PE1.55 Association of a single nucleotide polymorphisms in calpastatin gene with meat tenderness in Nellore cattle 346.00

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***Abstract*— Recently, the search for identification of the genes and single nucleotide polymorphisms associated with tenderness have been growth. Calpastatin is a potential candidate gene because of its inhibitory role in the calpain system that is involved in *post mortem* tenderization. Four hundred cattle of the Nellore breed with approximately 2 years old from Agro-Pecuária CFM Ltda. were genotyped for a single nucleotide polymorphism (SNP) in calpastatin gene (CAST). The association the CAST SNP with tenderness measured at 7, 14 and 21 days of maturation was studied. The CAST SNP allele C was more frequent in the Nellore cattle population than allele G. It was observed interaction between CAST and maturation at 7, 14 and 21 days on Warner-Bratzler shear force (WBSF; kgf). Animals inheriting the all genotype for *CAST* marker presented meat more tender from 7th to 21st days. However, animals inheriting the CC genotype for *CAST* produced meat more tender than the CG or the GG genotypes. The results of allelic distribution and association between genotypes for CAST marker and time of maturation have shown the potential for the utilization of this marker in programmers of genetic improvement in the Nellore cattle. In this case, the marked-assisted selection (MAS) in the direction of the C allele may be improvement the tenderness of beef matured for 14 and 21 days in Nellore cattle.**

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I. Introduction

Solving the problem of inconsistent meat tenderness is a top priority of the meat industry. This requires a greater understanding of the processes that affect meat tenderness and, perhaps more importantly, the adoption of such information by the meat industry [1].

Genetic improvement has long been considered an important factor in the competitiveness of beef cattle production for enhancing product quality. Identification of the genes and/or polymorphisms underlying quantitative/qualitative traits, and an understanding of how these genes/polymorphisms interact with the environment or with other genes affecting economic traits might be the keys to successful application of marker-assisted selection in the commercial animal population [2]. Past efforts to develop DNA markers have been limited by costs and available technology. New advancements in genotyping and sequencing have already led to positional cloning of mutations for single gene birth defects [3].

The ability to select genetically for animals that are superior and consistent for meat quality traits would benefit the industry. However, the genetic components for meat tenderness are complex and are controlled by multiple genes. Among the factors that have been identified as responsible for the *post mortem* meat tenderization process is the calpain proteolytic system. Two enzymes responsible for this process are the micromolar calcium-activated neutral protease -calpain (CAPN1), which is encoded by the *CAPN1* gene, and its inhibitor, calpastatin (CAST), which is encoded by the *CAST* gene [1].

A number of studies have shown that calpastatin plays a central role in regulation of calpains activity in the cell and is considered to be one of major modulators of postmortem protein turnover. Its role in meat ageing has been demonstrated [4],

especially by the strong genetic correlations between calpastatin activity and tenderness measured by Warner-Bratzler shear force [5]. These observations suggest that genes coding for calpains and calpastatin may be considered as candidate genes for beef tenderness [6].

Association studies of single nucleotide polymorphisms (SNPs) for the μ-calpain and calpastatin genes with tenderness have primarily been performed in *Bos indicus* and *Bos taurus* cattle [5,7]. Thus, the objective of this study was to assess the association of single nucleotide polymorphisms in the Calpastatin gene with meat tenderness in beef Nellore cattle.

II. MATERIALS AND METHODS

A. Animals

The data came from Agro-Pecuária CFM Ltda., located in northeast São Paulo State, Brazil. Agro-Pecuária CFM is a Nellore cattle operation consisting of close to 17,000 Nellore cows. This company sells around 2,000 young Nellore replacement bulls out of approximately 7,000 weaned calves per year. Both bulls and heifers were maintained on high-quality pasture (40% Brachiaria brizantha, 50% Panicum maximum, and 10% others) and provided only salt and mineral supplementation.

Four hundred cattle of the Nellore breed with approximately 2 years old were used in this experiment.

B. Experimental procedure

The slaughter was performed following humanity standard procedures at a local slaughterhouse. The captive bolt method was used to stun the animals. Carcasses were split, weighed and then chilled at 0-3ºC before processing on the following day after slaughter. At 24 hours post mortem, 4 beefs of 2.5 cm each were removed from *Longissimus dorsi* (LD) muscle at 12th rib toward cranial. The beefs were vacuum packaged and kept at 2-4°C for until 21 days. At 7, 14 and 21 days the packages were frozen and stored in freezer at -18°C until meat tenderness analysis.

C. Tenderness measurements

Initial, the beefs were removed from freezer and conditioned for thawing on refrigerate chamber at 8ºC for 24 hours.

