ELSEVIER

Meat Science 56 (2000) 229–237

MEAT SCIENCE

www.elsevier.com/locate/meatsci

Effects of growth-promoting implants on morphology of *Longissimus* and *Semitendinosus* muscles in finishing steers☆

Sonja Fritsche [a,1], Morse B. Solomon [a,*], Ernest W. Paroczay [a], Theron S. Rumsey [b]

[a] *Meat Science Research Laboratory, USDA-ARS, Building 201, BARC-EAST, Beltsville, MD 20705-2350, USA*
[b] *Growth Biology Laboratory, USDA-ARS, Beltsville, MD 20705-2350, USA*

Received 22 November 1999; received in revised form 25 April 2000; accepted 25 April 2000

Abstract

Growth-promoting implants lead to increased muscle accretion in ruminants. To elucidate the effects at a cellular level, muscle fiber distribution and cross-sectional area (CSA) of *longissimus* (LM) and *semitendinosus* (ST) muscles were compared in implanted and control steers. Sixty-four Charolais steers were assigned to one of four treatments (16 steers/treatment): (1) no implant, (2) Synovex-S® (estradiol benzoate + progesterone), (3) Ralgro® (zeranol) or (4) Revalor-S® (trenbolone acetate + estradiol-17β). The experiment was carried out using four slaughter groups (SGRP). Sixteen steers each were slaughtered after 48, 104, 160 and 175 days (four steers/treatment) on trial. Steers on an implant treatment were first implanted at 15 months of age (day 0) and re-implanted at 56 and 112 days. Muscle fibers in the LM and ST (for both live biopsy and post-mortem samples) were characterized as either slow-twitch oxidative (SO), fast-twitch oxidative-glycolytic (FOG) and fast-twitch glycolytic (FG) fibers. Fiber distribution was minimally affected by SGRP in these physiologically mature steers. Implantation with Synovex did not alter fiber distribution in either muscle compared with control steers. Both Synovex-implanted and control steers showed a decrease of FG and an increase of FOG fibers in the LM from day 0 to SGRP 2 followed by an increase of FG and a decrease of FOG fibers. Ralgro- and Revalor-implanted steers had an almost constant fiber distribution in the LM throughout the experiment resulting in higher precentages of FG fibers in SGRP 2 ($P < 0.05$) than SYN or CON steers. Biopsy samples of the LM muscle which were excised 51 days (SGRP 1–3) or 65 days (SGRP 4) before slaughter proved to be suitable for the determination of fiber distribution in live animals. Fiber area increased in post-mortem samples of both muscles from SGRP 1–3 in all treatment groups followed by a plateau. Implantation with Revalor led to an additional increase in fiber area from SGRP 3 and 4 ($P < 0.05$). Synovex did not affect fiber area compared with control steers whereas Ralgro and Revalor implants led to larger fibers in SGRP 3 and 4, respectively. It can be concluded that some growth-promoting implants result in noticeable differences in muscle hypertrophic responses which coincide with their different effectiveness to enhance lean mass accretion.

Keywords: Anabolic implant; Biopsy; Muscle fiber; Steer

1. Introduction

Muscle growth is most important for lean mass accretion in meat producing animals. Muscle accretion in ruminants as well as overall growth rate and feed conversion efficiency can be improved using growth-promoting agents (Hunt, Henricks, Skelley & Grimes, 1991; Rumsey, Hammond & McMurtry, 1992; Schmidely, 1993). Postnatal muscle growth at the cellular level is primarily due to muscle fiber hypertrophy because the total fiber number is fixed at or soon after birth (Pearson, 1990).

Muscle fiber population and muscle fiber growth can be influenced by hormonally active compounds (Vigneron, Dainat & Bacou, 1989). The effects are largely dependent on the hormone sensitivity of the particular muscle being investigated. Limited information is available on the effects of steroid hormones on fiber characteristics in cattle. Bulls, which produce high levels of testosterone, generally have larger muscle fibers than steers (Clancy, Lester & Roche, 1986; Dreyer, Naude, Henning & Rossouw, 1977; Ockerman, Jaworek, van

☆ Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by USDA and does not imply its approval to the exclusion of other products that may be suitable.

* Corresponding author. Tel.: +1-301-504-8400; fax: +1-301-504-8438.

E-mail address: msolomon@lpsi.barc.usda.gov (M.B. Solomon).

[1] Visiting scientist from the Department of Food Chemistry, University of Hamburg, Germany.

0309-1740/00/$ - see front matter
PII: S0309-1740(00)00047-4

Stavern, Parret & Pierson, 1984). Bulls have also been reported to have a higher percentage of FOG fibers accompanied by a lower percentage of FG fibers in the *longissimus* (LM) muscle than steers have (Clancy et al.; Young & Bass, 1984). Compared with heifers, steers have been shown to have higher percentages of SO fibers and lower percentages of FG fibers in the LM (Johnston, Moody, Boling & Bradley, 1981). However, other authors (Cornforth, Hecker, Cramer, Spindler & Mathias, 1980; Sink, Turgut & Mann, 1985) did not observe sex differences in fiber distribution.

Only few publications address the effect of exogenous sex hormones or their analogues on muscle fiber characteristics of steers. Ono, Solomon, Elsasser, Rumsey and Moseley (1996) observed an increase of FG fibers and a decrease of FOG fibers in the *supraspinatus* and *rectus femoris* but not in the LM and ST muscle of growing steers implanted with Synovex. The only effect on fiber area was for SO and FG fibers of the *psoas major* muscle. Hughes, Schelling, Garber, Eastridge, Solomon and Roeder (1998) reported an increase in number and CSA of FG fibers and a decrease of SO fiber percentage in the LM of Angus crossbred steers (BW 585 kg) implanted with Revalor. All three fiber types increased in CSA in the *supraspinatus* muscle of the implanted steers versus the controls (Hughes et al., 1998).

