percentage	53.3	54.8	.7
Biceps femoris[d]	1.22[b]	1.36[c]	.04
Actual fat thickness, mm	5.3[b]	3.7[c]	.3
Adjusted fat thickness, mm	5.7[b]	4.1[c]	.4
Kidney, pelvic, and heart fat, %	4.6	4.4	.5
Leg score	10.0[b]	11.8[c]	.3
Yield grade	3.8[b]	3.2[c]	.2
Longissimus muscle area, cm^2	12.0[b]	14.6[c]	.7
Marbling	192.5	195.0	.9
Lean color	4.1	4.5	.2
Lean firmness	5.6[b]	6.1[c]	.2

[a]$L_{644,969}$ = β-adrenergic agonist.

[b,c]Means within the same row lacking a common superscript letter differ ($P < .05$).

[d]Percentage of hot carcass weight.

BAA-fed animals. Similarly, the MFI value increased by 42.3% in control lambs and by only 3.3% in BAA-fed lambs. Although MFI is a direct index of the extent of postmortem proteolysis, to verify these findings myofibrils were purified from longissimus muscle of control and BAA-fed lambs after 0, 1, 7, and 14 d of postmortem storage at 4°C and analyzed by SDS-PAGE. In control lambs, changes typical of those that have been previously documented numerous times (Goll et al., 1983; Koohmaraie, 1988, 1991) were observed. These changes include the following (Figure 2): 1) disappearance of a high molecular weight polypeptide, probably titin (arrow A); 2) disappearance of a 55-kDa polypeptide,

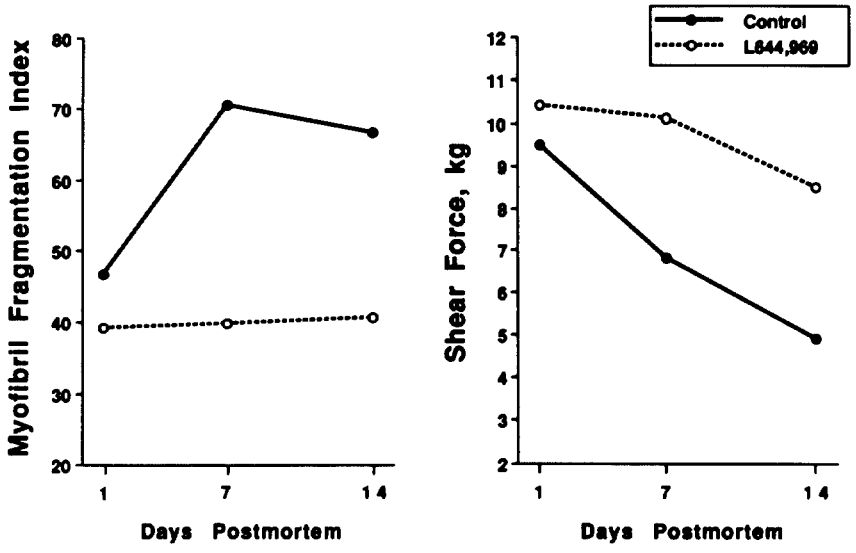

Figure 1. Effects of postmortem storage and β-adrenergic agonist ($L_{644,969}$) feeding on the shear force and myofibril fragmentation index (MFI) of longissimus muscle in wether lambs. The SE of the interaction were .8 and 2.9 for shear force and MFI, respectively.

probably desmin (arrow B); and 3) simultaneous disappearance of troponin-T and appearance of a group of polypeptides with molecular weights of 28 and 32 kDa (arrows C and D). The onset of these changes was apparent in control muscle after 1 d of storage and changes were completed by 7 d. However, none of these changes occurred in BAA-fed lambs by 7 d of storage, and some discernible changes were noted after 14 d.

Quantification of Calpains and Calpastatins. The calcium-dependent proteinases (CDP; calpains) are thought to play an important role in the turnover of myofibrillar proteins in live animals (Goll et al., 1989) and in their proteolysis during postmortem storage of carcasses (Koohmaraie, 1988, 1991). Therefore, it was of interest to determine the effects of BAA feeding on the components of this proteolytic system. The activities of μ-calpain (also called calpain-I and CDP-I; the form of the proteinase that is active at micromolar concentration of calcium), m-calpain (also called calpain-II and CDP-II: the form of proteinase that is active at millimolar concentration of calcium), and calpastatin (also called CDP inhibitor, an endogenous inhibitor of both forms of proteinases at their respective calcium requirement for activation) were measured after 0 h (within 35 min of slaughter) and 7 d of postmortem storage (Figure 3). At 0 h, total extractable μ-calpain was not different, m-calpain was increased by 24.6%, and calpastatin was increased by 62.8% with BAA feeding. After 7 d of storage, calpastatin (227.2%), μ-calpain (89.2%), and m-calpain (25.4%) activities were higher in BAA-fed than in control lambs. These results are in general agreement with those reported previously (Higgins et al., 1988; Kretchmar et al., 1989, 1990; Koohmaraie and Shackelford, 1991). However, Wang and Beermann (1988) reported that μ-calpain activity was decreased by 50% when clenbuterol was fed to lambs. This difference is probably the result of different methodology.

