EXPRESSION OF BEEF TENDERNESS MARKERS IN BEEF FROM NELLORE AND ANGUS CATTLE

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Abstract – The objective of the current study was to evaluate the mRNA expression of beef tenderness markers in Angus and Nellore longissimus muscle. A contemporary group of Nellore (n = 4) and Angus (n = 4) with 20 months of age and 400 ± 32 kg, reared under the exactly same conditions, were confined in individual pens and fed *ad libitum* the same diet for a total of 84 days during the finishing phase. At the end of the finishing phase (84 days) all the animals were slaughtered and longissimus muscle samples were quickly collected for further mRNA expression analysis of CAPN3 (calpain), CAST (calpastatin), CASP3 (caspase) and DNAJA1 (heat shock protein 40). No differences were observed (for mRNA expression of CAPN3 (P = 0.3194), CAST (P = 0.2849), and DNAJA1 (P = 0.0919) between Nellore and Angus muscle. Conversely, a greater mRNA expression of CASP3 was observed (P = 0.0443) in Angus than Nellore muscle. These data suggests that CASP3 contributes to discrepancy in beef tenderness between Nellore and Angus.

keywords: Bovine, breed, CAPN3, CAST, CASP3, DNAJA1.

I. INTRODUCTION

In a previous study we have found a greater calpastatin activity in beef from Nellore compared to Angus cattle, which has been one of the main explanations of the discrepancy of tenderness of beef among these breeds [1]. However, as these breeds differ in proteolysis postmortem, not only this enzyme activity but also expression of tenderness markers may also leads to differences in tenderness. Additionally, besides calpain-calpastatin complex, there are several other enzyme complexes that have been suggested to be involved in beef tenderization. One of them is the caspase enzyme complex, which causes the degradation of cellular protein compounds and initiates the cellular apoptosis being followed by other proteolytic enzymes including calpain [2]. On other hand, at the same time that those enzymes acts on myofibrillar degradation, there are an increase of the levels of heat shock proteins (HSP) that acts to preserve cell functions [3,4,5] and thus it would reduce the meat tenderization process. Therefore, we hypothesized that expression of several proteolytic enzymes and HSP differ in skeletal muscle of Nellore and Angus cattle contributing for the discrepancy in tenderness of beef among these breeds.

II. MATERIALS AND METHODS

A contemporary group of Nellore (n = 4) and Angus (n = 4) with 20 months of age and 400 ±32 kg, reared under the exactly same conditions, were confined in individual pens and fed *ad libitum* the same diet for a total of 84 days during the finishing phase. The feeding managements used were choosing to be as representative as possible to the feeding conditions commonly observed on Brazilian beef systems. Chemical composition and ingredient proportion of the experimental diets are presented in Table 1.

At the end of the finishing phase (84 days) all the animals were slaughtered. Pre-harvest handling was in accordance with good animal welfare practices, and slaughtering procedures followed the Sanitary and

Industrial Inspection Regulation for Animal Origin Products [6] .

Table1: Ingredient proportion and chemical composition of the experimental diets

Item	Concentrate : roughage ratio
	70 : 30
Ingredient proportion, % of dry matter	
Corn grain	-
Protein-mineral premix[1]	-
Corn silage	30.0
Corn meal	58.0
Soybean meal	10.0
Mineral mixture	2.0
Chemical composition, % of dry matter	
Dry matter	72.0
Crude protein	12.4
Neutral detergent Fiber	26.2
Total digestible nutrients	78.1
Starch	49.7

[1] Crude protein = 32.0%; Total digestible nutrients = 50.0%; Ca = 45.0 g/kg; Mg = 7.5 g/kg; P = 11.0 g/kg; Cu = 104 mg/kg; Zn = 344 mg/kg; Se = 0.83 mg/kg; Virginiamycin = 140.0 mg/kg; Monensin = 120.0 mg/kg

After the exsanguination samples of longissimus muscle (LM) were quickly collected placed in sterile tubes containing RNA*later*® (Qiagen, Austin, TX), stored at 4ºC overnight and then kept at -80ºC before RNA isolation [7].

Total RNA (1 µg) was extracted from 0.5 g of tissue samples using Trizol® reagent (Invitrogen, Carlsbad, CA), treated with DNase I, Amplification Grade (Invitrogen Carlsbad, CA) and reverse transcribed into cDNA using the GoScript™ Reverse Transcription System (Promega, Madison, WI). The primer sets used are shown in Table 1. RT-PCR was performed on a 7300 Real-Time PCR System (Applied Biosystems) using SYBR Green RT-PCR GoTaq® Master Mix from Promega and the following cycle parameters: 95°C for 10 min and 40 cycles at 95 °C for 15 s and 60 °C for 1 min. After amplification, a melting curve (0.01 C/s) was used to confirm product purity. Results are expressed relative to GAPDH using the $2^{\Delta\Delta Ct}$ [8].

Primers sets (Table 2) used in qPCR analysis were designed by using Primer Quest IDT software.

Table2: Forward and Reverse sequence of the primers.

