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Effects over Time of Feeding a β -Adrenergic Agonist to Wether Lambs on Animal Performance, Muscle Growth, Endogenous Muscle Proteinase Activities, and Meat Tenderness^{1,2}

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ABSTRACT: Forty wether lambs were used in a 2×4 factorial arrangement to determine the response of animal performance, muscle growth, proteinase activity, and meat tenderness to β -adrenergic agonist (BAA) supplementation. Lambs were fed a finishing diet with or without 4 ppm of L_{644,969} and slaughtered after 0, 2, 4, and 6 wk of treatment. The ADG was higher ($P < .05$) in the treated than in the control lambs after 2 wk and returned to control levels thereafter. Semitendinosus weight and calpastatin activity were higher and μ -calpain activity was lower in the treated than in the control lambs after 2, 4, and 6 wk. Cathepsin B activity was higher ($P < .01$) and cystatin-like activity was lower ($P < .05$) after 2 wk in treated than in control lambs but returned to control levels thereafter. Longissimus protein:DNA was higher after 4 ($P < .05$) and 6 ($P < .01$) wk in the

treated lambs than in the controls. The concentration of RNA and RNA:DNA ratio were higher ($P < .01$) in the longissimus and semitendinosus muscles in the treated lambs after 2 wk and remained higher throughout the study. Semitendinosus protein and RNA content were higher after 2, 4, and 6 wk and DNA content was higher after 2 and 6 wk in the treated than in the control lambs. Longissimus shear-force values were higher ($P < .001$) in the treated than in the control lambs at all slaughter end points. These data indicate a rapid alteration of muscle growth, activity of the calpain-calpastatin system, and meat tenderness during BAA treatment. It also seems that the BAA-stimulated muscle growth is closely related to the activity of the calpain-calpastatin system.

Key Words: Sheep, β -Adrenergic Agonist, Muscle, Proteinases, Meat Quality

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Introduction

Protein turnover involves two cellular processes, protein synthesis and degradation. Protein turnover decreases an animal's growth efficiency, because of energy lost during the breakdown and resynthesis of proteins. These processes are inefficient but necessary for maintaining available pools of amino acids, repairing erroneous proteins, and removing proteins not properly incorporated into their subcellular locations (Reeds, 1989). Young et al. (1975) suggested that 15

to 25% of the energy consumption of growing animals was used for protein turnover. Thus, a better understanding of the regulation of in vivo protein degradation could allow large alteration of animal growth efficiency and consequently reduce the cost of production.

The β -adrenergic agonists have been shown to increase muscle accretion and decrease fat accretion (Yang and McElligott, 1989). These compounds have been reported to regulate both protein synthesis (Maltin et al., 1987; Helferich et al., 1990) and protein degradation (Reeds et al., 1986; Bohorov et al., 1987). In most cases, the greatest effect is on protein degradation. Recently, the endogenous proteinases (the calpain system and the cathepsins) have been studied during β -adrenergic-agonist supplementation (Forsberg et al., 1989; Kretchmar et al., 1990; Koohmaraie et al., 1991). Most of the data indicate that activity of calpastatin, the endogenous inhibitor of μ - and m-calpain, increases substantially during the treatment period. Although data from laboratory animals have shown short-term responses to

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Table 1. Dry matter composition of the diet

Ingredient	% DM
Corn	64.83
Alfalfa hay	20.00
Molasses	6.00
Soybean meal (60% CP)	7.94
Limestone	.82
Dicalcium phosphate	.06
Salt	.30
Vitamin premix ^a	.02
Sheep trace mineral premix ^b	.03

^aVitamin A, 15,000 IU/g; vitamin D, 3,000 IU/g; vitamin E, 3.75 IU/g.

^b77.7% soyhulls; 12.3% MnSO₄; 7.0% ZnCO₃; 3.0% oil or fat.

β -adrenergic agonists (Reeds et al., 1986; Maltin et al., 1987), most of the data collected in livestock have been over long time periods. Therefore, this study was designed to investigate the response time of muscle growth, endogenous proteinase activity, and meat tenderness to β -adrenergic-agonist treatment in wether lambs.