Quantification of Cathepsins B and B + L and Cystatin-Like Activities. Like calpains, lysosomal cysteine proteinases have also been

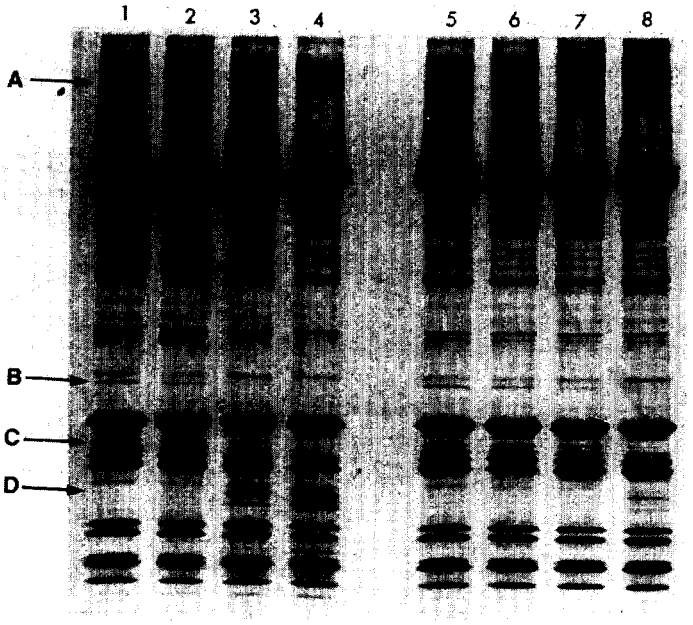

Figure 2. The SDS-PAGE of myofibrils isolated from longissimus muscle of control and β-adrenergic agonist (BAA)-fed wether lambs. Lanes 1, 2, 3, and 4 represent control myofibrils after 0, 1, 7, and 14 d of postmortem storage. Lanes 5, 6, 7, and 8 represent myofibrils from BAA-fed lambs after 0, 1, 7, and 14 d of postmortem storage. Arrows A, B, C, and D correspond to high molecular weight polypeptide (probably titin), 55-kDa polypeptide (probably desmin), troponin-T, and 28- to 32-kDa polypeptides. Samples (60 μg of purified myofibrillar proteins) were subjected to electrophoresis on a 7.5 to 15% gradient polyacrylamide gel and stained with Commassie Blue R-250.

TABLE 3. LEAST SQUARES MEANS FOR THE EFFECT OF TREATMENT OF LYSOSOMAL CYSTEINE PROTEINASES AND CYSTATIN-LIKE ACTIVITIES AT DIFFERENT POSTMORTEM TIMES IN WETHER LAMBS

Item	Cathepsin B[a]	Cathepsins B + L[a]	Cystatins[b]
Treatment			
Control	50.1[c]	250.9[c]	3.4[d]
$L_{644,969}$[f]	63.3[d]	276.9[d]	2.7[c]
SE	3.0	5.0	.1
Days postmortem			
0	52.6	245.4[c]	3.0
7	60.8	282.4[d]	3.1
SE	3.0	5.0	.1
Interaction			
Probability level	.54	.03	.36
Control, 0	44.7	224.0[e]	3.3
Control, 7	55.5	277.7[cd]	3.5
$L_{644,969}$, 0	60.6	266.8[d]	2.8
$L_{644,969}$, 7	66.0	287.1[c]	2.7
SE	4.4	7.2	.2

[a]Nanomoles of NMec (amido-methylcoumarin) released·minute^{-1}·gram of muscle^{-1}.

[b]Cathepsins B + L activity after affinity chromatography/cathepsins B + L activity before affinity chromatography.

[c,d,e]Means within the same column lacking a common superscript letter differ ($P < .05$).

[f]$L_{644,969}$ = β-adrenergic agonist.

implicated in the degradation of myofibrillar proteins before and after slaughter. We therefore measured the activities of cathepsins B and B + L and their endogenous inhibitor immediately after slaughter and after 7 d of postmortem storage at 4°C (Table 3). Results indicated that BAA-fed lambs had higher ($P < .05$) cathepsins B and B + L and lower ($P > .05$) cystatin-like activities. These results do not agree with those reported by Kretchmar et al. (1989, 1990). Kretchmar et al. (1989, 1990) used the same BAA used in this study and found that muscle from BAA-fed lambs had 30% less cathepsin B activity. The reason for this discrepancy is not apparent at this time. Possible explanations, such as the method of extraction and substrate concentration in the assay, have been discussed previously (Koohmaraie and Shackelford, 1991).