The aim of the present study was to compare the effects of three US approved anabolic implants (Synovex-S®: estradiol benzoate + progesterone, Ralgro®: zeranol, Revalor-S®: trenbolone acetate + estradiol-17β) on muscle fiber populations and muscle fiber hypertrophy in finishing steers. Our interest focused on the question if and/or how anabolic implants enable physiologically mature steers to enhance lean tissue accretion at the cellular level. Furthermore, we were interested in comparing the accuracy of samples obtained through a biopsy device on the live animal with that of excised muscle samples obtained postmortem.

2. Materials and methods

2.1. Animals

Sixty-four Charolais steers were randomly assigned to one of four ear-implant treatments (TRT); control (CON, no implant), Synovex-S® (SYN: 200 mg progesterone and 20 mg estradiol benzoate), Ralgro® (RAL: 36 mg zeranol) or Revalor-S® (REV: 120 mg trenbolone acetate and 24 mg estradiol). Steers assigned to an implant treatment were first implanted at 15 months of age (300 kg body weight, day 0). The steers used in the present study were part of a larger study which had a protocol requiring steers to be from Charolais breeding and at approximately 1.5 years of age at slaughter (Rumsey, Fritsche, Elsasser, Solomon, Mitchell & Kahl, 2000; Solomon & Berry, 2000). Sixteen steers (4 per TRT) were slaughtered 48 days after the first implantation: slaughter group one (SGRP 1). The remaining steers assigned to an implant treatment were reimplanted 56 days after the first implantation. Sixteen steers (4 per TRT) were slaughtered 48 days after the second implantation (SGRP 2). The remaining steers assigned to an implant treatment were reimplanted 56 days after the second implantation. Sixteen steers (4 per TRT) were slaughtered 48 days after the third implantation (SGRP 3). The remaining 16 steers (SGRP 4) were slaughtered 15 days after SGRP 3. The design of the study is illustrated in Table 1.

All steers were fed ad libitum amounts of a diet containing 30% silage (corn/grass: 50/50) and 70% concentrate (corn grain, soybean meal, cottonseed hulls, wheat straw, molasses, salt, minerals) from 2 weeks before day 0 of the study until 48 days prior to slaughter. Each steer during its respective period before slaughter was fed equal amounts of feed that allowed the control steers to gain 1.25 kg/day. (As part of another study, each steer, just before its slaughter, was challenged with thyrotropin releasing hormone and growth hormone releasing hormone to determine pituitary sensitivity to releasing growth hormone and thyroid stimulating hormone.)

2.2. Tissue collection

Biopsy samples (cylindrical bioptate, approximately 5×0.5 cm) were collected from the steers from the lumbar region (20 cm anterior to pin bone, 4 cm lateral from midline) of the *longissimus* muscle (LM) and from midlength (approximately 15 cm distal from the ischiatic tuber) of the *semitendinosus* muscle (ST) midway between the *semimembranosus* and *biceps femoris* muscles. Biopsy sampling was performed 3 days before the last implantation of each SGRP (i.e. 3 days before the first implantation in SGRP 1, 3 days before the second implantation in SGRP 2, and 3 days before the third implantation in SGRPs 3 and 4). The samples were taken with the biopsy device developed by Schöberlein (1976) set at a sampling depth of 6 cm. The instrument is an adaptation of a stunning gun and uses blank cartridges to drive a cannula with a precise cutting edge into tissues. A cylindrical plug of bioptate (skin + subcutaneous fat + muscle) of up to 2 g wet weight is removed from the live animal by means of a tissue retractor in less than 1 s. This technique provides muscle tissue samples with acceptable quality for morphological analyses while imposing only minimal stress to the steers and no need for anaesthesia (Cheah, Cheah & Just, 1997; Schöberlein, 1989). This technique has been successfully utilized for pigs (Schöberlein, 1989; Scholz, Mitchell, Solomon & Wangs, 1998) and calves (Vann,

Table 1
Design of the animal experiment (n = number of animals, LM = *Longissimus* muscle, ST = *Semitendinosus* muscle, pm = postmortem)

	No implant		Synovex		Ralgro		Revalor	
Slaughter group (SGRP)	*n*	Samples	*n*	Samples	*n*	Samples	*n*	Samples
SGRP 1 (1 implantation, slaughter: 48 days)	4	LM/ST biopsy/pm	4	LM/ST biopsy/pm	4	LM/ST biopsy/pm	4	LM/ST biopsy/pm
SGRP 2 (2 implantations, slaughter: 104 days)	4	LM/ST biopsy/pm	4	LM/ST biopsy/pm	4	LM/ST biopsy/pm	4	LM/ST biopsy/pm
SGRP 3 (3 implantations, slaughter: 160 days)	4	LM/ST biopsy/pm	4	LM/ST biopsy/pm	4	LM/ST biopsy/pm	4	LM/ST biopsy/pm
SGRP 4 (3 implantations, slaughter: 175 days)	4	LM/ST biopsy/pm	4	LM/ST biopsy/pm	4	LM/ST biopsy/pm	4	LM/ST biopsy/pm

Althen, Smith, Veenhuizen & Smith, 1998; Wegner & Schöberlein, 1984) with complete wound healing after 20 days.

The steers were slaughtered at the USDA abattoir (inspection No. 68) at Beltsville (MD) according to the guidelines of the Beltsville Animal Care and Use Committee and Food Safety Inspection Service humane slaughter procedures on days 48, 104, 160 and 175 of the experiment. Postmortem (pm) samples (5×1×1 cm) were excised from the LM and ST within 1 h after slaughter from the opposite side (of the biopsy samples) with an attempt to align corresponding locations and depths as the biopsy samples. Both biopsy and pm samples were restrained to a flat stick using twine (minimize muscle sample contraction), coated with talc powder and immediately frozen in liquid nitrogen. Samples were stored at −70°C in an ultra-low freezer until histochemical analysis. Biopsy samples were not restrained to the flat sticks at precise measurements of the exised samples the way pm samples were. Therefore, the contraction of muscle fibers was not controlled and bioptates were not used for CSA measurements. Contraction of muscle fibers after shot biopsy sampling has been reported to increase fiber diameter/area by 10 μm in ST muscle (compared to samples taken 24 h pm, Wegner & Schöberlein, 1984).

