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Gene expression analysis to evaluate differences in beef tenderness from nelore and Rubia gallega x nelore cattle (#206)

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Introduction

Beef tenderness is the most important attribute of eating sensory quality; however, beef herds (*Bos indicus*) adapted to tropical and subtropical regions presents low beef tenderness, which is a problem for product acceptability (Bressan et al., 2011). In the last decades, the use of *Bos taurus* x *Bos indicus* crossbred animals has increased in beef production systems in tropical and subtropical regions to assure a high beef quality (Bressan et al., 2011). Recently, the Rubia Gallega breed (*Bos Taurus*), already recognized in Europe for its quality, has been introduced in crossing with zebu in Brazil. Rubia Gallega animals have a higher growth rate and low development of fat depots, although without further details about their beef tenderness. In addition, Rubia Gallega may present mutations in myostatin gene (*MSTN*), the mutation results in inactivation of myostatin protein and, consequently, increase of muscle mass, providing the double-muscled phenotype (DM) (Wang & McPherron, 2012). According to Fiems (2012) DM cattle provides more tender meat than non-DM.

Thus, our hypothesis is that the crossbred Rubia Gallega x Nelore will affect meat tenderness and gene transcripts involved in beef tenderness attributes. The main objective of this study was to investigate the transcript levels of genes involved in proteolysis *post mortem* and muscle growth in *Longissimus* from Rubia Gallega x Nelore crossbred cattle.

Methods

Thirty two bulls from Nelore breed (N) (n=16) and Rubia Gallega x Nelore cross (RGN) (n=16) were selected (initial weight 280 kg ± 15 kg and 11 months of age ± 2). Bulls were fed with the same diet during 120 days of finishing. All bulls were harvested and *Longissimus thoracis et lumborum* (LTL) samples were collected for analysis of gene expression and immediately frozen in liquid nitrogen. All carcass were chilled for 24 h postmortem at 0-2ºC, and 2.5 cm thick steak was obtained between the 12th-13th ribs and the muscles were vacuum packed and kept at -18°C for Warner Bratzler Shear Force analysis (WBSF).

Samples were cooked according to AMSA (2015) and shear force was performed using Brooksfield® CT-3 Texture Analyzer, according to Wheeler et al. (2001) expressed in kilograms (kg).

RNA was extracted using TRIzol reagent® according to the manufacturer's recommendations. The target genes analyzed were myostatin (*MSTN*), μ-Calpaín 1 (*CAPN1*), m-Calpaín (*CAPN2*), calpastatin (*CAST*), and two normalizers Glyceraldehyde-3-phosphate Dehydrogenase (*GAPDH*), β-Actin. The reactions of qPCR were analyzed in Real-Time PCR QuantStudio 6 following cycle parameters: initial denaturation at 95°C for 2 min, followed by 45 cycles consisting of 95°C for 15 sec and 60°C for 1 min. To calculate relative expression, the cycle threshold (Ct) value of target genes were normalized to the geometric mean of the Ct values for GAPDH and β-actin, and the relative levels were represented as $2^{-\Delta Ct}$.

The experiment was completely randomized design, with 16 replicates per treatment, each animal being considered an experimental unit. The data were analyzed by the SAS program version 9.3. For gene expression results, normalized data were submitted to the Student's T test using the Excel OFFICCE tool.

Results

Beef tenderness was influenced by genetic group (P = 0.025) (Figure 1). RGN animals presented lower values of WBSF, when compared to N group, 5.41 kg and 6.00 kg, respectively. According to Fiems (2012) DM cattle can present meat more tender than non-DM, due to lower collagen content or a less mature collagen.

Genetic groups did not influence the transcript levels of *CAPN1* (P = 0.482), *CAPN2* (P = 0.1003) and *CAST* (P = 0.4309) (Figure 2). In the present study, it was expected to find greater gene expression of *CAST* in pure Nelore animals, due to the higher activity of calpastatin shown in previous studies in these animals when compared to *Bos taurus* (Koohmaraie, 1994; Wheller et al., 1990; Wright et al., 2018).

However, other proteolytic systems, besides calpain-calpastatin, may be involved in meat tenderization (Ouali et al., 2006), such as caspase systems (*CASP*) and heat shock proteins (*HSP*) (Ouali et al., 2013; Saccá et al., 2018).

Notes

Martins et al. (2017) when compared proteolysis of Angus and Nelore cattle, observed higher *post-mortem* proteolysis in *longissimus* muscle from Angus than Nelore, despite this, it was not found differences in gene expression of *CAST* or *CAPN1*, nor by the abundance of these enzymes, but due to the greater calpastatin activity in skeletal muscle of Nelore cattle than that from Angus.

RGN animals showed higher transcript levels of *MSTN* gene (P = 0.043) (Figure 3) when compared to N.