Nucleic Acids and Protein Concentrations and Contents. To gain some understanding of the mode of action of BAA, the DNA, RNA, and protein concentration were measured in longissimus and biceps femoris muscles (Tables 4 and 5). In addition, the weight of the biceps femoris was determined so that the DNA, RNA, and protein content of this muscle could be calculated (Table 5). Results indicated that in both of these muscles DNA concentration decreased ($P < .05$) but RNA and protein concentration increased in BAA-fed lambs ($P < .05$). However, the differences in RNA concentration for biceps femoris muscle were not statistically significant ($P >$

TABLE 4. LEAST SQUARES MEANS FOR THE EFFECT OF TREATMENT ON THE NUCLEIC ACID AND PROTEIN CONCENTRATIONS OF THE LONGISSIMUS MUSCLE IN WETHER LAMBS

Item	Control	$L_{644,969}$[a]	SE	% Change
Protein concentration, mg/g	222.2[b]	246.6[c]	4.3	+11.0
DNA concentration, μg/g	863.9[b]	790.0[c]	13.0	−8.6
RNA concentration, μ/g	615.3[b]	688.1[c]	18.8	+11.8
Protein/DNA, mg/mg	257.8[b]	312.4[c]	6.6	+21.1
Protein/RNA, mg/mg	364.0	358.9	10.8	−1.4
RNA/DNA, μg/μg	.71[b]	.87[c]	.02	+16.0

[a]$L_{644,969}$ = β-adrenergic agonist.

[b,c]Means within the same row lacking a common superscript letter differ ($P < .05$).

TABLE 5. LEAST SQUARES MEANS FOR THE EFFECT OF TREATMENT ON THE NUCLEIC ACID AND PROTEIN CONCENTRATION AND CONTENT OF THE BICEPS FEMORIS MUSCLE IN WETHER LAMBS

Item	Control	$L_{644,969}$[a]	SE	% Change
Biceps femoris wt, g	271.0[b]	320.2[c]	10.2	+18.2
Protein concentration, mg/g	238.3[b]	264.8[c]	5.8	+11.1
Protein content, g	64.8[b]	85.3[c]	4.1	+31.6
DNA concentration, μg/g	988.1[b]	896.4[c]	20.8	−9.3
DNA content, mg	266.9	286.9	9.5	+7.5
RNA concentration, μg/g	548.6	567.8	10.6	+3.5
RNA content, mg	148.8[b]	182.1[c]	7.1	+22.4
Protein/DNA, mg/mg	242.2[b]	296.5[c]	8.9	+22.4
Protein/RNA, mg/mg	435.8	466.8	12.2	+7.1
RNA/DNA, μg/μg	.56[b]	.64[c]	.02	+14.3

[a]$L_{644,969}$ = β-adrenergic agonist.

[b,c]Means within the same row lacking a common superscript letter differ ($P < .05$).

.5). In both muscles that protein:DNA ratio was significantly ($P < .05$) higher in BAA-fed lambs, indicating that cell size was altered with BAA feeding. The RNA:DNA ratio, which indicates transcriptional activity, was higher ($P < .05$) in both muscles. Though statistically not significant ($P > .05$), the RNA:protein ratio, which is an indication of translational activity, was higher in BAA-fed lambs. Total RNA and protein were significantly higher ($P < .05$) and total DNA was not affected with BAA feeding. These results are in agreement with those reported previously (Beermann et al., 1987; Kim et al., 1987; Kretchmar et al., 1990).

Quantification of α-Actin mRNA. Thus far, results have collectively indicated that feeding BAA induces hypertrophy of muscle. Because protein concentration and content were increased with BAA feeding, the amount of mRNA for α-actin (skeletal muscle specific protein) was quantified to determine whether the increase in protein content was related to increased protein synthesis. Northern blot analysis indicated that the cDNA probe used hybridized to a single species of mRNA of 1,650 basepairs (Figure 4), a size consistent with α-actin mRNA from other species. Slot-blot analysis of longissimus muscle mRNA, quantified by densitometry, indicated that BAA induced a 30% increase in α-actin mRNA in longissimus muscle (Table 6). Using similar analysis, but feeding ractopamine to pigs, Helferich et al. (1990) found a 100% increase in α-actin mRNA. Additionally, by using an in vitro translation assay, Helferich et al. (1990) demonstrated that the ractopamine effect was not specific for α-actin mRNA, because other mRNAs were similarly enhanced by ractopamine. Therefore, it can be concluded that similar results would be expected in this study.

