Effects of a β-Adrenergic Agonist (L-644,969) and Male Sex Condition on Muscle Growth and Meat Quality of Callipyge Lambs[1]

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ABSTRACT: The objective of this study was to determine the effects of dietary administration of a β-adrenergic agonist (BAA; L-644,969) and male sex condition (ram vs wether) on muscle growth and meat quality of Dorset × Romonov lambs believed to be heterozygous for the callipyge gene. At approximately 17 wk of age, lambs were blocked by weight within each sex condition and randomly assigned to BAA treatment group. The interaction of BAA and male sex condition was not significant for any of the traits measured. Rams had greater initial and final live weights, average daily gain, and hot carcass weight ($P < .01$). Rams did not differ ($P > .05$) from wethers with respect to any of the carcass traits, possibly because the wethers were so lean and heavily-muscled that there was little room for improvement. Kidney-pelvic fat weight was reduced 26% by BAA ($P < .05$). Knife separable lean weight and whole carcass proximate composition were not affected ($P > .05$) by BAA or male sex condition. Administration of BAA increased calpastatin activity at 20 d (1.1 vs 1.5 units/g), but not at 0 h (3.9 vs 4.8 units/g) postmortem, decreased myofibril fragmentation index (60.7 vs 44.9), and increased shear force (8.2 vs 10.9 kg) at 20 d postmortem ($P < .05$). These data suggest that muscle growth rates are near maximum in lambs expressing the callipyge gene, regardless of male sex condition or BAA treatment. Therefore, it seems that the callipyge gene exerts most, but not all, of its effect through intracellular events similar to those initiated by administering BAA.

Key Words: β-Adrenergic Agonists, Callipyge, Calpastatin, Castration, Muscles, Tenderness

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Introduction

Recently, empirical evidence for the existence of a gene in sheep causing extreme muscling has been identified. Preliminary evidence suggests that an autosomal gene (callipyge) may be responsible for this major effect on muscling and composition (Cockett et al., 1993).

Compared with normal animals (i.e., non-carriers), animals expressing the callipyge gene have superior feed efficiency, 32.3% more muscle (Jackson and Green, 1993; Jackson et al., 1993a,b), and decreased carcass fatness and longissimus tenderness (Koohmaraie et al., 1995). In addition, the effect of the callipyge gene on muscling is not consistent among muscles; all leg and loin muscles investigated are hypertrophied by the callipyge gene (18.8 to 42.1%) but certain shoulder muscles are not affected (Koohmaraie et al., 1995). We suspect that the callipyge gene exerts its effect on muscle growth by suppressing the rate of proteolysis via a dramatic increase in calpastatin activity and by increasing the capacity for protein synthesis (Koohmaraie et al., 1995).

It is now well documented that β-adrenergic agonists (**BAA**) such as clenbuterol, cimaterol, and L-644,969 improve animal growth performance and carcass characteristics in laboratory and meat-producing animals (for review see Yang and McElligott, 1989; Bergen and Merkel, 1991; Reeds and Mersmann, 1991). The effects of BAA on muscle protein metabolism are controversial. Evidence exists to support suppression of proteolysis, stimulation of protein synthesis, or both (for review see Reeds and Mersmann, 1991). Although the mechanism of action of BAA during growth remains controversial, feeding BAA undoubtedly reduces postmortem proteolysis, tenderization, and meat tenderness (Hamby et al., 1986; Fiems et al., 1990; Kretchmar et al., 1990;

[1]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 other products that may also be suitable. The authors greatly acknowledge the technical assistance of P. Ekeren, S. Hauver, S. Lonergan, C. Kurth, K. Mihm, P. Tammen, and K. Theer and the secretarial assistance of M. Bierman. We are grateful to Merck, Sharp and Dohme research laboratories for donating L-644,969.

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Table 1. Ingredients and composition of the finishing diet on an as-fed basis

Item	Content
Ingredient	
Alfalfa hay, %	20.0
Corn, %	59.3
Soybean meal, %	15.0
Liquid molasses, %	3.0
Limestone, %	1.0
Ammonium chloride, %	.5
Steambone meal, %	.5
Sodium chloride, %	.5
Vitamin ADE, ppm	500.0
Trace minerals, ppm	60.0
Rumensin 60, ppm	80.0
Aureomycin 50, ppm	500.0
Vitamin E, ppm	450.0
Composition	
DM, %	88.8
TDN, %	80.5
CP, %	17.5
Ca, %	.9
P, %	.5
Cu, ppm	9.5
Monensin, ppm	11.0
Chlortetracycline, ppm	55.0

Koohmaraie and Shackelford, 1991; Koohmaraie et al., 1991; Wheeler and Koohmaraie, 1992).