2.3. Histochemical analysis

A 1 cm^3 specimen of tissue was removed from each frozen sample and cut into 10 μm thick cross-sections at −17°C using a Cryostat 2800 Frigocut-E (Reichert-Jung, Cambridge Instruments, New York). Sections were allowed to air-dry for 30 min then stained according to the procedure of Solomon and Dunn (1988) for bovine skeletal muscle. Muscle fibers were classified into three types (SO: slow-twitch oxidative, FOG: fast-twitch oxidative-glycolytic, FG: fast-twitch glycolytic) on the basis of stain reactions (Peter, Barnard, Edgerton, Gillispie & Stempel, 1972). Photomicrographs from the stained muscle sections were used to determine muscle fiber distribution and cross-sectional area of each fiber type. Fiber type percentage was calculated counting an average of 424±153 cells photographed at 2–4 different locations within the muscle sections. Fiber area of at least 30 cells per fiber type (average: 74±25 cells/type) was measured with a Zeiss Interactive Digital Analysis System (Carl Zeiss, New York).

2.4. Statistics

Data obtained for LM bioptates, postmortem LM, ST bioptates and post-mortem ST of steers were analyzed separately using the General Linear Model procedure (SAS, 1990) with TRT and SGRP as main effects and the TRT×SGRP interaction. The analysis of variance (GLM) results are shown in Table 2. Appropriate group means were compared using the least significant difference. Additionally, least square means of bioptates were compared to their corresponding postmortem samples using analysis of variance (GLM).

3. Results and discussion

3.1. Introductory remarks

The present study provides information on the influence of growth-promoting implants on the ability of physiologically mature steers (15–22 months) to accrue muscle mass in the LM and ST at the cellular level. The selected histochemical analysis allows the monitoring of functional (slow-twitch vs. fast-twitch) and metabolic (oxidative vs. glycolytic) properties of muscle fiber cells as well as their radial growth. The fiber types (SO, FOG, FG), according to Peter et al. (1972), correspond to the following fiber types based on differentiation of myosin isoforms. SO fibers are identical to type I fibers. In bovine ST, FG fibers correspond to IIB fibers and FOG fibers to IIA + IIAB fibers (Jurie, Picard & Geay, 1998) whereas in LM, FG fibers correspond to IIB + IIAB and FOG fibers to IIA fibers (Picard, Duris & Jurie, 1998). This implies that immunohistochemically classified type IIA and IIAB fibers show different metabolic profiles in different muscles (Picard et al., 1998). Fibers containing myosin heavy chain (MyHC) IIX have not yet been detected in bovine species (Jurie et al., 1998). The percentage of IIC fibers (containing MyHC IIA and I) in adult bovine muscles is relatively small (0.5–5%, Jurie et al., 1998).

Table 2
Main effects[a] and their interaction[a] on percentage and cross-sectional areas of slow-twitch oxidative (SO), fast-twitch oxidative-glycolytic (FOG) and fast-twitch glycolytic (FG) muscle fibers in post-mortem *Longissimus* (LM) and *Semitendinosus* (ST) muscles of steers (biopsies in brackets; TRT: treatment[b], SGRP: slaughter group[c])

	Fiber percentage			Cross-sectional area		
	SO	FOG	FG	SO	FOG	FG
LM						
TRT	ns (ns)	ns (*)	ns (ns)	ns	ns	ns
SGRP	ns (ns)	** (***)	ns (**)	***	***	***
TRT×SGRP	ns (ns)	ns (ns)	ns (ns)	*	*	ns
ST						
TRT	ns (ns)	ns (ns)	* (ns)	**	ns	*
SGRP	*** (ns)	ns (ns)	*** (*)	*	*	***
TRT×SGRP	ns (ns)	ns (ns)	* (ns)	*	*	*

[a] Probability of effect: ns = not significant ($P > 0.1$), *$P < 0.1$, **$P < 0.01$, ***$P < 0.001$.
[b] Treatments: no implant, Synovex, Ralgro, Revalor.
[c] Slaughter groups: SGRP 1, SGRP 2, SGRP 3, SGRP 4.

There were no implant by feeding period or age interactions for carcass merit data (Rumsey et al., 2000) suggesting that the different implants had similar patterns of response over time on feed. Carcass merit results (not in tabular form), which give some indication of carcass lean yield showed that the greatest response to the ear implant products used in this study occurred during the first feeding period and that the combined androgen-estrogen formulation of Revalor gave the strongest response in carcass lean growth compared to the estrogenic implant formulations (Synovex and Ralgro). Longissimus muscle area (REA, cm^2), a carcass merit trait were as follows: SGRP 1 REA = Control 63.1, Synovex 67.9, Ralgro 61.6, Revalor 73.7; SGRP 2 REA = Control 76.6, Synovex 74.2, Ralgro 73.6, Revalor 84.2; SGRP 3 REA = Control 72.2, Synovex 78.5, Ralgro 83.9, Revalor 87.4; SGRP 4 REA = Control 76.5, Synovex 85.5, Ralgro 80.5, Revalor 84.6. Subcutaneous fat thickness measurements (FT, cm), a carcass merit trait were as follows: SGRP 1 FT = Control 0.23, Synovex 0.28, Ralgro 0.20, Revalor 0.23; SGRP 2 FT = Control 0.51, Synovex 0.53, Ralgro 0.53, Revalor 0.36; SGRP 3 FT = Control 0.84, Synovex 1.09, Ralgro 0.79, Revalor 0.99; SGRP 4 FT = Control 0.89, Synovex 1.04, Ralgro 1.12, Revalor 1.02.

