

# MUSCLE FIBER CHARACTERISTICS OF NELLORE AND F1 RED ANGUS X NELLORE FED DIETS CONTAINING CRUDE GLYCERIN

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**Abstract** – Our objective was to identify histochemically and perform of transverse section from *Longissimus dorsi* muscle fibers in two cattle genetic groups: Nellore and F<sub>1</sub> Red Angus x Nellore, fed with 5% or 15% of crude glycerin. Distinction of fiber type was performed based on sensibility of myofibrillar adenosine triphosphatase (m-ATPase) variations exposed to pH. We did not verify interactions between genetic group and diet, as well as there was no effect of them on muscle fiber frequency. F<sub>1</sub> Red Angus x Nellore steers had greater IIA fibers area ( $P = 0.0002$ ) compared to NE steers, we did not observed differences in I and IIB fibers ( $P = 0.0521$  and  $P = 0.0908$ , respectively) between genotypes. Steers fed with 15 % of CG had greater area for type I ( $P = 0.0244$ ) and IIA ( $P = 0.0068$ ) fibers, as compared to those fed with 5% of crude glycerin, we did not detected differences in IIB fibers ( $P = 0.0244$ ), for tested diets. Feedlot period did not allow muscle fibers modulation. Crude glycerin seems to promote muscle fiber hypertrophy differently.

**Key Words** – Hypertrophy, m-ATPase, Nellore

## I. INTRODUCTION

Overall, there is unacceptable variation in meat quality, suggesting a multifactor and complex issue. However, this situation is not surprise, since muscle is a highly organized structure comprised by various fiber types, defining meat proprieties as result of several interactions of these factors [1]. Thus, muscle fibers features are crucial base to explain difference among genotype and feed strategy.

Brazilian cattle herd is mainly composed by *Bos indicus* animals [2], our bovines are extremely adaptable, but also are associated with extensive grazing systems, this fact contributes with the bad impression of Brazilian meat; as originated from old animals with undesirable qualitative aspects. Given that, we observe growing attention about crosses between *Bos indicus* x *Bos taurus*, seeking, among other factors, improvement in beef production in terms of quality. The Red Angus breed has increased by 370.41% in semen trading in the past five years [3], clearly demonstrating its importance in the current scenario.

Regarding diet, with the growing demand for renewable resources, such as biodiesel, it is conjectured high availability of crude glycerin, byproduct of this agribusiness which stands as energy potential ingredient for ruminant diets.

In relation to changes in the meat quality, the use of crude glycerin is involved, among other factors, the effect of glycerol in the differential hypertrophy of muscle fibers. It is well established that at glucose absorption, levels of GLUT-4, insulin receptor number and insulin sensitivity are higher in type I fibers in relation to type II fibers, mainly in fibers of type IIB [4]. Thus, a possible hypothesis is that the use of glycerol, coupled with availability of gluconeogenic compounds and insulin may increase the availability of substrate for type I fibers and even fiber type IIA, providing differential hypertrophy thereof. According to [5], crude glycerin seems to induce meat sensorial quality changes, which still need to be investigated. Hence, we conducted this experiment in order to identify histochemically fibers and measure fiber

area at *Longissimus* muscle of Nellore and F<sub>1</sub> Red Angus x Nellore and the effect of crude glycerin use in the diet in relation to these characteristics.

## II. MATERIALS AND METHODS

This study was conducted at the Federal university of Viçosa, Brazil, with 24 non-castrated steers being 12 Nellore (NE) and 12 F<sub>1</sub> Red Angus x Nellore (NA), fed with 5% or 15% of crude glycerin (CG). The average age and initial body weight were 18 months and 320 kg.

Animals were confined individually in stalls with feeders and drinkers. Diets were formulated to be isonitrogenous with 14.6% of crude protein (DM basis). The average composition of the experimental diets is presented in Table 1.

Table 1. Average composition of the experimental diets

Ingredient (%)	Crude glycerin level (% total DM)	
	5	15
Corn Silage	50.00	50.00
Soybean meal	7.15	7.15
Corn meal	34.80	23.10
Mineral Mixture	0.25	0.30
Urea/Ammonium sulphate 9:1	1.0	1.0
Crude glycerin	5.0	15.0
Corn gluten meal	0.90	2.55
Limestone	0.65	0.60

We used completely randomized design in a 2 x 2 factorial scheme, with two genetic types (NE and NA) and two levels of crude glycerin (5% and 15% in substitution of corn on total DM).

