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Bovine Somatotropin Alters Myosin Heavy Chains and Beta Receptors in Skeletal Muscle of Feedlot Heifers with Little Impact on Live or Carcass Performance

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Abstract: The objective was to determine whether recombinant bovine somatotropin (rbST) enhanced live performance, skeletal muscle biological activity, and beta-adrenergic receptor expression of feedlot heifers during the finishing phase. Heifers ($n = 16$; initial body weight = 457 ± 3 kg) were randomly assigned to pens (4 pens/treatment; 2 heads/pen) and treatment: (1) no rbST (Control); (2) 500 mg/hd of sometribove zinc at day 0 and 14 (rbST; Posilac®; Elanco Animal Health, Greenfield, IN). *Longissimus* muscle biopsies for muscle chemistry were collected on day 0, 14, 28, 42, and 56. The rbST heifers had increased expression of AMP-activated protein kinase alpha and beta 3 adrenergic receptor ($P < 0.05$). Day of the study affected the expression of myosin heavy chain-IIA (*MHC-IIA*), *MHC-IIX*, beta 2 adrenergic receptor, peroxisome proliferator-activated receptor gamma, and stearoyl-CoA desaturase ($P < 0.05$). Day had a significant effect on muscle fiber cross-sectional area and proportion ($P < 0.05$). As days on feed increased, the area of MHC-I fibers decreased whereas MHC-IIA and IIX area increased ($P < 0.05$). The rbST heifers had decreased proportions of MHC-I fibers and increased proportions of MHC-IIX fibers ($P < 0.05$). The greatest density of Paired Box 7-positive cells was on day 0, 28, and 42 ($P < 0.05$), and the greatest density of Myogenic factor 5-positive cells was on day 42 and 56 ($P < 0.05$). Also, the greatest density of cells positive for Paired Box 7:Myogenic factor 5 was measured on day 28 ($P < 0.05$). These data indicate that, as days on feed increase, the effects of skeletal muscle biological activity are not dependent on rbST administration but may be more due to physiological changes occurring as the animal reaches physiological maturity.

Key words: β-adrenergic receptor, myosin heavy chain, recombinant bovine somatotropin
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Introduction

Somatotropin is a hormone produced in the hypothalamus by somatotrophs and lactosomatotrophs that is secreted into the anterior lobe of the pituitary gland and that functions in a myriad of physiological processes, including the regulation of growth (Baumen, 1992). Several studies have reported increases in catecholamine-induced lipolysis and increased numbers of beta-adrenergic receptors (βAR) in adipocytes administered growth hormone (GH) (Watt et al., 1991; Kamel et al., 2000; Yang et al., 2004). Watt et al. (1991) found that, in sheep adipose tissue, chronic exposure to GH increased the response and sensitivity to beta-adrenergic agonists (βAA), reporting that the response is partly due to increased ligand binding by βAR because a saturating concentration of the ligand was used increasing the number of βAR per adipocyte. To further complement this, lipolysis was increased in GH-treated rat adipocytes partly through βAR function and having increased numbers of beta 1

adrenergic receptor (β1AR) and beta 3 adrenergic receptor (β3AR) (Yang et al., 2004). Kamel et al. (2000) reported that the lipolysis effect of GH is related to the stimulatory effect of beta 2 adrenergic receptor (β2AR) in adipocytes.

Bovine somatotropin (bST) was originally approved for use in the dairy industry in 1993 to increase the production of marketable milk (FDA, 1993). Studies have reported that bST stimulates growth in immature cattle, improves average daily gain (ADG) (Sandles and Peel, 1987; Vestergaard et al., 1995), and improves feed efficiency (Grings et al., 1990; Maltin et al., 1990; Dalke et al., 1992; Moseley et al., 1992). Early et al. (1990a, 1990b) reported that weight gains of steers administered 20.6 mg/d recombinant bST (rbST) were primarily accrued by noncarcass components. However, Vestergaard et al. (1995) reported increased carcass weights and decreased fat trim of heifers administered 15 mg/d rbST for 15 wk. Dalke et al. (1992) reported an increase in percent protein and a decrease in percent fat from the 9-10-11 rib section of steers as a weekly dose of rbST increased in concentration.

These studies evaluating the effects of rbST on growth and carcass performance in cattle were done before the approval of βAA. βAA were not approved for use in cattle until 2003. Studies that have examined the effect of GH on βAR response have focused on lipolysis in adipocytes and not on the effects that GH may have on βAR in skeletal muscle. While we know that bST administration positively affects growth and carcass performance, we have not fully elucidated the effects of rbST on muscle biological activity and skeletal muscle βAR response throughout the finishing phase. Based on previous studies, we hypothesize that rbST may positively interact with βAR within the skeletal muscle possibly increasing the number of βAR and their sensitivity to βAA. This study differs from previous studies as rbST was only administered twice at a concentration of 500 mg/head. Furthermore, muscle growth and performance were monitored throughout the finishing phase. The original design of the study was to administer rbST 56 and 42 d prior to βAA supplementation to boost the expression of the βAR, increasing the efficacy of the βAA. Unfortunately, we did not receive approval to supplement with a βAA after the heifers received rbST, so we could not carry out that part of the study. In addition, rbST was only administered twice to try and make it more feasible for real-world practices if it were to catch on. Thus, the objective of this study was to determine whether rbST enhanced performance and biological activity and βAR in the skeletal muscle of heifers during the finishing phase when 500 mg/hd of rbST was administered on day 0 and 14 of the experiment.

Materials and Methods

This study was conducted at the Texas Tech University Burnett Center (New Deal, TX). All animal procedures in the following experiment were reviewed and approved by the Texas Tech University Institutional Animal Care and Use Committee (approval #T11043).

Animals and management

Fed British and British × Continental crossbred heifers ($n = 16$) were sourced from 2 locations in the Texas panhandle. Heifers were received at the Texas Tech University Burnett Center at New Deal, Texas, on June 20, 2013. Heifers were provided access to water and a moderate-concentrate mixed diet upon arrival and were placed in dirt pens. Initial processing occurred on June 23, 2013, at which point each heifer was weighed and tagged. Heifers were assigned to pen based on weight and source and moved to confinement slated floor pens, where they were allowed to acclimate for 14 d prior to trial initiation. Diets were formulated to meet or exceed the National Research Council (1996) requirements for finishing beef cattle (Table 1) and were fed ad libitum throughout the study.

Ration samples were obtained from the feed bunks at feedings to assess dry matter (DM) diet composition on a weekly basis. A monthly composite was put together from a portion of these samples that was submitted for nutrient analysis (Servi-Tech Laboratories, Amarillo, TX). Ration samples were analyzed for moisture, crude protein, acid detergent fiber, calcium (Ca), and phosphorus (P) (Table 1). During the weighing period, orts were collected and weighed and samples were dried to analyze DM content. Pens were observed daily by trained personnel to identify and remove heifers with observable signs/symptoms of health and/or lameness issues.

Experimental design and treatments

Heifers were stratified by body weight (BW) and sorted into 4 BW blocks, each block consisting of 2 pens (2 heifers per pen). Heifers within a block were obtained from the same source. Each pair of pen replicates within a block was randomly assigned to rbST treatments: (1) no rbST (Control) or (2) 500 mg·heifer^{-1} of sometribove zinc at day 0 and 14 (rbST; Posilac®; Elanco Animal Health, Greenfield, IN).

Table 1. Ingredient composition (%, DM basis) of the experimental design[1]

	Control and rbST
Ingredient	
Corn	83.05
Alfalfa	4.48
Cotton seed hulls	4.62
Supplement	2.24
Ca	1.55
Urea	0.72
Fat	3.13
Analyzed Composition	
DM, %	77.60
CP, %	13.60
ADF, %	8.20
TDN, %	86.50
Fat, %	5.20
Ca, %	0.49
P, %	0.39

[1]Diets were formulated to meet or exceed NRC (1996) requirements for growing/finishing beef cattle.

ADF, acid detergent fiber; CP, crude protein; DM, dry matter; NRC, National Research Council; rbST, recombinant bovine somatotropin; TDN, total dietary nutrients.

The experimental design yielded 4 BW blocks and 4 pen replicates per treatment.

On treatment initiation date, heifers were individually weighed (initial BW was reduced by 4% to represent a standard industry shrink), and *longissimus* muscle (LM) biopsy samples were taken in addition to blood collection. Biopsy samples and blood were collected with a certified squeeze chute at approximately 0700 Central Standard Time on day 0, 14, 28, 42, and 56. Heifers were administered rbST after blood collection on day 0 and 14 of the trial. Approval for administering rbST and βAA was not received, so the effect of rbST on skeletal muscle βAR sensitivity to βAA was not tested.

Biopsy brocedure

To collect biopsies, animals were harnessed in a hydraulic chute; their backs were shaved over the LM between the 10th and 13th rib, and the area was disinfected with a 70% ethanol solution. Local anesthetic (lidocaine hydrochloride; 20 mg/mL; 8 mL per biopsy) was administered subcutaneously in a 2.54 cm^2 diamond pattern (4 injection sites; 2 mL per site), and 10 min was allotted to ensure numbness. The area was disinfected with 70% ethanol solution, sterile gauze was placed over the biopsy site, and an approximately 1-cm incision was made with a sterile scalpel. The *longissimus* tissue sample was extracted utilizing a sterile 4-mm Bergstrom biopsy needle. The tissue taken from the animal was then placed in a plastic container (Rubbermaid, Mogadore, OH) on sterile gauze. The sample was aliquoted into 3 samples: one was intended for real-time quantitative reverse transcription polymerase chain reaction (PCR), and the second was used for protein quantification via sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). These samples were placed in whirl-pack bags, snap frozen in liquid nitrogen, and placed into a cooler of dry ice for storage and transportation. The final, third sample was used for immunohistochemical analysis. The sample was placed under magnifying glass, and muscle fibers were taken from the sample following fiber direction. The fibers were placed in clear frozen section compound (VWR International, West Chester, PA) on a 1 × 1.5 cm piece of cork board, frozen using dry ice and chilled 2-methyl-butane, placed in a whirl-pack bag, and then placed in a cooler of dry ice. All samples were transported to Texas Tech University prior to analysis and stored at −80°C. After tissue extraction, the biopsy site was cleaned and disinfected. The incision was sealed with Vetbond™ tissue adhesive (3M Animal Care Products, St. Paul, MN) and sprayed with AluShield aerosol bandage (Neogen Corp., Lexington, KY) to reduce the risk of infection and to protect the site from foreign contaminants. All heifers were monitored for 72 h post-biopsy for signs of infection and swelling. None of the heifers were removed from the trial due to infection/swelling. This procedure was performed on day 0, 14, 28, 42, and 56. The initial biopsy was taken from between the 12th and 13th rib on the left side of the animal, and on day 14 the biopsy was taken from between the 12th and 13th rib of the right side of the heifer. The biopsy site was then moved up between the 11th and 12th ribs on the left side on day 28, on the right side on day 42, and finally between the 10th and 11th rib on the left side on day 56.