Thus, the objective of the present experiment was to determine the effects of dietary administration of a BAA and male sex condition (ram vs wether) on measures of muscle growth and meat quality of lambs heterozygous for the callipyge gene.

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. Twenty-nine Dorset × Romonov lambs believed to be heterozygous for the callipyge gene were used. At birth, one-half of the lambs were randomly castrated. At approximately 17 wk of age, lambs were blocked by weight within each sex condition and randomly assigned to BAA treatment group (wethers: eight control and eight BAA-fed; rams: seven control and six 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. The diet contained 17.5% CP on an as-fed basis. After 6 wk, the animals were slaughtered according to standard procedures. At slaughter, the head, pelt, heart, lungs, liver, spleen, kidneys, kidney-pelvic fat, and viscera were weighed. Hot carcass weight was then determined and carcasses were chilled for 22 h (−1°C).

Carcass and Muscle Measurements. At 22 h postmortem, carcasses were ribbed between the 12th and 13th ribs and leg score, actual and adjusted fat thickness, body wall thickness (at a point 7.6 cm from the lateral end of the longissimus), longissimus area, longissimus depth, marbling score (100 = Traces^0, 200 = Slight^0, 300 = Small^0, 400 = Modest^0), and lean color score (1 = dark red, 8 = pale pink) were recorded.

The right side of each carcass was dissected and individual weights of the adductor, biceps femoris, infraspinatus, longissimus, psoas group (psoas major and psoas minor combined), quadriceps femoris (rectus femoris, vastus medialis, vastus intermedius, and vastus lateralis combined), semimembranosus, semitendinosus, supraspinatus, and gluteus group (gluteus medius, gluteus profundus, and gluteus accessorius combined) were recorded. Muscle nomenclature was based on Tucker et al. (1952).

Semitendinosus Analyses. The left semitendinosus was trimmed of any external fat, weighed, frozen in liquid nitrogen, pulverized, and stored (−70°C). Aliquots of the frozen powder were analyzed for DNA, RNA, and protein concentration.

Longissimus Analyses. The left side of all carcasses was used for those determinations made immediately after slaughter. Immediately after slaughter, a section of longissimus was removed for the following determinations: quantification of calpain and calpastatin activity, protein, RNA, and DNA concentration, and SDS-PAGE analysis of myofibrillar proteins. At 24 h postmortem, the entire longissimus was removed from the right side of each carcass, cut into 2.54-cm-thick chops, vacuum-packaged, and aged (4°C).

Calpains and Calpastatin Activities. Immediately after slaughter, calpains and calpastatin were extracted from 10 g of fresh longissimus and quantified as described by Koohmaraie (1990b).

Heated Calpastatin Activity. At 7 and 20 d postmortem, calpastatin was extracted from 10 g of unfrozen longissimus and quantified as described by Shackelford et al. (1994) with the following modifications: 100 mg/L of ovomucoid, 2 m*M* phenylmethylsulfonyl fluoride (**PMSF**), and 6 mg/L of leupeptin were added to the extraction solution and samples were dialyzed after first centrifugation.

DNA, RNA, and Protein Quantification. The RNA concentration was determined by the method of Munro and Fleck (1969). The DNA concentration was determined according to Labarca and Paigen (1980) using Hoechst 33258 reagent. Protein concentration was determined by the biuret method (Gornall et al., 1949).

Postmortem Metabolism. Longissimus pH and temperature were determined at 0, 3, 6, and 9 h postmortem with a digital handheld pH meter (Model 05669-00, Cole-Palmer, Chicago IL). Additionally, pH of longissimus chops was measured at 1, 7, and 20 d postmortem.

Osmotic Pressure and Water-Holding Capacity. Water-holding capacity and osmotic pressure were measured on longissimus chops at 1, 7, and 20 d