Experimental Procedures

Experimental Design. Forty wether lambs (1/2 Finnsheep, 1/4 Dorset, 1/4 Ramboillet) with an average weight of $35.2 \pm .6$ kg were assigned to a factorial arrangement with two dietary treatments (control and treated) and four treatment periods (0, 2, 4, and 6 wk). The lambs were ranked by weight, divided into five groups of eight, and randomly assigned to the eight treatments. The control diet was a corn, alfalfa hay diet containing 14% CP (Table 1). The treated diet consisted of 4 ppm of the β -adrenergic agonist L_{644,969} (Merck, Sharp and Dohme Research Laboratories, Rahway, NJ) added to the control diet. Daily feed consumption and biweekly live weights were recorded. The lambs were weighed on three consecutive days to start the test. After the assigned treatment period, the lambs were weighed, transported to the University of Nebraska Meat Laboratory, and slaughtered. Within 45 min postmortem, the longissimus muscle was removed from one randomly chosen side and sampled for determination of activity of the calpain system and cathepsins B and L as well as concentrations of DNA, RNA, and protein. Within 1 to 2 h postmortem, the semitendinosus muscle from the same side was removed intact, weighed, and sampled for determination of DNA, RNA, and protein concentration.

The longissimus muscle from the other side of the carcass was removed after it had been stored for 48 h at 4°C and divided in half, and the halves were randomly assigned to either 2 or 7 d of aging. A 5-g slice of the loin was removed for sarcomere length determination and stored at -20°C. The loin halves

were vacuum-packaged and stored at 4°C until completion of the assigned aging time. The loin halves were then frozen at -35°C and stored at -20°C until shear force was measured.

Determination of Calpain Activity. Activity of μ -calpain, m-calpain, and calpastatin were determined on unfrozen, prerigor muscle according to the procedures of Koohmaraie (1990), with some slight modifications. Longissimus muscle samples (50 g) were homogenized in 2.5 volumes of extraction buffer (100 mM Tris, 5 mM EDTA, and 10 mM β -mercaptoethanol [MCE], pH 8.3). The samples were then centrifuged, filtered, and dialyzed overnight in dialysis buffer (20 mM Tris, 5 mM EDTA, 10 mM β -MCE, pH 7.5). After dialysis, the samples were loaded onto anion exchange (2-[diethylamino] ethyl ether [DEAE]-Sephacel; Sigma Chemical, St. Louis, MO) columns and washed with three volumes of elution buffer (20 mM Tris, .1 mM EDTA, and 10 mM β -MCE, pH 7.35). The samples were then eluted using a linear NaCl gradient (0 to 400 mM), and 130 fractions (6 mL) were collected. Activity of the proteinases and the inhibitor were determined using a casein solution (100 mM Tris, 1 mM NaN₃, 5 mM CaCl₂, 1 μ L/mL of β -MCE, and 5 mg/mL of casein, pH 7.5). One unit of proteinase activity was defined as the amount of enzyme necessary to cause an increase of 1.0 optical density unit at 278 nm. One unit of inhibitor activity was defined as the amount of calpastatin necessary to inhibit one unit of DEAE-purified m-calpain activity.

Cathepsin B and L and Cystatin-like Activity. Activity of the cathepsins and their inhibitor were determined on frozen muscle samples according to method D of Koohmaraie and Kretchmar (1990). Longissimus samples (5 g) were homogenized in seven volumes of extraction buffer (50 mM sodium acetate, 1 mM EDTA, 100 mM NaCl, and .2% Triton X-100, pH 5.0). After homogenization, the samples were centrifuged and filtered and 2 mL of the extracts was passed through a papain-Sepharose affinity column to remove the cystatins, endogenous inhibitors of the cathepsins. The samples, pre- and postcolumn, were assayed using synthetic substrates (cathepsin B, Z-Arg-Arg-NMec; cathepsin B + L, Z-Phe-Arg-NMec; Bachem Fine Chemicals, Torrance, CA). The substrate used for cathepsin L is also a substrate for cathepsin B; therefore, the specific activity is reported as B + L. Specific activity of the cathepsins was reported as the nanomoles of NMec released·minute⁻¹·gram of tissue. Cystatin-like activity was reported as the ratio of post:precolumn cathepsin B + L activity.