Harvest and carcass evaluation

The morning of shipping (88 d after treatment initiation date), heifers were individually weighed (final BW was reduced by 4% to represent a standard industry shrink). Heifers were then transported approximately 180 km and harvested under US Department of Agriculture Food Safety and Inspection Service inspection at a commercial abattoir.

Carcasses were chilled approximately 25 h prior to grading. Individual carcass measurements included hot carcass weight (HCW); 12th rib back fat (BF); LM area; kidney, pelvic, and heart fat percentage;

and marbling score (MS) determined via Texas Tech University trained personal. Dressing percentage for each heifer was calculated as the HCW/shrunk (4% pencil shrink) live weight × 100.

RNA isolation and real-time quantitative reverse transcription PCR

RNA from the *longissimus* tissue biopsy was isolated with ice-cold buffer containing TRIreagent (Sigma, St. Louis, MO). Approximately 0.5–1 g of frozen tissue was homogenized with TRIreagent at a ratio of 0.5:1 grams of tissue to milliliters of reagent. The homogenate was then pipetted into 2 microcentrifuge tubes (1-mL sample per tube), and 200 µL of chloroform was added to each tube, vortexed for 30 s, and incubated for 5 min. The sample was then centrifuged at 15,000 × *g* for 15 min, separating the sample into 3 layers. The top supernatant layer was pipetted off and placed into new microcentrifuge tubes. Two hundred and fifty microleters of ice-cold isopropyl alcohol was added to the supernatant, shaken, and incubated for 10 min on the bench top. The samples were then centrifuged at 15,000 × *g* for 10 min. The supernatant was poured off the RNA pellet as the bottom of each tube was allowed to dry, and then 500 µL of 75% ethanol was added to each tube to rinse and resuspend the RNA pellet. Tubes were then placed in a −80°C freezer. One tube was then removed from the freezer and thawed on ice. Tubes were then centrifuged at 215 × *g* for 10 min, ethanol was poured off, and the pellet was air dried. Thirty microleters of nuclease-free water was then added to each tube to dissolve the RNA pellet. The concentration of RNA was determined with an ultraviolet-visable spectrophotometer at an absorbance of 260 nm and 280 nm to determine the 260/280 ratio, using a NanoDrop 1000 (NanoDrop Products, Wilmington, DE). An acceptable range of 1.76 to 2.05 was used for the 260/280 ratio. Samples were then treated with DNAse to remove any DNA contaminants using a DNA-free kit (Life Technologies, Grand Island, NY). The RNA was then subjected to reverse transcription, and complementary DNA (cDNA) was produced. The cDNA was then used for real-time quantitative reverse transcription PCR to measure the quantity of AMP-activated protein kinase alpha (*AMPKα*), *β1AR*, *β2AR*, *β3AR*, myosin heavy chain-I (*MHC-I*), *MHC-IIA*, *MHC-IIX*, insulin-like growth factor-I (*IGF-I*), G-protein coupled receptor 43 (*GPR43*), *GPR41*, glucose transporter type 4 (*Glut4*), stearoyl-CoA desaturase (*SCD*), C-enhancer binding protein beta, and peroxisome proliferator-activated receptor gamma (*PPARγ*) messenger RNA (mRNA) relative to the quantity of ribosomal protein S9 (*RPS9*) mRNA in total RNA. Expression of *RPS9* was not different across bovine tissue samples. Therefore, *RPS9* was used as the endogenous control in order to normalize the expression of genes of interest. Bovine primers and probes for *AMPKα*, *β1AR*, *β2AR*, *β3AR*, *IGF-I*, *MHC-I*, *MHC-IIA*, *MHC-IIX*, *GPR43*, *GPR41*, *Glut4*, *SCD*, C-enhancer binding protein beta, and *PPARγ* are presented in Table 2. Assays were performed in triplicate using the GeneAmp 7900HT Sequence Detection System (Applied Biosystems, Life Technologies) using thermal cycling parameters recommended by the manufacturer (40 cycles of 15 s at 95°C and 1 min at 60°C). Titration of mRNA primers against increasing amounts of cDNA yielded linear responses with slopes between −2.8 and −3.0. Real-time quantitative values, based on change in cycle threshold (ΔΔCT), were analyzed by RQ Manager (Applied Biosystems).

Protein extraction and SDS-PAGE

Protein from *longissimus* tissue was isolated with whole muscle extraction buffer (WMEB; 2% SDS, 10 mM phosphate, pH 7.0). The tissue sample was homogenized with the WMEB at a ratio of 1:5 grams of tissue to milliliters of extraction buffer. The homogenized samples were centrifuged at 15,000 × *g* for 15 min, separating the sample into 3 layers. The middle supernatant layer was pipetted off and placed into microcentrifuge tubes. The protein samples were then diluted with WMEB to determine protein concentration using the Pierce™ BCA™ protein assay (Thermo Fisher Scientific, Fairlawn, NJ). Protein concentration was then determined using a NanoDrop 1000 spectrophotometer (NanoDrop Technologies) at 562 nm. All samples were then diluted to the same concentration. MHC tracking dye (50% glycerol, 2% SDS, 0.1% bromophenol blue, 60 mM Tris-HCl, pH 6.8) was added to SDS-PAGE samples. Samples were denatured with β-mercaptoethanol and incubated for 2 min at 95°C. Six percent acrylamide separating gels, with 4% acrylamide stacking gels, were made and set at 4°C for 4–24 h for SDS-PAGE. Samples were then loaded onto the gels, and protein was separated by gel electrophoresis. The gels were run for approximately 72 h at 100 V. The gel was placed in 300 mL Coomassie® Fluor Orange (Life Technologies) for 30 min at 25°C in an opaque container. The Coomassie® Fluor Orange was drained off the gel, and the gel was briefly rinsed in 7.5% acetic acid followed by NanoPure water. The gels were then visualized using Imager Scanner II and ImageQuant TL software (BioRad, Hercules, CA). The MHC bands were the only

Table 2. Sequence of bovine-specific PCR primers and TaqMan probes to be used for determination of expression of mRNA of AMPKα, MHC-I, MHC-IIA, MHC-IIX, β1AR, β2AR, β3AR, and RPS9

Primer	Sequence (5′ to 3′)
AMPk*α* (accession #NM_001109802)	
Forward	ACCATTCTTGGTTGCTGAAACTC
Reverse	CACCTTGGTGTTTGGATTTCTG
TaqMan probe	6FAM-CAGGGCGCGCCATACCCTTG-TAMRA
MHC-I (accession #AB059400)	
Forward	CCCACTTCTCCCTGATCCACTAC
Reverse	TTGAGCGGGTCTTTGTTTTTCT
TaqMan probe	6FAM-CCGGCACGGTGGACTACAACATCATAG-TAMRA
MHC-IIA (accession #AB059398)	
Forward	GCAATGTGGAAACGATCTCTAAAGC
Reverse	GCTGCTGCTCCTCCTCCTG
TaqMan probe	6FAM-TCTGGAGGACCAAGTGAACGAGCTGA-TAMRA
MHC-IIX (accession #AB059399)	
Forward	GGCCCACTTCTCCCTCATTC
Reverse	CCGACCACCGTCTCATTCA
TaqMan probe	6FAM-CGGGCACTGTGGACTACAACATTACT-TAMRA
***β*1AR (accession #AF188187)**	
Forward	GTGGGACCGCTGGGAGTAT
Reverse	TGACACACAGGGTCTCAATGC
TaqMan probe	6FAM-CTCCTTCTTCTGCGAGCTCTGGACCTC-TAMRA
***β*2AR (accession #NM_174231)**	
Forward	CAGCTCCAGAAGATCGACAAATC
Reverse	CTGCTCCACTTGACTGACGTTT
TaqMan probe	6FAM-AGGGCCGCTTCCATGCCC-TAMRA
***β*3AR (accession #X85961)**	
Forward	AGGCAACCTGCTGGTAATCG
Reverse	GTCACGAACACGTTGGTCATG
TaqMan probe	6FAM-CCCGGACGCCGAGACTCCAG-TAMRA
IGF-I (accession #X15726)	
Forward	TGTGATTTCTTGAAGCAGGTGAA
Reverse	AGCACAGGGCCAGATAGAAGAG
TaqMan probe	6FAM-GCCCATCACATCCTCCTCGCA-TAMRA
CEBP*β* (accession #NM_176788)	
Forward	CCAGAAGAAGGTGGAGCAACTG
Reverse	TCGGGCAGCGTCTTGAAC
TaqMan probe	6FAM-CGCGAGGTCAGCACCCTGC-TAMRA
GPR43 (accession #FJ562212)	
Forward	GGCTTTCCCCGTGCAGTA
Reverse	ATCAGAGCAGCCATCACTCCAT
TaqMan probe	6FAM-AAGCTGTCCCGCCGGCCC-TAMRA
GPR41 (accession #FJ562213)	
Forward	TGCTCCTCAGCACCCTGAA
Reverse	TTGGAACCCAGATGATGAGAAA
TaqMan probe	6FAM-TCCTGCGTCGACCCCCTTGTCTAC-TAMRA
Glut4 (accession #D63150)	
Forward	CCTCGGCAGCGAGTCACT
Reverse	AAACTGCAGGGAGCCAAGAA
TaqMan probe	6FAM-CCTTGGTCCTTGGCGTATTCTCCGC-TAMRA
PPAR*γ* (accession #NM_181024)	
Forward	ATCTGCTGCAAGCCTTGGA
Reverse	TGGAGCAGCTTGGCAAAGA
TaqMan probe	6FAM-CTGAACCACCCCGAGTCCTCCCAG-TAMRA
SCD (accession #AB075020)	
Forward	TGCCCACCACAAGTTTTCAG
Reverse	GCCAACCCACGTGAGAGAAG
TaqMan probe	6FAM-CCGACCCCCACAATTCCCG-TAMRA
RPS9 (accession #DT860044)	
Forward	GAGCTGGGTTTGTCGCAAAA
Reverse	GGTCGAGGCGGGACTTCT
TaqMan probe	6FAM-ATGTGACCCCGCGGAGACCCTTC-TAMRA

