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Effects of Recombinant Bovine Somatotropin (ST) and Protein on Growth and Muscle Fiber Profiles in Early-Weaned Beef Steers

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Abstract: The objective of this study was to determine effects of somatotropin (ST) and nutrition on growth, myofiber profiles, serum ST and IGF-1 values in beef calves. At 100 day of age, 48 crossbred steers were weaned and trained on the Calan Feeding System. At 155 day of age, steers were assigned to treatments in a 2×2 factorial design testing the effects of protein fed at levels according to NRC (NP) vs a high protein diet (125% of NRC, HP) and ST (Posilac, 2.2 mg×kg^{-1}×14 day^{-1} s.c., ST) vs no ST (C). Blood samples were taken every 14 day for IGF-1 and ST analysis. Treatments continued until 255 day of age, when semitendinosus muscle biopsy samples were collected to determine myofiber morphology. The C-NP, C-HP and ST-NP steers consumed 7.7 kg×hd^{-1}×d^{-1}, which was 19% more than that consumed by ST-HP steers (6.52 kg×hd^{-1}×d^{-1}). The ST-treated steers had improved feed efficiency when compared to controls (0.17 vs 0.15 gain/feed). As expected, steers administered ST had increased serum ST concentrations over time and IGF-1 concentrations increased by 11% after week 4 compared to controls, however, cross-sectional areas for myofiber types and percentage distribution of slow- twitch-oxidative fibers were not different between treatments. The ST treatment resulted in a decreased percentage of fast-twitch-oxidative-glycolytic (26 vs 31%) and increased percentage of fast-twitch-glycolytic (58 vs 52%) fibers when compared to C steers. This data substantiates earlier findings and demonstrates that ST treatment alters myofiber morphology in beef calves, regardless of normal or high protein diets.

Key words: rbST, beef cattle, muscle fiber types, nutrition, protiens

INTRODUCTION

For commercial cattle production, skeletal muscle growth represents a major economic factor and improving production efficiency requires developing methods for enhancing the efficiency of producing muscle tissue. The use of Somatotropin (ST) in growing steers from 28 day of age increases muscling, as indicated by larger longissimus muscle areas, greater conformation scores and more separable muscle at 205 day of age (Vann *et al*., 1998). However, in a follow-up experiment, steers that were younger and maintained in the same pasture in which competition for creep feed may have existed, did not exhibit the magnitude of difference in muscling between treatments by subjective evaluation at 205 day of age (Moulton *et al*., 1998).

Muscle fiber morphology is divided into three primary types: Slow-twitch-oxidative (SO), fast-twitch-oxidative-glycolytic (FOG) and fast-twitch-glycolytic (FG) (Ashmore and Doerr, 1971). The distribution of FG fibers, which possess a larger Cross-Sectional Area (CSA) than FOG and SO fibers was increased in ST treated steers, which may be involved in the increased muscling reported earlier (Vann *et al*., 2001).

The objective of this study was to determine the interaction of ST treatment and protein on growth performance traits, myofiber profiles and serum ST and IGF-1 profiles in growing cattle.

MATERIALS AND METHODS

Animal selection and treatment: Forty-eight crossbred steers were weaned and trained on the Calan Feeding System (American Calan, Northwood, NH) at 100 day of age. At 155 day of age, steers were randomly assigned to treatments to test the effects of dietary protein concentration on the response to bST. Commercial diets were mixed by Nutrena Feeds (Cargill, Minneapolis, MN; Table 1) and fed at levels according to the National Research Council (NRC) recommendation for dietary CP (16% dietary CP; NP) or at 125% of NRC recommendations (HP) and rbST (Posilac, Monsanto, St Louis, MO; ST)

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Table 1: Guaranteed tag analysis of diets for steers fed at 100% (NP) and 125 % (HP) of the NRC requirements for protein

	NP		HP	
Ration Component	Starter	Finisher	Starter	Finisher
Crude Protein, Min (%)	16	12	20	15
Crude Fat, Min (%)	3.5	3.5	3.5	3.5
Crude Fiber, Max (%)	13.5	16	13.5	16
Calcium, Min (%) to Max (%)	0.5-1.0	0.4-0.9	0.5-1.0	0.4-0.9
Phosphorous, (%)	0.3	0.3	0.3	0.3
Salt, Min (%) to Max (%)	0.3-0.8	0.3-0.8	0.3-0.8	0.3-0.8
Sodium, Min (%) to Max (%)	0.26-0.43	0.26-0.43	0.26-0.43	0.26-0.43
Potassium, (%)	0.8	0.8	0.8	0.8
Vitamin A, IU kg^{-1}	3,038	3,038	3,038	3,038