Nucleic Acid and Protein Concentrations. Concentrations of DNA were determined fluorometrically using Hoechst 33258 (Sigma Chemical; Labarca and Paigen, 1980). Concentrations of RNA were measured spectrophotometrically using the procedures of Munro and Fleck (1969), and protein concentrations were

Table 2. Changes in average daily gain, hot carcass weight, dressing percentage, and longissimus cathepsin B, B + L, and cystatin-like activity of lambs after β -adrenergic agonist (BAA) treatment

Trait	Time on feed, wk			
	0	2	4	6
Average daily gain, kg				
Control	—	.23 \pm .02**	.25 \pm .02	.11 \pm .03
BAA ^a	—	.34 \pm .02	.26 \pm .02	.14 \pm .03
Hot carcass wt, kg				
Control ^a	20.2 \pm 1.2	21.8 \pm 1.2	24.1 \pm 1.2	25.2 \pm 1.2
BAA ^a	20.2 \pm 1.2	23.3 \pm 1.2	25.6 \pm 1.2	27.4 \pm 1.2
Dressing percentage, %				
Control	57.1 \pm .9	55.2 \pm .9**	56.9 \pm .9**	57.1 \pm 1.2**
BAA ^a	56.9 \pm .9	58.6 \pm .9	61.5 \pm .9	61.6 \pm .9
Cathepsin B, nmol NMec·min ⁻¹ ·g ⁻¹				
Control	73.2 \pm 7.0	69.9 \pm 7.0**	74.7 \pm 7.0	72.5 \pm 7.0
BAA ^b	88.8 \pm 7.0	118.7 \pm 7.0	84.7 \pm 7.0	81.3 \pm 7.0
Cathepsin B + L, nmol NMec·min ⁻¹ ·g ⁻¹				
Control	123.5 \pm 9.0	135.4 \pm 9.0	143.3 \pm 9.0	135.1 \pm 9.0
BAA	136.5 \pm 9.0	154.1 \pm 9.0	142.9 \pm 9.0	133.4 \pm 9.0
Cystatin-like activity ^c				
Control	2.75 \pm .19	2.63 \pm .19*	2.88 \pm .19	2.85 \pm .19
BAA	2.42 \pm .19	2.01 \pm .19	2.51 \pm .19	2.51 \pm .19

^aLinear effect, $P < .01$.

^bQuadratic effect, $P < .05$.

^cSee text for description.

*, **Means within a trait, at a specific time on feed, differ, $P < .05$ and $.01$, respectively.

determined using the biuret procedure of Gornall et al. (1949).

Determination of Sarcomere Length. Sarcomere lengths were measured according to the procedure of Cross et al. (1981), modified for use with a Zeiss microscope (LEP, Scarsdale, NY) attached to a BioQuant System VI digitizer (Houston Instrument, Austin, TX). Longissimus samples (5 g) were homogenized in eight volumes of 250 mM sucrose and measurements recorded for 10 adjacent sarcomeres on each of 10 different myofibrils.

Determination of Warner-Bratzler Shear Force. Loin roasts used for Warner-Bratzler shear (WBS) force determination were thawed at 4°C for 24 h and oven-roasted to an internal temperature of 70°C. Internal temperatures were monitored using copper-constantan thermocouples. Six to eight cores (1.3 cm) were removed randomly from each sample, parallel to the longitudinal orientation of the fiber. Cores were sheared once using the WBS blade attached to an Instron Universal Testing Machine (Model 1123, Instron, Canton, MA).

Statistical Analyses. The data were analyzed using ANOVA for a completely randomized design (Steel and Torrie, 1980). The treatment means for animal performance, muscle weight, proteinase activity, nucleic acid and protein concentration, and sarcomere length of treated and control lambs at specific slaughter end points were separated using single df

contrasts. The WBS means for treated and control loin portions at specific slaughter end points and at specific aging times were separated using single df contrasts. The means for 2 and 7 d of aging at specific slaughter end points in treated and control loin portions were also separated using single df contrasts. The data are presented as means \pm SEM. Different values for SE within a trait are due to differences in the number of observations for means.

Results

Animal Performance and Muscle Growth. Average daily gain was higher ($P < .05$) after 2 wk of treatment and returned to control levels thereafter (Table 2). Daily feed intake and feed efficiency were not affected ($P > .30$ and $> .45$, respectively) by BAA treatment (data not shown). Hot carcass weight was numerically higher ($P > .10$; Table 2) and backfat was numerically lower ($P > .10$; data not shown) in the BAA lambs than in control lambs. In accordance with most BAA studies, dressing percentage was increased by 6% after 2 wk ($P < .05$) and by 8% after 4 and 6 wk ($P < .05$) of BAA treatment. Muscle accretion, indicated by semitendinosus weight (Figure 1), was higher in BAA-treated lambs than in control lambs after 2 wk ($P < .05$) and remained higher after 4 and 6 wk ($P < .01$) of BAA supplementation. This

