Regular Paper

HISTOCHEMISTRY AND GROWTH CHARACTERISTICS OF BOVINE *SEMITENDINOSUS* MUSCLE EXPOSED TO RECOMBINANT BOVINE SOMATOTROPIN (rbST)

Fernanda Regina Carani[1], Paulo Sérgio Andrade Moreira[2], Robson Francisco Carvalho[1], Carlos Roberto Padovani[3], Luiz Artur Loyola Chardulo[4] and Maeli Dal Pai Silva[1]

[1]Department of Morphology, Institute of Biosciences (IB), [2]Department of Animal Improvement and Production, Faculty of Veterinary Medicine and Zootechnology (FMVZ), [3]Department of Biostatistics, Institute of Biosciences (IB), [4]Department of Chemistry and Biochemistry, Institute of Biosciences (IB), Paulista State University (UNESP), Botucatu, SP, Brazil.

ABSTRACT

Bovine skeletal muscle growth characteristics and muscle fiber type frequency are of primary interest because they both play a fundamental role in modeling meat quality and tenderness, although the precise relationship remains undefined. Growth promoters like rbST have been reported to have varying effects on muscle growth performance. The objective of this experiment was to evaluate the histochemistry and growth characteristics of bovine *semitendinosus* muscle treated with rbST. Animals were divided into two groups: control (saline-injected; n=8) and rbST-injected (15 μg/kg; n=8). Heifers were injected every 14 days from day 210 until day 285 of age. Muscle samples were collected (day 210 and 360) and frozen in liquid nitrogen. Histological sections (10 μm) underwent morphological and histochemical analysis (HE, NADH-TR and mATPase), morphometry (fiber area and distribution), and biochemical analysis. Fibers were classified as SO, FOG, and FG. FOG fiber percentage distribution decreased and cross-sectional area increased in rbST-treated animals. Recombinant bST caused a greater animal body weight gain and FOG fiber hypertrophy, while contributing to a decrease in FOG fiber distribution. We conclude that the phenotypic modulation seen in this muscle fiber suggests a potential role of this muscle in modeling the meat quality.

Key words: Beef cattle, fiber types, histochemistry, muscle growth, rbST

INTRODUCTION

In animal breeding and husbandry extensive efforts have been made to efficiently produce leaner meat. Increased muscle mass can be achieved by increasing muscle fiber number and size, and (or) fiber modulation.

The investigation of muscle fiber characteristics is therefore of practical importance to give meat scientists, breeders, and the meat industry a better understanding of muscle fiber involvement in determining muscle growth and final meat quality traits [28]. Studies have explored the relationships between muscle fiber size and fiber types frequency in bovines [7,25,32,48,50], and the relationship between total muscle fiber number, growth and meat quality [6,47,51], but the precise relationship in cattle remains undefined.

Correspondence to: Dr. Maeli Dal Pai Silva
Departamento de Morfologia, Instituto de Biociências (IB), Universidade Estadual Paulista (UNESP), CEP: 18618-000, Botucatu, SP, Brazil. Tel/Fax: (55) (14) 3811-6264. E-mail: dpsilva@ibb.unesp.br

Bovine skeletal muscles mainly consist of three fiber types: SO (slow oxidative), with oxidative metabolism and slow contraction; FG (fast glycolytic), with glycolytic metabolism and fast contraction; and FOG (fast oxidative-glycolytic), with fast contracting fibers and intermediate resistance to fatigue [19,34,39,42]. There is a correlation between muscle contraction speed and myosin ATPase activity [4]. The different myosin ATPase-based fibers correspond to different myosin heavy chain isoforms (MyHC) [15,43]. The main MyHC isoforms identified are MyHC I, MyHC IIa, MyHC IId/x and MyHC IIb which correspond to fiber types I, IIA, IID/X and IIB, respectively [41,43,44]. The classification systems based on reactions for enzymes involved in oxidative metabolism and m-ATPase activity appear to be incompatible [34]. The SO fiber corresponds to type I, but FOG and FG fibers do not fully match fiber type IIA and IIB [35]. Bovine muscle fibers have been classified into three types by histochemical and/or immunohistochemical techniques [12,18,38].

Some authors have demonstrated that muscle fiber type IIB is not present in bovine muscle [9,26]. However, this does not mean that this kind of muscle fiber is not present. Muscle fibers can co-express multiple MyHC isoforms and IIb isoforms present in muscle fiber type IIB, is expressed in the latest stage of muscle development [9]. Thus, the different methods used for classification of muscle fibers have resulted in a wide spectrum of different nomenclatures.

Growth promoters such as rbST have been used to enhance muscle fiber size and improve meat quality by increasing the skeletal muscle area and changing the fiber types in bovines; however these have produced variable and sometimes contradictory results due to factors which are intrinsic and extrinsic to the animal, such as hormone dose and administration period [10,30], animal sex [21], age [7,13,40,48], and diet [20,27].

Therefore, the objective of this study was to determine the effect of rbST treatment on *semitendinosus* muscle fiber morphology and histochemistry in crossbred heifers during growth.

MATERIALS AND METHODS

Experimental Animals

This experiment was approved by Ethics Committee of Instituto de Biociências, UNESP, Botucatu, SP, Brazil (Protocol N° 1273).