Discussion

It has been demonstrated that BAA are potent growth promoters in many species of animals (for a review see Yang and McElligott, 1989). Dietary administration of BAA to

TABLE 6. LEAST SQUARES MEANS FOR THE EFFECT OF TREATMENT ON THE RELATIVE ABUNDANCE OF α-ACTIN mRNA IN WETHER LAMBS[a]

Item	Control	$L_{644,969}$[b]	SE	% Change
.1 μg RNA	132,478	166,528	12,268	+25.7
.2 μg RNA	223,736	284,647	24,304	+27.2
.3 μg RNA	271,314[c]	372,049[d]	16,379	+37.1
.4 μg RNA	284,706[c]	392,070[d]	16,502	+37.7

[a]Data are arbitrary units from densitometry of autoradiographs of slot-blots.

[b]$L_{644,969}$ = β-adrenergic agonist.

[c,d]Means within the same row lacking a common superscript letter differ ($P < .05$).

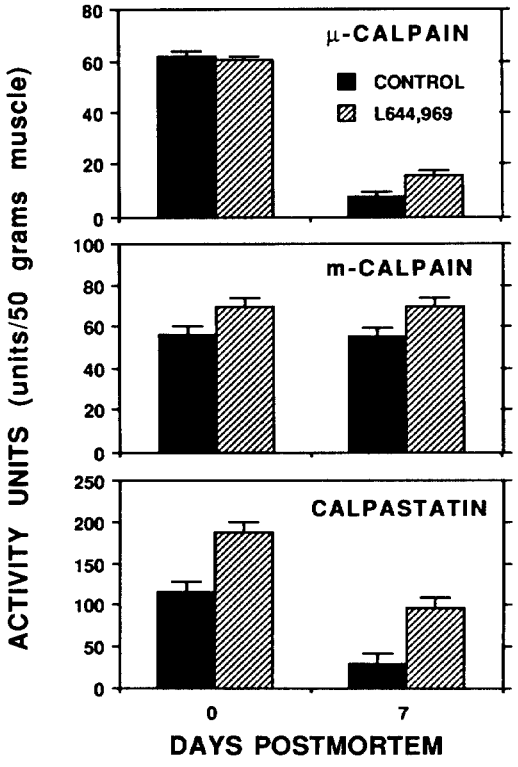

Figure 3. The μ-calpain, m-calpain, and calpastatin activity (total activity/50 g of muscle) isolated from longissimus muscle of control and β-adrenergic agonist ($L_{644,969}$)-fed lambs immediately after slaughter (d 0) and after 7 d of postmortem storage.

lambs results in improvement in feed efficiency and carcass composition (Baker et al., 1984; Hamby et al., 1986; Beermann et al., 1987; Bohorov et al., 1987; Kim et al., 1987; Higgins et al., 1988; Kretchmar et al., 1990; Koohmaraie and Shackelford, 1991). The anabolic effects of BAA probably are at least partly mediated by a reduction in protein degradation (Bohorov et al., 1987), possibly mediated by the calpain proteolytic system (Harper et al., 1990). It has been demonstrated that feeding BAA to lambs results in a significant increase in the activities of calpastatin (endogenous inhibitor of calpains) and m-calpain but a slight decrease in the activity of α-calpain (Higgins et al., 1988; Kretchmar et al., 1989, 1990; Koohmaraie and Shackelford, 1991). The BAA-induced muscle hypertrophy could also be mediated by an increase in protein synthesis independent of the degradation or concomitant with a decrease in degradation. Ractopamine has been shown to increase the rate of muscle α-actin synthesis in vivo by 50% and to increase α-actin mRNA by 100% (Helferich et al., 1990). Although numerous experiments have been conducted to examine the effects of feeding BAA to lambs, each of these experiments has examined limited measurements of growth, carcass characteristics, and meat quality. We have attempted to measure the effect of BAA ($L_{644,969}$) on 1) carcass characteristics and selected growth traits (i.e., muscle protein, DNA and RNA concentration, and content), 2) the expression of a muscle-specific protein (i.e., α-actin), 3) activities of two proteolytic systems thought to be involved in antemortem and postmortem degradation of myofibrillar proteins, and 4) postmortem proteolysis and meat tenderness.