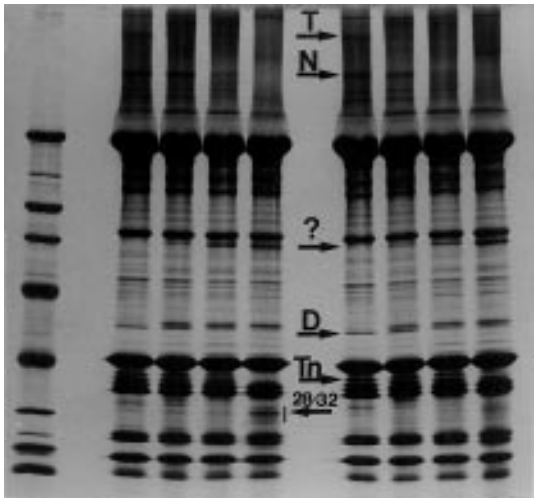

Figure 1. Effect of postmortem storage on longissimus myofibrillar proteins from callipyge lambs fed a diet with 0 or 4 ppm L-644,969. Lane 1 is molecular weight standards and contains myosin heavy chain, 200 kDa; *E. coli* β-galactosidase, 116.3 kDa; rabbit muscle phosphoralase b, 97.4 kDa; bovine serum albumin, 66.2 kDa; hen egg-white ovalbumin, 45 kDa; bovine carbonic anhydrase, 31 kDa; soybean trypsin inhibitor, 21.5 kDa; and hen egg-white lysozyme, 14.4 kDa. Lanes 2, 3, 4, and 5 are control at 0, 1, 7, and 20 d postmortem, respectively, and lanes 6, 7, 8, and 9 are β-adrenergic agonist at 0, 1, 7, and 20 d postmortem, respectively. Each lane was loaded with 80 μg of protein. T = Titin; N = Nebluin; ? = Unknown degradation product; D = Desmin; Tn = Troponin-T; 28–32 = degradation products with molecular mass of 28 to 32 kDa.

postmortem as described by Bonnet et al. (1992). Briefly, 10 to 15 g of muscle (connective tissue and fat-free) were minced with a household coffee grinder (Braun, Model KSM2). A 5-g sample was centrifuged at 50,400 × *g* for 30 min. The supernatant was weighed to determine water-holding capacity and its osmotic pressure was determined by using the Advanced Micro-Osmometer (Model 3MO plus; Advanced Instruments, Needham Heights, MA).

Myofibril Fragmentation Index. At 1, 7, and 20 d postmortem, myofibril fragmentation index (**MFI**) was determined on fresh longissimus according to the procedure of Culler et al. (1978).

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis. Myofibrils were isolated after 0, 1, 7, and 20 d postmortem and analyzed by SDS-PAGE as described by Koohmaraie (1990a). The SDS-PAGE analysis was conducted on muscles from all animals. The animals whose shear force and MFI values were nearest to the mean of each treatment were reanalyzed, photographed, and reported as Figure 1.

Warner-Bratzler Shear Force. Longissimus Warner-Bratzler shear force was determined at 1, 7, and 20 d postmortem. After the appropriate aging period, three fresh chops per animal were broiled to an internal temperature of 40°C, turned, and broiled to an internal temperature of 75°C. Chops were chilled (4°C) overnight before removal of six cores (1.27 cm diameter) parallel to the muscle fibers. Each core was sheared once with a Warner-Bratzler shear attachment on an Instron Universal Testing Machine (Instron, Canton, MA) with a 50-kg load cell, a full-scale load setting of 20 kg, and a crosshead speed of 5 cm/min.

Muscle Histochemistry. Samples of longissimus, psoas major, and semitendinosus were obtained at 24 h postmortem. Several .7-cm^3 samples were frozen on cork in liquid nitrogen-cooled isopentane and stored at –70°C. Transverse cryostat sections, 10μm 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 β-red, α-red, or α-white 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.

Apparent fiber number (Swatland, 1984) was determined on psoas major and semitendinosus at two locations (approximately 3 cm from the origin or insertion) within the portion of maximal cross-sectional area. At each sample location, the area of the muscle was measured and two samples were obtained and frozen as described above. Fiber density was determined on four contiguous fields (10× objective) from each location (approximately 1,200 and 700 fibers per semitendinosus and psoas major, respectively, were counted). Fiber density and muscle area data were used to calculate apparent fiber number.

Statistical Analysis. An ANOVA was conducted for a 2 (BAA treatment) × 2 (male sex condition) factorially arranged completely randomized design using the GLM procedures of SAS (1988). Due to limited variation in initial weight, weight blocks did not account for a significant amount of variation in any trait. The interaction of BAA treatment and male sex condition was not significant for any trait. Thus, only the main effect means are presented.

Results and Discussion

Growth and Carcass Traits. The literature is mixed with regard to the effects of BAA on growth rate of lamb (Kim et al., 1987; Kretchmar et al., 1990; Koohmaraie and Shackelford, 1991; Koohmaraie et al., 1991; Lee and Kim, 1994); however, previously, we have not seen an effect of L-644,969 on growth rate of normal wethers (Koohmaraie and Shackelford, 1991; Koohmaraie et al., 1991). Similarly, in this experiment, L-644,969 did not affect growth rate of callipyge

wethers and rams (Table 2). As expected (Reeds et al., 1986), BAA decreased heart weights, but contrary to previous data in our lab (Koohmaraie and Shackelford, 1991; Koohmaraie et al., 1991), BAA reduced kidney-pelvic fat.

