Effect of production factors on muscle fiber type and dimensions in the *m. semimembranosus* of crossbred steer carcasses

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Introduction

The use of steroids, the beta-adrenergic agonist ractopamine and selection for residual feed intake (RFI) are common beef production management tools to increase cattle growth rate and feed efficiency. Characteristics of muscle fibers can affect meat quality by affecting meat color, water-holding capacity, marbling, and texture. Examining and analyzing the influence of production practices on these factors can help understand the relationship between production practices, muscle fibers and meat quality. The objective of the research was to examine the effects of growth-promoting steroids and ractopamine and selection for low residual feed intake on different aspects of meat quality such as toughness, collagen solubility and muscle fiber types and dimensions. The research presented is specific to one muscle, the *semimembranosus* (SM), from crossbred steer carcasses.

Materials & Method

Materials

For the study, 48 crossbred angus steers were used, 12 for each of the following treatment groups: no steroid + no ractopamine (control); no steroid + ractopamine; steroid + no ractopamine; and steroid + ractopamine.

Creating muscle sections

From each SM muscle, 1-inch thick steaks were cut (Fig. 1a) and then 1 cm^3 cubes were cut from the steaks (Fig. 1b), that were frozen in acetone chilled in dry ice and stored at -80^{0}C until they were sectioned. For sectioning, the cubes were removed from the freezer and placed in a cryostat with a moderated temperature of -25ºC. In the cryostat, transverse serial sections of 10 µm were cut and mounted onto dry slide glass. The slide glasses were stored at -80ºC until staining for myosin ATPase activity. After staining, images were captured of the muscle fiber sections with the three different types of staining that the samples undergo. All the muscle fiber dimensions were measured using the software program ImageJ.

Figure 1: Semimembranosus muscle from experimental steers (a) indicates steak sampling position for muscle fiber types and diameter. Meat steak from Semimembranosus muscle (b) indicates the muscle cubes (1cm x 1cm x 1cm) used for muscle fiber types and diameter determination.

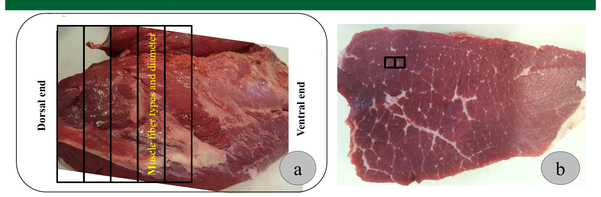
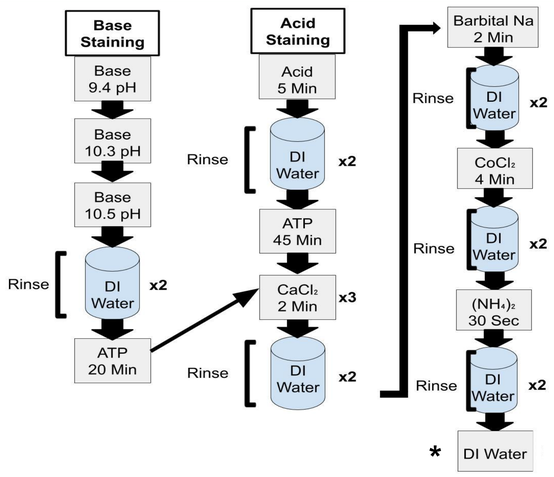


Figure 2: Myosin ATPase staining method flowchart of frozen muscle section



Materials & Method Cont'd

Staining

There are three different staining processes that the slides undergo to help identify the different types of muscle fibers in the samples. The NADH-TR (Nicotinamide adenine dinucleotide tetrazolium reductase) staining (Fig. 3a) is a method to identify different muscle fiber types whether oxidative or glycolytic (metabolic pathways).The alkali pre-incubation myosin ATPase staining (Fig. 3b) will react with type II muscle fibers (IIA and IIB) rather than type. The acid pre-incubation myosin ATPase staining (Fig. 3c) is stable for type I muscle fibers but IIA and IIB are unstable. These two staining methods identify twitch speed (contractions) in the muscle fibers. The muscle fibers with nearly no color at all are type IIB. Different methods of staining are used to easily and with certainty identify all three different types of muscle fibers (Table 1).

Table 1: Muscle fiber staining reactions by muscle fiber type

Methods	Type I	Type IIA	Type IIB
Myosin ATPase Alkali (b)	-	++	+++
Myosin ATPase Acid (c)	+++	-	-
NADH-TR (a)	+++	+	-

Figure 3: Histochemistry of muscle fiber typing in semimembranosus muscle. (a); NADH-TR, Myosin ATPase activity (b); at alkaline pre-incubation (pH 10.5) and (c); at acid pre-incubation (pH 4.3). Bar = 200µm

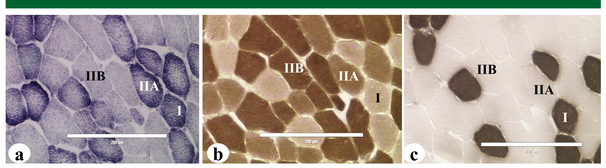


Table 2: Least squares means (±SEM) of different carcass characteristics of crossbred steers subjected to different growth promotants.