It is suggested that increasing levels of active myostatin protein decrease their own expression and promoter activity, representing a classic example of negative feedback. However, the same does not occur when has nonfunctional myostatin, allowing higher expression of this gene.

Conclusion

The use of Rubia Gallega x Nelore crossbred suggests its potential application to improvement of meat tenderness; however, this result is not caused by differences in expression of *CAPN1*, *CAPN2* and *CAST* genes, but due to greater levels of *MSTN* gene.

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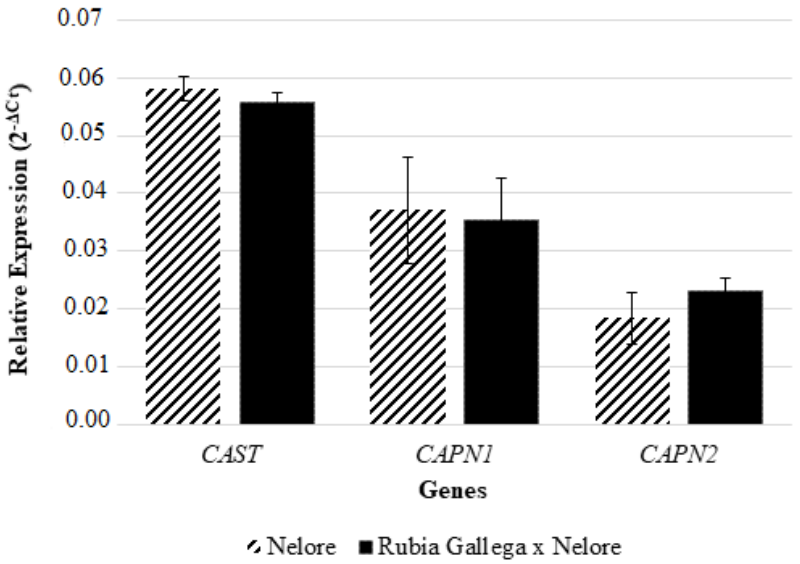

Figure 2. Gene expression of CAST, CAPN1 and CAPN2 from Nelore and Rubia Gallega x Nelore

Notes

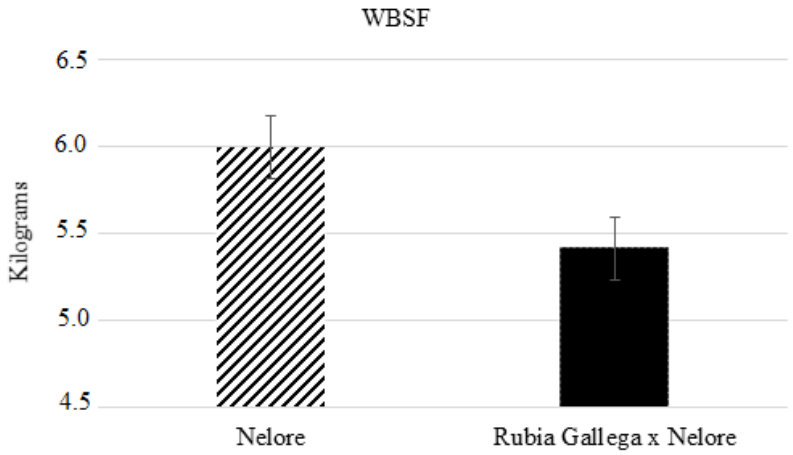

Figure 1. Warner Bratzler Shear Force (WBSF) of beef from Nelore and Rubia Gallega x Nelore

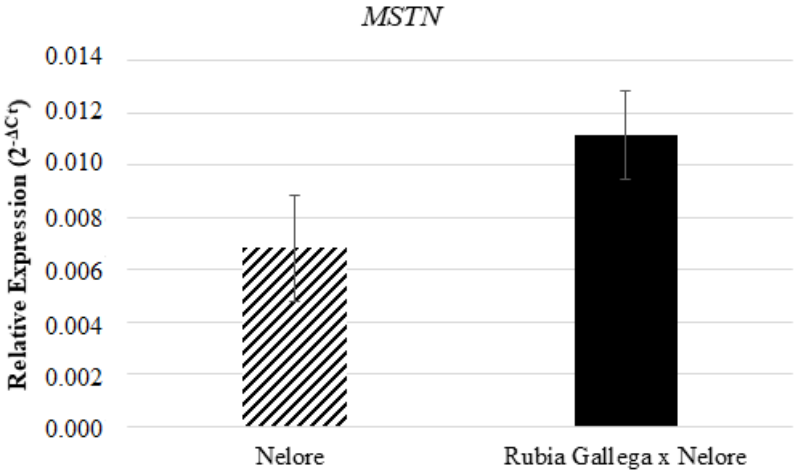

Figure 3. Gene expression of myostatin (MSTN) from Nelore and Rubia Gallega x Nelore

Notes