Our data agree with the wealth of literature (Field, 1971; Crouse et al., 1981; Wynn and Thwaites, 1981; Seideman et al., 1982) establishing that intact males have increased rates of gain and weights of non-carcass body components and decreased dressing percentage compared with male castrates (Table 2). However, in contrast to other genotypes, the callipyge wethers and rams used in this experiment did not differ with respect to carcass cutability traits (Table 3). This was likely due to the fact that there was little room for improvement beyond the leanness and extreme muscling of the callipyge wether lambs. Despite the expectation that a Dorset × Romanov genotype would produce fat, thinly muscled carcasses (Gallivan et al., 1993), the callipyge lambs used in this experiment were consistently lean and heavily muscled. In normal lambs, feeding a BAA would be expected to decrease fat thickness and increase longissimus area and dissected muscle weights; however, because the callipyge gene dramatically improves carcass composition (Koohmaraie et al., 1995), carcass traits were not affected by BAA. Of the 10 muscles dissected, weights of only two muscles (quadriceps femoris and supraspinatus) were increased by BAA and weights of only three muscles (infraspinatus, psoas group, and supraspinatus) were increased for rams as compared to wethers. Interestingly, of those same 10 muscles, infraspinatus and supraspinatus are the only muscles not affected by the callipyge gene and quadriceps femoris and psoas groups were affected to a lesser extent than the other muscles (Koohmaraie et al., 1995).

In normal lambs, intact males would be expected to have lower marbling scores than male castrates (Field, 1971; Seideman et al., 1982) and lambs fed a BAA would be expected to have lower marbling scores than control lambs (Koohmaraie and Shackelford, 1991). However, we believe that because the callipyge gene decreases marbling score (Koohmaraie et al., 1995), there was no further effect of BAA or sex condition on marbling score (Table 3).

Postmortem Metabolism and Muscle Functionality. Neither BAA nor sex condition affected longissimus pH and temperature decline, water-holding capacity, or osmotic pressure of muscle from callipyge lambs (Table 4). Typically, intact males would be expected to produce meat with a greater ultimate pH (Purchas, 1990; Purchas and Aungsupakorn, 1993). Additionally, feeding a BAA has been shown to affect the pattern of pH decline and result in a greater ultimate pH (Beermann, 1985; Lee and Kim, 1994), although some workers have found that BAA did not affect ultimate pH (Fiems et al., 1990; Berge et al., 1993; Vestergaard et al., 1994). Previously, we (Koohmaraie et al., 1995) reported that the callipyge gene did not affect longissimus pH and temperature decline.

It is well established that the osmotic pressure of muscle increases with rigor and will reach its maximum value (corresponding to ionic strength ranging from .2 to .3) at rigor completion (for review see Ouali, 1990). It has been suggested (Ouali, 1990) that this increase in ionic strength can influence postmortem tenderization mechanisms in two ways: 1) it enhances enzymatic activity directly or indirectly by modifying the substrates (i.e., myofibrils and[or] myofibrillar proteins), and(or) 2) it causes weakening of myofibrils directly and, thus, induces tenderization. The values obtained for osmotic pressure (Table 4) are comparable to those reported by others (for review see Ouali, 1990). In callipyge longissimus (with and without BAA feeding), the osmotic pressure increased as expected, but postmortem tenderization was minimal; therefore, we suggest that osmotic pressure does not affect postmortem tenderization by directly modifying myofibrils (alternative 2, above). The other alternative (enhanced enzymatic activity directly or indirectly) remains the most likely role for osmotic pressure in postmortem tenderization. Based on these and other results (for review see Koohmaraie, 1994), we conclude that osmotic pressure by itself cannot play a direct role in postmortem tenderization and, thus, it cannot explain animal-to-animal variation in tenderness.

Postmortem Proteolysis and Meat Tenderness. In normal animals, MFI is slightly lower (Morgan et al., 1993b) and shear force slightly greater in intact males than in castrated males (Field, 1971; Seideman et al., 1982). However, in the present experiment, MFI values were lower in wethers than in intact males (Table 5). This would suggest that there is an interaction between callipyge genotype and male sex condition on postmortem proteolysis. Normally, BAA feeding decreases MFI and increases shear force (Beermann, 1985; Fiems et al., 1990; Kretchmar et al., 1990; Koohmaraie and Shackelford, 1991; Koohmaraie et al., 1991; Wheeler and Koohmaraie, 1992; Berge et al., 1993; Lee and Kim, 1994; Vestergaard et al., 1994). However, because callipyge lambs have extremely tough meat, as indicated by high shear force (Koohmaraie et al., 1995), BAA only had a small effect on MFI and shear force in this experiment. Our data agree with previous findings that the reduced tenderness of BAA occurred without any effect on sarcomere length (Berge et al., 1993; Lee and Kim, 1994).

