

CALPASTATIN SNP POLYMORPHISM ASSOCIATED WITH MYOFIBRILLAR FRAGMENTATION INDEX IN BEEF FROM EXCITABLE *BOS INDICUS* CATTLE

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I. INTRODUCTION

Calpastatin (CAST) expression and/or activity is determinant for proteolysis in beef from *Bos taurus indicus* [1]. Its activity is regulated by phosphorylation [2], which is up regulated when animals are stressed. Non-castrated Nellore cattle are prone to stress. Testosterone influences cattle temperament, and more excitable animals are related to inferior beef quality [3]. Immunocastration can be an option. Since there is a relationship between CAST SNP and instrumental tenderness [4], as well as the evidence that the rate of beef tenderisation is already limited by CAST in this type of cattle [5], it was hypothesized that excitable Nellore males, independently of gender, have reduced myofibrillar fragmentation index (MFI) during beef ageing and that CAST SNP would be important in the proteolysis. The objective was to associate the CAST SNP with MFI as well as verify the interaction of sexual condition and temperament on proteolysis during beef ageing.

II. MATERIALS AND METHODS

All experimental procedures involving animal care were conducted in accordance with the FZEA/USP Animal Care and Use Committee Guidelines (6493190121). From a group of 72 Nellore males, a sub-group of 23 was selected based on temperament tests. Chute score and flight speed were determined and used to calculate temperament index [5], that was used to classify animals either as excitable or calm. Care was taken to select progenies from several bulls, as well as to represent two genders (non-castrated and immunocastrated), and hair was used to genotype for the SNP (single nucleotide polymorphism) in the CAST *locus* by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) with restriction enzyme Ddel [4]. At 24h *postmortem* the *Longissimus lumborum* (LL) steaks were *vacuum* packaged, aged during three times *postmortem* (48, 360 and 696 hours) and used to measure MFI [6]. To characterize the MFI according to CAST, the model included the gender as a block. The effect of gender and temperament on MFI was analysed as a factorial design [2 × 2; 2 genders (non-castrated or immunocastrated) and 2 temperaments (excitable or calm)], using a mixed model with random effect of slaughter date and fixed effects of gender (G), temperament (T), time *postmortem* (time; repeated measure), as well as its interactions.

III. RESULTS AND DISCUSSION

Calpastatin genotype frequency between genders and temperaments is shown in Table 1. Since the GG genotype was not present in all sub-groups, it was removed from further characterization. The MFI according to CAST genotype and during the *postmortem*, revealed that the AA was favourable to earlier fragmentation (Fig. 1). There was a gender effect (P = 0.007) for MFI, with non-castrated animals showing lower MFI than immunocastrated (Fig. 2). It was observed an interaction effect between temperament and time (P = 0.03), with excitable animals showing lower MFI value at the 360

and 696 h *postmortem*. Beef from excitable and non-castrated animals showed the lowest MFI values (91.85 ± 5.393), which is related with impairment in proteolysis promoted by calpastatin inhibitory activity early *postmortem*.

Table 1. Calpastatin genotype frequency (%) in Nellore males selected according to gender and temperament.

	Immunocastrated		Non-castrated		Total
	Calm (n = 6)	Excitable (n = 6)	Calm (n = 5)	Excitable (n = 6)	
AA	18.2	9.09	4.55	9.09	40.9
AG	4.55	18.2	13.6	13.6	50.0
GG	4.55	-	-	4.55	9.09
				Total	100

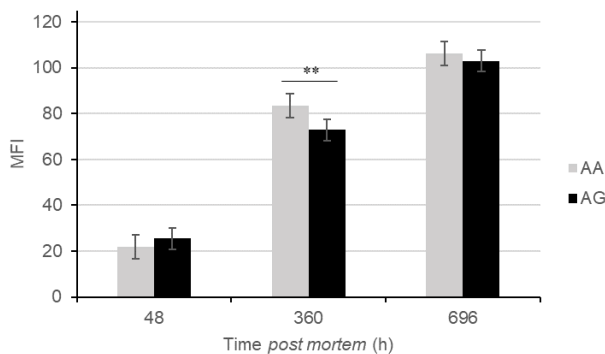


Figure 1. Myofibrillar fragmentation index (MFI) in *Longissimus lumborum* muscle from Nellore males feedlot finished and divided according to calpastatin (CAST) genotype. **P = 0.04

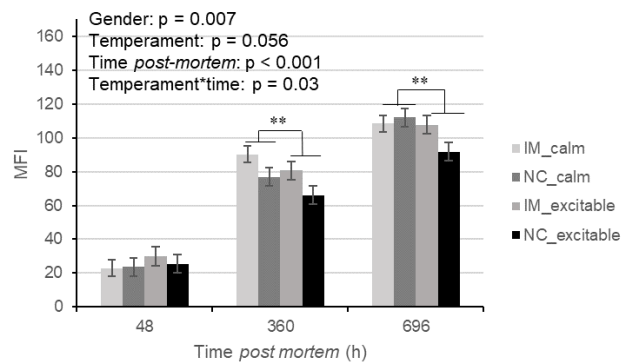


Figure 2. Myofibrillar fragmentation index (MFI) in *Longissimus lumborum* muscle from Nellore males feedlot finished and divided according to gender (IM = immunocastrated; NC = non-castrated) and temperament. **P < 0.03

IV. CONCLUSION

The presence of the allele G in the calpastatin genotype is related with reduced proteolysis evidenced by the fragmentation index. Immunocastration is a suitable alternative for improvement of the myofibrillar fragmentation, especially when animals are calm.

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