The study was performed in the Department of Animal Improvement and Production, FMVZ, UNESP – Botucatu, SP. Twenty-eight 210 day-old crossbred heifers (*Bos indicus* X *Bos taurus*) were allowed *ad libitum* access to water and creep feed based on corn silage and dry alfafa, with a concentrate: Dry Matter: 58.5%; Crude Protein: 16.3% and Total Digestive Nutrients: 71.3% to compensate for any increased protein and energy requirements due to rbST treatment.

In our study we used sixteen heifers that were randomly assigned to two treatment groups: control (saline-injected; n=8), and rbST-treated (BOOSTIN® 500 mg - Coopers do Brasil Ltda – 15 $\mu g.Kg^{-1}$; n=8). Animals were given a single rbST injection on alternating sides of the tail base every 14 days of age, starting on average at day 210 of age and continuing until day 285. *Semitendinosus* muscle biopsies were collected in two phases: on day 210 before the first injection, and on day 360 (75 days after the last hormone or saline application), when the animals were slaughtered. Animals were weighed at birth (B_0), at before treatment (BT – day 210), and at the end of treatment (ET – day 360).

Morphological and morphometric analysis

Semitendinosus muscle was chosen for this study because of its accessibility for biopsy. On day 210 and 360, eight muscle samples were collected from each group. Tissue samples (approximately 1 cm long and 0.5 cm diameter) were obtained from the *semitendinosus* muscle in the middle of the central portion. Samples were frozen in n-hexane, cooled in liquid nitrogen [8], and stored in cryo-vials at -80ºC until sectioned.

Histochemical analysis was performed on biopsy and post-mortem tissues. Cross serial frozen sections (10 μm) were obtained in cryostat and stained with Haematoxylin-Eosin (HE). To evaluate the contraction ability and the oxidative activity of muscle fibers, further sections were submitted to histochemical myosin ATPase (m-ATPase) (pH 4.6 and 10.4) and to NADH-TR reactions, respectively. We classified the muscle fibers as: SO (slow-twitch-oxidative), FOG (fast-twitch-oxidative-glycolytic) and FG (fast-glycolytic), according to Peter *et al.* [34].

The identification of the *myosin heavy chain* (MyHC) isoforms was determined by *polyacrylamide gel electrophoresis* (SDS-PAGE). Small quantities (10 μl) of the samples were submitted to electrophoresis in 7% polyacrylamide gel [49] with 4% stacking gel for 19-21 h at 70 V. The gel was stained with Coomassie Blue (Brilliant Blue R). MyHC isoforms I and II were identified according to their molecular weights.

The morphometric analysis involved calculation of the muscle fiber cross-sectional area (CSA) and frequency. Approximately 120 fibers (SO, FOG, and FG) from each animal were analyzed through the use of five photomicrographs (40x) per tissue section. A Digital Image Analysis System (Leica QWin) was used to perform the CSA measurements as per Dubowitz [11].

This was a randomized experimental design. Animal weight data were analyzed for repeated measurements in independent groups using ANOVA [24]. Muscle fibers analysis used non-parametric ANOVA for repeated measurements in independent groups [31]. Results were statistically significant at the $P < 0.05$ level.

RESULTS

Morphology and biochemistry of muscle fibers

During the experiment both animal groups showed a significant body weight gain ($P<0.01$). However, a higher body weight gain was observed within the treated animals group (Table 1). Morphological analysis (HE staining) showed normal multinucleated fibers, with peripheral nuclei separated by the endomysium and grouped in fascicles by the connective tissue of the perimysium (Figs. 1A, 2A and 2D).

Histochemical analysis (NADH-TR) revealed two fiber types in a mosaic pattern in *semitendinosus* muscle: (1) fibers with intense or moderate enzyme activity, with reaction product clusters distributed in the subsarcolemmal or intermyofibrillar regions were oxidative, and (2) fibers with weak enzyme activity and small clusters of reaction product in fiber sarcoplasm were glycolytic. Reaction profiles were similar in both groups (Figs. 1B, 2B and 2E).

Acid and alkaline pH m-ATPase reactions revealed three types of muscle fibers: fibers with intense reaction after acid (pH 4.6) pre-incubation (acid-stable and alkali-labile) (Fig. 1C), fibers with intense reaction after alkaline (pH 10.4) pre-incubation (alkali-stable and acid-labile) (Fig. 2C and 2F), and fibers with moderate reaction in both acid and alkaline pH (Figs. 1C, 2C and 2F). Reaction profiles were similar in both groups. Based on metabolism analysis and m-ATPase reaction characteristics, the *semitendinosus* muscle fibers were classified as SO, FOG, and FG.

Biochemical analysis of *semitendinosus* muscle showed separation of myosin heavy chain (MyHC) isoforms into well-defined bands, confirming the presence of MyHC I (in SO fibers) and II (in FOG and FG fibers) (Fig. 3A and 3B).

Muscle fiber frequency

In this study FG fiber frequency was the highest ($P<0.05$), followed by FOG and SO fibers. During the experiment SO fibers frequency significantly increased ($P<0.05$) in the control group. There was no significant difference between groups in SO fiber frequency at the end of the experiment ($P>0.05$). FOG fiber frequency increased in controls and was reduced in treated animals, giving a significant difference between groups at the end of the experiment ($P<0.05$). Although FG fiber frequency was significantly reduced in both groups ($P<0.05$), there was no significant difference between groups at the end of the experiment (Table 2).