Koohmaraie et al. (1995) reported that the callipyge gene reduced the rate and extent of postmortem proteolysis (as determined by SDS-PAGE, western blots, and MFI) such that MFI and shear force values after 21 d postmortem were 63 and 248%, respectively, of those of non-callipyge lambs. Because the interaction between BAA and sex condition was not signifi-

Table 2. Effect of β-agonist treatment and male sex condition on growth rate, body weight, hot carcass weight, and weights of dress-off items

	β-agonist treatment		Male sex condition		
Trait	Control (n = 15)	$L_{644,969}$ (n = 14)	Ram (n = 13)	Wether (n = 16)	SEM
Starting body wt, kg	35.2	34.8	36.6[a]	33.5[b]	1.0
Final body wt, kg	44.9	45.5	48.5[a]	41.9[b]	1.3
Average daily gain, kg	.23	.25	.28[a]	.20[b]	.01
Heart wt, g	206.5[a]	174.6[b]	203.2[a]	177.9[b]	7.6
Lungs wt, g	535.7	492.1	516.5	511.3	20.2
Liver wt, g	845.1	767.2	884.8[a]	727.5[b]	38.5
Spleen wt, g	59.7	56.8	60.5	56.0	2.4
Viscera wt, g	9,556.0	9,346.5	9,986.0[a]	8,916.5[b]	351.4
Kidney-pelvic fat wt, g	674.3[a]	499.7[b]	592.2	581.7	48.3
Kidney wt, g	109.7	106.6	115.1[a]	101.2[b]	3.6
Head wt, g	2,044.7	2,056.3	2,287.3[a]	1,813.7[b]	41.5
Pelt wt, g	5,271.3	5,227.7	5,797.9[a]	4,701.1[b]	198.4
Hot carcass wt, g	24.8	25.4	26.5 [a]	23.6[b]	.7

[a,b]Within each row and main effect, means with uncommon superscripts differ ($P < .05$).

cant, only the effect of BAA on the pattern of postmortem degradation of myofibrillar proteins is shown (Figure 1). Consistent with previous findings (Fiems et al., 1990; Kretchmar et al., 1990; Koohmaraie et al., 1991; Wheeler and Koohmaraie, 1992), BAA treatment significantly reduced degradation of

Table 3. Effect of β-agonist treatment and male sex condition on carcass measurements, dissected muscle weights, and carcass chemical composition

	β-agonist treatment		Male sex condition		
Trait	Control (n = 15)	$L_{644,969}$ (n = 14)	Ram (n = 13)	Wether (n = 16)	SEM
Carcass measurements					
Leg score	12.8	13.2	12.9	13.1	.3
Actual fat thickness, mm	3.3	3.2	3.0	3.5	.4
Adjusted fat thickness, mm	3.2	3.0	2.8	3.4	.3
Bodywall thickness, mm	23.4	22.9	22.8	23.5	.9
Longissimus area, cm^2	17.8	19.1	18.8	18.0	.9
Longissimus depth, mm	33.1	35.5	34.3	34.3	1.1
Marbling score[a]	232.1	221.4	235.7	217.9	22.0
Lean color score[b]	4.7	5.2	4.9	5.0	.2
Dissected muscle weights					
Adductor wt, g	165.9	173.2	165.5	173.6	7.1
Biceps femoris wt, g	423.3	431.5	436.1	418.8	17.8
Gluteus group wt, g	324.4	336.2	339.2	321.5	11.7
Infraspinatus wt, g	181.0	189.8	197.5[c]	173.3[d]	6.3
Longissimus wt, g	751.0	781.8	787.0	745.8	32.5
Psoas group wt, g	176.8	184.5	190.8[c]	170.6[d]	6.2
Quadriceps femoris wt, g	522.3[d]	573.5[c]	568.5	527.3	17.4
Semimembranosus wt, g	390.7	406.0	398.5	398.2	17.3
Semitendinosus wt, g	151.9	165.5	166.2	151.2	5.6
Supraspinatus wt, g	127.6[d]	144.0[c]	144.7[c]	126.9[d]	4.9
Total muscle wt, g	3,215.0	3,386.1	3,394.0	3,207.0	112.0
Whole-carcass chemical composition					
Moisture, %	56.9	58.1	57.7	57.2	.8
Fat, %	21.5	19.8	20.2	21.2	1.1
Protein, %	17.3	17.7	17.7	17.4	.3
Ash, %	4.3	4.3	4.4	4.3	.1

[a]100 = traces^0, 200 = slight^0, 300 = small^0, 400 = modest^0.
[b]1 = dark red, 8 = pale pink.
[c,d]Within each row and main effect, means with uncommon superscripts differ ($P < .05$).