β1AR, beta 1 adrenergic receptor; β2AR, beta 2 adrenergic receptor; β3AR, beta 3 adrenergic receptor; AMPKα, AMP-activated protein kinase alpha; CEBPβ, C-enhancer binding protein beta; Glut4, glucose transporter type 4; GPR41, G-protein coupled receptor 41; GPR43, G-protein coupled receptor 43; IGF-I, insulin-like growth factor-I; MHC-I, myosin heavy chain-I; MHC-IIA, myosin heavy chain-IIA; MHC-IIX, myosin heavy chain-IIX; mRNA, messenger RNA; PCR, polymerase chain reaction; PPARγ, peroxisome proliferator-activated receptor gamma; RPS9, ribosomal protein S9; SCD, stearoyl-CoA desaturase.

bands present in the separating gels, with the larger proteins still being trapped in the stacking gels and all the smaller proteins being run off the gel. Densitometry measurements were made on the bands corresponding to MHC-II and MHC-I.

Immunohistochemical analysis

Twenty-four hours prior to sectioning, embedded muscle samples were moved from −80°C to a −20°C freezer to thaw. Muscle fiber distribution, area, βAR, and satellite cell abundance was determined on 10-µm-thick cross sections. The sections were cut at −20°C using a Leica CM1950 cryostat (Lieca Biosystems, Buffalo Grove, IL) from the embedded muscle samples. The sections were then mounted on positively charged glass slides (5 slides per sample/5 cryosections per slide; Superfrost Plus; VWR International). Cryosections were fixed using 4% paraformaldehyde (Thermo Fisher Scientific) for 10 min at 25°C followed by 2 brief rinses and a single 5-min rinse in phosphate buffered saline (PBS). All dilutions for slide staining can be found in Table 3. Cryosections were incubated with 5% horse serum (Invitrogen, Carlsbad, CA), 2% bovine serum albumin (MP Biomedical, Solon, OH), 0.2% Triton-X100 (Thermo Fisher Scientific) in PBS for 30 min at 25°C to block nonspecific antibody binding. Cryosections were then incubated for 1 h at 25°C in the following primary antibodies:

- Slide 1: 1:100 α-dystrophin, rabbit, immunoglobulin G (IgG; Thermo Fisher Scientific); 1:100 supernatant anti-MHC-I, IgG2b (BA-D5; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA); and 1:100 supernatant anti-MHC (all but type IIX IgG1; BF-35, Developmental Studies Hybridoma Bank)
- Slide 2: 1:750 α-β1AR, rabbit, IgG (Abcam, Cambridge, UK); 1:750 α-β2AR, chicken, IgY (Abcam); 1:500 α-β3AR, goat, IgG (Abcam)
- Slide 3: 1:10 supernatant anti-Paired Box 7 (Pax7), mouse α-chicken (Developmental Studies Hybridoma Bank); 1:100 anti-Myogenic factor 5 (Myf5), rabbit, IgG (Santa Cruz Biotechnology, Dallas, TX)

Slides were then rinsed 3 times for 5 min in PBS. Cryosections were incubated for 30 min at 25°C in opaque boxes in the following secondary antibodies:

- Slide 1: 1:1,000 goat α-rabbit, IgG, Alexa-Fluor 488 (Invitrogen); 1:1,000 goat α-mouse, IgG1, Alexa-Fluor 546 (Invitrogen); 1:1,000 goat α-mouse, IgG2b, Alexa-Fluor 633 (Invitrogen)

Table 3. Dilutions for staining slides with primary and secondary antibodies for MHC, beta-adrenergic receptors, and satellite cells

Antibody	Concentration	PBS
Blocking Buffer		92.8%
Horse serum	5%	
Bovine serum albumin	2%	
Triton-X100	0.2%	
MHC Staining		97%
α-Dystrophin	1%	
Supernatant anti-MHC type I	1%	
Supernatant anti-MHC all but type IIX	1%	
Beta-Adrenergic Agonist Staining		
α-Beta 1 adrenergic receptor	1 µL	750 µL
α-Beta 1 adrenergic receptor	1 µL	750 µL
α-Beta 1 adrenergic receptor	1 µL	500 µL
Satellite Cell Staining		
Supernatant anti-paired box 7	1 µL	10 µL
Anti-myogenic factor 5	1 µL	100 µL
Secondary Staining, MHC		
Goat α-rabbit, IgG, Alexa-Flour 488	1 µL	1,000 µL
Goat α-mouse, IgG1, Alexa-Flour 546	1 µL	1,000 µL
Goat α-mouse, IgG2b, Alexa-Flour 633	1 µL	1,000 µL
Secondary Staining, Beta-Adrenergic Agonist		
Goat α-chicken, IgY, H&L, Alexa-Flour 488	1 µL	1,000 µL
Donkey α-rabbit, IgG, Alexa-Flour 546	1 µL	1,000 µL
Donkey α-goat, IgG, Alexa-Flour 633	1 µL	1,000 µL
Secondary Staining, Satellite Cells		
Goat α-rabbit, IgG, Alexa-Flour 488	1 µL	1,000 µL
Goat α-mouse, IgG1, Alexa-Flour 546	1 µL	1,000 µL

H&L, heavy and light chains; IgG, immunoglobulin G; IgY, immunoglobulin Y; MHC, myosin heavy chain; PBS, phosphate buffered saline.

- Slide 2: 1:1,000 goat α-chicken, IgY, heavy and light chains, Alexa-Fluor 488 (Abcam); 1:1,000 donkey α-rabbit, IgG, Alexa-Fluor 546 (Invitrogen); 1:1,000 donkey α-goat, IgG, Alexa-Fluor 633 (Invitrogen)
- Slide 3: 1:1,000 goat α-rabbit, IgG, Alexa-Fluor 488 (Invitrogen); 1:1,000 goat α-mouse, IgG1, Alexa-Fluor 546 (Invitrogen)

Slides were then rinsed 3 times for 5 min in PBS. Finally, cryosections were incubated in 1 µg/mL 4′,6-diamidino-2-phenylindole (Thermo Fisher Scientific) for 1 min followed by 2 brief PBS rinses. Slides were cover-slipped with mounting media (Aqua Mount; Lerner Laboratories, Pittsburgh, PA) and thin glass cover slips (VWR International) and were left to dry at 4°C for 24 h.

All slides were imaged within 48 h of staining. All slides were imaged at 200× working difference

magnification using an inverted fluorescence microscope (Nikon Eclipse, Ti-E; Nikon Instruments, Inc., Mellville, NY) with a ultraviolet light source (Intensilight C-HGFIE; Nikon Instruments, Inc.) and a CoolSnap ES^2 monochrome camera (Photometrics, Tucson, AZ). Images were artificially colored and analyzed using NIS Elements Imaging software (Nikon Instruments, Inc.).

Five random images were taken of cryosections from each slide of the *longissimus*. All MHC-I, -IIA, and -IIX muscle fibers in each image were identified and expressed as a percentage of the number of muscle fibers counted. The cross-sectional area of each fiber in each image was measured using NIS Elements Imaging software (Nikon Instruments, Inc.) and expressed on a square-millimeter basis. The total number of 4′,6-diamidino-2-phenylindole-stained cells in each image were enumerated to determine the nuclear density on a per-square-millimeter basis. All βAR, Pax7, Myf5, and PAX7:Myf5 satellite cells were identified on the respective slides stained for them and counted, and densities are reported on a square-millimeter basis.

Blood samples

Jugular blood was collected using 18-gauge needles and 10-mL vacuum-sealed glass tubes containing no additive (BD Vacutainer®; BD, Franklin Lakes, NJ) and placed in a cooler of ice for transportation. Blood was allowed to clot at 4°C for 24 h. The blood was then centrifuged at 1,250 × *g* for 20 min at 4°C to obtain serum. Serum was stored at −20°C for IGF-I analysis. Serum IGF-I concentrations were determined by triplicate aliquots on 96-well microtiter plates with a colorimetric enzyme-linked immunoassay following the manufacturer's procedures (Immunodiagnostic Systems, Inc., Fountain Hills, AZ); intra- and interassay coefficient of variation were less than 10%. The results were read with a Spectra Max 380pc plate reader and Softmax Pro software.

Statistical analysis

All data were analyzed using SAS version 9.2 (SAS Institute, Inc., Cary, NC). Initial weight was used as a covariate when analyzing performance parameters, and day 0 values were used as a covariate for all other analysis. For growth performance responses, treatment was included as a fixed effect, block was considered random, and pen served as the experimental unit. For all gene, protein, immunohistochemical, and sera analyses, treatment, day and their interaction were included as fixed effects; block served as random effect; heifer served as the experimental unit; and the Kenward-Roger adjustment was used to correct degrees of freedom. Data were analyzed as repeated measures using the MIXED procedure of SAS (SAS Institute). The covariance structure with the lowest Akaike information criterion was used (Littell et al., 1998). Means were generated using the LSMEANS procedure in SAS, and if a significant preliminary F-test was detected, means were separated using the pairwise comparisons (PDIFF) option of SAS. Means were considered different at $P \leq 0.05$. Tendencies for differences among treatment means were declared when $0.06 \leq P \leq 0.10$.