Lasalocid and Oxytetracycline were included in all rations at 54 g mt^{-1} and 11 g mt^{-1}, respectively

given s.c. at 2.2 mg kg^{-1} per 14 day vs no ST (sham-injected by needle stick only, C) until 217 day of age. At 218 day of age only the diets changed to (NRC, 12% CP; NP) vs high protein levels (125% of NRC, 15% CP; HP) until 255 day of age. Treatments continued until 255 day of age when ST treatment was terminated. During the study, four steers were removed as a result of bloating problems.

Sample collection and analysis: Steers were weighed every 14 day to calculate and analyze growth performance and Feed Efficiency (FE). Blood samples were collected every 14 day from 155-255 day. Serum was harvested and frozen (-20°C) until analyzed for IGF-1 and ST concentrations. IGF-1 was dissociated from binding proteins using a modified glycyl-glycine extraction procedure (Underwood *et al*., 1982). The concentration of IGF-1 was determined using a double antibody RIA by the method (Vicini *et al*., 1991). Serum ST concentrations were determined by a double antibody RIA procedure (Althen and Gerrits, 1976). The intra-assay coefficient of variation was 6.12% and the interassay coefficient of variation was 12.9%. On day 255, semitendinosus muscle biopsies were collected for muscle fiber morphology using the shot biopsy method, which includes a biopsy cannula allowing muscle samples (of up to 1.5 g) independent of animal size to be collected quickly from live animals without restraint or anesthesia (Wegner *et al*., 1997; Schoberlein, 1989) (Approved by Mississippi State University IACUC committee). A sample of the semitendinosus muscle was collected via biopsy, due to the accessibility of this muscle. Muscle fiber morphology determination was performed by histochemical analysis according to the procedure of Solomon and Dunn (1988) and muscle fibers were classified into three types slow-twitch-oxidative (SO), fast-twitch-oxidative-glycolytic (FOG) and fast-glycolytic (FG) (Solomon and Dunn, 1988; SAS, 1996; Dalke *et al*., 1991; Holzer *et al*., 2000). The fiber type percentages were computed using two to three muscle fiber bundles (approximately 50-75 fibers per bundle). Fiber Cross-sectional area (CSA) was measured on a minimum of 20 fibers of each type.

Statistical methods: Growth performance and fiber morphology data were analyzed in a 2×2 factorial arrangement in a Randomized Complete Block Design by using the Mixed Procedure SAS® (1996). The two levels of treatment were C and ST and the two levels of nutrition were NP and HP. Means were compared ($p<0.05$) using the method of Least Squares Means. In addition, IGF-1 and ST were analyzed by repeated measures in time using the Mixed Procedure of SAS® (1996).

RESULTS AND DISCUSSION

Somatotropin and IGF-1 serum concentrations: Prior to subcutaneous injections of ST, serum ST concentrations averaged 10.0±1.0 μg mL^{-1} and were not different among treatment groups (Fig. 1). Serum ST concentrations remained unchanged in the C steers throughout the trial, but began to increase in ST-treated steers after the first injection, as expected. After 3 injections, the ST concentrations in ST-treated steers averaged 14.7±1.0 μg mL^{-1} greater than in C steers ($p<0.05$) and remained elevated throughout the remainder of the trial ($p<0.001$), averaging 55% above that in controls from day 42-112. Other scientists have reported a similar increase in plasma ST concentrations in response to ST treatment in finishing steers (Dalke *et al*., 1991). In the present study serum was collected for ST measurement at the end of each 14 day injection period, yet, the elevated ST values agree with other research (Holzer *et al*., 2000), in which ST levels were measured in the middle of the injection period, indicating that at 1 week post-injection ST levels are higher and remain higher at 14 day post-injection.