Results of this study indicated that dietary administration of $L_{644,969}$ to lambs results in alterations in carcass composition similar to those reported previously (Kim et al., 1987;

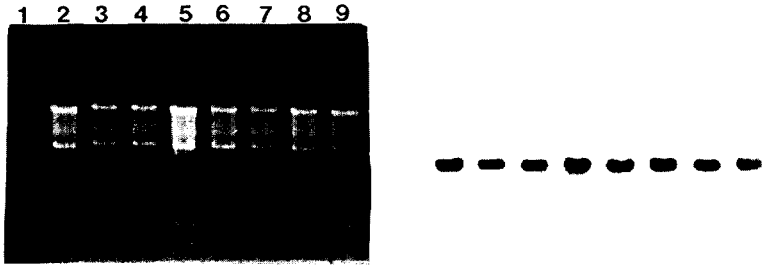

Figure 4. Agarose gel electrophoresis (.8%) of total cellular RNA (15 μg) of longissimus muscle of control (Lanes 2, 3, 4, 5) and β-adrenergic agonist-fed (6, 7, 8, 9) lambs (left panel). Northern blots were hybridized to a ^{32}P nick-translated α-actin cDNA.

Kretchmar et al., 1990). Determination of protein concentration (longissimus and biceps femoris muscles) and content (biceps femoris muscle) indicated that these traits were increased by 11 and 31.6%, respectively. Consistent with other results using other BAA in lambs (Beermann et al., 1987; Kim et al., 1987), these results clearly demonstrate that feeding BAA causes muscle hypertrophy. Because muscle growth is the balance between protein synthesis and degradation, the BAA-induced muscle hypertrophy could result from a decrease in protein degradation, an increase in protein synthesis, or both.

To determine whether BAA induces its effect through a reduction in protein degradation, we determined the activities of two classes of proteinases (calpains and lysosomal) thought to be involved in the regulation of muscle protein degradation during growth and postmortem storage (Goll et al., 1989; Koohmaraie, 1988, 1991). Results indicated that BAA feeding (specifically ($L_{644,969}$) significantly altered both of these proteolytic systems. The most significant effect of BAA was a substantial increase in the activity of calpastatin both at 0 h and 7 d postmortem. Results of several experiments indicate that calpastatin is one of the principal regulators of the calpains in postmortem muscle. First, infusion of carcasses with zinc chloride, which prevented the postmortem proteolysis and tenderization process, completely blocked the inactivation of calpastatin (Koohmaraie, 1990a). Second, infusion of carcasses with calcium chloride, which results in acceleration of the postmortem proteolysis and tenderization process, also accelerates the process of calpastatin inactivation (Koohmaraie et al., 1988, 1989). Third, the rate of inactivation of calpastatin has been shown to be the principal factor affecting the rate of postmortem proteolysis and tenderness in meat from *Bos taurus* vs *Bos indicus* breeds of cattle (Whipple et al., 1990; Shackelford et al., 1991). Finally, the differences in the rate of postmortem proteolysis and tenderization of meat from different species are negatively correlated with their calpastatin activity (Ouali and Talmant, 1990; Koohmaraie et al., 1991). Because calpastatin is very specific for calpains and does not inhibit the activity of any other proteinases, our results suggest that the proteolytic capacity of the calcium-dependent proteinase system in skeletal muscle is reduced with BAA feeding. The activity of μ-calpain was not changed with BAA feeding; however, the activity of m-calpain was significantly increased. This increase in the activity of m-calpain is not consistent with the hypothesis that BAA treatment induces a decrease in proteolytic capacity of the muscle; however, because m-calpain requires a millimolar concentration of calcium for activation and this concentration is not present under physiological conditions, the biological significance of the increased m-calpain activity cannot be determined at this time. We have conducted numerous experiments on the mechanism of postmortem proteolysis and have concluded that calpains play a major role in the regulation of degradation of myofibrillar proteins during postmortem storage (for reviews, see Koohmaraie, 1988, 1991). Results of this experiment indicated that meat tenderness and degradation of myofibrillar proteins during 14 d of postmortem storage was significantly reduced in animals fed BAA.

Unlike the results for the effect of BAA on calpains (Higgins et al., 1988; Wang and Beermann, 1988; Forsberg et al., 1989; Kretchmar et al., 1989, 1990; Koohmaraie and Shackelford, 1991), the results for the effects of BAA on lysosomal proteinases are very inconsistent; some authors have reported a decrease (Kretchmar et al., 1989, 1990; Morgan et al., 1989; Béchet et al., 1990), others have reported an increase (McElligott et al., 1987, 1989; Koohmaraie and Shackelford, 1991), and others have reported no effect (Forsberg et al., 1987; McElligott et al., 1987, 1989) on the activity of cathepsins with BAA feeding. In this study we found that feeding BAA increased cathepsins B and B + L but decreased cystatin-like activities. Because the results for cathepsin activities are so inconsistent, a role for cathepsins in BAA-induced muscle hypertrophy cannot be determined at this time.