3.2. Influence of implant treatment and time on feed on fiber distribution

Using physiological mature steers proportions of SO and FOG fibers were on average 20–30% and FG fibers 45–55% in the LM throughout the experiment (Table 3). These results are in agreement with published values for slaughter-weight steers (e.g. Hunt & Hedrick, 1977: 29% SO, 25% FOG, 46% FG). No change in SO percentage over time on feed could be observed in the LM of any of the TRT groups. However, there seems to be a conversion between the two types of fast-twitch fibers. Although the FG percentage did not change significantly with age in the LM of all implant groups, a temporary decrease (SGRP 2) was observed in the control steers (Fig. 1) accompanied by an increase of FOG fibers (Fig. 2). After that the percentage of FOG fibers decreased from SGRP 2 (19 months of age) to SGRP 3 (21 months of age) in the CON steers. These changes were not observed in RAL- and REV-treated steers which lead to higher FG and lower FOG percentages in these steers compared to CON steers in SGRP 2. One of the main active ingredients in REV is trenbolone which has glucocorticoid antagonist properties. Corticosteroids have been shown to decrease fast fibers (type IIB) and increase intermediate fibers (IIA) in rat muscles (Nava et al., 1996). Hughes et al. (1998) similarly found higher FG proportions in LM of REV-treated steers (BW 585 kg) compared with control steers but at the expense of SO fibers. Synovex had no effect on fiber distribution in our experiment, nor was a response from Synovex implantation observed by Ono et al. (1996) in LM and ST fiber distribution of growing steers.

Although the contractile differentiation of muscle fibers is practically complete in the early postnatal life of cattle (Geay & Picard, 1995), muscle fibers can react to a variety of signals including hormonal signals with transformation from fast to slow or vice versa. This transition proceeds stepwise by myofibrillar protein isoform exchanges through changes in gene expression (Adams, Mather, Baldwin & Dudley, 1993; Carpenter, Greaser & Cassens, 1987; Pette & Staron, 1997). During growth of steers, a shift from oxidative to glycolytic fibers has been observed (Spindler, Mathias & Cramer, 1980: *biceps femoris*, 1–11 months, Vann, Althen, Solomon, Eastridge & Veenhuizen, 1998: ST, 3.5–7 month, Cornforth, Schwarz & Cramer, 1973: *biceps femoris* and LM, 90–550 kg) although some authors detected an increase in SO fibers in some muscles (Johnston et al., 1981: LM, 7–10 months) while others indicated no changes with age (Cornforth et al., 1980: *biceps femoris*, 40 kg to slaughter weight, Sink et al., 1985: ST, 8–20 months). Changes in metabolism do not necessarily have to be accompanied by alterations in the myofibrillar system (Pette & Staron, 1997). Solomon, West and Hentges (1986) monitored fiber distribution in LM of Angus and Brahman bulls from 60 to 100% physiological maturity. The percentage of SO fibers increased as physiological maturity increased from 60 to 80% followed by a plateau. The percentage of FOG fibers decreased continually from 60 to 100% maturity whereas FG% increased. This transformation from the smaller FOG to the larger FG fibers was suggested to contribute to muscle enlargement.

Table 3
Percentage of slow-twitch oxidative (SO), fast-twitch oxidative-glycolytic (FOG), and fast-twitch glycolytic (FG) fibers in *Longissimus* muscle of steers, as affected by treatment and time on feed (least square means[c])

	Postmortem samples				Biopsy samples			
	CON	SYN	RAL	REV	CON	SYN	RAL	REV
SO fibers								
SGRP 1	25.8	25.1	23.3	23.7	23.1	26.5	25.0	20.7
SGRP 2	26.4	25.8	24.2	23.6	22.2	27.4	26.8	24.6
SGRP 3	29.1	26.6	23.5	27.3	25.6	26.5	24.5	32.4
SGRP 4	24.4	23.3	24.3	27.8	25.5	24.9	24.5	28.0
FOG fibers								
SGRP 1	26.2ab[b]	25.5a,b	27.6a	25.2	21.7bc	22.6	19.9	22.0ab
SGRP 2	30.5a	27.8a	22.4b[a]	23.7[a]	27.7a	25.6	23.8	24.7a
SGRP 3	21.9bc	22.3b	24.9ab	21.8	25.7ab	25.2	20.7*	18.5b[a]
SGRP 4	21.2c	22.8b	21.0b	22.5	18.3c	22.3	19.5	20.5ab
FG fibers								
SGRP 1	48.0ab	49.5	49.2	51.1	55.2ab	51.1	55.2	57.4a
SGRP 2	43.2b	47.1	53.4[a]	52.7[a]	50.2ab	47.1	49.5	50.7ab
SGRP 3	49.0ab	52.0	51.7	50.9	48.7b	48.3	54.8	49.1b
SGRP 4	54.5a	50.6	54.8	49.7	56.3a	52.8	56.0	51.5ab

[a] Treatment effect for implanted vs. control steers ($P < 0.05$) within one SGRP and within either pm or biopsy.
[b] Time on feed effect: values with a different letter within a sub-column differ from each other ($P < 0.05$).
[c] Standard error (overall): SO: 2.4%, FOG: 1.7%, FG: 2.5%.

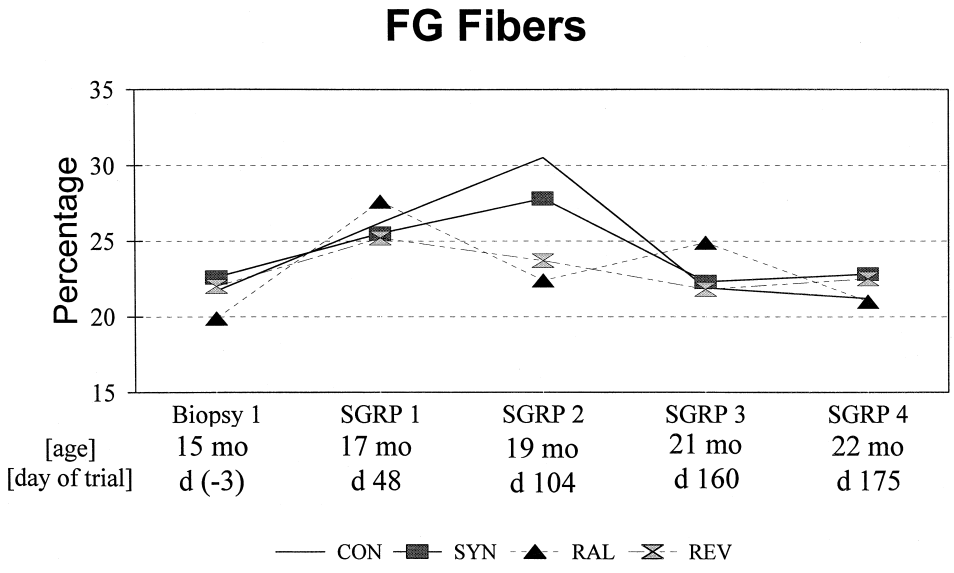

Fig. 1. Changes in percentage of fast-twitch glycolytic (FG) fibers in the *Longissimus* muscle over time on feed as influenced by treatment (CON: control, SYN: Synovex, RAL: Ralgro, REV: Revalor, SGRP: slaughter group).