Table 4. Effect of β-agonist treatment and male sex condition on longissimus pH, temperature, water-holding capacity, and osmotic pressure

Trait and time postmortem	β-agonist treatment		Male sex condition		SEM
	Control (n = 15)	$L_{644,969}$ (n = 14)	Ram (n = 13)	Wether (n = 16)	
pH					
0 h	6.48	6.44	6.45	6.47	.04
3 h	6.21	6.32	6.27	6.26	.04
6 h	6.06	6.15	6.08	6.13	.05
9 h	5.98	6.04	5.99	6.04	.05
24 h	5.79	5.83	5.83	5.79	.02
168 h	5.62	5.67	5.63	5.66	.02
480 h	5.68	5.72	5.70	5.69	.02
Temperature, °C					
0 h	37.6	38.5	38.1	38.0	.5
3 h	13.2	14.0	13.5	13.7	.6
6 h	4.1	4.6	4.3	4.4	.3
9 h	.5	.6	.5	.6	.2
Water-holding capacity, %					
1 d	20.5	18.0	19.3	19.2	1.1
7 d	15.0	14.6	13.8	15.8	1.5
20 d	15.1	14.6	13.6	16.1	1.1
Osmotic pressure, m0sm					
1 d	524.1	520.1	521.0	523.2	4.5
7 d	554.9	551.1	556.5	549.5	4.5
20 d	541.2	535.6	537.9	539.0	3.9

myofibrillar proteins during a 20-d postmortem storage. It is well documented that the following changes can be observed with SDS-PAGE analysis of longissimus myofibrils at different times postmortem (for review see Goll et al., 1983; Koohmaraie, 1988, 1992a,b, 1994): 1) disappearance of a high-molecular-weight polypeptide, probably titin (T in Figure 1); 2) disappearance of a 55-kDa polypeptide, probably desmin (D in Figure 1); and simultaneous disappearance of troponin-T (Tn in Figure 1) and appearance of a group of polypeptides with molecular masses of 28 to 32 kDa (28 to 32 in Figure 1). In myofibrils from a normal lamb, these changes are apparent at 1 d postmortem and these changes are completed by 3 to 7 d postmortem. Of the above changes, only titin degradation and partial degradation of desmin and troponin-T were detected in BAA-fed animals. Those changes were only detected at 20 d postmortem. Whether fed BAA or not, there was no proteolysis detected through 7 d postmortem. However, by 20 d postmortem, a greater amount of degradation of titin, troponin-T, and desmin occurred in the control callipyge lambs. Thus, some postmortem proteolysis occurred in callipyge longissimus, regardless of BAA feeding; however, the proteolysis was minimal and greatly delayed (> 2 wk) relative to non-callipyge lambs. These results are consistent with our previous findings comparing callipyge and non-callipyge lambs (Koohmaraie et al., 1995).

Calpains and Calpastatin. It is now well established that calpain-induced proteolysis of certain myofibrillar proteins is responsible for postmortem tenderization of meat. Furthermore, it is hypothesized that the calpain proteolytic system also plays a key role in muscle protein degradation in living animals (for review see Goll et al., 1989) and perhaps muscle growth and development through calpain-mediated degradation of the transcription factors c-Fos and c-Jun (Hirai et al., 1991; Carillo et al., 1994). With remarkable agreement, results from several laboratories (for review see Koohmaraie et al., 1991) have indicated that feeding BAA to sheep or cattle has the following effects on the components of the calpain proteolytic system: no effect or slight reduction in the activity of μ-calpain, 10 to 20% increase in the activity of m-calpain, and 60 to 100% increase in the activity of calpastatin. The callipyge gene causes similar effects on the components of the calpain system (Koohmaraie et al., 1995). Therefore, it was of interest to examine the effects of BAA on the components of the calpain system in callipyge lambs (Table 6). Whereas Koohmaraie et al. (1991) demonstrated that BAA (4 ppm L-644,969, 6 wk) increased longissimus calpastatin activity by 63% in normal lambs, for these callipyge lambs, BAA (4 ppm L- 644,969, for 6 wk) only increased longissimus calpastatin activity by 23% (Table 6). The results are consistent with the hypothesis that the callipyge gene had increased the calpastatin activity to near maximal levels such that BAA had a limited effect. Furthermore, calpastatin activity remained higher in BAA-fed lambs throughout postmortem storage. With regard to sex effect,

Table 5. Effect of β-agonist treatment and male sex condition on longissimus myofibril fragmentation index, Warner-Bratzler shear force, and sarcomere length

Trait	β-agonist treatment		Male sex condition		SEM
	Control (n = 15)	$L_{644,969}$ (n = 14)	Ram (n = 13)	Wether (n = 16)	
Myofibril fragmentation index					
1 d postmortem	44.3[a]	36.0[b]	39.6	40.7	1.6
7 d postmortem	52.7[a]	37.0[b]	50.7[a]	38.9[b]	3.8
20 d postmortem	60.7[a]	44.9[b]	59.7[a]	45.9[b]	2.7
Warner-Bratzler shear force, kg					
1 d postmortem	11.5	12.3	11.7	12.2	.5
7 d postmortem	10.3	12.0	10.6	11.7	.8
20 d postmortem	8.2[b]	10.9[a]	9.0	10.1	.6
Sarcomere length, μm	1.66	1.62	1.60[b]	1.68[a]	.03

[a,b]Within each row and main effect, means with uncommon superscripts differ ($P < .05$).

wether lambs had higher m-calpain at death and heated calpastatin at 7 and 20 d postmortem. Morgan et al. (1993a,b) reported that castration in beef decreased calpastatin activity at death and at 24 h postmortem.

