Effect of anabolic steroid (trenbolone acetate) and β-adrenergic agonist (ractopamine hydrochloride) on semimembranosus muscle fiber characteristics in crossbred steers

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Introduction

- Due to population increases and a need increased meat production, it is critical for beef cattle to be more efficient with increasing animal performance. (Reference 1)
- To increase animal production and performance, growth promotants are important.
- Growth Promotants also help lower production costs and aid in reducing methane production from cattle.
- Anabolic implants which are available to use include RAC (ractopamine), trenbolone acetate, progesterone and estradiol.

Objectives

Our objective was to assess the effect of growth promotants on muscle fiber characteristics, carcass characteristics and the most important meat tenderness characteristics.

Material and Methods

Animal Management and Experimental Design

Anabolic Steroid		Ractopamine Hydrochloride		Residual Feed Intake (RFI)	
Yes	No	Yes	No	Low	High
N = 8	N = 8	N = 8	N = 8	N = 8	N = 8

- Muscle isolated from carcass 24 hours after harvesting, vacuum packaged & transported to lab on ice.
- Sampling is done 3 days post-mortem after being stored at 4 °C.
- Muscle is cut into 1.5 cm × 1 cm × 1 cm cubes & frozen in dry ice cooled acetone, then stored in -80 °C.

Fig 1. A) Location of samples for muscle fiber type and area in experimental *semimembranosus* steaks is indicated by the black rectangles; B) Acetone cooling; and C) Cryostat sectioning

Sectioning of frozen muscle cubes in a Cryostat:

- Frozen muscle cubes in vials placed in cryostat chamber (-25 °C) for 30 minutes to allow to evaporate of excess acetone.
- Small portion of sample cube was cut off the end.
- We assessed for oval shape of muscle fibers in transverse position sectioning for correct longitudinal orientation.

Histochemistry for muscle fiber typing:

- NADH-TR (nicotinamide adenine dinucleotide tetrazolium reductase) method: Slides incubated at 30 °C for 20 minutes, rinsed with distilled water, fixed in acetate buffer for 5 minutes and rinsed again with distilled water.
- Myosin ATPase (adenosine triphosphate) after alkali pre-incubation: Slides dried for 1 hour at room temp and alkaline incubation solutions equilibrated in water bath at 21°C.

Materials and Methods

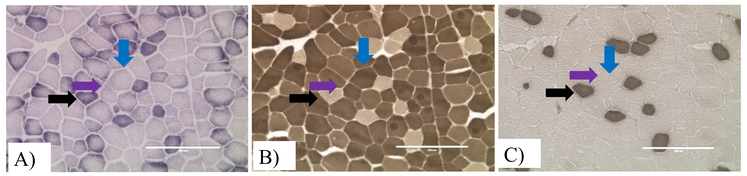
Histochemistry for muscle fiber typing (Continued):

- Slides were in incubation solutions at each respective pH for 10 minutes (pH 9.4, 10.3, 10.5) then placed in the 9.4 pH ATP incubation solution for 20minutes.
- Myosin ATPase after acid pre-incubation: same steps as above however slides placed in acid incubation at pH of 4.3 for 5 minutes instead of alkali, rinsed with distilled water and placed in ATP incubation solution for 45 minutes.
- Next all slides were placed in CaCl2, barbital sodium, cobalt chloride, ammonium sulphide, rinsing in between treatments with distilled water, mounted with cover slips and allowed to dry at room temp for 30 minutes.

Table 1: Muscle fiber typing of semimembranosus muscle based on histochemical reactions