The results obtained with regard to the effect of BAA on the calcium-dependent proteinase system and postmortem proteolysis suggest that BAA-induced muscle hypertrophy could, at least in part, be due to a reduction in protein degradation. To determine whether BAA feeding induces muscle protein synthesis, we determined the mRNA abundance of a muscle-specific protein (α-actin) in longissimus muscle obtained from control and BAA-fed animals. Longissimus muscle α-actin

mRNA abundance was approximately 30% greater in lambs fed BAA. Because contractile protein mRNA accumulate coordinately and because the synthesis of myofibrillar proteins is regulated by changes in levels of mRNA (Devlin and Emerson, 1979), one would expect that mRNA for other myofibrillar proteins would also be elevated with BAA feeding. However, the increase in other myofibrillar protein mRNA is expected to be much lower than that of α-actin. Studying the stoichiometry of contractile proteins, Devlin and Emerson (1978, 1979) reported that all the contractile proteins were synthesized at virtually identical molar rates except for α-actin, which was synthesized at approximately three times the molar rate of the other contractile proteins. Assuming that the BAA-induced mRNA are translated into their respective proteins, the results of the present study suggest that BAA feeding may also increase muscle protein accretion by stimulating protein synthesis. However, it has been demonstrated that this may not be the case (i.e., increased transcription of mRNA may not result in increased protein synthesis). Endo and Nadal-Ginard (1987) showed that Ca^{2+}-deprivation specifically inhibits the translation of most muscle-specific mRNA despite their abundant presence in the cells. They (Endo and Nadal-Ginard, 1987) also demonstrated that this translational control is restricted to muscle-specific mRNA and is not applicable to other mRNA present in the same cells. Therefore, at least under certain conditions, muscle-specific gene expression is also regulated at the level of translation. It is important to bear in mind that cell-free translation systems do not reproduce this translational regulation of muscle-specific gene expression (Endo and Nadal-Ginard, 1987). For this reason we did not attempt to use cell-free translation systems to translate RNA from control and BAA-fed animals. Therefore, it seems that the only acceptable method to determine the effect of BAA on muscle protein synthesis must include an in vivo method. Although Helferich et al. (1990) demonstrated increased protein synthesis with ractopamine feeding, such results should not be extrapolated to include all forms of BAA. Different BAA could potentially have different modes of action depending on the form of BAA and the species used.

To determine the effect of BAA on translational and transcriptional activity, RNA and DNA concentration (biceps femoris and longissimus muscle) and content (biceps femoris muscle) were measured. Consistent with the effects of other BAA in lambs, the DNA concentration was decreased, whereas total DNA was not affected, suggesting that the BAA-induced muscle hypertrophy does not require satellite cell incorporation as a prerequisite. This effect of BAA is in contrast to other forms of accelerated muscle growth such as stretch-induced hypertrophy (Barnett et al. 1980) and compensatory growth (Beermann, 1983). In agreement with results obtained by Reeds et al. (1986) and Beermann et al. (1987), the RNA:DNA ratio was greater in both longissimus and biceps femoris muscle from BAA-fed lambs, suggesting that the transcriptional capacity is increased, which is supported by α-actin mRNA abundance data.

Implications

This experiment was conducted to determine the effect of dietary administration of a β-adrenergic agonist on muscle growth and meat quality in lambs. Results indicated that β-agonist feeding results in muscle hypertrophy and that the increase in protein accretion is due to decreased protein degradation and possibly to increased protein synthesis. Feeding the specific β-agonist investigated in this research produces meat that is significantly tougher than that from control lambs. However, we have previously demonstrated that infusion of β-agonist-fed carcasses with calcium chloride is an effective method to overcome the β-agonist-induced meat toughness.

Literature Cited

Baker, P. K., R. H. Dalrymple, D. L. Ingle and C. A. Ricks. 1984. Use of a β-adrenergic agonist to alter muscle and fat deposition in lambs. J. Anim. Sci. 59:1256.

Barnett, J. G., R. G. Holly and C. R. Ashmore. 1980. Stretch-induced growth in chicken wing muscles: biochemical and morphological characterization. Am. J. Physiol. 239:C39.

Béchet, D. M., A. Listrat, C. Deval, M. Ferrara, and J. F. Quirke. 1990. Cimaterol reduces cathepsin activities but has no anabolic effect in cultured myotubes. Am. J. Physiol. 259:E822.

Beermann, D. H. 1983. Effects of maternal dietary restriction during gestation and lactation, muscle, sex and age on various indices of skeletal muscle growth in the rat. J. Anim. Sci. 57:328.

Beermann, D. H., W. R. Butler, D. E. Hogue, V. K. Fishell, R. H. Dalrymple, C. A. Ricks and C. G. Scanes. 1987. Cimaterol-induced muscle hypertrophy and altered endocrine status in lambs. J. Anim. Sci. 65:1514.