Nucleic Acids and Protein. Previously, it has been shown that longissimus DNA concentration was greater in bulls than in steers (Morgan et al., 1993a), indicating that greater satellite cell proliferation may occur in intact males. Additionally, we (Koohmaraie et al., 1995) have shown that the callipyge gene increases total muscle RNA, DNA, and protein content. In contrast, Koohmaraie et al. (1991) reported that feeding a BAA to normal lambs increased total biceps femoris RNA and protein content without affecting DNA content. In the present experiment, neither sex condition nor BAA affected nucleic acid or protein parameters (Table 7). Collectively, these studies suggest that there are interactions between callipyge genotype, BAA, and male sex condition on nucleic acid parameters. It would seem that an increase in the capacity for muscle protein synthesis is partially responsible for the increased muscle mass of callipyge lambs.

Muscle Histochemistry. Longissimus from BAA-treated lambs had increased α-white fiber areas (22.2%), but these were not affected by male sex condition (Table 8). The distribution of fiber types was not affected by either BAA treatment or male sex condition in longissimus. This is in contrast to a report by Moody et al. (1980) indicating that longissimus of ram lambs had larger β-red fibers and a higher percentage of β-red fibers and lower percentage of α-red fibers than wether lambs. The fiber characteristics (Table 8) were consistent with the small, nonsignificant changes in longissimus weight due to BAA treatment or male sex condition (Table 3). Psoas major had larger β-red fibers in both BAA-treated lambs (12.5%) and ram lambs (13.8%) than in control or wether lambs, respectively. Ram lambs also had a greater percentage of β-red fibers (20%) than wether lambs (16%). The BAA treatment increased α-white fibers (20.4%) in the psoas major. However, none of these effects resulted in an overall increase in

Table 6. Effect of β-agonist treatment and male sex condition on longissimus activities of the components of the calpain proteolytic system

Trait	β-agonist treatment		Male sex condition		SEM
	Control (n = 15)	$L_{644,969}$ (n = 14)	Ram (n = 13)	Wether (n = 16)	
At death					
μ-calpain activity	.7	.7	.7	.6	.1
m-calpain activity	1.7	1.7	1.5[b]	1.9[a]	.1
Calpastatin activity	3.9	4.8	4.0	4.8	.4
Heated calpastatin activity[c]					
7 d postmortem	2.0[b]	2.4[a]	1.8[b]	2.6[a]	.3
20 d postmortem	1.1[b]	1.5[a]	1.2[b]	1.4[a]	.1

[a,b]Within each row and main effect, means bearing uncommon superscripts differ ($P < .05$).
[c]Heated calpastatin data was converted to column equivalents by using the following equation: column calpastatin activity = .2 + .53 × heated calpastatin activity.

Table 7. Effect of β-agonist treatment and male sex condition on longissimus nucleic acid and protein concentration and abundance

	β-agonist treatment		Male sex condition		
Trait	Control (n = 15)	$L_{644,969}$ (n = 14)	Ram (n = 13)	Wether (n = 16)	SEM
DNA concentration, μg/g	1,256.3	1,182.7	1,267.4	1,171.6	34.9
RNA concentration, μg/g	502.7	497.5	501.2	499.0	18.0
Protein concentration, mg/g	234.9	228.6	238.4	225.2	8.6
DNA content, mg	946.4	920.8	998.3	868.9	47.0
RNA content, mg	376.4	389.7	394.3	371.8	21.5
Protein content, mg	175.6	180.5	188.2	167.9	10.4
RNA:DNA, mg/mg	.40	.42	.39	.43	.01
Protein:DNA, mg/mg	189.1	196.1	190.0	195.2	9.7
Protein:RNA, mg/mg	475.1	463.3	481.6	456.8	21.6

Table 8. Effect of β-agonist treatment and male sex condition on muscle fiber sizes and distributions and apparent fiber number