By the end of the experiment, all animals were slaughtered then bled. It occurred after the 16-h fast and were performed by cerebral concussion followed by jugular and carotid venesection, per the Normative Instruction n<sup>o</sup>.3 of 01/13/2000 (Technical Regulation of Methods for Humane Slaughtering of Livestock).

At the end of slaughter, each animal carcass were divided into identical longitudinal halves and

chilled at 4°C for 24 h. After 24 h *postmortem* chill, muscles samples of *Longissimus dorsi* (LD) were taken from both halves of the carcass of each animal.

To perform fibers histological analysis, the samples were transferred from ultrafreezer to cryomicrotome Leica® CM 1850TM (Leica Microsystems, Wetzlar, Germany) where they were fixed in resin *Optimal Critical Temperature compound* – OCT Tissue-Tek® (Sakura, Finetek, Zoeterwoude, The Netherlands) on cryostat metallic supports. We made several perpendicular fibers sections to direction of 12 µm, after cutting, they were bonded to slides; they were at room temperature and were previously soaked in a solution of gelatin and chromium potassium sulfate. The slides were stained based on variations in sensitivity to adenosine myofibrillar triphosphatase activity (m-ATPase) exposed to different pH values. [6]. After temperature setting, they were subjected to pre-incubation at pH 9.4 for m-ATPase activation. Thereafter, half of the slides were subjected to 4.2 and 4.7 pH incubations. The fibers were classified into I, IIA and IIB according to [6]. After staining, we captured 20 images of each slide using Olympus® U-CMAD-2 camera (Olympus Corporation Tokyo Japan) directly coupled to the light microscope Olympus® BX-60™ (Olympus Corporation, Tokyo, Japan), with 10X objective and aid of Image-Pro® Plus v.4.5.0.29 software (Media Cybernetics, Maryland, USA). Fibers relative frequency (%) and area (µm<sup>2</sup>) were calculated from points located in voids in the grid mask of 65 points. we used best 15 images within 20 captured for each animal.

All statistical procedures were carried out using SAS 9.2 (Statistical Analysis System Institute, Inc., Cary, NC, USA).

The data for frequency and area were analyzed as repeated measures following the model:

$$Y_{ij} = \mu + D_i + G_j + (DG)_{ij} + e_l$$

Where:

$Y_{ij}$  is the measured response variable;  $\mu$  is the overall constant;  $D_i$  is the fixed effect of the  $i^{\text{th}}$  level of diet;  $G_j$  is the fixed effect of the  $j^{\text{th}}$  level of genetic group;  $(DG)_{ij}$  is the fixed effect of the interaction between the levels of D and G;  $e_l$  is the random error associated with  $Y_{ij}$ ,  $NID$

(0,  $\sigma^2_{el}$ ). When first order interaction effects were significant ( $P < 0.05$ ), the Tukey-Kramer multiple comparison method was used to test differences among the levels.

### III. RESULTS AND DISCUSSION

We did not observe interaction ( $P = 0.3254$ ) between genetic group and level of glycerin, so data were discussed separately.

Our data reveal no influence of genetic group on fiber frequency; type I, type IIA, and type IIB (Table 2). NA steers had greater IIA fibers area compared to NE steers, we did not observed differences in I and IIB fibers between genotypes (Table 2).

Table 2. Average of frequency and cross sectional area of fiber muscle types of *Longissimus dorsi* muscle of Nellore and F<sub>1</sub> Red Angus x Nellore

Item	Genetic Group		<i>P</i> - Value
	NA	NE	
Frequency (%)			
I	30.14±0.65	29.38±0.73	0.4821
IIA	26.81±1.35	23.37±1.42	0.1241
IIB	43.01±1.37	46.63±1.43	0.1109
Cross Sectional Area (μm <sup>2</sup> )			
I	1852.46±70.36	1621.40±73.72	0.0521
IIA	2371.17±65.00	1896.82±68.11	0.0002
IIB	3033.25±80.17	2807.04±84.00	0.0908

Tested levels of CG did not affect the frequency of muscle fiber type I, type IIA and type IIB. Steers fed with 15 % of CG had greater area for type I and IIA fibers compared to those fed with 5% of CG, we did not detected differences in IIB fibers, for tested diets (Table 3).