Results and Discussion

There was a treatment by day effect on *AMPk*α mRNA concentrations ($P < 0.05$; Table 4). The greatest concentration of *AMPk*α was expressed by rbST heifers on day 0, and the lowest concentration was expressed by Control heifers on day 0. However, rbST significantly increased *AMPk*α mRNA concentration ($P < 0.05$). No treatment effects were observed when analyzing the mRNA concentrations of *MHC-IIA*, *MHC-IIX*, *β2AR*, *PPARγ*, and *SCD*; however, a day effect was revealed ($P < 0.05$). These changes with day were expected as the animal reached physiological maturity and the muscles underwent hypertrophy increasing cell size and the expression of *MHC-IIA* and *IIX* (Johnston et al., 1975). All heifers had the greatest concentration of *MHC-IIA* mRNA on day 56 and the greatest concentration of *MHC-IIX* mRNA on day 14, 28, and 42 ($P < 0.05$). Concentrations of *β2AR* mRNA were the greatest on day 56 ($P < 0.05$), whereas the greatest concentration of *PPARγ* and *SCD* mRNA were on day 0 ($P < 0.05$). The rbST heifers had a greater concentration of *β3AR* mRNA ($P < 0.05$) compared to the Control heifers. The increased expression of *β3AR* may be due to increased exposure to GH. Yang et al. (2004) discovered that GH affected the function of β1 and β3AR, with the effect seeming to be from the direct exposure of GH in vitro. There was not a significant increase observed in *IGF-I* mRNA concentrations ($P > 0.05$) between treatments. Taaffe et al. (1996) reported no changes in *IGF-I* mRNA concentrations after a 14-wk pretreatment or after treatment of elderly men administered recombinant human GH. However, Turner et al. (1988) reported an 8-fold increase in *IGF-I* mRNA concentrations in skeletal muscle on day 80 of rats implanted with GH-secreting GH3 cells. No differences ($P > 0.05$) were observed in mRNA

Table 4. Effect of rbST on relative mRNA concentrations of AMPKα, IGF-I, MHC-I, MHC-IIA, MHC-IIX, β1AR, β2AR, β3AR, CEBPβ, GPR43, GPR41, Glut4, PPARγ, and SCD genes in *longissimus* tissue

	Treatment[1]													
	Control					rbST						P Value		
Gene[2]	Day 0	Day 14	Day 28	Day 42	Day 56	Day 0	Day 14	Day 28	Day 42	Day 56	SEM[3]	Trt	Day	Trt × Day
AMPKα	1.97^{c}	2.34^{bc}	2.74^{bc}	2.77^{bc}	3.50^{ab}	4.59^{a}	2.49^{bc}	3.43^{ab}	3.44^{ab}	2.48^{bc}	0.71	0.049	0.450	0.011
IGF-I	1.04^{x}	0.79^{yz}	0.41^{z}	0.45^{z}	0.99^{xy}	1.53^{x}	0.84^{yz}	0.68^{z}	0.48^{z}	1.08^{xy}	0.31	0.185	0.002	0.821
MHC-I	1.69	1.97	2.02	2.45	2.31	2.60	1.78	2.03	2.89	2.18	0.44	0.291	0.113	0.345
MHC-IIA	2.46^{z}	3.50^{z}	2.15^{z}	2.94^{z}	6.19^{y}	3.77^{z}	2.98^{z}	3.59^{z}	3.46^{z}	5.97^{y}	1.19	0.350	0.001	0.658
MHC-IIX	0.30^{z}	0.83^{xy}	1.00^{x}	1.16^{x}	0.74^{yz}	0.72^{z}	1.10^{xy}	1.39^{x}	1.09^{x}	0.72^{yz}	0.24	0.077	0.001	0.465
β1AR	76.71	8.45	31.49	3.98	186.59	97.85	53.34	51.83	117.48	100.84	60.15	0.388	0.058	0.213
β2AR	0.41^{z}	0.74^{y}	0.48^{yz}	0.60^{yz}	1.13^{x}	0.51^{z}	0.79^{y}	0.66^{yz}	0.67^{yz}	0.90^{x}	0.17	0.682	0.001	0.525
β3AR	182.76^{p}	33.23^{p}	281.45^{p}	94.08^{p}	221.73^{p}	175.97^{o}	486.32^{o}	365.88^{o}	279.74^{o}	608.45^{o}	213.31	0.006	0.253	0.221
CEBPβ	2.07	2.47	2.22	2.70	3.96	1.38	2.85	2.45	2.99	3.99	1.14	0.919	0.059	0.955
GPR43	0.27	0.07	1.54	0.06	3.45	0.21	1.37	1.53	1.83	1.60	1.23	0.660	0.064	0.223
GPR41	5.23	0.10	1.95	0.04	4.08	0.37	1.76	2.29	2.76	2.89	3.01	0.837	0.709	0.370
Glut4	0.67	0.67	0.56	0.65	0.53	0.58	0.52	0.67	0.75	0.69	0.15	0.669	0.868	0.452
PPARγ	2.07^{x}	0.46^{z}	0.12^{z}	0.19^{z}	0.97^{y}	2.00^{x}	0.40^{z}	0.17^{z}	0.78^{z}	1.40^{y}	0.51	0.386	0.001	0.813
SCD	1.62^{x}	0.39^{yz}	0.03^{z}	0.08^{yz}	0.25^{y}	1.48^{x}	0.26^{yz}	0.07^{z}	0.21^{yz}	0.87^{y}	0.35	0.493	0.001	0.486

[1]Control = 0 mg/hd rbST; rbST = 500 mg/hd sometribove zinc at day 0 and 14 (Posilac®; Elanco Animal Health, Greenfield, IN).

[2]Relative abundance of the AMPKα, MHC-I, MHC-IIA, MHC-IIX, β1AR, β2AR, β3AR, CEBPβ, GPR43, GPR41, Glut4, PPARγ, and SCD genes were normalized with the RPS9 endogenous control by using the change in cycle threshold (ΔΔCT).

[3]Pooled standard error of the mean.

[a–c]Means in the same row having different superscripts are significant at $P \leq 0.05$ due to Trt × Day interaction.

[o,p]Means in the same row having different superscripts are significant at $P \leq 0.05$ due to Trt.

[x–z]Means in the same row having different superscripts are significant at $P \leq 0.05$ due to Day.

β1AR, beta 1 adrenergic receptor; β2AR, beta 2 adrenergic receptor; β3AR, beta 3 adrenergic receptor; AMPKα, AMP-activated protein kinase alpha; CEBPβ, C-enhancer binding protein beta; Glut4, glucose transporter type 4; GPR43, G-protein coupled receptor 43; IGF-I, insulin-like growth factor; MHC-I, myosin heavy chain-I; MHC-IIA, myosin heavy chain-IIA; MHC-IIX, myosin heavy chain-IIX; mRNA, messenger RNA; PPARγ, peroxisome proliferator-activated receptor gamma; rbST, recombinant bovine somatotropin; RPS9, ribosomal protein S9; SCD, stearoyl-CoA desaturase; Trt, treatment.

between treatments for *MHC-I*, *GPR41*, or *Glut4*. Maltin et al. (1990) also reported no increase in mRNA abundance of various genes associated with growth of steers administered GH.

There were no significant changes in blood serum IGF-I concentrations ($P > 0.05$; Figure 1). The lack of significant change in IGF-I serum concentrations was not expected as several studies have reported increased serum IGF-I with increased rbST administration (Dalke et al., 1992; Moseley et al., 1992; Preston et al., 1995). The absence of detecting a difference in IGF-I serum concentrations may be due to the fact that blood was collected prior to rbST injection and on collection dates, and rbST was only administered on day 0 and 14 instead of throughout the study. Previous studies would draw blood at the time of injection and 1–2 h after injection (Moseley et al., 1992; Vestergaard et al., 1995). Other studies drew blood similarly to the current study; however, in those studies, steers were implanted with time-release bST implants (Dalke et al., 1992; Preston et al., 1995). Vestergaard et al. (1995) reported that rbST treatment of heifers increased serum IGF-I concentrations. Draghia-Akli et al. (1999) reported that pigs administered protease-resistant porcine GH-releasing hormone enhanced GH secretion and serum IGF-I levels by 3- to 6-fold in 3-wk-old piglets. Furthermore, serum concentrations of GH and IGF-I were unchanged during a 4-wk control period or the course of a 21-wk strength training period of elderly women (Hakkinen et al., 2001).

There was not a significant treatment effect on MHC-I, MHC-IIA, and MHC-IIX protein concentrations appearing within muscle fibers; however, there was a day effect ($P < 0.05$; Table 5). The greatest concentrations of MHC-I, MHC-IIA, and MHC-IIX were on day 28, and the lowest concentrations were on day 0 ($P < 0.05$).