Muscle fiber area

In this study FG fiber area was always greater ($P<0.05$) than FOG or SO fibers. There was a significant increase ($P<0.05$) in fiber areas in both groups except for FG fibers in the treated group. At the end of the experiment, FOG fiber area was significantly higher ($P<0.05$) in treated animals, but was not significant for SO and FG fibers (Table 3).

DISCUSSION

The present study evaluated the effect of rbST treatment on *semitendinosus* muscle fibers size (cross-sectional area), fiber type frequency, and myosin heavy chain (MyHC) isoforms in crossbred heifers during growth. FOG fiber percentage distribution decreased and cross-sectional area increased in rbST-treated animals. Recombinant bST caused a greater weight gain and FOG fiber hypertrophy.

Histochemical analysis of the *semitendinosus* muscle revealed three fiber types according to metabolism and ATPase myofibrillar activity [34]: SO (*Slow Oxidative*), FOG (*Fast Oxidative-Glycolytic*), and FG (*Fast Glycolytic*), distributed in a mosaic pattern with a predominance of glycolytic fibers (FOG and FG fibers). Fiber type distribution within muscles is of importance when studying fiber type composition in relation to meat quality. Muscles involved in resistance movements, such as postural muscles, have a more oxidative metabolism than those involved in strength movements [22,46]. In most animals, the deepest limb muscles generally have the highest percentage of oxidative fibers, while the superficial muscles have the highest percentage of glycolytic fibers [1,3]. The finding of the present study corroborates with previous results [5,7,25,48] in so far as s*emitendinosus* muscle showed a predominance of glycolytic fibers. The pattern of muscle fiber type distribution in s*emitendinosus* muscle, revealed by mATPase reaction, was partially correlated with MyHC distribution by electrophoresis analysis, and confirmed the presence of MyHC types I and II [16,17,44].

In this study, 15 $\mu g.Kg^{-1}$ rbST administered every 14 days for three months, given to approximately 7-month-old calves, promoted a significant reduction in FOG fiber frequency. This differs from Vestergaard *et al.* [50], where growth hormone did not affect the proportions of fiber types in Friesian calves, and from Cervieri *et al.* [7], who applied 1.4 $mg.kg^{-1}$

Table 1. Mean ± S.E. of body weight (Kg) in the control (**Sal-C**) and rbST-treated (**rbST-T**) animals at birth (B_0), before treatment (**BT**), and at the end of treatment (**ET**).

Groups	Stages of evaluation		
	B_0	BT	ET
Sal-C (n=8)	98.0 ± 8.9 ^{Aa}	207.4 ± 13.5 ^{Ba}	343.4 ± 40.3 ^{Ca}
rbST-T (n=8)	120.3 ± 11.3 ^{Ab}	241.0 ± 25.6 ^{Bb}	386.0 ± 37.3 ^{Cb}

Uppercase letters: Intra-group body weight comparisons among the stages of evaluation;
Lowercase letters: Inter-group body weight comparisons at a fixed stage of evaluation.
(p value < 0.05) . Values with the same uppercase letters did not differ significantly among the various stages of evaluation. Values with the same lowercase letters did not differ significantly between the two groups.