In the first 4 week of the trial, serum IGF-1 concentrations were not altered by ST treatment (Fig. 2), but in the final 5 week, IGF-1 concentrations in ST-treated steers were 17±5.7 ng mL^{-1} (11%) above that in C-steers (linear contrast of ST effect in week 5 through 9, $p<0.001$). The response in IGF-1 concentration is less than that noted by scientist (Schlegel *et al*., 2006), who reported ST treatment increased IGF-1 concentrations 55% in Holstein steers (185 kg) compared to controls; yet, these data confirm similar findings in sheep (McLaughlin *et al*., 1993), steers (Dalke *et al*., 1991; Mosely *et al*., 1992; Enright *et al*., 1990; Preston *et al*., 1995) and bulls (Holzer *et al*., 2000). As with the present study, others (Holzer *et al*., 1999) reported no change in serum IGF-1 concentrations in Holstein-Friesian bulls (165 day old and 193 kg) treated with 3 injections of 500 mg ST at 2-week intervals. In the previous studies, the interval between the

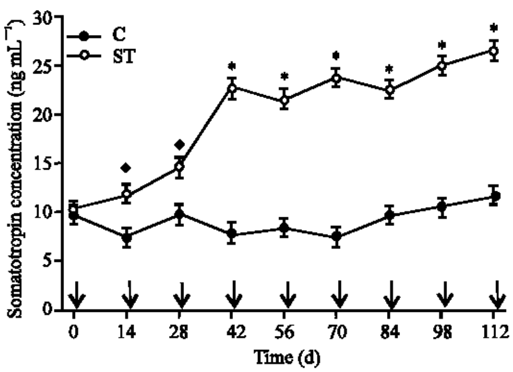

Fig. 1: Somatotropin concentrations in beef steers given recombinant bovine somatotropin (ST) or sham injections (C) every 14 day from 156 day of age. In ST-treated steers, the somatotropin (Posilac, Monsanto, St Louis) was injected s.c. at 2.2 mg kg^{-1} per 14 day (arrows). Data represents the least squares mean±SEM of 22 observations per group. Differences between groups at each time point are indicated as $^{\prime}$ p = 0.07 and * p<0.05

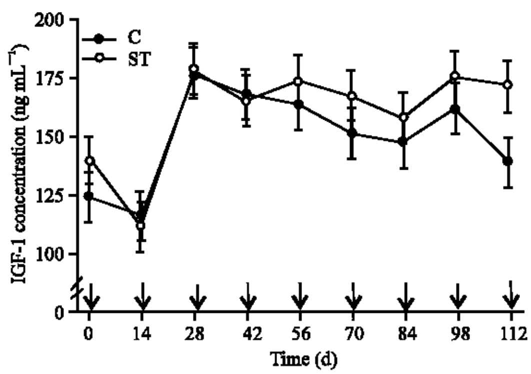

Fig. 2: Serum concentrations of IGF-1 in beef steers given recombinant bovine somatotropin (ST) or sham injections (C) every 14 day from 156 day of age. In ST-treated steers, the somatotropin (Posilac, Monsanto, St Louis) was injected s.c. at 2.2 mg kg^{-1} per 14 day (arrows). Data represents the least squares mean±SEM of 22 observations per group

ST injection and blood sampling may have a role in the observed magnitude of the IGF-1 response; yet, other scientist (Neathery *et al*., 1991) found no change in serum IGF-1 concentrations in Holstein calves (75 kg) injected daily with ST (10 mg) for 42 day.

Growth Performance: Initial (155 day) body weight of the steers was 167±3.5 kg and was similar for calves in all treatment groups (Table 2). Steers gained 134±4.5 kg during the 100 day trial and the ADG was not affected by either ST treatment or dietary protein level. The present results agree with those who reported similar gains between control and rbST-treated calves (Vann *et al*., 1998) and with those who reported ST treatment did not alter ADG in young Holstein calves (Neathery *et al*., 1991). These results also agree with studies of older finishing cattle in which ST-treatments had no effect on ADG (Perston *et al*., 1995; Stine, 1994).

However, other scientist reported that Holstein bull calves treated with ST had increased ADG and final BW compared to controls (Groenewegan *et al*., 1990). Feed intake averaged 7.73±0.12 kg×hd^{-1}×day^{-1} for steers in the C-NP, C-HP and ST-NP groups; yet, there was an interaction of ST treatment and dietary protein ($p<0.05$), in which ST-HP steers consumed 16% less feed (6.52±0.22 kg×hd^{-1}×day^{-1}) than steers in the other treatment groups. This finding is in agreement with those who reported rbST-treated calves (182 day of age) tended to eat less than controls (Holzer *et al*., 2000). Conversely, injections of 10 mg sometribove (recombinant methionyl bST) for 6 week to Holstein calves (59 day of age) did not influence feed intake compared to controls (Neathery *et al*., 1991).

