Effect of beta-agonists and immunocastration on expression of CAPN1, CAPN2 and CAST in feedlot finished Zebu cattle

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Abstract –This study was developed to evaluate the effect of beta-adrenergic agonists (βAA) and immunocastration on gene expression of meat tenderness of *Bos indicus* cattle. Forty-eight Nellore males received two doses of immunocastration vaccine (IM), and 48 Nellore males were kept non-castrated (NC). In the last 30 days of feeding they were assigned to one of the following treatments: control diet without βAA (CON), 80mg/day zilpaterol hydrochloride (ZIL) or 300mg/day ractopamine hydrochloride (RAC). At slaughter a sample of *Longissimus dorsi* (LD) at 12th rib level was taken for CAPN1, CAPN2, and CAST gene expression analysis. Samples of LD were collected 48h after slaughter and aged for 0, 7, 14 or 21 days for Warner-Bratzler shear force (WBSF) determinations. Sexual condition and βAA treatments did not affect gene expression. The NC had smaller WBSF 48 h after slaughter with no differences in the subsequent days of ageing. The WBSF was not affected by treatments and showed low correlations with gene expression. Sexual condition and use of BAA had no effect on CAPN1, CAPN2 and CAST gene expression in *Bos indicus* Nellore catlle.

Key Word – Calpain, calpastatin, meat quality.

1- INTRODUCTION

Beta-adrenergic agonists (βAA) are a class of pharmacological compounds that have similar chemical structure to a group of natural compounds called catecholamines, which show potent anabolic effects on skeletal muscle. βAA have been used to increase weight gain and to improve feed efficiency of feedlot cattle. This process increases protein synthesis, while decreasing protein degradation and lipolytic action, causing a reduction in the amount of fat in the carcass. Immunocastration of cattle, on the other hand, improve fat deposition and beef quality, and has smaller impact on animal performance since it does not cause pain.

An increase in m-calpain (CAPN2) and Calpastatin (CAST) activities in cattle fed βAA at 1 and 24h post-mortem was verified [1]. In the same work μ-calpain (CAPN1) activity decreased after 23h post mortem and the authors attributed the negative effect on meat tenderness to βAA. Meat tenderness from animals fed Zilpaterol was attributed to the effect of calpastatin activity.

Changes of mRNA can reflect in an increase of m-calpain and calpastatin activity [2]. When evaluating the effect of βAA on the gender condition, authors noted an increase of calpastatin in non-castrated cattle, thus decreasing calpain activity when compared with castrated cattle [3, 4].

The objective of this study was to evaluate the effect of immunocastration and βAA treatment on mRNA expression of CAPN1, CAPN2 and CAST genes in meat from feedlot finished *Bos indicus* cattle.

2- MATERIALS AND METHODS

Ninety-six Nellore bulls (409 ± 50 kg LW; 20 mo old) were divided in two groups. Half of them received two doses of immunocastration vaccine (IM; Bopriva® - Zoetis Veterinary Products Industry LTDA, São Paulo, SP, Brazil) within a 30 day interval, and the other half were kept non-castrated (NC). Cattle were fed a common diet containing 76% concentrate and 24% roughage (corn silage) for 70 days. Following this they were divided in three groups (n=32), and fed 30 more days one of the following treatments: control diet without βAA (CON), 80 mg/d zilpaterol hydrochloride (ZIL; Zilmax® - MSD Animal

Health, São Paulo, SP, Brazil) or 300 mg/d of ractopamine hydrochloride (RAC; Optaflexx® - Elanco Animal Health, São Paulo, SP, Brazil). After this period, cattle were slaughtered according to humanitarian slaughter procedures required by Brazilian law.

Longissimus dorsi (LD) samples were collected between the 12th and 13th ribs immediately after harvest. Samples were snap frozen in liquid nitrogen, and stored at -80°C to extract RNA. Twenty-four hours after slaughter, four samples of LD muscle were taken, vacuum-packed and aged for 0, 7, 14 or 21 days for posterior Warner-Bratzler Shear Force (WBSF), according to AMSA [5].

Total RNA was extracted according to the manufacturer's protocol (RNeasy Fibrous Tissue Quiagen, USA). Briefly, the samples were macerated by liquid nitrogen with the pestle in a crucible placed in a 1.5 ml tube and added to reagents according to the manufacturer's protocol. Total RNA concentration of samples was estimated by spectrophotometry (Nano Drop, BioPhotometer, Eppendorf, Hamburg, Germany), and purity was estimated by the ratio of absorbance at 230, 260, 280 nm. Only samples with A260 / 280 between 1.8 and 2.0 and A230 / 260 above 2.0 were used. After integrity of these samples was evaluated by RIN (RNA Integrity Number), following the manufacturer's protocol (RNA Nano chip, Bioanalyser, USA), samples that presented an RIN value lower than 8 were excluded. Taqman system with primer pairs and probes (Life Technologies, USA) was used to evaluate gene expression. Tests used were Calpain -1 (Bt03213558_g1), Calpain -2 (Bt03817738_m1), and calpastatin (Bt03212306_m1), to evaluate 18S ribosomal RNA expression as an endogenous control (4318413E - VIC MGB). The reactions were performed in a thermocycler StepOne® (Life Technologies, USA) using the reagents DNase I (RNase - free) and FG RNA to cDNA (4,387,408) (Life Technologies, USA). The calculation of relative gene expression was performed according to described by Livak and Schmittgen [6].