Birnboim, H. C. 1988. Rapid extraction of high molecular weight RNA from cultured cells and granulocytes for Northern analysis. Nucleic Acids Res. 16:1487.

Bohorov, O., P. J. Buttery, J.H.R.D. Correia and J. B. Soar. 1987. The effect of the β 2-adrenergic agonist clenbuterol or implantation with oestradiol plus trenbolone acetate on protein metabolism in wether lambs. Br. J. Nutr. 57:99.

Chomczynski, P. and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162: 156.

Culler, R. D., F. C. Parrish, Jr., G. C. Smith and H. R. Cross. 1978. Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. J. Food Sci. 43:1177.

Devlin, R. B. and C. P. Emerson, Jr. 1978. Coordinate regulation of contractile protein synthesis during myoblast differentiation. Cell 13:599.

Devlin, R. B. and C. P. Emerson, Jr. 1979. Coordinate accumulation of contractile protein mRNAs during myoblast differentiation. Dev. Biol. 69:202.

Endo, T. and B. Nadal-Ginard. 1987. Three types of muscle-specific gene expression in fusion-blocked rat skeletal muscle cells: translation control in EGTA-treated cells. Cell 49:515.

Forsberg, N. E., M. A. Ilian, A. Ali-Bar, P. R. Cheeke and N. B. Wehr. 1989. Effects of cimaterol on rabbit growth and myofibrillar protein degradation and on calcium-dependent proteinase and calpastatin activities in skeletal muscle. J. Anim. Sci. 67:3313.

Forsberg, N. E., A. R. Nassar, R. H. Dalrymple and C. A. Ricks. 1987. Cimaterol reduces Cathepsin B activity in sheep skeletal muscle. Fed. Proc. Fed. Am. Soc. Exp. Biol. 46:1176A.

Goll, D. E., W. C. Kleese and A. Szpacenko. 1989. Skeletal muscle proteases and protein turnover. In: D. R. Campion, G. J. Hausman and R. J. Martin (Ed.) Animal Growth Regulation. p 141. Plenum Publishing, New York.

Goll, D. E., Y. Otsuka, P. A. Nagainis, J. D. Shannon, K. S. Sheridhar and M. Muguruma. 1983. Role of muscle proteinases in maintenance of muscle integrity and mass. J. Food Biochem. 7:137.

Gornall, A. G., C. J. Bardawill and M. M. David. 1949. Determination of serum-protein by means of the biuret reaction. J. Biol. Chem. 177:751.

Hamby, P. L., J. R. Stouffer and S. B. Smith. 1986. Muscle metabolism and real-time ultrasound measurement of muscle and subcutaneous adipose tissue growth in lambs fed diets containing a beta-agonist. J. Anim. Sci. 63:1410.

Harper, J.M.M., I. Mackinson and P. J. Buttery. 1990. The effects of beta agonists on muscle cells in culture. Domest. Anim. Endocrinol. 7:477.

Helferich, W. G., D. B. Jump, D. B. Anderson, D. M. Skjaerlund, R. A. Merkel and W. G. Bergen. 1990. Skeletal muscle α-actin synthesis is increased pretranslationally in pigs fed the phenethanolamine ractopamine. Endocrinology 126:3096.

Higgins, J. A., Y. V. Lasslett, R. G. Bardsley and P. J. Buttery. 1988. The relation between dietary restriction or clenbuterol (a selective β-2 agonist) treatment on muscle growth and calpain proteinase (EC 3.4.22.17) and calpastatin activities in lambs. Br. J. Nutr. 60:645.

Kim, Y. S., Y. B. Lee and R. H. Dalrymple. 1987. Effect of the repartitioning agent cimaterol on growth, carcass and skeletal muscle characteristics in lambs. J. Anim. Sci. 65:1392.

Koohmaraie, M. 1988. The role of endogenous proteases in meat tenderness. Recip. Meat Conf. Proc. 41:89.

Koohmaraie, M. 1990a. Inhibition of postmortem tenderization in ovine carcasses through infusion of zinc. J. Anim. Sci. 68:1476.

Koohmaraie, M. 1990b. Quantification of Ca^{2+}-dependent protease activities by hydrophobic and ion-exchange chromatography. J. Anim. Sci. 68:659.

Koohmaraie, M. 1991. The role of Ca^{2+}-dependent proteases (calpains) in postmortem proteolysis and meat tenderness. Biochimie (In press).

Koohmaraie, M., A. S. Babiker, A. L. Schroeder, R. A. Merkel and T. R. Dutson. 1988. Acceleration of postmortem tenderization in ovine carcasses through activation of Ca^{2+}-dependent proteases. J. Food Sci. 53:1638.