In the present study, feed efficiency (gain to feed ratio) was improved 13% ($p<0.01$) in response to ST treatment, from 0.15±0.004 in C steers to 0.17±0.004 for ST-treated steers.

Scientists reported that Friesian heifers showed no change in feed intake in response to ST, but increased ADG (27%) and thereby improved the feed to gain ratio (25%) (Vestergaard *et al*., 1995). Similarly, others reported administration of ST to bull calves increased ADG 9% and thereby improved the efficiency of gain 10% (Holzer *et al*., 1999). Yet, others reported ST treatment did not alter FE in young Holstein calves (Neathery *et al*., 1991).

Muscle fiber distribution and areas: The effect of ST-treatment on the percentage distribution of fiber types was to decrease FOG while increasing FG fibers ($p<0.05$) without affecting SO fibers in growing steers (Table 3). These results agree with those who reported that the percentage of FG fibers increased, while the percentage FOG fibers decreased in growing cattle treated with ST compared to controls (Vann *et al*., 2001).

However, unlike the study of others, there were no differences in fiber CSA for SO, FOG and FG fibers ($p>0.10$) (Vann *et al*., 2001). The increase in size of individual muscles due to exogenous administration of porcine ST as evaluated by histochemical methods is a result of increased muscle fiber size (Beermann *et al*., 1990; Solomon *et al*., 1994; Ono *et al*., 1995). However, it has been previously reported that some muscles respond

Table 2: Effect of ST and dietary protein on feed intake for growing beef steers

	Treatment group					
	Control ©		Somatotropin (ST)			
Growth trait	NP (n = 12)	HP (n = 12)	NP (n = 12)	HP (n = 12)	SEM	p-value
Initial (155 day) body wt, kg	164	165	169	170	7	> 0.10
Final (255 day) body wt, kg	296	295	312	301	9	> 0.10
ADG, kg d^{-1}	1.32	1.30	1.43	1.31	.09	> 0.10
Feed Intake, kg×hd^{-1}×d^{-1}	7.66[a]	7.91[a]	7.63[a]	6.52[b]	0.22	< 0.006

Values are represent least squares means and SEM of 10 to 12 observations. [ab]Means in the same row with different superscripts are significantly different ($p<0.05$)

Table 3: Percentage distribution of fiber types and cross-sectional areas for control and ST-treated steers at 255 day of age

	Treatment group			
Fiber type	C (n = 22)	ST (n = 22)	SEM	p-value
Muscle fiber distribution				
SO (%)	17	16	1	< 0.310
FOG (%)	31	26	1	< 0.010
FG (%)	52	58	1	< 0.009
Cross-sectional areas				
SO, μm^2	1602	1718	63	< 0.23
FOG, μm^2	1987	2074	117	< 0.62
FG, μm^2	3414	3766	131	< 0.08

Values are represent least squares means±SEM

differently to exogenous administration of bovine ST (Maltin *et al*., 1990). Unlike the current study, muscle fiber proportions or relative areas were unchanged by ST administration compared to controls in the longissimus muscle (Vestergaard *et al*., 1995), which may illustrate difference in response to ST by different muscles.

There was no effect of dietary protein on muscle fiber distribution. The muscles of newborn calves exhibit dense glycogen deposits and are nearly devoid of lipid droplets. In addition, glycogen phosphorylase activity is high in newborn bovine muscles and apparently FOG fiber transformation begins early (Ashmore and Doerr, 1971). Energy-producing enzymes appear to be the primary focus of transformation of FOG fibers from an aerobic state of metabolism to an anaerobic state found in the FG fibers. As mitochondrial density decreases, glycogen phosphorylase (indicative of glycolytic enzymes) in general increases (Bass *et al*., 1969). The increased fiber size and reduction of blood flow to the fiber are characteristics of FG fibers. Since FG fibers are significantly larger on the average than the SO and FOG fibers, the size of a given muscle is going to vary directly with the proportion of and the degree to which FOG fibers transform to FG fibers (Ashmore *et al*., 1972).

CONCLUSION

Higher percentages of FG fibers in ST-treated calves could be attributed to the increased rate of transformation from FOG to larger FG myofibers. However, the level of IGF-1 may have been near maximal in the young growing steers since the IGF-1 hormone profiles were not dramatically different between control and ST-treated animals, despite the differences in the ST profiles. This may indicate that ST is generating additional energy needed for a more rapid myofiber transformation by repartitioning energy from adipose tissue to muscle.

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