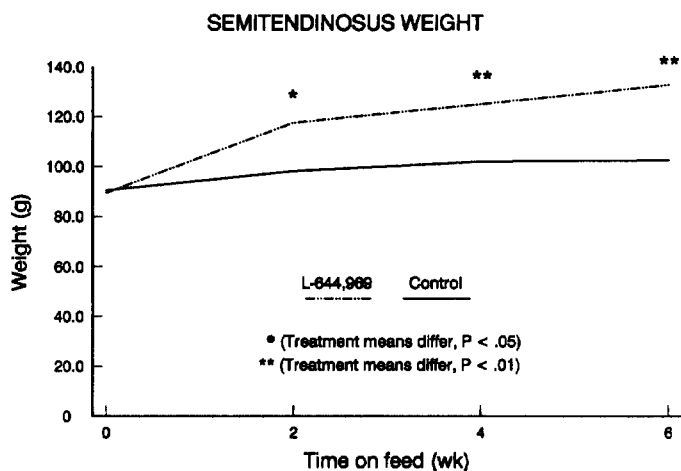


Figure 1. The response of semitendinosus weight to β -adrenergic agonist feeding in lambs.

indicates that the increased growth rate of the BAA-treated lambs is primarily due to increased muscling.

Calpains and Calpastatin Activities. Activity of m-calpain was not significantly affected ($P > .60$) by BAA treatment (data not shown). Activity of μ -calpain was significantly lower after 2 and 6 wk of BAA treatment and tended to be lower ($P < .20$) after 4 wk of treatment (Figure 2). Conversely, calpastatin activity was 73% higher in BAA-treated lambs than in control lambs after 2 wk ($P < .01$) and remained significantly higher throughout the study (Figure 3). Collectively, these data indicate a decrease in the proteolytic capacity of BAA-treated muscle; the greatest response occurred within 2 wk.

Cathepsins B and B + L and Cystatin-like Activities. Cathepsin B activity was higher ($P < .01$) and cystatin-like activity was lower ($P < .05$) in BAA-treated lambs than in control lambs after 2 wk and

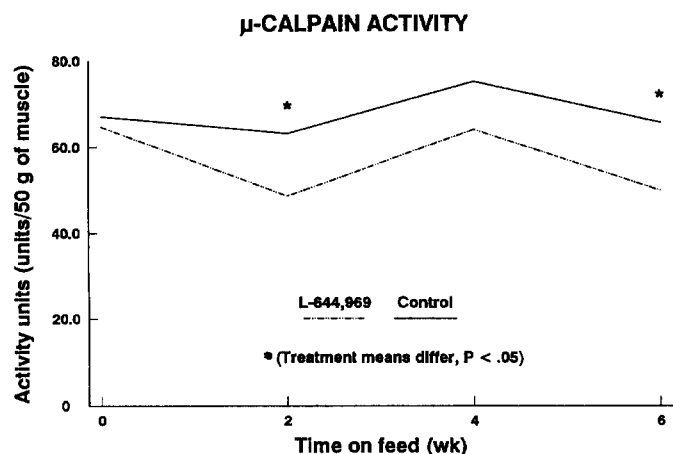


Figure 2. The response of longissimus muscle μ -calpain activity to β -adrenergic agonist feeding in lambs.

returned to control levels after 4 wk (Table 2). Cathepsin B + L activities did not differ ($P > .25$) between treated and control lambs (Table 2). Thus, it seems unlikely that the increased muscle accretion (Figure 1) of the treated lambs was due to altered activity of lysosomal proteinases.

Longissimus Nucleic Acids and Protein Concentrations. The protein:DNA ratio (Table 3), an indicator of cell mass or size, was greater in the BAA-treated than in the control lambs after 2, 4 ($P < .05$), and 6 wk ($P < .01$). Longissimus protein concentration increased linearly in both the treated ($P < .05$) and control ($P < .10$) lambs but did not differ significantly at any slaughter end point. The increased protein:DNA ratio in the treated lambs is consistent with previous data on BAA treatment (Kim et al., 1987; Koochmarai et al., 1991) and agrees with the theory that BAA-stimulated muscle growth is hypertrophic.

Longissimus RNA concentrations in the control lambs decreased progressively during the 6-wk feeding trial (Table 3). The RNA concentrations in the BAA-treated lambs were 21% higher than in the control lambs after 2 wk ($P < .01$) and remained higher throughout the study. Longissimus RNA:DNA ratio, an indicator of transcriptional activity, exhibited the same pattern of changes as RNA concentration during the study. Conversely, the protein:RNA ratio, an indicator of translational activity, decreased to a value less than that in control lambs after 2 wk ($P < .05$) of BAA treatment and remained lower through 4 and 6 wk (Table 3).