Gene		Sequence
CAPN3	Forward	CTACGAGGTTCCCAAAGAGATG
	Reverse	ACCTCCCGCATGTTAATGTAG
CAST	Forward	GTCGGATCCAATGAGTTCTACC
	Reverse	CCTGCGATCCCTTCTTCTTTAT
CASP3	Forward	CGTCCCTTTCTGCCATCC
	Reverse	CAGACCATTAGGCCACACTC
DNAJA1	Forward	AATGTGAAGGCCGAGGTG
	Reverse	CTGAACCATTCCAGGTCCTATC
GAPDH	Forward	AGATAGCCGTAACTTCTGTGC
	Reverse	ACGATGTCCACTTTGCCAG

Gene expression results were correlated to Warner-Bratzler shear force and calpastatin activity values obtained in a previously study published by our research group using the same animals [1].

Statistical analysis was performed using SAS 9.2 (Statistical Analysis System Institute, Inc., Cary, NC, USA). Gene expression and, a bilateral T-test were used in the GLM procedure. Additionally, Pearson's correlation coefficients of gene expression and Warner-Bratzler shear force were evaluated. Statistical significances were considered at $P < 0.05$.

III. RESULTS AND DISCUSSION

Despite of the difference found in calpastatin activity between Angus and Nellore in our previous study [1], no differences were found for mRNA expression of calpain-calpastatin proteolytic system (Figure 1). Although post-transcriptional changes may occur involving this enzyme complex, our data suggests that the mRNA expression of calpain and

calpastatin may not play a pivotal role in regulation of proteolysis postmortem in Nellore compared to Angus beef.

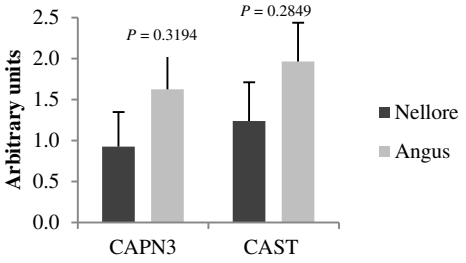

Figure 1. mRNA expression of calpastatin (CAST) and calpain (CAPN3) in longissimus muscle of Nellore and Angus cattle.

As previously mentioned, besides calpain-calpastatin proteolytic system, another mechanisms of meat tenderization have been studied lately. Among them, the caspases groups appears as the first proteolytic system involved in the conversion of muscle to meat being responsible for cellular apoptosis [9], leading to a weakness of skeletal muscle structure, and consequently to a tenderization of meat.

Our data shows a greater mRNA expression of CASP3 (P = 0.0443) in Angus than Nellore muscle (Figure 2), which may also contribute to the discrepancy in tenderness of beef from *Bos taurs* and *Bos indicus* cattle.

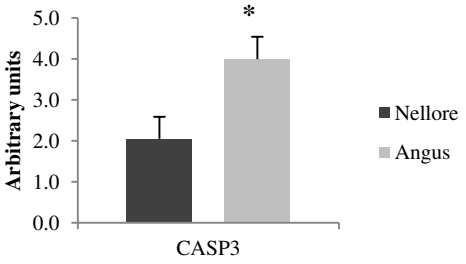

Figure 2. mRNA expression of CASP3 (caspase) in longissimus muscle of Nellore and Angus cattle.

Moreover, we investigated the existence of a correlation between mRNA expression of CASP3 and meat tenderness the analysis. Pearson`s correlation coefficient among Warner-Bratzler shear force (WBSF) values observed in our previously study using the same animals [1], and mRNA expression of CASP3 showed a tendency of a negative correlation between these variables (r = -0.62; P = 0.0977). Such results suggest that the increase WBSF values in Nellore beef may be partially explained by the greater expression of mRNA of CASP3 in Angus beef.

Heat shock proteins (HSP) have been also suggested as a group of protein that controls the meat tenderization. Those proteins play a pivotal role in maintenance of cellular protein integrity avoiding denaturation and likely loss of function [9]. Therefore, HSP may decrease the caspases activity [3,4,5] and increasing levels of HSP would lead to a reduction in meat tenderization. However, no differences were found for mRNA expression of DNAJA1 (HSP40) between Nellore and Angus muscle (P = 0.0919; Figure 3).

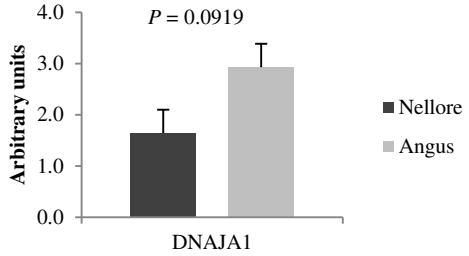

Figure 3. mRNA expression of DAJA1 (HSP40) in longissimus muscle of Nellore and Angus cattle.

Heat shock proteins tend to enhance expression and/or activity as a consequence of physiological imbalance. However, as cells recover their regular activity the demand of HSP is diminished. As such, the expression of DNAJA1 (HSP40) is likely affected by the time required by the tissue to reach postmortem homeostasis.

IV. CONCLUSION

These data suggest that besides the activity of calpain-calpastatin proteolytic system, caspases may also contribute for discrepancy in tenderness of beef from Nellore and Angus. Additionally, the effects of heat shock proteins on meat tenderness require further investigations.

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