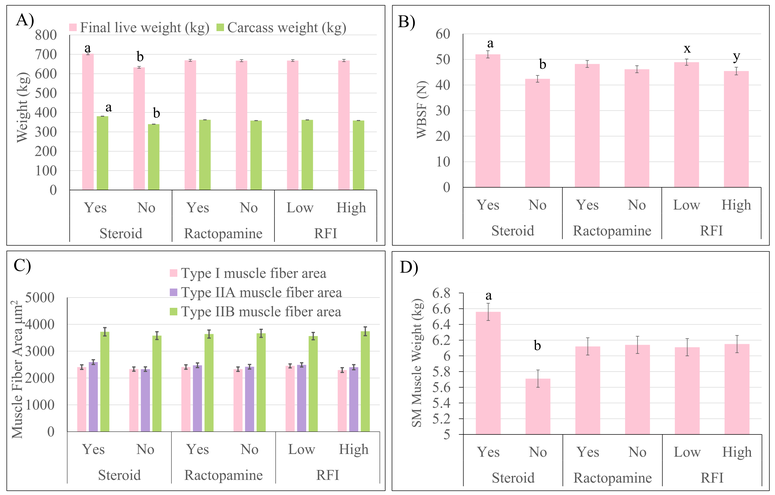
Muscle fiber types	NADH-TR	Myosin ATPase	
		Alkali pre-incubation (pH 10.3)	Acid pre-incubation (pH 4.3)
Type 1	+ + +	-	+ + +
Type 2A	+	+ +	-
Type 2B	-	+ + +	-

Fig 2. Histochemical staining of *semimembranosus muscle*



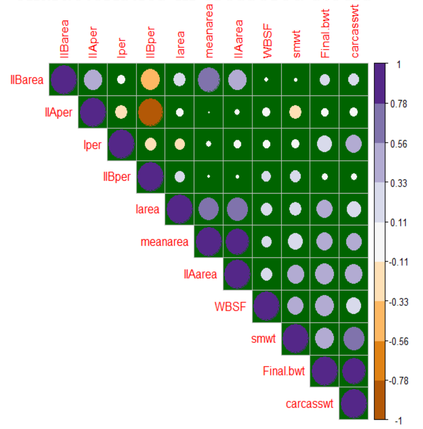
Black arrow: Type 1, **Blue arrow:** Type 2A, **Purple arrow:** Type 2B

Fig 3. Least squares means (± SEM) of final live, & carcass weight (A), Warner-Bratzler shear force (B), muscle fiber area (C) and percentages (D) muscle weight of crossbred steers subjected to different growth promotants. [a, b] means differences at $P < 0.05$; [x, y] means differences at $P < 0.10$



Results

Fig 4. Pearson Correlations between carcass and muscle fiber characteristics in crossbred steers



Carcasswt = Carcass weight
Finalbwt = Final body weight
Smwt = *Semimembranosus* muscle weight
WBSF = Warner-Bratzler shear force
IIAarea = Type IIA muscle fiber area
Meanarea = Mean muscle fiber area
Iarea = Type I muscle fiber area
IIBper = Type IIB muscle fiber percentage
Iper = Type I muscle fiber percentage
IIAper = Type IIA muscle fiber percentage
IIBarea = Type IIB muscle fiber area

Statistical Analysis

All data were analyzed using R Studio (Version 3.5.1) as 2 × 2 × 2 factorials, where RFI, steroid and ractopamine served as fixed sources of variation. Tukey's Honest Significant Difference was used to compare least square means of different combination of treatments and significance was declared at $P \leq 0.05$ and tendencies from $0.05 < P < 0.1$. Pearson's correlation coefficient analysis was performed to know the linear relationships between carcass characteristics and muscle fiber characteristics using the RColorBrewer package in R Studio.

Conclusions

- Non efficient RFI animals with no steroid or RAC had a higher percentage of type 1 muscle fibers.
- Efficient RFI animals with no steroid or RAC had a lower perecentage of type 1 muscle fibers.
- From these results we can determine that the use of steroids in cattle can positively impact muscle weight.
- This is significant because ranchers can now use steroids to aid in producing more efficient cattle which helps reduce the methane footprint.
- These results also show that cattle with high RFI have a lower Warner-Bratzler shear force (N) than low RFI steers which means that their muscles are more tender.

Acknowledgements

The authors would like to thank Canada Summer jobs for their funding for this summer internship. They would also like to thank WISEST coordinators: Fervone Goings, Haleema Pannu, Bridget Fraser, Hannah Bayne, Clara Vicera, and Helen Yip, for their support.

References

Reference 1: Gonzalez et al. 2007. Effect of ractopamine-hydrochloride and trenbolone acetate on longissimus muscle fiber area, diameter and satellite cell numbers in cull beef cows J. Anim. Sci. 2007. 85:1893–1901 doi:10.2527/jas.2006-624