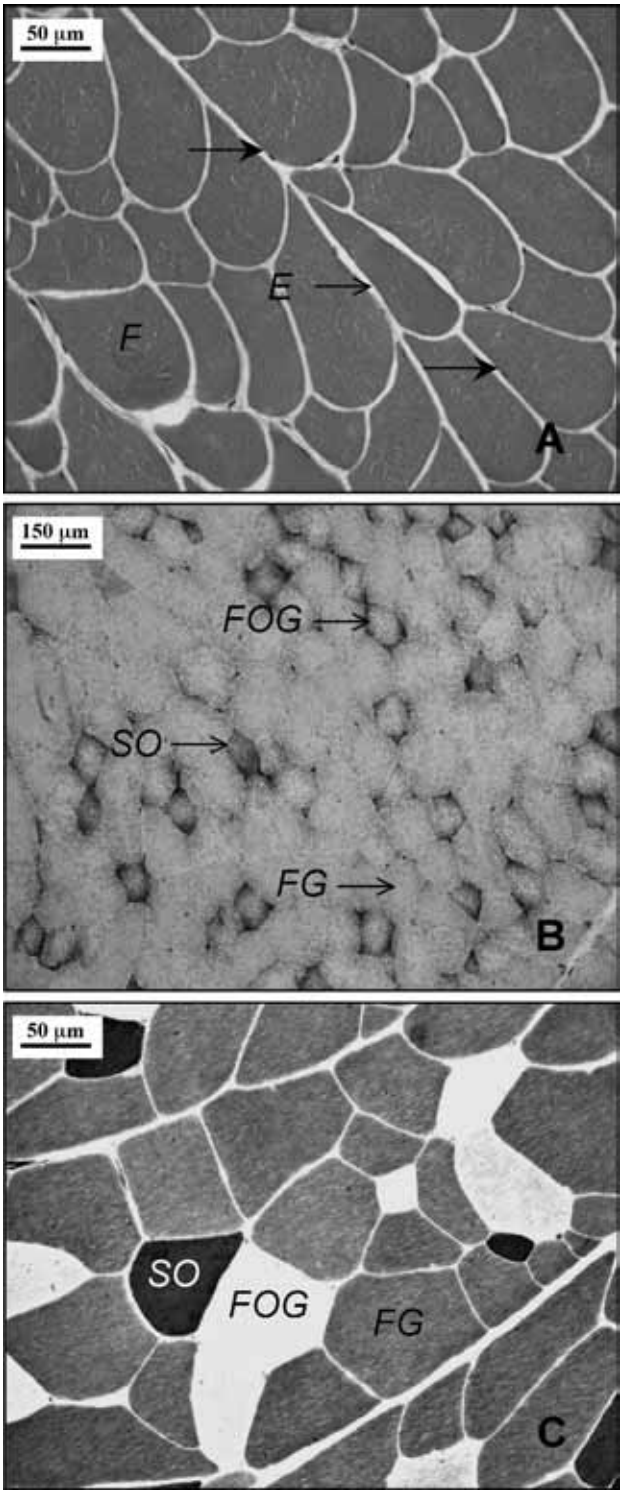

Figure 1. Cross sections of the *semitendinosus* muscle at the beginning of experiment. **A:** HE. **B:** NADH-TR. **C:** Acid mATPase, pH 4.6. ***F*** - Muscle fiber; Peripheral nuclei (**arrows**); ***E*** -Endomysium; **SO** - Slow oxidative; **FOG -** Fast oxidative-glycolytic and **FG** - Fast glycolytic fibers.

rbST to crossed ½ Aberdeen Angus X ½ Nelore until weaning.

In finishing steers, exogenous growth hormone (GH) supplement in the form of recombinant bovine somatotropin (rbST) increased *Longissimus dorsi* muscle area, reduced subcutaneous and intramuscular fat, and increased Insulin - Like Growth Factor - I (IGF-I) plasma levels [10,13,30]. However, the effects of rbST on bovine muscle fibers are still contradictory [51], which may be accounted by hormone dose and time used [10,30], animal gender and age [21,40,48], and diet [20,27].

In male growing and finishing crossed *Bos indicus* X *Bos taurus* steers, muscle fibers were evaluated after treatments with 250 mg.animal^{-1} rbST every 14 days over a 210 day period. A negative effect from rbST supplement was observed between 12 and 14 months of age, when slow contraction (SO) fiber diameter showed a 14.7% reduction, even though frequency did not alter [Furlan LR, 1998, PhD thesis, Paulista State University (UNESP), Jaboticabal, Brazil]. However, European calves (28 day-old) treated with 0.09 mg.kg^{-1} rbST showed reduced FOG and increased FG fibers frequencies, which indicates modulation from FOG to FG fibers in *semitendinosus* muscle [48]. This is, in part, similar to our results; however, we were unable to demonstrate an increase in FG fiber frequency.

A transition of muscle fiber phenotypic profiles causes changes in myosin heavy chain (MyHC) isoforms. During muscle fiber modulation from slow to fast fibers, there is an increase in hybrid fibers, which have two myosin heavy chain isoforms [33,36,37]. This transition in muscle fiber phenotypic profiles may justify the reduction in FOG fiber frequency observed in our study.

During post-natal muscular growth there is also a change in the frequency of different fiber types, varying according to muscle type, species, and age [14,23,25,45,51].

In this study, although an increase in SO fiber frequency was seen at the end of the experiment, this could not be attributed to the modulation occurring during growth because *semitendinosus* muscle has a predominance of glycolytic fibers [5,7]. Also, the significant increase in SO fibers in the control animals could be attributed to variations in the muscle sample collection site. In the first evaluation, a biopsy sample was removed from the muscle surface, where glycolytic fibers predominate; the second sample,