Semitendinosus Nucleic Acid and Protein Concentrations and Contents. Unlike the longissimus muscle, the semitendinosus protein:DNA ratio was not significantly affected ($P > .5$) by BAA treatment (Table 4). Likewise, BAA treatment did not affect ($P > .5$) the protein concentration (Table 4). Changes in the RNA concentration of the semitendinosus muscle were similar to those in the longissimus (Table 4); RNA

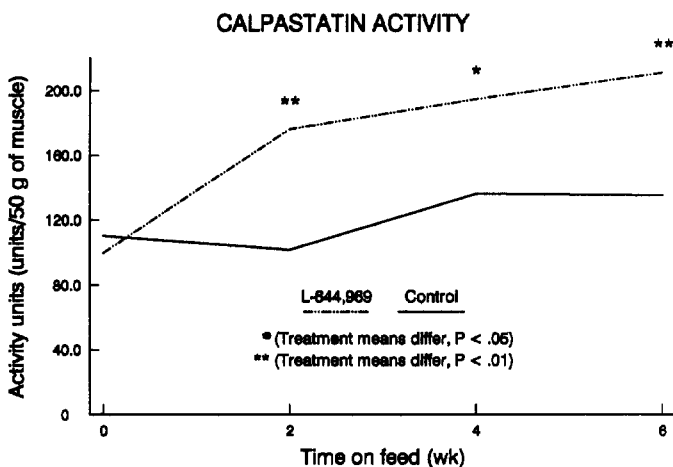


Figure 3. The response of longissimus muscle calpastatin activity to β -adrenergic agonist feeding in lambs.

Table 3. Changes in longissimus protein:DNA ratio, protein and RNA concentration, and RNA:DNA and protein:RNA ratios of lambs after β -adrenergic agonist (BAA) treatment

Trait	Time on feed, wk			
	0	2	4	6
Protein:DNA, mg/mg				
Control	252.8 \pm 9.0	248.5 \pm 9.0	256.2 \pm 9.0*	256.3 \pm 9.0**
BAA ^a	257.5 \pm 10.1	266.1 \pm 11.7	282.2 \pm 9.0	294.7 \pm 9.0
Protein, mg/g of tissue				
Control	253.1 \pm 5.6	248.5 \pm 5.6	258.4 \pm 5.6	266.6 \pm 5.6
BAA ^b	257.8 \pm 6.3	255.8 \pm 7.3	270.2 \pm 5.6	274.3 \pm 5.6
RNA, mg/g of tissue				
Control ^a	653.2 \pm 23.0	592.9 \pm 23.0**	585.5 \pm 23.0**	567.1 \pm 23.0*
BAA ^c	656.6 \pm 23.0	715.6 \pm 25.7	669.6 \pm 23.0	639.1 \pm 23.0
RNA:DNA, mg/mg				
Control ^a	.65 \pm .02	.59 \pm .02**	.58 \pm .02**	.54 \pm .02**
BAA ^d	.65 \pm .02	.75 \pm .03	.70 \pm .02	.69 \pm .02
Protein:RNA, mg/mg				
Control ^a	388.5 \pm 18.9	422.1 \pm 18.9*	445.9 \pm 18.9	471.6 \pm 18.9
BAA ^b	382.6 \pm 21.1	357.9 \pm 24.3	408.6 \pm 18.9	430.0 \pm 18.9

^aLinear effect, $P < .01$.

^bLinear effect, $P < .05$.

^cQuadratic effect, $P < .10$.

^dQuadratic effect, $P < .05$.

*, **Means within a trait, at a specific time on feed, differ at $P < .05$ and $.01$, respectively.

concentration in the control lambs decreased progressively during the feeding period, whereas RNA concentration in the BAA-fed lambs increased after 2 wk and then decreased to levels found in the 0-wk treated lambs. As previously noted in the longissimus muscle, the semitendinosus RNA:DNA ratio was higher in the treated animals than in the control animals after 2 (P

$< .05$) and 4 ($P < .01$) wk. However, in contrast to the longissimus data, semitendinosus RNA:DNA ratio in BAA-fed lambs was similar to controls after 6 wk of treatment (Table 3 and 4). Although lower at all end points, the protein:RNA ratio of the semitendinosus muscle was only significantly lower ($P < .10$) after 4 wk of BAA treatment (Table 4).