For immunohistochemistry fiber area and fiber proportion, there was a treatment effect ($P < 0.05$; Figures 2–5), day effect ($P < 0.05$), and treatment by day effect ($P < 0.05$). Control heifers had the greatest MHC-I cross-sectional fiber area on day 0 ($P < 0.05$; Figure 2). However, on day 42 and 56, Control heifers had the smallest MHC-I cross-sectional area ($P < 0.05$). On day 28, rbST heifers had the greatest

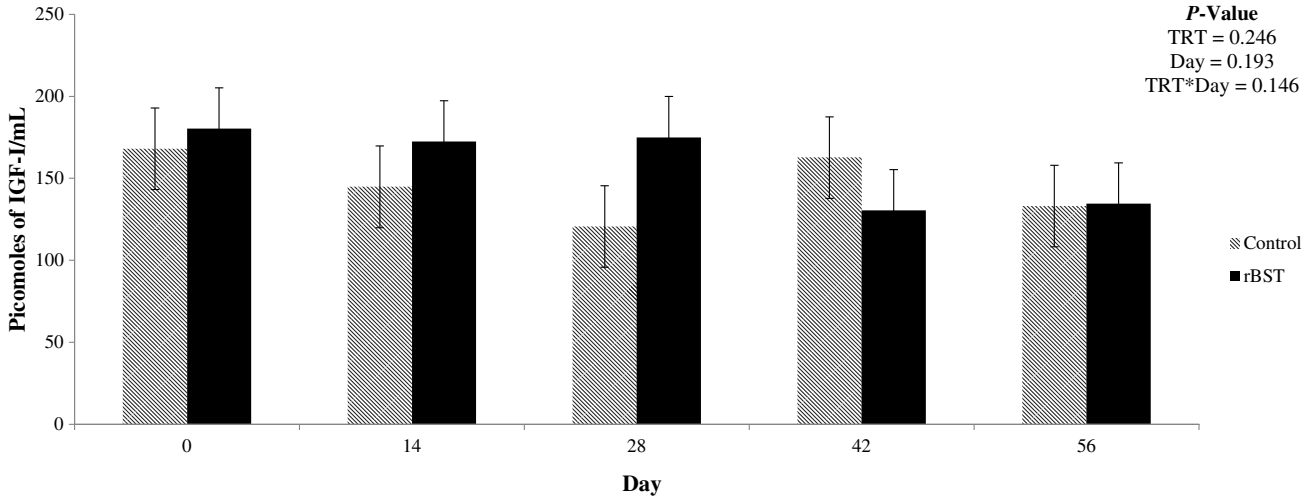

Figure 1. Effect of recombinant bovine somatotropin (rbST) on insulin-like growth factor-I (IGF-I) concentration in blood serum. Control = 0 mg/hd rbST; rbST = 500 mg/hd sometribove zinc at day 0 and 14 (Posilac®; Elanco Animal Health, Greenfield, IN). TRT, treatment.

Table 5. Effect of rbST on relative protein concentration of myosin heavy chain type I and II in *longissimus* tissue

	Treatment[1]													
	Control					rbST					P Value			
MHC	Day 0	Day 14	Day 28	Day 42	Day 56	Day 0	Day 14	Day 28	Day 42	Day 56	SEM[2]	Trt	Day	Trt × Day
Type I	14,854^{c}	23,057^{ab}	28,578^{a}	21,771^{b}	26,946^{ab}	15,391^{c}	21,890^{ab}	26,707^{a}	22,655^{b}	25635^{ab}	2,989.36	0.702	0.001	0.964
Type II	22,984^{d}	40,952^{ab}	42,908^{a}	29,860^{cd}	36,240^{bc}	24,551^{d}	40,295^{ab}	50,816^{a}	29,643^{cd}	32967^{bc}	2,959.03	0.595	0.001	0.389

[1]Control = 0 mg/hd rbST; rbST = 500 mg/hd sometribove zinc at day 0 and 14 (Posilac®; Elanco Animal Health, Greenfield, IN).

[2]Pooled standard error of the mean.

MHC, myosin heavy chain; rbST, recombinant bovine somatotropin; Trt, treatment.

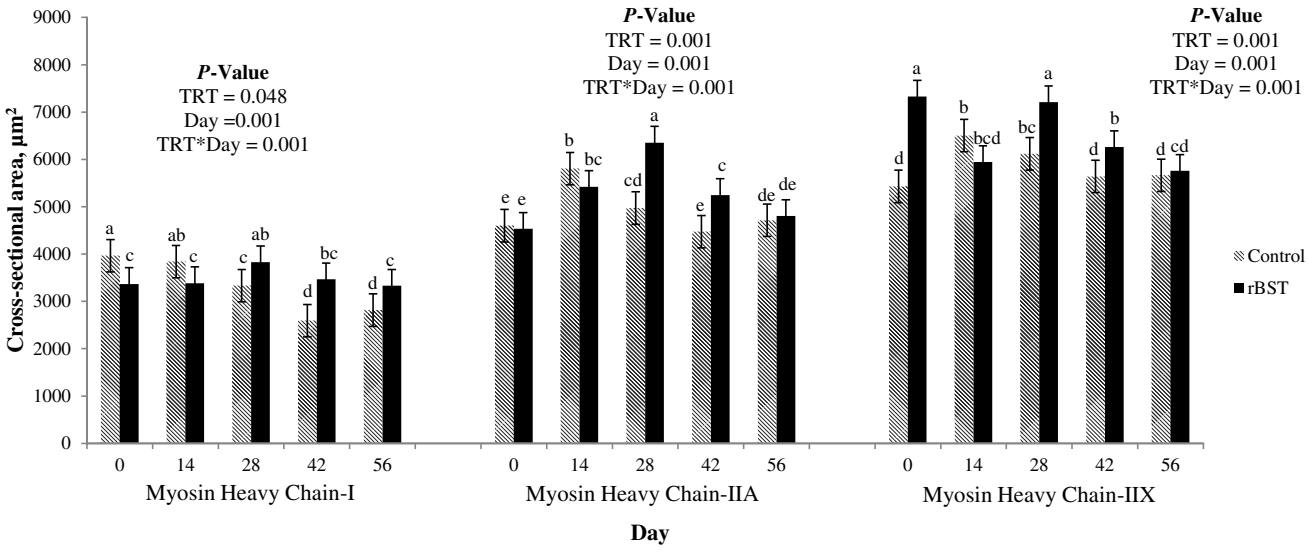

Figure 2. Effect of recombinant bovine somatotropin (rbST) on fiber cross-sectional area (square micrometers) in *longissimus* tissue. [a–e]Means in the same row having different superscripts are significant at $P \leq 0.05$. Control = 0 mg/hd rbST; rbST = 500 mg/hd sometribove zinc at day 0 and 14 (Posilac®; Elanco Animal Health, Greenfield, IN). TRT, treatment.

MHC-IIA fiber cross-sectional area ($P < 0.05$), whereas on day 0 and 42 Control heifers—and on day 42 rbST heifers—had the smallest MHC-IIA fiber cross-sectional areas ($P < 0.05$). On day 28 through day 56 of the finishing phase, rbST heifers had a greater fiber cross-sectional area in MHC-IIX fibers ($P < 0.05$).

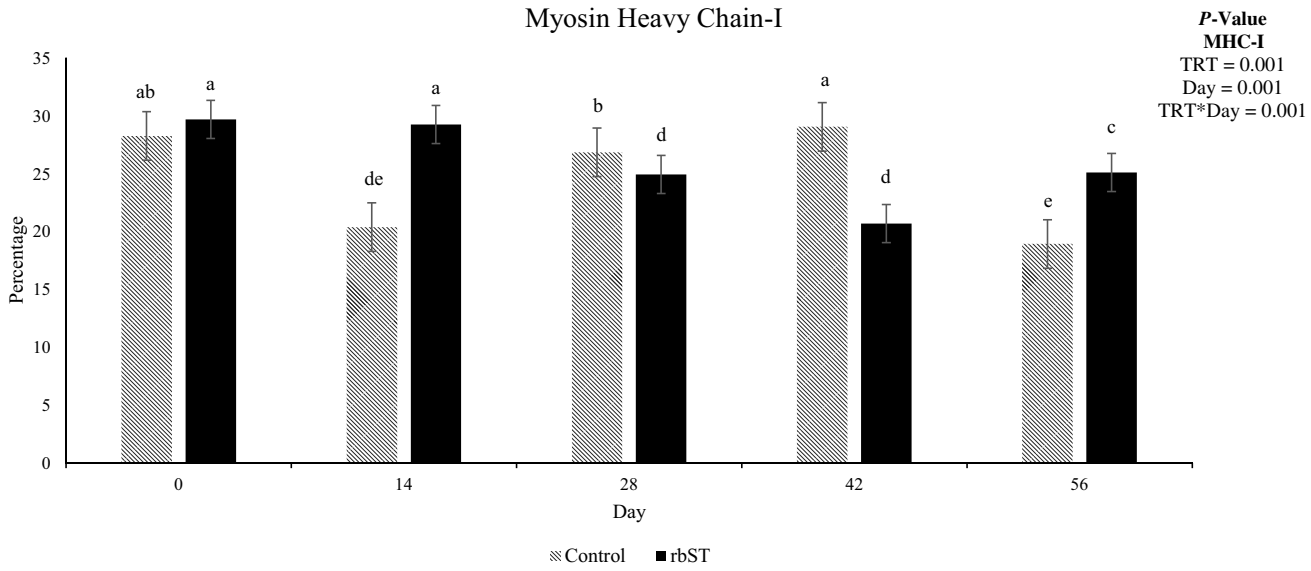

Figure 3. Effect of recombinant bovine somatotropin (rbST) on myosin heavy chain type I composition in *longissimus* tissue. [a–e]Means over different columns having different superscripts are significant at $P \leq 0.05$. Control = 0 mg/hd rbST; rbST = 500 mg/hd sometribove zinc at day 0 and 14 (Posilac®; Elanco Animal Health, Greenfield, IN). MHC, myosin heavy chain; TRT, treatment.