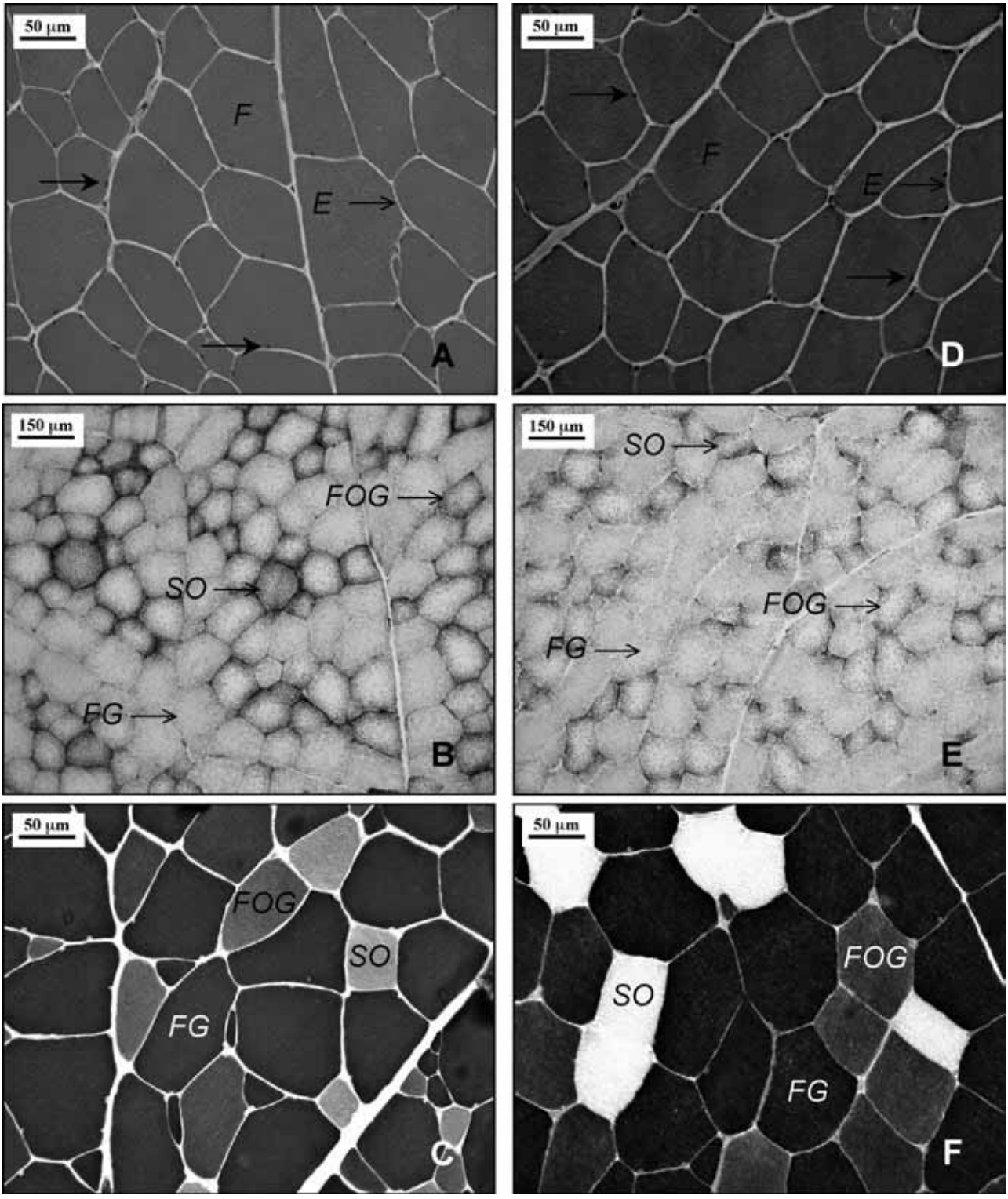

Figure 2. Cross sections of the *semitendinosus* muscle at the end of the experiment in the control and rbST-injected groups. **A, B, C:** control group. **D, E, F:** rbST-injected group. **A, D:** HE. **B, E:** NADH-TR reaction. **C, F:** Alkaline mATPase, pH 10.4. ***F*** - Muscle fibers; Peripheral nuclei **(arrows); *E*** - Endomysium; **SO -** Slow Oxidative; **FOG -** Fast Oxidative-Glycolytic and **FG**-Fast Glycolytic fibers.

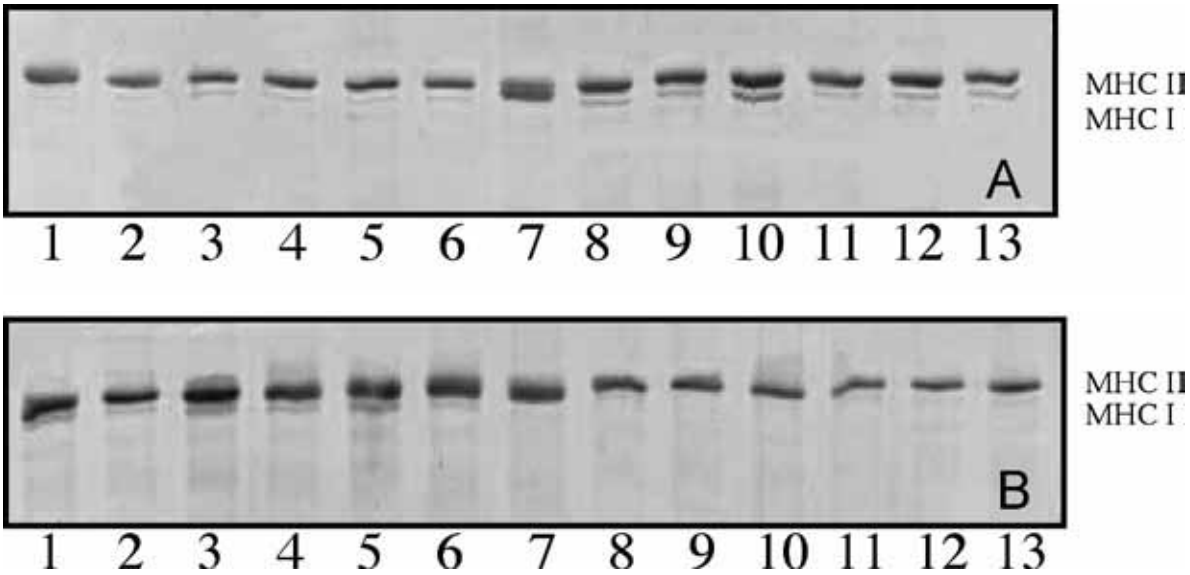

Figure 3. Electrophoretic separation of myosin heavy chain isoforms (MyHC I and II) of s*emitendinosus* muscle. **A:** control group; beginning (1-5) and end (8-13) of the experiment. **B:** rbST-injected group; beginning (1-5) and end (8-13) of the experiment. (6 and 7): rat *soleus* and EDL muscles, respectively, used as pattern.