The results in the ST muscle (Table 4) were inconsistent because post-mortem samples of ST showed a high variability in fiber distribution, especially with regard to SGRP 1 where significantly higher percentages of SO fibers and lower percentages of FG fibers (in SYN steers: FOG) were calculated. Proportions of SO fibers were 18–23% in SGRPs 2–4 (SGRP 1: 30–40%), FOG 20–33% in all SGRPs and FG 45–60% in SGRPs 2–4 (SGRP 1: 30–45%). The different fiber distribution in SGRP 1 may be due to a sampling location effect. Differences in fiber distribution along the longitudinal axis of the bovine ST have been reported with decreasing glycolytic and increasing oxidative capacity towards the distal end (Brandstetter, Picard & Geay, 1997). This difference was pronounced in young bulls and steers and diminished by the age of 16 months (Brandstetter et al., 1997), which could account for only SGRP 1 showing a pronounced deviation. The bovine ST muscle is also

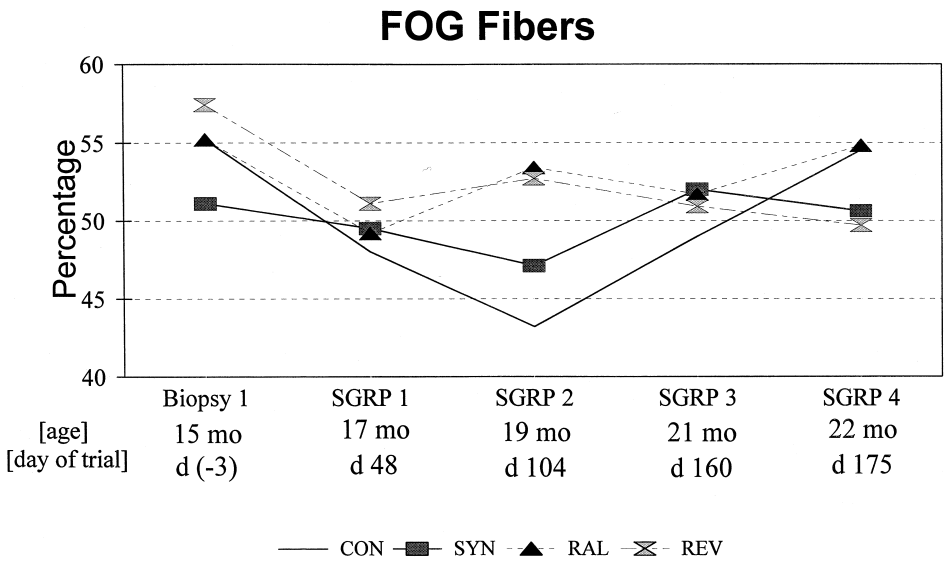

Fig. 2. Changes in percentage of fast-twitch oxidative-glycolytic (FOG) fibers in the *Longissimus* muscle over time on feed as influenced by treatment (CON: control, SYN: Synovex, RAL: Ralgro, REV: Revalor, SGRP: slaughter group).

Table 4
Percentage of slow-twitch oxidative (SO), fast-twitch oxidative-glycolytic (FOG), and fast-twitch glycolytic (FG) fibers in *Semitendinosus* muscle of steers, as affected by treatment and time on feed (least square means[c])

	Postmortem samples				Biopsy samples			
	CON	SYN	RAL	REV	CON	SYN	RAL	REV
SO fibers								
SGRP 1	40.5a	32.5a[a]	37.5a	31.2a[a]	14.9	16.8	15.3	12.7
SGRP 2	18.6b	23.4b	23.0a	18.0b	11.7	11.8	10.9	14.0
SGRP 3	16.7b	18.5b	22.5b	18.5b	13.5	12.9	12.3	14.0
SGRP 4	21.8b	19.7b	16.6b	16.4b	11.5	14.4	12.7	13.3
FOG fibers								
SGRP 1	31.1	23.5b[a]	33.8a[b]	28.6	33.5	33.8	35.3	35.9a
SGRP 2	32.0	26.5ab	31.7ab	29.9	34.5	34.9	34.2	29.0b
SGRP 3	29.3	30.6a	31.8ab	24.6	31.1	*35.5*	32.2	33.4ab
SGRP 4	27.5	29.4ab	26.5b	30.3	34.5	36.4	36.9	35.5a
FG fibers								
SGRP 1	28.4b	44.0a	28.7c	40.2b[a]	51.6	49.5	49.4	51.5
SGRP 2	49.4a	50.2	45.4b	52.2a	53.8	53.3	54.9	57.1
SGRP 3	54.0a	50.9	45.7b[a]	57.0a	55.4	51.6	55.6	52.5
SGRP 4	50.7a	51.0	56.9a	53.4a	54.0	49.2	50.4	51.2

[a] Treatment effect for implanted vs. control steers ($P < 0.05$) within one SGRP and within either pm or biopsy.
[b] Time on feed effect: values with a different letter within a sub-column differ from each other ($P < 0.05$).
[c] Standard error (overall): SO: 2.6%, FOG: 2.2%, FG: 2.9%.

heterogeneous with regard to a histochemically red inner part and a white outer part showing a parallel increase in the volume fraction of SO fibers (10–30%) and a decrease in the volume fraction of FG fibers (58–34%) from superficial to deep layers (Hunt & Hedrick, 1977). The values in Table 4 for pm samples of SGRP 2–4 are similar to those for the outer portion of the ST (12% SO, 21% FOG, 67% FG) whereas the values for pm ST in SGRP 1 correspond to the fiber distribution reported for the inner portion of the ST muscle (36% SO, 25% FOG, 39% FG, Hunt & Hedrick, 1977). This heterogeneity obviously overlays the TRT effect. A maximum percentage of FG fibers was reached in SGRP 4 of the RAL-treated steers.