Data was analyzed by ANOVA as a randomized complete block (initial LW) design in 2 x 3 factorial arrangement (sexual condition x treatments) using the MIXED procedure of SAS software. Statistical differences were considered significant at $P < 0.05$.

3- RESULTS AND DISCUSSION

There was no gender condition x βAA interaction. Sexual condition did not affect gene expression (Figure 1).

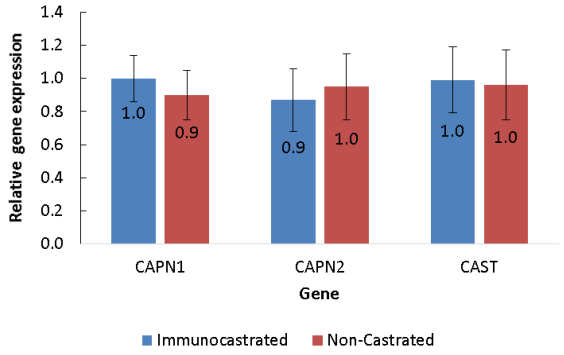

Figure 1 – m-calpain (CAPN1), μ-calpain (CAPN2) and calpastatin (CAST) gene expression according to sexual condition of Bos indicus cattle.

These results differ from those reported by Koohmaraie *et al.* [3] which found that wether lambs had higher m-calpain at slaughter and calpastatin at 7 and 20 days postmortem when compared with Rams. It was also observed a decrease in calpastatin activity in castrated animals at death and 24h postmortem. Androgenic hormones can increase calpastatin activity in bulls when compared to steers [4].

There was no effect of βAA on gene expression (Figure 2). These results were different than those reported in literature, where βAA have been reported to increase calpastatin activity and decrease calpain activity [1,3]. Many factors such as species, breed, gender and other genetic variants, can affect calpastatin activity [7] and mRNA expression [2]. On the other hand, some authors have not observed this effect of βAA in m- and μ-calpain [8].

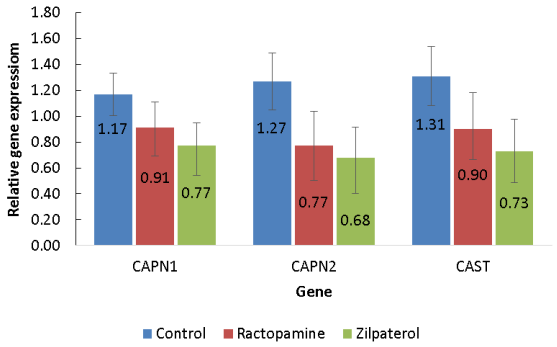

Figure 2 – m-calpain (CAPN1), µ-calpain (CAPN2) and calpastatin (CAST) gene expression according to treatments in Bos indicus cattle.

In this work it was evaluated the gene expression and not activity. Maximal response of βAA depend on dose and duration of animal exposure, since constant exposure of receptors will cause desensitization or inactivation [9], causing an eventual negative effect of mRNA.

The NC animals had a smaller value of WBSF 48h after slaughter (day 0; P=0.0321; Table 1). There was no effect of gender on WBSF for any other period. Similar findings were reported by Miguel *et al.* [10] who did not observe an effect of castration on meat tenderness.

Table 1. Least square means of Warner-Bratzler shear force and standard error of mean (SEM) according to sexual condition and treatments.

Ageing period	Sexual condition			Treatments			
	IM	NC	SEM	CON	RAC	ZIL	SEM
0	6.4[a]	5.3[b]	0.35	5.7	5.7	6.1	0.43
7	4.4	4.1	0.28	3.9	4.5	4.5	0.34
14	2.9	3.3	0.21	3.2	3.1	3.1	0.28
21	2.9	2.9	0.25	2.6	2.7	3.5	0.30

[a,b] Different letters within sexual condition or treatments differ (P<0.05) by Student T Test.

Likewise, the no effect on gene expression of βAA in this study, but there was differences in tenderness, according authors, that the beef of animals fed with ZH required the most force to shear, RH steaks were intermediate and CON were lowest (more soft) [11].

The correlation coefficients among gene expression and WBSF at different ageing period were low (Table 2)

Table 2: Correlation coefficients of µ-calpain (CAPN1), m-calpain (CAPN2) and calpastatin (CAST) expression with Warner-Bratzler Shear force values at different days of ageing.

	Ageing days			
	0	7	14	21
CAPN1	-0.03	-0.18	0.02	-0.21
Pr>F	*0.7853*	*0.1487*	*0.8923*	*0.1018*
CAPN2	-0.05	-0.21	0.01	-0.27
Pr>F	*0.7037*	*0.0808*	*0.945*	*0.0341*
CAST	0.01	-0.16	0.05	-0.23
Pr>F	*0.932*	*0.1892*	*0.7086*	*0.0675*

The low correlation between tenderness and gene expression of CAPNs and CAST was not expected, because CAST has been associated with lower and calpain with increase in tenderness, although the calpain action is only significant 150h after the onset of *rigor mortis*, the same results were observed for other authors, and a possible explanations for this results is the fact that animals were young and had others expression of enzymes involved in the process of protein turnover [12].

The correlation between CAPN1 and CAST was greater than 0.90 (P<.0001) and CAPN2 and CAST greater than 0.96 (P<.0001), which seems to indicate there are calpain and calpastatin available in muscle.

4- CONCLUSION

Sexual condition and βAA did not affect expression of CAPN1, CAPN2 and CAST gene. No correlation between tenderness and genes expression was found, only between calpain and calpastatin expression.

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