Measurements	Steroid		Ractopamine		RFI	
	Yes	No	Yes	No	Low	High
Final live weight (kg)	703.30 ± 5.60a	632.76 ± 4.99b	668.87 ± 5.16	667.19 ± 5.44	667.86 ± 4.66	668.21 ± 5.88
Carcass weight (kg)	380.65 ± 4.14a	339.69 ± 3.88b	362.34 ± 4.02	358.01 ± 4.02	361.71 ± 4.37	358.64 ± 3.63
Semimembranosus muscle weight (kg)	6.56 ± 0.17a	5.71 ± 0.16b	6.12 ± 0.16	6.14 ± 0.16	6.11 ± 0.18	6.15 ± 0.15
Warner-Bratzler shear force (N)	51.98 ± 1.42a	42.39 ± 1.33b	48.21 ± 1.37	46.16 ± 1.37	48.91 ± 1.24x	45.45 ± 1.50y

[a, b] means differences at P < 0.05
[x, y] means differences at P < 0.10

Table 3: Three-way interaction between steroid, ractopamine and RFI for type I muscle fiber (%) of SM muscle of crossbred steers subjected to different growth promotants

Steroid	Ractopamine	Residual Feed Intake	Type I (%)
Yes	No	H (non-efficient)	14.03 ± 2.24b
		L (efficient)	17.04 ±1.69 b
	Yes	H (non-efficient)	17.04 ±1.69 b
		L (efficient)	16.73 ± 1.84b
No	No	H (non-efficient)	19.56 ± 2.00a
		L (efficient)	10.51 ± 1.83c
	Yes	H (non-efficient)	16.13 ± 2.03b
		L (efficient)	15.25 ± 1.69b

[a, b, c] means differences at P < 0.05

Statistical Analysis

The data collected was analysed by R (version 3.3.1) using the package lm as a mixed model. Steroid, ractopamine and RFI and their interaction were fixed effects. Initial body weight was included as a covariate for the analysis. Differences between means (P<0.05) were determined by least square mean differences.

Results and Conclusion

The final live weight, carcass weight, SM muscle weight and shear force were increased with steroid use, which indicated that although steroids increase steer growth, they also increase meat toughness (Table 2). Selection for low RFI (increased efficiency) tended to increase shear force (Table 2). Type I fiber proportion was highest in those steers that did not receive steroids or ractopamine and were not selected for low RFI. Growth enhancement regardless of method decreased Type I proportion, with steers selected for low RFI having decreased Type I fibre proportion but increased Type I fibre size. Selection for low RFI can affect Type I fibres in the SM, which may contribute to the metabolic efficiency observed in these cattle.

Table 4: Least squares means (±SEM) of different muscle fiber characteristics of crossbred steers.

Measurements	Steroid		Ractopamine		RFI	
	Yes	No	Yes	No	Low	High
Mean muscle fiber diameter (µm)	29.9 ± 0.5	29.1 ± 0.4	29.6 ± 0.5	29.4 ± 0.5	29.6 ± 0.4	29.4 ± 0.5
Type I mean muscle fiber diameter (µm)	26.9 ± 0.4	26.5 ± 0.4	26.9± 0.4	26.5 ± 0.4	27.4 ± 0.4a	26.0 ± 0.5b
Type IIA mean muscle fiber diameter (µm)	27.5± 0.5	26.3± 0.5	27.2 ± 0.4	26.7 ± 0.5	27.2 ± 0.4	26.7 ± 0.5
Type IIB mean muscle fiber diameter (µm)	33.9 ± 0.8	33.3 ± 0.7	33.8± 0.8	33.4± 0.7	34.3 ± 0.8	32.9 ± 0.7
Type I muscle fiber (%)	19.7 ± 1.1	15.5 ± 1.1	16.7 ± 1.1	15.5 ± 1.1	14.9 ± 1.0y	17.3 ± 1.2x
Type IIA muscle fiber (%)	39.6 ± 2.7	40.3 ± 2.6	40.0 ± 2.7	39.8 ± 2.6	37.4 ± 2.3	42.5 ± 3.0
Type IIB muscle fiber (%)	43.7 ± 2.9	44.3 ± 2.7	43.2 ± 2.9	44.7 ± 2.7	47.7 ± 2.4	40.3 ± 3.1

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