3.3. Influence of implant treatment and time on feed on fiber cross-sectional area (CSA)

The cross-sectional areas of SO, FOG, and FG fibers are presented in Tables 5 (LM) and 6 (ST). Generally the fiber cross-sectional area enlarged with increasing time on feed which was associated with an increase in

age, weight and number of implants. The enlargement was most pronounced between SGRP 1 (17 months of age) and SGRP 3 (21 months of age). *Longissimus* muscle area, a carcass merit measurement that reflects lean yield in the US yield grading formula increased from 66.6 to 80.5 cm^2 from SGRP 1 to 3 (Rumsey et al., 2000). The animals of SGRP 4 were only 2 weeks older than those of SGRP 3 and did not receive an additional implant. Cross-sectional areas of SO and FG fibers increased in the LM of CON steers from SGRP 1 to 3 followed by a plateau.

Synovex did not affect fiber area compared to CON steers in any slaughter group, which is in accordance with the results of Ono et al. (1996) for growing steers (7–9 months of age). The CSA of all fiber types increased in the RAL-treated group from SGRP 1 to 3 and in the REV-treated group continually from SGRP 1 to 4. This resulted in larger SO and FOG cells ($P < 0.05$) in RAL-treated steers of SGRP 3 and in larger SO and FOG cells ($P < 0.01$) and slightly larger FG cells ($P = 0.11$) in REV-treated steers of SGRP 4. Hughes et al. (1998) similarly detected larger FG fiber CSA ($P < 0.1$) in REV-treated steers compared with control steers. The CSA fiber results for the implant treatments are in agreement with the carcass lean accretion data and longissimus muscle area measurements for these carcasses reported by Rumsey et al. (2000). Johnson, Halstead, White, Hathaway, DiCostanzo and Dayton (1998) reported that muscle tissue from Revalor-S-implanted steers may produce more IGF-I than non-implanted steers which in turn may activate and (or) maintain satellite cells in a proliferative state that would enhance muscle growth. This may explain the improved responses seen as a result of Revalor implant treatment in our present study.

The ST showed a similar response to the growth-promoting implants as the LM with minor differences. Cross-sectional areas of SO fibers increased only in the REV-treated group with increasing time on feed (SGRP 1–4). Implantation with REV led to larger SO cells in SGRPs 3 and 4, RAL achieved larger SO cells in SGRP 3 compared with control steers. CSA of FOG fibers increased from SGRP 1 to 3 in all implant groups followed by a plateau (or a decrease in the SYN-treated group). All implant treatments led to significantly larger FOG cells in SGRP 3. Cross-sectional area of FG fibers increased slightly in the CON group (SGRP 1–4), and was most pronounced in the REV-treated group (SGRP 1–4). Revalor led to larger FG cells in SGRPs 3 and 4 compared to CON steers. In the SYN- and RAL-treated group, maximum FG areas (significantly larger than in CON steers) were reached in SGRP 3. The decrease in fiber area in SGRP 4 may indicate the depletion of the exogenous hormone supply. Due to a down-regulation

Table 5
Cross-sectional area (μm^2) of slow-twitch oxidative (SO), fast-twitch oxidative-glycolytic (FOG), and fast-twitch glycolytic (FG) fibers in *Longissimus* muscle of steers, as affected by treatment and time on feed (least square means[c])

	Postmortem samples			
	CON	SYN	RAL	REV
SO fibers				
SGRP 1	1810b[b]	1740b	2190b	l470c
SGRP 2	2080ab	2040ab	1990b	2200b
SGRP 3	2470a	2490a	3170a[a]	2740ab
SGRP 4	2240ab	2370ab	2400b	3240a[a]
FOG fibers				
SGRP 1	2270	2370	2690ab	1840c
SGRP 2	2610	2790	2390b	2420bc
SGRP 3	2830	2790	3630a[a]	3120ab
SGRP 4	3030	2940	3010ab	3960a[a]
FG fibers				
SGRP 1	3550b	3900	4050b	3140c
SGRP 2	4380ab	4440	3940b	4320bc
SGRP 3	4900a	4570	5540a	4870ab
SGRP 4	4810ab	4750	4380ab	5800a

[a] Treatment effect for implanted vs. control steers ($P < 0.05$) within one SGRP.

[b] Time on feed effect: values with a different letters within a sub-column differ from each other ($P < 0.05$).

[c] Standard error (overall): SO: 230 μm^2, FOG: 350 μm^2, FG: 480 μm^2.

Table 6
Cross-sectional area (μm^2) of slow-twitch oxidative (SO), fast-twitch oxidative-glycolytic (FOG), and fast-twitch glycolytic (FG) fibers in *Semitendinosus* muscle of steers, as affected by treatment and time on feed (least square means[c])

	Postmortem samples			
	CON	SYN	RAL	REV
SO fibers				
SGRP 1	2310	2300	2620	2340b[b]
SGRP 2	2120	2340	2310	2540b
SGRP 3	2210	2760	2880[a]	2830ab[a]
SGRP 4	2380	2350	2480	3310[a]
FOG fibers				
SGRP 1	2910	2470b	3180ab	2460b
SGRP 2	2720	2930b	2700b	3030ab
SGRP 3	2540	3660a[a]	3610a[a]	3430a[a]
SGRP 4	3190	2930b	3030ab	3550a
FG fibers				
SGRP 1	3690b	3800b	4250b	3790c
SGRP 2	4460ab	4640ab	3770b	4630bc
SGRP 3	3850ab	5300a[a]	5520a[a]	5370ab
SGRP 4	4710a	4450ab	4370b	5800a[a]

[a] Treatment effect for implanted vs. control steers ($P < 0.05$) within one SGRP.