Table 4. Changes in semitendinosus protein:DNA ratio, protein and RNA concentration, and RNA:DNA and protein:RNA ratios of lambs after β -adrenergic agonist (BAA) treatment

Trait	Time on feed, wk			
	0	2	4	6
Protein:DNA, mg/mg				
Control	290.2 \pm 15.6	301.0 \pm 15.6	276.2 \pm 17.5	333.2 \pm 15.6
BAA	279.8 \pm 15.6	306.2 \pm 17.5	328.5 \pm 15.6	311.5 \pm 17.5
Protein, mg/g of tissue				
Control	251.2 \pm 8.4	252.3 \pm 8.4	253.0 \pm 8.4	266.6 \pm 8.4
BAA ^a	246.3 \pm 8.4	257.0 \pm 9.4	256.9 \pm 8.4	275.6 \pm 8.4
RNA, mg/g of tissue				
Control ^b	563.9 \pm 20.0	507.2 \pm 17.9**	491.1 \pm 17.9*	476.9 \pm 17.9*
BAA ^c	537.5 \pm 17.9	581.8 \pm 17.9	548.6 \pm 17.9	528.0 \pm 17.9
RNA:DNA, mg/mg				
Control	.66 \pm .04	.60 \pm .03*	.56 \pm .04**	.60 \pm .03
BAA ^d	.61 \pm .03	.70 \pm .03	.70 \pm .03	.61 \pm .04
Protein:RNA, mg/mg				
Control ^b	445.7 \pm 21.9	497.5 \pm 19.6	519.7 \pm 19.6 [†]	561.4 \pm 19.6
BAA ^a	459.1 \pm 19.6	449.5 \pm 21.9	470.0 \pm 19.6	522.3 \pm 19.6

^aLinear effect, $P < .05$.

^bLinear effect, $P < .01$.

^cQuadratic effect, $P < .10$.

^dQuadratic effect, $P < .01$.

[†], *, **Means within a trait, at a specific time on feed, differ at $P < .10$, $.05$, and $.01$, respectively.

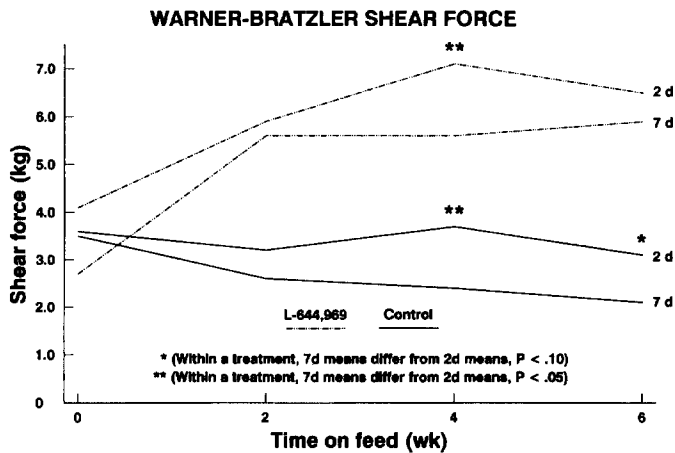


Figure 4. The response of Warner-Bratzler shear force to β -adrenergic agonist feeding and subsequent aging in lamb longissimus muscle. Within a time period, $L_{644,969}$ means were higher ($P < .001$) than controls.

Nucleic acid and protein contents were calculated by multiplying the concentrations of each by the weight of the semitendinosus muscle. Although the concentrations of DNA were lower in the BAA-treated animals than in the controls (data not shown), the total amount of DNA (Table 5) was higher in the treated lambs after 2 wk ($P < .10$) and 6 wk ($P < .01$). Similar data have been reported in lambs (Beermann et al., 1987) and rabbits (Forsberg et al., 1989); however, Koohmaraie et al. (1991) and Kim et al. (1987) reported a decrease in the concentrations of DNA in treated animals without a change in the total amount of DNA. Because of the combined responses in muscle weight and RNA and protein concentrations, the RNA and protein content of the semitendinosus muscle in BAA-treated lambs was higher ($P < .05$) than in control lambs at all end points.

Longissimus Tenderness and Aging. The Warner-Bratzler shear force values for longissimus muscle aged 2 d (**WBS2**) and 7 d (**WBS7**) are presented in Figure 4. Both the WBS2 and WBS7 values were higher ($P < .001$) in the treated lambs than in the control lambs after 2, 4, and 6 wk. Sarcomere length was not affected ($P > .25$) by BAA treatment (data not shown). The tenderness data agree with the findings of Hamby et al. (1986), Miller et al. (1988), Morgan et al. (1989), and Koohmaraie and Shackelford (1991), who also found that BAA treatment decreased muscle tenderness. Our data also concur with the observation of Merkel (1988) that the decreased tenderness in BAA-treated animals could not be explained by muscle shortening.