Table 3. Average of frequency and cross sectional area of fiber muscle types of *Longissimus dorsi* muscle of animals fed with crude glycerin

Item	Genetic Group		<i>P</i> - Value
	NA	NE	
Frequency (%)			
I	30.11±0.63	29.42±0.63	0.4512
IIA	26.06±1.28	24.11±1.23	0.2899
IIB	43.86±1.30	45.78±1.24	0.3025
Cross Sectional Area (μm <sup>2</sup> )			
I	1622.52±66.82	1851.34±63.91	0.0244
IIA	2002.39±61.73	2265.60±59.05	0.0068
IIB	2939.54±72.82	2900.75±76.13	0.7189

Just as in the current work, previous study did not proved differences in the proportions of muscle fibers between Nellore and Nellore x Red Angus crossbred [7]. Muscles from different genetic groups may have the same fiber composition, noting that possible differences between these animals are related to distinct metabolic profile of these fibers [8].

Regarding the crude glycerine inclusion, it is known that feed and more directly the diet energy level are able to promote muscle fibers modulation [8]. Given the above and considering that both diets allowed even intake of metabolizable energy (2.70 Mcal / kg DM,  $P = 0.3394$ ), we expected that there would be variation in frequency of fibers between diets, which was observed.

Additionally, it is worth to notice that, probably before modulation, fiber area varies [7]. Hence, possibly feeding period adopted in this experiment was not enough to promote the modulation on frequency muscle fibers between genotypes and diets.

As the proteins that make up muscle fibers, the size and number of fibers are factors that determine muscle mass and influence on meat quality [9]. In cattle, unlike suine, there is no muscle hypertrophy with high growth potential due to the higher total number of fibers [10], that may explain, among other factors, the absence of differences in type IIB fibers area, that being higher, would have reached maximum growth potential.

Moreover, the greater fiber type I and IIA areas at *Longissimus dorsi* muscle of animals fed with 15% GB, no effect of dietary fibers in the type IIB is in agreement with our initial hypothesis that differential hypertrophy fibers using the glycerol in the diet would occur. Type I fibers have greater glucose uptake, higher levels of GLUT-4, insulin receptor number and insulin sensitivity compared to type II fibers, but mainly in relation to IIB fibers [4], what allows that diets with a higher content of glycerol increases the availability of substrate for type I and IIA fibers and differential hypertrophy of them.

#### IV. CONCLUSION

Feedlot period adopted did not allow muscle fibers modulation. Crude glycerin seems to promote muscle fiber hypertrophy differently.

#### REFERENCES

1. Zamora, F., Aubry, L., Sayd, T., Lepetit, J., Lebert, A., Sentandreu, M.A., & Ouali, A. (2005). Serine peptidase inhibitors, the best predictor of beef ageing amongst a large set of quantitative variables. *Meat Science* 71: 730-742.
2. Ferraz, J. B. S. & Felício, P. E. D. (2010). Production systems - an example from Brazil. *Meat Science* 84: 238-243.
3. Associação Brasileira de Inseminação Artificial, 2011. Relatórios de Comercialização de Sêmen (Online). <http://www.asbia.org.br> (accessed 8.15.2012).
4. Lefaucheur, L. (2010). A second look into fibre typing-Relation to meat quality. *Meat Science* 84: 257-270.
5. Drouillard, J.S. (2008). Glycerin as a feed for ruminants: using glycerin in high-concentrate diets. *Journal of Animal Science* 86 (E-Suppl.2): 392.
6. Brooke, M.H. & Kaiser, K.K. (1970). Three myosin adenosine triphosphatase system: the nature of their pH liability and sulphydryl dependence. *Journal of Histochemistry and Cytochemistry* 18: 670-672.
7. Oliveira, I.M., Paulino, P.V.R., Marcondes, M.I., Valadares Filho, S.C., Cavali, J., Prados, L.F., Duarte, M.S., & Detmann, E. (2011). Beef quality traits of Nellore, F1 Simmental × Nellore and F1 Angus × Nellore steers fed at the maintenance level or *ad libitum* with two concentrate levels in the diet. *Revista Brasileira de Zootecnia* 40: 2894-2902.
8. Choi, Y.M., & Kim, B.C. (2009). Muscle fiber characteristics, myofibrillar protein isoforms, and meat quality. *Livestock Science*, 122: 105-118.
9. Rehfeldt, C., & Kuhn, G. (2006). Consequences of birth weight for postnatal growth performance and carcass quality in pigs as related to myogenesis. *Journal of Animal Science*, 84(Suppl.13): 113-123.
10. Picard, B., Juire, C., Duris, M.P., & Renand, G. (2006). Consequences of selection for higher growth rate on muscle fibre development in cattle. *Livestock Science*, 102: 107-120.