	β-agonist treatment		Male sex condition		
Muscle and fiber type	Control (n = 15)	$L_{644,969}$ (n = 14)	Ram (n = 13)	Wether (n = 16)	SEM
	Mean fiber area, μm^2				
Longissimus					
β-red	871	944	883	932	76
α-red	1,870	1,890	1,664	2,096	158
α-white	2,101[b]	2,567[a]	2,279	2,389	129
Overall	1,850	2,091	1,874	2,067	117
Psoas major					
β-red	839[b]	944[a]	949[a]	834[b]	36
α-red	1,083	1,120	1,068	1,135	49
α-white	1,268[b]	1,526[a]	1,413	1,380	55
Overall	1,118	1,245	1,170	1,193	54
Semitendinosus					
β-red	1,104	1,155	1,159	1,101	80
α-red	1,732	1,697	1,637	1,793	90
α-white	1,964	2,244	2,083	2,125	104
Overall	1,781	1,926	1,805	1,902	88
	Fiber type distribution, %				
Longissimus					
β-red	17	18	19	17	2
α-red	27	27	27	27	1
α-white	56	55	54	56	2
Psoas major					
β-red	18	18	20[a]	16	1
α-red	40	39	39	40	1
α-white	42	43	41	44	2
Semitendinosus					
β-red	13	13	14	12	1
α-red	30	30	31	29	1
α-white	57	56	55[b]	59[a]	1
	Apparent fiber no.				
Psoas major	342,444	314,534	346,608	310,370	12,801
Semitendinosus	598,449	621,754	625,719	594,484	21,505

[a,b]Within each row and main effect, means with uncommon superscripts differ ($P < .05$).

average fiber size in psoas major for either treatment. Fiber size was not affected by treatment in semitendinosus, but the percentage of α-white fibers was lower in ram than in wether lambs in this muscle (Table 8). Apparent fiber number also tended ($P < .31$) to be greater in semitendinosus for ram lambs. These changes in fiber characteristics may have contributed to the tendency ($P < .08$) for heavier semitendinosus in ram lambs. The most consistent documented change in muscle fiber traits resulting from BAA is increased white fast-twitch fiber areas (Beermann et al., 1987; Kim et al., 1987; Maltin et al., 1990; Wheeler and Koohmaraie, 1992; Vestergaard et al., 1994). The much smaller effect of BAA on muscle fiber traits in these lambs than has been previously reported for BAA is consistent with the smaller effects on muscle growth (Table 3 and Beermann et al., 1987; Kim et al., 1987; Koohmaraie et al., 1991) and was probably because muscle hypertrophy was already near maximum due to the callipyge gene (Koohmaraie et al., 1995) and had little capacity for additional muscle growth.

Though the effects of BAA administration are well defined, the mechanisms through which BAA exerts its effects are not well characterized. Evidence has been presented to support suppression of proteolysis, increased protein synthesis, and both (for review see Bergen and Merkel, 1991; Reeds and Mersmann, 1991). The mode of action of the callipyge gene, which is a recently discovered gene in Dorset sheep, has not been fully characterized. However, the callipyge gene apparently exerts its effect by affecting both mechanisms involved in muscle protein accretion, that is, by decreasing proteolysis and increasing the capacity for protein synthesis (Koohmaraie et al., 1995). The purpose of this experiment was to determine whether BAA treatment and male sex condition, two factors that are known to affect the rate of muscle growth in normal lambs, would further affect the rate of muscle growth in lambs heterozygous for the callipyge gene.

It seems that growth rate of most muscles is maximized in callipyge wethers; however, some muscles exhibit additional growth in response to BAA and testosterone (intact males vs wethers). Those muscles that were affected by BAA and sex condition were muscles whose weight was increased < 21% by the callipyge gene (Koohmaraie et al., 1995). Thus, those muscles had capacity for growth above and beyond that induced by the callipyge gene.

When growth hormone and BAA were administered together, there clearly was an additive effect, suggesting that BAA and growth hormone do not use the same mechanisms to modify growth (Maltin et al., 1990; Bates and Bell, 1991; Link et al., 1991). In the present experiment, BAA and male sex condition had little effect on weight of muscles from callipyge lambs. This could be interpreted to mean either 1) muscles from callipyge lambs were growing at their maximum capacity and, thus, could not be enhanced by any treatment or 2) callipyge, sex condition, and BAA use the same mechanisms to modify growth. The data collected in this experiment do not allow us to confirm or refute either of these two possibilities.

Although control lambs had very low MFI values, BAA further decreased postmortem proteolysis. Concomitantly, although control lambs had very high (8.2 kg) Warner-Bratzler shear force values at 20 d postmortem, BAA administration resulted in even greater shear values. These results indicate that the decline in longissimus tenderness due to the callipyge gene was not as close to maximum as was muscle growth.

Implications

Muscle growth in callipyge wethers was not enhanced by either sex condition or dietary administration of β-adrenergic agonist (L-644,969). Thus, use of intact males and β-adrenergic agonists would not greatly improve muscle growth or carcass composition of callipyge lambs. In order for the industry to fully benefit from this germplasm, meat tenderizing technologies must be used.

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