The response to aging, indicated by the difference between WBS2 and WBS7, varied with treatment (Figure 4). There were no significant differences between WBS2 and WBS7 in the BAA-treated lambs after 2 and 6 wk of treatment; however, after 4 wk of BAA treatment WBS7 was 21% lower ($P < .05$) than WBS2. This was due mainly to an increase in the WBS2 value at that end point and not to a reduction in WBS7. In the control lambs, WBS7 was 35 and 32% lower than WBS2 after 4 ($P < .05$) and 6 ($P < .10$) wk of the study, respectively. The decreased response to aging in BAA-treated lambs has also been reported by Kretchmar et al. (1990), Koohmaraie and Shackelford (1991), and Koohmaraie et al. (1991).

Discussion

The effects of BAA on animal performance vary. In our study, $L_{644,969}$ supplementation increased ADG during the first 2 wk of treatment and had no effect thereafter. Other studies have shown early increases in ADG (Beermann et al., 1986; Moloney et al., 1990), longer-term effects on ADG (Kim et al., 1987;

Table 5. Changes in DNA, RNA, and protein content of lamb semitendinosus muscle after β -adrenergic agonist (BAA) treatment

Trait	Time on feed, wk			
	0	2	4	6
DNA, mg				
Control	78.3 \pm 6.1	82.7 \pm 6.1 [†]	89.3 \pm 6.1	82.8 \pm 6.1**
BAA ^a	78.9 \pm 6.1	98.5 \pm 6.1	98.6 \pm 6.1	113.7 \pm 6.8
RNA, mg				
Control	50.7 \pm 4.2	49.7 \pm 3.7**	50.2 \pm 3.7**	48.9 \pm 3.7**
BAA ^{ab}	48.7 \pm 3.7	68.3 \pm 3.7	68.3 \pm 3.7	70.3 \pm 3.7
Protein, g				
Control	22.9 \pm 1.8	24.8 \pm 1.8*	26.0 \pm 1.8*	27.3 \pm 1.8**
BAA ^a	22.0 \pm 1.8	31.0 \pm 2.1	31.9 \pm 1.8	36.7 \pm 1.8

^aLinear effect, $P < .01$.

^bQuadratic effect, $P < .05$.

[†], *, **Means within a trait, at a specific time on feed, differ at $P < .10$, $.05$, and $.01$, respectively.

Anderson et al., 1989; Koohmaraie et al., 1991), and no BAA effect on ADG (Bohorov et al., 1987; Claeys et al., 1989). The varied response in ADG may be due to the type and dose of BAA, animal age, or the length of treatment. Beermann et al. (1986), Claeys et al. (1989), and Williams et al. (1989) have all reported that young lambs (< 20 kg) do not show a response in ADG or feed/gain during BAA treatment. This may be caused by a lower number of β -adrenergic receptors (BAR) or by a reduction in the ability to stimulate postreceptor events upon binding of the BAA to the BAR (for review see Stiles et al., 1984).

The most consistent response noted in BAA-treated animals is an increase in the weight of various muscles (Yang and McElligott, 1989), with the most prominent increase in muscles dominated by Type II fibers (Beermann et al., 1987). Based on the results of this study and other studies using lambs (Wang and Beermann, 1988) and rats (Reeds et al., 1986), it seems that BAA administration has its greatest effect on muscle growth during the first 2 to 3 wk of treatment. This muscle accretion could occur from increased protein synthesis, as reported for pigs fed ractopamine (Helferich et al., 1990) and rats fed clenbuterol and fenoterol (Emery et al., 1984) or from decreased protein degradation, as reported for lambs (Bohorov et al., 1987) and rats (Reeds et al., 1986) fed clenbuterol. Alternatively, Anderson et al. (1991) suggested a biphasic response of muscle to BAA treatment, whereby protein degradation was initially reduced followed by increased protein synthesis with chronic treatment.