[b] Time on feed effect: values with a different letter within a sub-column differ from each other ($P < 0.05$).

[c] Standard error (overall): SO: 210 μm^2, FOG: 250 μm^2, FG: 340 μm^2.

of endogenous hormones, fibers may actually shrink. Picard, Gagniere, Geay, Hocquette and Robelin (1995) have shown that fibers can shrink as a response to physiological signals such as temporary stress at weaning.

3.4. Comparison of biopsy bioptates with post-mortem samples

The number of countable muscle cells was comparable in biopsy and post-mortem samples because biopsy samples were almost the same size as pm samples. The biopsy samples were collected 51 days before slaughter in SGRPs 1, 2 and 3, and 65 days before slaughter in SGRP 4. Steers of SGRP 2 at the time of biopsy sampling were of the same age as steers of SGRP 1 at the time of slaughter. Steers of SGRP 3 and 4 were at the time of biopsy sampling of the same age as steers of SGRP 2 at slaughter.

The biopsy samples of the LM show a similar fiber distribution as the corresponding post-mortem samples (Table 3). No statistical differences ($P < 0.05$) were observed between biopsy and pm samples of the same animals (comparisons within SGRP) with two exceptions (SGRP 1/RAL/FOG and SGRP 1/CON/FG). Furthermore, similar values were also obtained if fiber distributions in steers of the same age were compared (biopsy SGRP 2 vs. pm SGRP 1, biopsy SGRP 3,4 vs. pm SGRP 2). It can be concluded that the shot biopsy technique used in the present study enables the reliable determination of muscle fiber distributions in the LM of live steers.

Fiber distribution in ST bioptates was constant throughout the experiment with 10–15% SO, 30–35% FOG and 50–55% FG fibers and did not show the differences observed for the pm samples (Table 4). Due to the heterogeneous observation of the ST, which displays a region of predominantly red and a region of predominantly white fibers, it was too difficult to compare biopsy and post-mortem samples in this experiment.

4. Conclusion

Of the three growth-promoting implants used in this study, only Revalor and Ralgro led to noticeable differences in muscle hypertrophic responses (muscle fiber size) in finishing beef steers. Both Revalor and Ralgro buffered the temporary decrease in FG fiber percentage in the LM, thereby also contributing to muscle enlargement. All three implants had minimal effects on muscle fiber distribution. LM biopsy samples excised from the live animal during the implant trial of this study proved to be suitable for the characterization of muscle fiber type profiles. It can be concluded that some growth-promoting implants result in noticeable differences in muscle hypertrophic responses which coincide with their different effectiveness to enhance lean mass accretion.

Acknowledgements

The study was made possible by support of a scholarship from the German Academic Exchange Service (DAAD) within the framework of the "Gemeinsames Hochschulsonderprogramm III" of the German Federal Government and its Länder. The authors wish to express their gratitude to Janet Eastridge, Ray Zephir and James Piatt for their assistance with harvesting of the animals and muscle tissue sampling.

References

Adams, G. R., Hather, B. M., Baldwin, K. M., & Dudley, G. A. (1993). Skeletal muscle myosin heavy chain composition and resistance training. *Journal of Applied Physiology, 74*, 911–915.

Brandstetter, A. M., Picard, B., & Geay, Y. (1997). Regional variations of muscle fiber characteristics in *m. semitendinosus* of growing cattle. *Journal of Muscle Research and Cell Motility, 18*, 57–62.

Carpenter, C. E., Greaser, M. L., & Cassens, R. G. (1987). Accumulation of newly synthesized myosin heavy chain during thyroxine-induced myofiber transition. *Journal of Animal Science, 64*, 1574–1587.

Cheah, K. S., Cheah, A. M., & Just, A. (1997). A simple and rapid biopsy technique for obtaining fat and muscle samples from live animals for predicting meat quality. *Dansk Veterinaertidsskrift, 80*, 775–777.

Clancy, M. J., Lester, J. M., & Roche, J. F. (1986). The effects of anabolic agents and breed on the fibers of the *Longissimus* muscle of male cattle. *Journal of Animal Science, 63*, 83–91.

Cornforth, D. P., Schwartz, W. C., & Cramer, D. A. (1973). Growth and differentiation of muscle fibers in cattle. *Journal of Animal Science*, *36*, 1196 (Abstr.).

Cornforth, D. P., Hecker, A. L., Cramer, D. A., Spindler, A. A., & Mathias, M. M. (1980). Maturity and its relationship to muscle characteristics of cattle. *Journal of Animal Science, 50*, 75–80.

Dreyer, J. H., Naude, R. T., Henning, J. W., & Rossouw, E. (1977). The influence of breed, castration and age on muscle fiber type and diameter in Friesland and Afrikaner cattle. *South African Journal of Animal Science, 7*, 171–180.

Geay, Y., & Picard, B. (1995). Significance of muscle fibre types in development of muscle and meat quality. *Reciprocal Meat Conference Proceedings, 48*, 51–57.

Hughes, N. J., Schelling, G. T., Garber, M. J., Eastridge, J. S., Solomon, M. B., & Roeder, R. A. (1998). Skeletal muscle morphology alterations due to Posilac® and Revalor-S® treatments alone or in combination in feedlot steers. *Journal of Animal Science (Proceedings Western Section), 49*, 90–93.

Hunt, D. W., Henricks, D. M., Skelley, G. C., & Grimes, L. W. (1991). Use of trenbolone acetate and estradiol in intact and castrated male cattle: effects on growth, serum hormones, and carcass characteristics. *Journal of Animal Science, 69*, 2452–2462.

Hunt, M. C., & Hedrick, H. B. (1977). Profile of fiber types and related properties of five bovine muscles. *Journal of Food Science, 42*, 513–517.