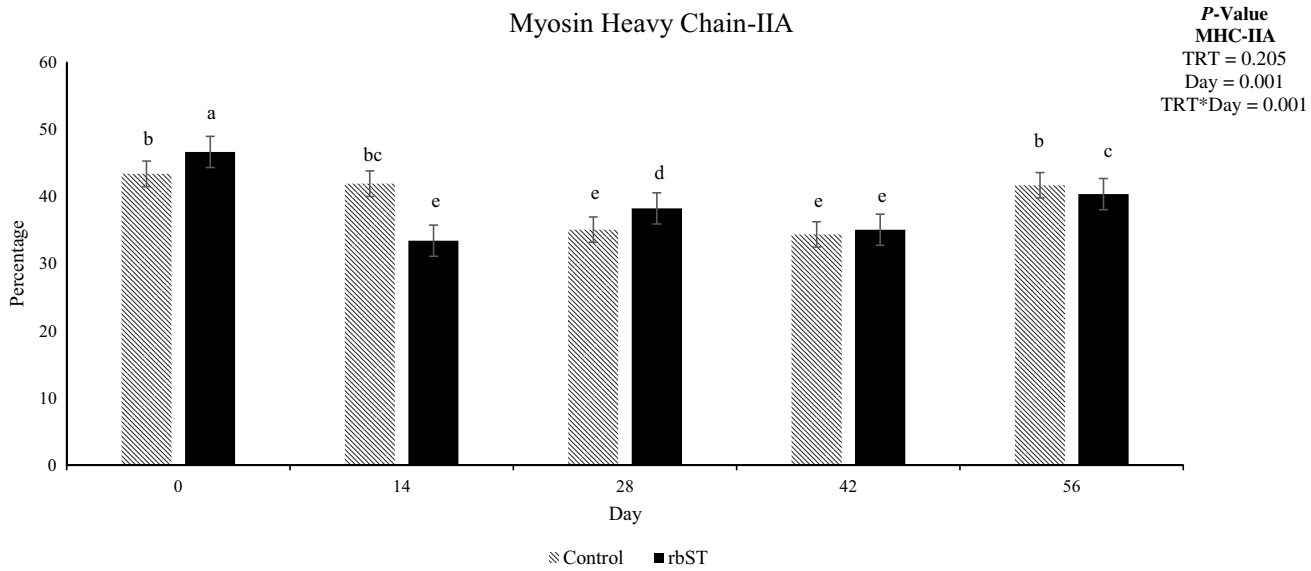

Figure 4. Effect of recombinant bovine somatotropin (rbST) on myosin heavy chain type IIA composition in *longissimus* tissue. [a–e]Means over different columns having different superscripts are significant at $P \leq 0.05$. Control = 0 mg/hd rbST; rbST = 500 mg/hd sometribove zinc at day 0 and 14 (Posilac®; Elanco Animal Health, Greenfield, IN). MHC, myosin heavy chain; TRT, treatment.

The proportion of MHC-I fibers decreased ($P < 0.05$; Figure 3) in Control and rbST heifers as days on feed increased. Control heifers had the least MHC-I fibers on day 56 ($P < 0.05$). This trend was also seen with the proportion of MHC-IIA fibers, with the greatest proportions on day 0 in Control and rbST heifers ($P < 0.05$; Figure 4). Conversely, as days on feed increased, the proportion of MHC-IIX fibers increased ($P < 0.05$; Figure 5)—the greatest proportion of MHC-IIX fibers were in rbST heifers on day 42 ($P < 0.05$). The physiological change in fiber type can be seen in Figures 6–7. Pette and Staron (2000) reported that changes can induce myosin isoform expression in the direction of fast-to-slow or slow-to-fast (I → IIA → IIX → IIB and vice versa) depending on the conditions such as increased or decreased neuromuscular activity, mechanical loading and unloading, altered hormonal profiles, and aging. This is commonly seen as animals grow and mature; satellite cells are active aiding in muscle hypertrophy and increasing the glycolytic

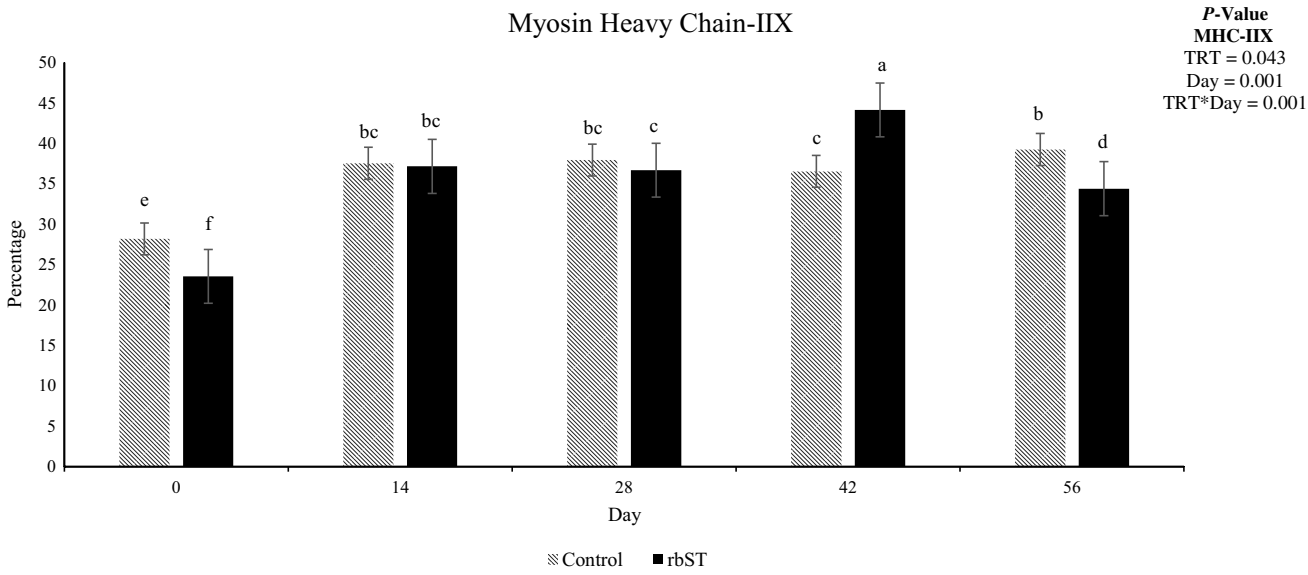

Figure 5. Effect of recombinant bovine somatotropin (rbST) on myosin heavy chain type IIX composition in *longissimus* tissue. [a–e]Means over different columns having different superscripts are significant at $P \leq 0.05$. Control = 0 mg/hd rbST; rbST = 500 mg/hd sometribove zinc at day 0 and 14 (Posilac®; Elanco Animal Health, Greenfield, IN). MHC, myosin heavy chain; TRT, treatment.

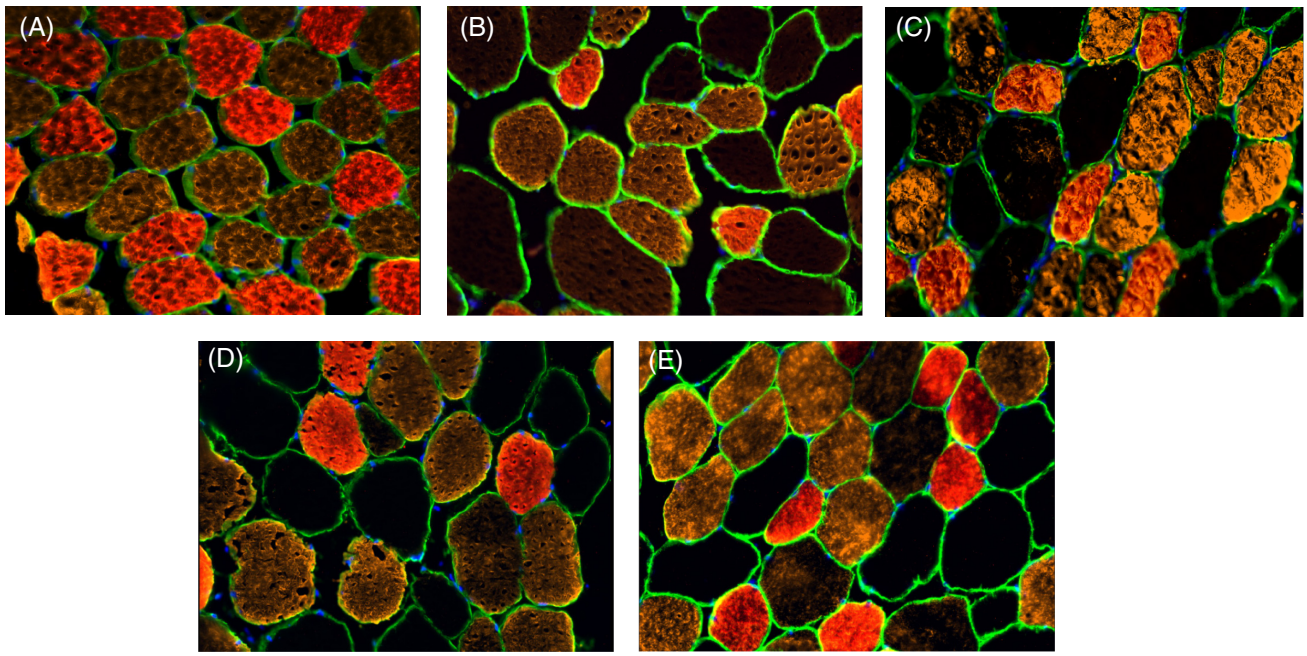

Figure 6. Immunohistochemical detection on a muscle cross section of the *longissimus* biopsy from heifers administered recombinant bovine somatotropin demonstrates sarcolemma by dystropin in green, myosin heavy chain type I positive muscle fibers in red, myosin type I and IIA in orange, and myosin type IIX in gray (negative for myosin heavy chain type I and IIA), (A) day 0, (B) day 14, (C) day 28, (D) day 42, and (E) day 56.

properties of myosin isoforms (Schultz et al., 1978; Rhoads et al., 2009). Maltin et al. (1990) reported no change in cross-sectional area of fast-twitch glycolytic fibers, and fast-twitch oxidative glycolytic fibers, in the *semimembranosus* of veal calves administered GH at 3–5 mg for ~105 d; however, FOG fibers in the *triceps brachii* were increased by GH treatment. Vestergaard et al. (1995) reported no differences in MHC fiber area when heifers were administered 15 mg rbST for 15 wk. The proportion of fibers in the *longissimus dorsi*, *semimembranosus*, and *triceps brachii* was not affected by rbST administration for 15 wk in heifers or by GH treatment for ~105 d in veal steers (Maltin et al., 1990; Vestergaard et al., 1995).