Table 2. The median and total semi-amplitude of the SO, FOG and FG fiber frequencies in control (**Sal-C**) and rbST-treated (**rbST-T**) animals, before (**BT**) and at the end (**ET**) of the treatment.

Fiber types	Sal-C		rbST-T	
	BT	**ET**	**BT**	**ET**
SO	16 ± 7 ^{Aa} (9.14%)	24 ± 11 ^{Ba} (17.27%)	20 ± 13 ^{Aa} (12.20%)	23 ± 15 ^{Aa} (19.01%)
FOG	28 ± 19 ^{Aa} (16.00%)	34 ± 10 ^{Aa} (24.46%)	25 ± 12 ^{Aa} (15.24%)	21 ± 20 ^{Ab} (17.35%)
FG	131 ± 29 ^{Aa} (74.86%)	81 ± 25 ^{Ba} (58.27%)	119 ± 43 ^{Aa} (72.56%)	77 ± 32 ^{Ba} (63.64%)
Total	**175 (100%)**	**139 (100%)**	**164 (100%)**	**121 (100%)**

Uppercase letters: Intra-group comparisons among the stages of evaluation;
Lowercase letters: Inter-group comparisons at a fixed stage of evaluation.
(p value < 0.05) . Values with the same uppercase letters did not differ significantly among the various stages of evaluation. Values with the same lowercase letters did not differ significantly between the two groups.

Table 3. Mean ± S.E. of the SO, FOG and FG fiber cross-sectional area (μm^2**)** in control (**Sal-C**) and rbST-treated (**rbST-T**) animals before (**BT**) and at the end (**ET**) of the treatment.

Fiber types	Sal-C		rbST-T	
	BT	**ET**	**BT**	**ET**
SO	1587±266^{Aa}	2258±369^{Ba}	1348±443^{Aa}	2746±530^{Ba}
FOG	1600±264^{Aa}	2584±467^{Ba}	1817±579^{Aa}	3337±702^{Bb}
FG	3046±506^{Aa}	3628±6^{Ba}	3010±631^{Aa}	3963±1089^{Aa}

Uppercase letters: Intra-group comparisons among the stages of evaluation;
Lowercase letters: Inter-group comparisons at a fixed stage of evaluation.
(p value < 0.05) . Values with the same uppercase letters did not differ significantly among the various stages of evaluation. Values with the same lowercase letters did not differ significantly between the two groups.

taken during slaughter, may have been removed from a deeper region, where muscles normally have a higher proportion of oxidative fibers [2,5].

During the experiment, both groups showed a significant increase in fiber cross-sectional area (except in FG) accompanied by a significant weight gain. At the end of the experiment, FOG fiber areas were significantly larger in the treated group. Concurrently, SO and FG fibers did not have significantly increased fiber area. In newborn Friesian calves, 3-5 mg rbST administered daily produced little change in *semimembranosus* muscle fiber cross-sectional area [27]. In pre-puberal Holstein heifers, rbST tended to increase SO fiber area [50]. Vann *et al.* [48] reported an increase in SO, FOG, and FG fiber areas in calves treated with rbST; however this effect was only significant in FG fibers; also seen by Cervieri *et al.* [7]. On the other hand, Moreira *et al.* [29] did not confirm differences in SO, FOG, and FG fibers diameter in *semitendinosus* muscle of male Simmentals treated with 0.15 mg/Kg/day rbST between 150 and 210 days old. Even though in the present study rbST had promoted a reduction in FOG fiber frequency, they also had hypertrophy, which could be reflected in muscle growth. Moreover, this muscle fiber phenotypic modulation represents a potential role of the *semitendinosus* muscle in modeling the meat quality.

ACKNOWLEDGMENTS

This study was supported by grants from FAPESP, Proc. Nº 05/51152-9, RUNESP, Proc. N° 1306/50/1/2001 and FUNDUNESP, Proc. N° 557-01-DCP.