Koohmaraie, M., J. D. Crouse and H. J. Mersmann. 1989. Acceleration of postmortem tenderization in ovine carcasses through infusion of calcium chloride: Effect of concentration and ionic strength. J. Anim. Sci. 67: 934.

Koohmaraie, M. and D. H. Kretchmar. 1990. Comparisons of four methods for quantification of lysosomal cysteine proteinase activities. J. Anim. Sci. 68:2362.

Koohmaraie, M. and S. D. Shackelford. 1991. Effect of calcium chloride infusion on the tenderness of lambs fed a β-adrenergic agonist. J. Anim. Sci. 69:2463.

Koohmaraie, M., G. Whipple, D. H. Kretchmar, J. D. Course, H. J. Mersmann. 1991. Postmortem proteolysis in longissimus muscle from beef, lamb and pork carcasses. J. Anim. Sci. 69:617.

Kretchmar, D. H., M. R. Hathaway, R. J. Epley and W. R. Dayton. 1989. *In vivo* effect of a β-adrenergic agonist on activity of calcium-dependent proteinases, their specific inhibitor, and cathepsins B and H in skeletal muscle. Arch. Biochem. Biophys. 275:228.

Kretchmar, D. H., M. R. Hathaway, R. J. Epley and W. R. Dayton. 1990. Alterations in postmortem degradation of myofibrillar proteins in muscle of lambs fed a β-adrenergic agonist. J. Anim. Sci. 68:1760.

Labarca, C. and K. Paigen. 1980. A simple, rapid, and sensitive DNA assay procedure. Anal. Biochem. 102: 344.

McElligott, M. A., A. Barreto, Jr. and L.-Y. Chaung. 1989. Effect of continuous and intermittent clenbuterol feeding on rat growth rate and muscle. Comp. Biochem. Physiol. 92C:135.

McElligott, M. A., J. E. Mulder, L.-Y. Chaung and A. Barreto, Jr. 1987. Clenbuterol-induced muscle growth: investigation of possible mediation by insulin. Am. J. Physiol. 253:E370.

Miller, M. F., D. K. Garcia, M. E. Coleman, P. A. Ekeren, D. K. Lunt. K. A. Wagner, M. Procknor, T. H. Welsh, Jr. and S. B. Smith. 1988. Adipose tissue, longissimus muscle and anterior pituitary growth and function in clenbuterol-fed heifers. J. Anim. Sci. 66:12.

Morgan, J. B., S. J. Jones and C. R. Calkins. 1989. Muscle protein turnover and tenderness in broiler chickens fed cimaterol. J. Anim. Sci. 67:2646.

Munro, H. N. and A. Fleck. 1969. Analysis of tissue and body fluids for nitrogenous constituents. In: H. N. Munro (Ed.) Mammalian Protein Metabolism. Vol. 3. p 423. Academic Press, New York.

Ouali, A. and A. Talmant. 1990. Calpains and calpastatin

distribution in bovine, porcine and ovine skeletal muscles. Meat Sci. 28:331.

Reeds, P. J., S. M. Hay, P. M. Dorwood and R. M. Palmer. 1986. Stimulation of muscle growth by clenbuterol: lack of effect on muscle protein biosynthesis. Br. J. Nutr. 56:249.

Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual (2nd Ed.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SAS. 1985. SAS User's Guide: Statistics. SAS Inst., Inc., Cary, NC.

Shackelford, S. D., M. Koohmaraie, M. F. Miller, J. D. Crouse and J. O. Reagan. 1991. An evaluation of tenderness of the longissimus muscle of Angus by Hereford versus Brahman crossbred heifers. J. Anim. Sci. 69:171.

Steel, R.G.D. and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach (2nd Ed.). McGraw-Hill Book Co., New York.

USDA. 1982. Official United States Standards for Grades of Carcass Lambs, Yearling Mutton and Mutton Carcasses. Agric. Marketing Serv., USDA, Washington, DC.

Wang, S.-Y. and D. H. Beermann. 1988. Reduced calcium-dependent proteinase activity in cimaterol-induced muscle hypertrophy in lambs. J. Anim. Sci. 66:2545.

Whipple, G., M. Koohmaraie, M. E. Dikeman, J. D. Crouse, M. C. Hunt and R. D. Klemm. 1990. Evaluation of attributes that affect longissimus muscle tenderness in *Bos taurus* and *Bos indicus* cattle. J. Anim. Sci. 68: 2716.

Yang, Y. T. and M. A. McElligott. 1989. Multiple actions of β-adrenergic agonists on skeletal muscle and adipose tissue. Biochem. J. 261:1.