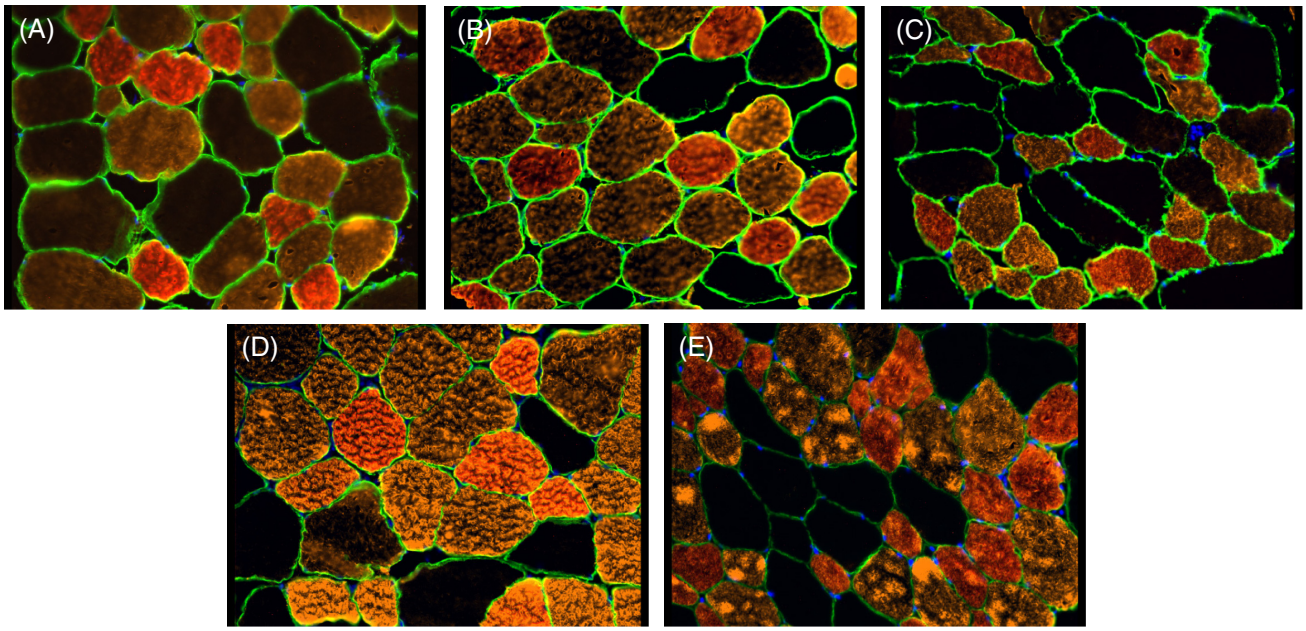

Figure 7. Immunohistochemical detection on a muscle cross section of the *longissimus* biopsy from control heifers demonstrates sarcolemma by dystropin in green, myosin heavy chain type I positive muscle fibers in red, myosin type I and IIA in orange, and myosin type IIX in gray (negative for myosin heavy chain type I and IIA), (A) day 0, (B) day 14, (C) day 28, (D) day 42, and (E) day 56.

The administration of rbST in this study decreased the proportion of MHC-I fibers ($P < 0.05$), increased the proportion of MHC-IIX fibers ($P < 0.05$), and increased the cross-sectional area of MHC-I, -IIA, and -IIX ($P < 0.05$).

rbST heifers had the greatest density of nuclei on day 28 ($P < 0.05$; Table 6). Day had a significant effect on the density of β1AR, β2AR, and β3AR, as well as satellite cell populations. The greatest density of β1AR was on day 28 and 42 ($P < 0.05$), β2AR on day 42 and 56 ($P < 0.05$), and β3AR on day 56 ($P < 0.05$). In addition, rbST heifers numerically had a greater density of β2AR and β3AR on day 56. Staining of βAA is shown in Figure 8. In satellite cells, the greatest density of PAX7-positive cells was on day 0, 28, and 42 ($P < 0.05$; Table 7), the greatest density of Myf5-positive cells was on day 42 and 56 ($P < 0.05$), and the greatest density of cells positive for PAX7:Myf5 was on day 28 ($P < 0.05$). The Control heifers had the greater Myf5 ($P < 0.05$) expression on day 42 and 56 when compared to rbST heifers on similar days. The PAX7:Myf5 ratio was greatest in rbST heifers on day 14 ($P < 0.05$), whereas it was the greatest in Control heifers on day 28 ($P < 0.05$).

Feedlot growth performance was not affected by rbST treatment ($P > 0.05$; Table 8). There was no effect on ADG, final BW, and gain-to-feed ratio due to treatment ($P > 0.05$). These results are not common with

Table 6. Effect of rbST on nuclei and β-adrenergic receptor density in *longissimus* tissue

	Treatment[1]													
	Control					rbST					P Value			
Item, mm[2]	Day 0	Day 14	Day 28	Day 42	Day 56	Day 0	Day 14	Day 28	Day 42	Day 56	SEM[2]	Trt	Day	Trt × Day
β_1AR	129.19^{bc}	64.67^{f}	143.10^{ab}	161.62^{a}	98.31^{de}	129.19^{bc}	92.46^{e}	144.58^{ab}	142.85^{ab}	110.33^{cde}	10.80	0.712	0.001	0.012
β_1ARI	0.00	7.81^{b}	11.15^{b}	0.00	0.00	0.00	17.68^{a}	0.38^{c}	0.00	0.00	3.22	0.776	0.005	0.003
β_2AR	141.94^{cd}	95.74^{f}	146.46^{c}	172.39^{ab}	157.91^{bc}	117.17^{e}	123.79^{de}	151.75^{c}	151.95^{c}	182.35^{a}	10.57	0.579	0.001	0.001
β_2ARI	7.94	9.52	10.40	0.00	0.00	9.27	11.97	22.74	0.00	0.00	6.99	0.714	0.159	0.925
β_3AR	45.61^{cd}	31.94^{f}	44.27^{cde}	49.99^{c}	58.75^{b}	33.11^{f}	38.87^{def}	37.92^{ef}	46.00^{cd}	66.58^{a}	3.89	0.333	0.001	0.001

[1]Control = 0 mg/hd rbST; rbST = 500 mg/hd sometribove zinc at day 0 and 14 (Posilac®; Elanco Animal Health, Greenfield, IN).

[2]Pooled standard error of the mean.

[a–f]Means in the same row having different superscripts are significant at $P \leq 0.05$ due to Trt × Day interaction.

β_1AR, beta-1-adrenergic receptor; β_1ARI, beta-1-adrenergic receptor, internalized; β_2AR, beta-2-adrenergic receptor; β_2ARI, beta-2-adrenergic receptor, internalized; β_3AR, beta-3-adrenergic receptor; rbST, recombinant bovine somatotropin; Trt, treatment.

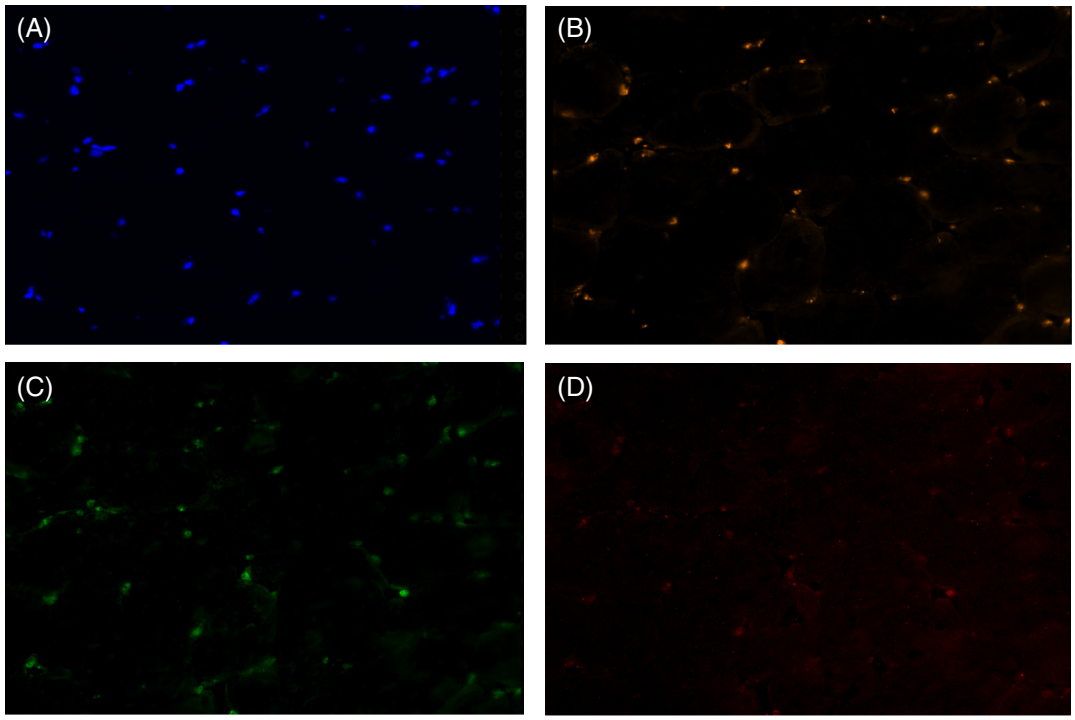

Figure 8. Immunohistochemical detection of beta-adrenergic receptors on a muscle cross section of the *longissimus* biopsy from heifers demonstrates (A) nuclei, (B) beta-1 adrenergic receptors, (C) beta-2 adrenergic receptors, and (D) beta-3 adrenergic receptors.