Johnson, B. J., Halstead, N., White, M. E., Hathaway, M. R., DiCostanzo, A., & Dayton, W. R. (1998). Activation state of muscle satellite cells isolated from steers implanted with a combined trenbolone acetate and estradiol implant. *Journal of Animal Science, 76*, 2779–2786.

Johnston, D. M., Moody, W. G., Boling, J. A., & Bradley, N. W. (1981). Influence of breed type, sex, feeding system, and muscle bundle size on bovine fiber type characteristics. *Journal of Food Science, 46*, 1760–1765.

Jurie, C., Picard, B., & Geay, Y. (1998). Influences of the method of housing bulls on their body composition and muscle fibre types. *Meat Science, 50*, 457–469.

Nava, S., Gayan-Ramirez, G., Rollier, H., Bisschop, A., Dom, K., de Bock, V., & Decramer, M. (1996). Effects of acute steroid administration on ventilatory and peripheral muscles in rats. *American Journal of Respiratory and Critical Care Medicine, 153*, 1888–1896.

Ockerman, H. W., Jaworek, D., van Stavem, B., Parret, N., & Pierson, C. J. (1984). Castration and sire effects on carcass traits, meat palatability and muscle fiber characteristics in Angus cattle. *Journal of Animal Science, 59*, 981–990.

Ono, Y., Solomon, M. B., Elsasser, T. H., Rumsey, T. S., & Moseley, W. M. (1996). Effects of Synovex-S® and recombinant bovine growth hormone (Somavubove®) on growth responses of steers: II Muscle morphology and proximate composition of muscles. *Journal of Animal Science, 74*, 2929–2934.

Pearson, A. M. (1990). Muscle growth and exercise. *Critical Reviews in Food Science and Nutrition, 29*, 167–196.

Peter, J. B., Barnard, R. J., Edgerton, V. R., Gillispie, C. A., & Stempel, K. E. (1972). Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. *Biochemistry, 11*, 2627–2633.

Pette, D., & Staron, R. S. (1997). Mammalian skeletal muscle fiber type transitions. *International Review of Cytology, 170*, 143–223.

Picard, B., Duris, M. P., & June, C. (1998). Classification of bovine muscle fibers by different histochemical techniques. *Histochemical Journal, 30*, 473–479.

Picard, B., Gagniere, H., Geay, Y., Hocquette, J. F., & Robelin, J. (1995). Study of the influence of age and weaning on the contractile and metabolic characteristics of bovine muscle. *Reproduction Nutrition Development, 35*, 71–84.

Rumsey, T. S., Hammond, A. C., & McMurtry, J. P. (1992). Response to reimplanting beef steers with estradiol benzoate and progesterone: Performance, implant absorption pattern, and thyroxine status. *Journal of Animal Science, 70*, 995–1001.

Rumsey, T. S., Fritsche, S., Elsasser, T. H., Solomon, M. B., Mitchell, A., & Kahl, S. (2000). Growth and carcass responses to different anabolic hormonal ear implants in beef steers. *Expt Biol 2000 (FASEB* 1) p. 62 No. 342.1.

SAS (1990). *SAS user's guide: statistics*. Cary, NC: SAS Inst.

Schmidely, P. (1993). Quantitative review on the use of anabolic hormones in ruminants for meat production. *Annales de Zootechnie, 42*, 333–359.

Schöberlein, L. (1976). Gun biopsy — a new approach to muscle sampling. *Monatshefte für Veterinärmedizin, 12*, 457–460.

Schöberlein, L. (1989). Shot biopsy — method to determine quality and structure parameters on live animals. *Archiv für Tierzüchtung, 32*, 235–244.

Scholz, A. M., Mitchell, A. D., Solomon, M. B., & Wang, P. C. (1998). In vivo muscle fiber characteristics, muscle metabolism and body composition of pigs with different ryanodine receptor genotypes. *Journal of Animal Science, 76* (Suppl. 1), 151 (Abstr.).

Sink, J. D., Turgut, H., & Mann, O. M. (1985). Dynamics of physiological changes in bovine myofibers during growth and sexual development. *Experimental Cell Biology, 53*, 170–179.

Solomon, M. B., & Dunn, M. C. (1988). Simultaneous histochemical determination of three fiber types in single sections of ovine, bovine and porcine skeletal muscle. *Journal of Animal Science, 66*, 255–264.

Solomon, M. B., & Berry, B. W. (2000). Comparison of two different containers for performing the hydrodynamic pressure process. *Journal of Animal Science (Suppl 1)* (in press).

Solomon, M. B., West, R. L., & Hentges Jr., J. F. (1986). Growth and muscle development characteristics of purebred Angus and Brahman bulls. *Growth, 50*, 51–67.

Spindler, A. A., Mathias, M. M., & Cramer, D. A. (1980). Growth changes in bovine muscle fiber types as influenced by breed and sex. *Journal of Food Science, 45*, 29–31.

Vann, R. C., Althen, T. G., Smith, W. K., Veenhuizen, J. J., & Smith, S. B. (1998). Recombinant bovine somatotropin (rbST) administration to creep-fed beef calves increases muscle mass but does not affect satellite cell number or concentration of myosin light chain-1f mRNA. *Journal of Animal Science, 76*, 1371–1379.

Vann, R. C., Althen, T. G., Solomon, M. B., Eastridge, J. S., & Veenhuizen, J. J. (1998). Effects of recombinant bovine somatotropin administration on muscle fiber morphology of creep-fed beef calves. *Journal of Animal Science 76* (Suppl. 2), 11 (Abstr.).

Vigneron, P., Dainat, J., & Bacon, F. (1989). Properties of skeletal muscle fibers. II. Hormonal influences. *Reproduction Nutrition Development, 29*, 27–53.

Wegner, J., & Schöberlein, L. (1984). Suitability of shot bioptate for morphometric and histochemical examination of muscle tissue. *Monatshefte für Veterinärmedizin, 39*, 665–667.

Young, O. A., & Bass, J. J. (1984). Effect of castration on bovine meat composition. *Meat Science, 11*, 139–156.