Administration of BAA has been reported to decrease the proteolytic capability of the muscle through alteration of the calpain-calpastatin complex (Higgins et al., 1988; Wang and Beermann, 1988; Forsberg et al., 1989; Kretchmar et al., 1989; Koohmaraie et al., 1991). We found that the greatest changes in activity of this system occurred during the first 2 wk of treatment and involved a decrease in μ -calpain activity, an increase in calpastatin activity, and no change in m-calpain activity. The similarity between the response of calpastatin activity (Figure 3) and muscle weight (Figure 1) suggests that an increase in calpastatin activity may be related to BAA-induced muscle growth.

The results from BAA treatment on the cathepsins vary, with reports of increased activity (McElligott et al., 1987; Koohmaraie et al., 1991), decreased activity (Kretchmar et al., 1989; Morgan et al., 1989; Béchet et al., 1990), or no change in activity (Forsberg et al., 1989). In our study, activity of the catheptic system increased, suggesting an increase in the proteolytic capability of the longissimus muscle (Table 2). Although the role of the catheptic proteinases in skeletal muscle turnover is unknown, the increased cathepsin activity in BAA-treated animals in the present study did not prevent an increased rate of net muscle protein accumulation. Goll et al. (1989) suggested a role for this system in the control of sarcoplasmic protein turnover.

Most researchers have proposed a hypertrophic model for BAA-induced muscle growth (Beermann et al., 1987; Kim et al., 1987; Koohmaraie et al., 1991), as opposed to models for compensatory muscle growth (Beermann, 1983) or somatotropin-induced muscle growth (for review see Boyd and Bauman, 1989). Our longissimus data are consistent with these findings. The semitendinosus data (Table 4) also agree with the hypertrophic model of growth up to 4 wk of treatment. However, after 6 wk of treatment, the semitendinosus muscle seemed to grow through some mechanism other than strict hypertrophy. Although the incorporation of satellite cells or hyperplastic muscle growth is not consistent with most of the BAA data, it has been suggested as a possibility in rabbits fed cimaterol (Forsberg et al., 1989) and may partially explain the long-term differences noted in semitendinosus weight.

In agreement with previous studies (Beermann et al., 1987; Williams et al., 1989; Koohmaraie et al., 1991), the present study finds that transcriptional activity (RNA:DNA ratio) is higher in muscle from BAA-fed animals than in muscle from control animals. There was attenuation in the transcriptional activity of muscle from BAA-treated lambs after 4 and 6 wk. Attenuation to BAA treatment has been reported previously (Eisemann et al., 1988; Yang and McElligott, 1989) and may be due to down-regulation of the BAR. Kim et al. (1992) reported this phenomenon in rat skeletal muscle, noting a 26.8% decrease in the number of β -adrenergic binding sites with 3 d of BAA treatment. Our data are consistent with the fact that translational activity, protein:RNA ratio, of muscle is generally not altered by BAA treatment. Collectively, the nucleic acid and protein data in our study and previous studies suggest that BAA treatment increases the protein synthetic capacity of muscle. As suggested by Anderson et al. (1991), our data imply that the decreased muscle protein degradation, due to changes in the calpain/calpastatin complex, coupled with the increased muscle protein synthetic capacity may account for the lean growth of BAA-treated animals.

Our tenderness data agree with previous findings (Morgan et al., 1989; Koohmaraie and Shackelford, 1991; Koohmaraie et al., 1991) that the meat from BAA-treated animals is consistently tougher than untreated meat and responds less favorably to aging. Responses of shear force (Figure 4) and calpastatin activity (Figure 3) were similar, and elevated calpastatin activity has been implicated in BAA-induced toughening of meat (Koohmaraie et al., 1991). These data imply that the increase in calpastatin activity may be related to both the decreased tenderness and decreased response to aging in meat from BAA-treated animals.

Because this study monitored the effects of BAA administration over a 6-wk period, it was possible to

show the rapid response and subsequent attenuation elicited by these compounds. Measurable changes occur in muscle weight and metabolism within 2 wk of treatment. Also, there seems to be a differential response of the longissimus and semitendinosus muscles to BAA treatment. Kim et al. (1987) and Beermann et al. (1987) reported that muscle fiber type influenced the response of muscle to BAA treatment. They noted a larger growth in the Type II (glycolytic) fibers than in the Type I (oxidative) fibers, which may explain some of the differential responses in the two muscles measured in this study.

Implications

This study shows that the β -adrenergic agonists may act on the calpain-calpastatin system to stimulate muscle growth through a decrease in protein degradation. Decreased protein degradation has been suggested as the most efficient means of increasing muscle protein accretion. Therefore, a better understanding of the mechanisms that control these proteinases and protein degradation may allow improvement of animal growth efficiency.

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