Table 7. Effect of recombinant bovine somatotropin on nuclei density and satellite cell density in *longissimus* tissue

	Treatment[1]											P Value		
	Control					rbST								
Item, mm^2	Day 0	Day 14	Day 28	Day 42	Day 56	Day 0	Day 14	Day 28	Day 42	Day 56	SEM[2]	Trt	Day	Trt × Day
Nuclei, mm^2	473.88^{bc}	388.06^{e}	456.06^{cd}	524.58^{ab}	439.22^{cde}	450.60^{cd}	406.80^{de}	543.69^{a}	457.49^{cd}	513.10^{ab}	30.79	0.156	0.001	0.001
PAX7	4.49	1.54	5.04	5.72	2.86	4.20	1.30	4.21	4.98	2.29	1.29	0.322	0.001	0.995
Myf5	39.37^{bcde}	35.92^{de}	42.25^{bc}	44.31^{b}	41.75^{bcd}	34.96^{e}	35.35^{e}	36.60^{cde}	35.83^{de}	56.41^{a}	3.32	0.528	0.001	0.001
PAX7:Myf5	4.81^{bc}	1.75^{d}	7.91^{a}	3.03^{bcd}	1.00^{d}	1.66^{d}	5.30^{ab}	4.67^{bc}	4.67^{bc}	2.36^{cd}	1.46	0.956	0.001	0.001

[1]Control = 0 mg/hd recombinant bovine somatotropin (rbST); rbST = 500 mg/hd sometribove zinc at day 0 and 14 (Posilac®; Elanco Animal Health, Greenfield, IN).

[2]Pooled standard error of the mean.

[a–e]Means in the same row having different superscripts are significant at $P \leq 0.05$ due to Trt × Day interaction.

Myf5, myogenic factor 5; PAX7, anti-paired box 7; Trt, treatment.

what has been previously reported. This lack of effect is likely because the heifers in this study were only administered 500 mg/hd rbST twice instead of continuously for extended periods, which is in contrast to previous studies (at 7-d age, daily injection of 3.5 mg bovine pituitary GH harvested at 150–170 kg [Maltin et al., 1990]; 15 mg/d GH for 15 wk [Vestergaard et al., 1995]; 80 or 160 mg/wk of rbST for 12–17 wk [Preston et al., 1995]). However, the dose of rbST administered to the heifers based on the 56-d period was 18 mg/d. The heifers were also housed on slatted floors at heavy BW and worked every 2 wk resulting in possible stressors from handling and biopsy procedures. Studies reported cattle treated with bST exhibited increased ADG from 6% to 27% (Sejrsen et al., 1986; Dalke et al., 1992; Preston et al., 1995; Vestergaard et al., 1995). Dalke et al. (1992), Moseley et al. (1992), and Preston et al. (1995) reported that DM intake decreased when rbST was administered to steers. In our study, we saw no difference in DM intake ($P > 0.05$) between

Table 8. Growth performance response of finishing heifers administered recombinant bovine somatotropin (kg)

	Treatments[1]			
	Control	rbST	SEM[2]	P Value
Initial Weight	459.32	454.24	14.91	0.740
Day 56	487.55	498.43	20.22	0.602
Final Weight	534.95	543.66	20.26	0.676
ADG, D0–56	0.50	0.78	0.18	0.159
ADG, D0–88	0.86	1.01	0.10	0.154
DMI	7.39	7.39	0.04	0.965
G:F, D56	0.09	0.11	0.02	0.478
G:F, D88	0.12	0.14	0.01	0.159

[1]Control = 0 mg/hd recombinant bovine somatotropin (rbST); rbST = 500 mg/hd sometribove zinc at day 0 and 14 (Posilac®; Elanco Animal Health, Greenfield, IN).

[2]Pooled standard error of the mean.

ADG, average daily gain; D, day; DMI, dry matter intake; G:F, gain:feed.

Table 9. Carcass trait response of finishing heifers administered recombinant bovine somatotropin

	Treatments[1]			
	Control	rbST	SEM[2]	P Value
HCW, kg	337.81	344.59	12.55	0.600
LMA, cm²	88.38	94.83	4.84	0.223
FT, cm	1.62	1.87	0.23	0.275
KPH, %	1.9	2.1	0.10	0.088
Marbling	498	441	28.46	0.073
Yield Grade	2.9	3.0	0.40	0.890
Dress, %	63.19	63.38	0.60	0.760

[1]Control = 0 mg/hd recombinant bovine somatotropin (rbST); rbST = 500 mg/hd sometribove zinc at day 0 and 14 (Posilac®; Elanco Animal Health, Greenfield, IN).

[2]Pooled standard error of the mean.

FT, fat thickness; HCW, hot carcass weight; KPH, kidney, pelvic and heart fat; LMA, loin muscle area.

treatments. Moreover, feed efficiency in previous studies was improved from 3% to 25% (Fabry et al., 1987; Early et al., 1990a; Enright et al., 1990; Maltin et al., 1990; Moseley et al., 1992). Grings et al. (1990) reported that heifers treated with rbST gained weight 0.18 kg/d faster than control heifers during a 5-mo treatment period; however, after that treatment period, the control heifers gained 0.12 kg/d more than non-treated heifers. Similar to the results of this study, Peters (1986) reported no difference in growth performance of steers during a 4-wk trial when steers were administered 38 IU/d for 29 d.

Carcass traits were not affected by rbST treatment ($P > 0.05$; Table 9). There was no difference in dressing percentage, HCW, LM area, BF, and yield grade ($P > 0.05$). However, Control heifers tended to have a greater MS ($P < 0.10$) and a decreased kidney, pelvic, and heart fat percentage ($P < 0.10$). Several studies reported no change in HCW between control and rbST-treated steers (Early et al., 1990a; Dalke et al., 1992; Moseley et al., 1992). However, Vestergaard et al. (1995) and Sandles and Peel (1987) reported an increase in HCW in prepubertal heifers administered rbST. Additionally, several studies reported that rbST decreased BF and MS (Peters, 1986; McShane et al., 1989; Early et al., 1990a; Dalke et al., 1992; Moseley et al., 1992; Preston et al., 1995; Vestergaard et al., 1995). Moseley et al. (1992) reported a 4% to 15% increase in LM area of rbST steers. While there was not a significant difference in this study, LM area was increased by 6.8% in the rbST heifers.

Studies have reported that adipocytes treated with GH in vitro or adipocytes collected from animals or patients administered GH had increases in catecholamine-induced lipolysis and increased numbers of βAR (Sechen et al., 1990; Watt et al., 1991; Kamel et al., 2000; Yang et al., 2004). Yang et al. (1996) found that hypophysectomized male rats had decreased isoproterenol and norepinephrine-stimulated lipolysis. However, when these rats were treated with GH, there was a significant increase in isoproterenol and norepinephrine-stimulated lipolysis, but not to the same degree as the increases observed in control rats (Yang et al., 1996). In addition to increased lipolysis partly through βAR function, GH-treated rat adipocytes had increased numbers of β1 and β3AR (Yang et al., 1996, 2004). Kamel et al. (2000) reported that the lipolysis effect of GH is related to the stimulatory effect of β2AR in adipocytes. Sheep adipose tissue exposed to chronic GH treatment increased their response and sensitivity to βAA (Watt et al., 1991). The response was partly due to increased βAR ligand binding because saturating concentrations of ligand were used, thus increasing the number of βAR per adipocyte (Watt et al., 1991). Sechen et al. (1990) reported that lactating cows administered bST had a significantly enhanced response to the lipolytic action of epinephrine regardless of energy balance. Cows administered bST had greater circulating non-esterified fatty acids and glycerol concentrations at every dose of epinephrine given (Sechen et al., 1990). Furthermore, Yang et al. (1996) reported that GH inhibited phosphodiesterase, activating cyclic adenosine monophosphate-dependent protein kinase and hormone-sensitive lipase, leading to a decrease in fat storage by the hydrolysis of triglycerides to free fatty acids and glycerol.

We observed an increase in *AMPkα* mRNA concentrations in rbST-treated heifers, which may explain

the decreased MS and increased fiber cross-sectional area. Increase in *AMPkα* can increase fatty acid synthesis as well as cell growth and protein synthesis (Mihaylova and Shaw, 2011). Furthermore, rbST treatment increased *β3AR* mRNA concentration, with the greatest concentration occurring on day 56 of the trial. rbST treatment did not increase the mRNA concentration of *β1AR* or *β2AR*; however, the greatest concentrations were also observed on day 56. While we were unable to test whether rbST treatment increased the sensitivity of the βAR to a βAA, we may conclude that cattle may exhibit a greater response to βAA based on previous studies (Watt et al., 1991; Yang et al., 1996).

The increased density of PAX7-postive satellite cells on day 0, 28, and 42 indicated an increase in cells able to proliferate or begin the differentiation process. As the density of PAX7-positive satellite cells decreases, Myf5-positive satellite cell densities increase, suggesting an increase in the population of satellite cells preparing to fuse with the muscle fiber, allowing for greater increases in fiber size. With the addition of nuclei to the muscle fiber, the density of βAR will also increase.

Overall, we may conclude that the administration of rbST at 500 mg/hd as 2 distinct doses early in the feeding period may not be the most effective supplementation strategy to improve feedlot and carcass performance as well as profitability. Conversely, the administration of rbST prior to feeding βAA may be beneficial. The greatest mRNA concentrations of βAR occurred on day 56, and rbST heifers had a greater density of β2AR and β3AR on day 56, possibly allowing for increased efficacy. Furthermore, the rbST heifers had a greater density of Myf5- and PAX7:Myf5-positive satellite cells on day 56 compared to Control heifers. This may indicate that the rbST heifers could have a greater response to the administration of a βAA. The rbST heifers had a greater abundance of βAR and possibly an increased sensitivity of βAR to βAA, as well as increased satellite cell numbers capable of fusing with the muscle fiber, allowing for increased hypertrophy. However, further investigation is needed to elucidate interactions between concentration and dosage intervals of rbST given to heifers that most effectively enhance muscle metabolism during the finishing period through enhancement of βAR functionality.

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