REFERENCES

1. Armstrong RB, Delp MD, Goljan EF, Laughlin MH (1987) Distribution of blood flow in muscles of miniature swine during exercise. *J. Appl. Physiol.* **62**, 1285-1298.
2. Armstrong RB, Phelps RO (1984) Muscle fiber type composition of rat hindlimb. *Am. J. Anat.* **171**, 259-272.
3. Ashmore CR, Doerr L (1971) Comparative aspects of muscle after types in different species. *Exp. Neurol.* **31**, 408-418.
4. Barany M (1967) ATPase activity of myosin correlated with speed of muscle shortening. *J. Gen. Physiol.* **50**, 197-218.
5. Brandstetter AM, Picard B, Geay Y (1997) Regional variations of muscle fibre characteristic in m. semitendinosus of growing cattle. *J. Muscle Res. Cell Motil*. **18**, 57-62.
6. Calkins CR, Dutson TR, Smith GC, Carpenter ZL, Davis GW (1981) Relationship of fiber type composition to marbling and tenderness of bovine muscle. *J. Food Sci.* **46**, 708-715.
7. Cervieri RC, Arrigoni MB, Chardulo LAL, Silveira AC, Oliveira HN, Martins CL, Silva MDP (2005) Caracterização das fibras musculares do músculo *semitendinosus* de bezerros mestiços Angus-Nelore recebendo somatotropina bovina recombinante (rbST) até a desmama. *R. Bras. Zootec.* **34**, 907-914.
8. Chayen J, Bitensky L, Butcher RG (1969) *A Guide to Practical Histochemistry.* Oliver & Boyd: Edimburgh.
9. Chikuni K, Muroya S, Nakajima I (2004) Myosin heavy chain isoforms expressed in bovine skeletal muscles. *Meat Sci.* **67**, 87-94.
10. Dalke BS, Roeder RA, Kasser TR, Veenhuizen JJ, Hunt CW, Hinman DD, Schelling GT (1992) Dose-response effects of recombinant bovine somatotropin implants on feedlot performance in steers. *J. Anim. Sci.* **70**, 2130-2137.
11. Dubowitz V (1985) Normal muscle. In: *Muscle biopsy. A Practical Approach.* pp. 41-48. 2nd edn. Bailliere Tindall: London.
12. Duris MP, Picard B, Geay Y (2000) Specificity of different anti-myosin heavy chain antibodies in bovine muscle. *Meat Sci.* **55**, 67-78.
13. Elsasser TH, Rumsey TS, Hammond AC (1989) Influence of diet on basal and gowth hormone-estimulated plasma concentrations of IGF-I in beef cattle. *J. Anim. Sci.* **67**, 128-141.
14. Finkelstein DI, Andrianakis P, Luff AR, Walker DW (1992) Developmental changes in hindlimb muscles and diaphragm of sheep. *Am. J. Physiol.* **263**, R900-R908.
15. Fry AC, Allemeier CA, Staron RS (1994) Correlation between percentage fiber type area and myosin heavy chain content in human skeletal muscle. *Eur. J. Appl. Physiol. Occup. Physiol.* **68**, 246-251.
16. Gauthier GF, Lowey S (1977) Polymorphism of myosin among skeletal muscle fiber types. *J. Cell Biol.* **74**, 760-779.
17. Gorza L (1990) Identification of a novel type 2 fiber population in mammalian skeletal muscle by combined use of histochemical myosin ATPase and anti-myosin monoclonal antibodies. *J. Histochem. Cytochem.* **38**, 257-265.
18. Gotoh T, Iwamoto H, Nakanishi Y, Umetsu R, Ono Y (1999) Histochemical properties of skeletal muscles in different body parts of young Japanese black steers. *Anim. Sci. J.* **70**, 497-509.
19. Guth L, Samaha FJ (1970) Procedure for the histochemical demonstration of actomyosin ATPase. *Exp. Neurol.* **28**, 365-367.
20. Hall JB, Schillo KK, Fitzgerald BP, Bradley NW (1994) Effects of recombinant bovine somatotropin and dietary energy intake on growth, secretion of luteinizing hormone, follicular development and onset of puberty in beef heifers. *J. Anim. Sci.* **72**, 709-718.
21. Hannon K, Gronowski A, Trenkle A (1991) Relationship of liver and skeletal muscle IGF-1 mRNA to plasma

GH profile, production of IGF-1 by liver, plasma IGF-1 concentrations, and growth rates of cattle. *Proc. Soc. Exp. Biol. Med.* **196**, 155-163.
22. Henckel P (1995) Perimortal metabolic events and consequences for meat quality. In: *Proceedings 2nd Dummerstorf Muscle Workshop Muscle Growth and Meat Quality.* pp. 77-82, Rostock.
23. Ishihara A, Inoue N (1989) Histochemical profiles of fibers in the rat tibialis anterior muscle during early postnatal development. *Jpn. J. Physiol.* **39**, 617-622.
24. Johnson RA, Wichern DW (1998) *Applied Multivariate Statistical Analysis.* 4th edn. Prentice-Hall: New Jersey.
25. Jurie C, Robelin J, Picard B, Geay Y (1995) Post-natal changes in the biological characteristics of *semitendinosus* muscle in male Limousin cattle. *Meat Sci.* **41**, 125-135.
26. Maccatrozzo L, Patruno M, Toniolo L, Reggiani C, Mascarello F (2004) Myosin heavy chain 2B isoform is expressed in specialized eye muscles but not in trunk and limb muscles of cattle. *Eur. J. Histochem.* **48**, 357-366.
27. Maltin CA, Delday MI, Hay SM, Innes GM, Williams PE (1990) Effects of bovine pituitary growth hormone alone or in combination with the β-agonist clenbuterol on muscle growth and composition in veal calves. *Br. J. Nutr.* **63**, 535-545.
28. McComas AJ (1996) *Skeletal Muscle: Form and Function*. Human Kinetics: Champaign.
29. Moreira PSA, Chardulo LA, Silveira AC, Arrigoni MDB, Dal Pai V (2002) Desempenho e crescimento tecidual de bezerros Simbrasil suplementados com somatotropina bovina recombinante (rbST). In: *XXXIX Reunião Anual da Sociedade Brasileira de Zootecnia*. Recife (PE), Brazil, July 29 – August 1. v. 1, p.2.
30. Moseley WM, Paulissen JB, Goodwin MC, Alaniz GR, Claflin WH (1992) Recombinant bovine somatotropin improves growth performance in finishing beef steers. *J. Anim. Sci.* **70**, 412-425.
31. Norman GR, Streiner DL (1994) *Biostatistics - The Bare Essentials.* Mosby Year Book: St. Louis.
32. Ono Y, Solomon MB, Elsasser TH, Rumsey TS, Moseley WM (1996) Effects of Synovex-S® and recombinant bovine growth hormone (Somavubove®) on growth responses of steers: II. Muscle morphology and proximate composition of muscles. *J. Anim. Sci.* **74**, 2929-2934.
33. Parcell AC, Sawyer RD, Craig Poole R (2003) Single muscle fiber myosin heavy chain distribution in elite female track athletes. *Med. Sci. Sports Exerc.* **35**, 434-438.
34. Peter JB, Barnard RJ, Edgerton VR, Gillespie CA, Stempel KE (1972) Metabolic profiles of the three fiber types of skeletal muscle in guinea pigs and rabbits. *Biochemistry* **11**, 2627-2633.
35. Pette D, Staron RS (1990) Cellular and molecular diversities of mammalian skeletal muscle fibres. *Rev. Physiol. Biochem. Pharmacol*. **116**, 1-76.
36. Pette D, Staron RS (2000) Myosin isoforms, muscle fiber types and transitions. *Microsc. Res. Tech.* **50**, 500-509.
37. Pette D, Staron RS (2001) Transitions of muscle fiber phenotypic profiles. *Histochem. Cell Biol.* **115**, 359-372.
38. Picard B, Duris MP, Jurie C (1998) Classification of bovine muscle fibers by different histochemical techniques. *Histochem. J.* **30**, 473-479.
39. Sanger AM, Stoiber W (2001) Muscle fiber diversity and plasticity. In: *Muscle Development and Growth. Fish Physiology Series.* (Johnston IA, ed). pp. 187-250. Academic Press: San Diego.
40. Schwarz FJ, Schams D, Röpke R, Kirchgessner M, Kögel J, Matzke P (1993) Effects of somatotropin treatment on growth performance, carcass traits, and endocrine system in finishing beef heifers. *J. Anim. Sci.* **71**, 2721-2731.
41. Scott W, Stevens J, Binder-Macleod SA (2001) Human skeletal muscle fiber type classifications. *Phys. Ther*. **81**, 1810-1816.
42. Sillau AH, Banchero N (1978) Skeletal muscle fiber size and capillarity. *Proc. Soc. Exp. Biol. Med.* **158**, 288-291.
43. Staron RS (1997) Human skeletal muscle fiber types: delineation, development, and distribution. *Can. J. Appl. Physiol.* **22**, 307-327.
44. Staron RS, Pette D (1986) Correlation between myofibrillar ATPase activity and myosin heavy chain composition in rabbit muscle fibers. *Histochemistry* **86**, 19-23.
45. Suzuki A, Cassens RG (1980) A histochemical study of myofiber types in muscle of the growing pig. *J. Anim. Sci.* **51**, 1449-1461.
46. Totland GK, Kryvi H (1991) Distribution patterns of muscle fiber types in major muscles of the bull (*Bos taurus*). *Anat. Embryol.* **184**, 441-450.
47. Tuma HJ, Venable JH, Wuthier PR, Henrickson RL (1962) Relationship of fiber diameter to tenderness and meatiness as influenced by bovine age. *J. Anim. Sci.* **21**, 33-36.
48. Vann RC, Althen TG, Solomon MB, Eastridge JS, Paroczay EW, Veenhuizen JJ (2001) Recombinant bovine somatotropin (rbST) increases size and proportion of fast-glycolytic muscle fibers in *semitendinosus* muscle of creep-fed steers. *J. Anim. Sci.* **79**, 108-114.
49. Vescovo G, Ceconi C, Bernocchi P, Ferrari R, Carraro U, Ambrosio GB, Libera LD (1998) Skeletal muscle myosin heavy chain expression in rats with monocrotaline-induced cardiac hypertrophy and failure. Relation to blood flow and degree of muscle atrophy. *Cardiovasc. Res.* **39**, 233-241.
50. Vestergaard M, Purup S, Henckel P, Tonner E, Flint DJ, Jensen LR, Sejrsen K (1995) Effects of growth hormones and ovariectomy on performance, serum hormones, insulin-like growth factor-binding proteins, and muscle fiber properties of prepubertal Friesian heifers. *J. Anim. Sci.* **73**, 3574-3584.
51. Wegner J, Albrecht E, Fiedler I, Teuscher F, Papstein H-J, Ender K (2000) Growth- and breed-related changes of muscle fiber characteristics in cattle. *J. Anim. Sci.* **78**, 1485-1496.

Received: May 2, 2006
Accepted: May 20, 2006