The Warner-Bratzler shear force (WBSF) determinations, which are a measure of the force required to pass a blunt blade through a core sample of cooked meat perpendicular to the muscle fibers, were performed according to AMSA (1995) recommendations [8]. Each sample was cooked in electric broiler cookery to an internal temperature of 40°C, flipped, and cooked to a final internal temperature of 71°C. Steaks were stored overnight at 8°C, subsequently eight 1.27-cm-diameter cores were removed from each steak parallel to the fiber direction. Each core was sheared once perpendicular to the muscle fibers using the WBSF equipment with a crosshead speed of 250 mm/min.

D. Marker Used

The single nucleotide polymorphism (**SNP**) developed at the *CAST* gene was reported by Schenkel et al. [9]. The marker is a transition from a guanine to a cytosine. The marker will be referred to as *UOGCAST1* (GenBank accession AY008267).

E. Genotyping

Blood samples were collected and DNA prepared from these animals was storage -80ºC. The UOGCAST1 SNP were genotyped on 400 animals by Real Time PCR (ABI Prism® 7500 Sequence Detection System – *Applied Biosystem*). The PCR master mix was 0.25μl Assay Mix® (Applied Biosystem), 5μl Taqman® Master Mix Universal PCR (Applied Biosystem), and 15 ng of DNA for 10μl total volume.

The PCR cycling condition was 95^{o}C (10.0 min) for 1 cycle; then 92^{o}C (15 s) for 45 cycles and 1 to 60^{o}C (60s) maintaining 4^{o}C thereafter.

F. Statistical Methods

Individual association of marker with WBSF was evaluated using the Mixed procedure of SAS (SAS Inst., Inc., Cary, NC) with repeated measures in animal effect and shear force as the dependent variable. The following model was used:

$$Y_{ijklm} = \mu + C_i + A_j + M_k + T_l + MT_{kl} + \beta_1\left(I_{ijklm} - I\right) + e_{ijklm}$$

where Y_{ijklm} = phenotypic tenderness observations; μ = common constant for all observations (overall mean); C_i = is the fixed effect for contemporary group, A_j = is the random effect of animal; M_k = is the fixed effect of marker genotypes (CAST), T_l = is the fixed effect for time of maturation (7, 14 and 21 days); MT_{kl}= is the interaction effect when marker is at the *k*th level and time of maturation is at the *l*th level; β_1 = coefficient of linear regression

of characteristics Y_{ijklm} on the age of animal at slaughter; I_{ijklm}= age of animal at slaughter; I = mean age of animals at slaughter and e_{ijklm}= random effect associated at characteristics Y_{ijklm} with mean 0 and variance σ_e^2.

III. RESULTS AND DISCUSSION

A. Genotypic and Allelic Frequencies

A total of 374 animals were used in the study. Samples from 26 of the 400 animals in the population were unable to amplify any product to be genotyped, and were thus excluded from the study. The genotypic frequencies for the 374 cattle in the population obtained with DNA samples are presented in Table 1.

The GG genotype was present at low frequency in these animals being observed 12.7% animals homozygous GG for marker *CAST*. However, 40.0% and 47.2% animals had the genotypes CC and CG, respectively.

Table1. Genotypic frequencies for UOGCAST1

Marker	Alleles	Frequency	Percent
CAST	GG	47	12.7
	CG	177	47.2
	CC	150	40.0
	Total	374	100

In relation of the allelic frequency observed a greater frequency of allele C (frequency = 0.64) than allele G (frequency = 0.36).

The magnitude of the genotypic and allelic frequency in this study was similar to that reported for the Schenkel et al. [9], which genotyped animals of several breed. The animals showed the lowest genotype frequency for the genotype GG and the highest allelic frequency for the allele C.

B. Marker Associations

Least squares means and standard errors are reported in Table 2 for the effect of *CAST* genotypes and time of maturation on WBSF in the population evaluated. The marker at the *CAST* gene and time of maturation at 7, 14 and 21 days was associated (P<0.05) with WBSF.

Table 2. Least squared means for Warner-Bratzler shear force (**WBSF**) according to genotypic for CAST marker and time of maturation at 7, 14 and 21 days in the Nellore beefs

Marker	**Genotype**	**WBSF** (kg)		
		7th day	14th day	21st day
CAST	GG	5.9±0.2$^{a;A}$	5.0±0.2$^{a;B}$	4.6±0.28$^{a;B}$
	CG	5.8±0.1$^{a;A}$	4.8±0.1$^{a,b;B}$	4.3±0.1$^{a,b;C}$
	CC	5.7±0.1$^{a;A}$	4.6±0.1$^{b;B}$	4.1±0.1$^{b;C}$

[+] Means in the same column and followed at same minuscule letter superscript are not differ significantly (P>0.05);

[++]Means in the same line and followed at same majuscule letter superscript are not differ significantly (P>0.05).

It is possible observed overall increase of tenderness during the maturation period independent of genotypes. A significant association was observed for tender in the C allele frequency, where animals with the CC and the CG genotypes produced beefs more tender when compared with animals with the GG genotype, particularly at 14th and 21th days of maturation.

Calpain is responsible for the breakdown of myofibrillar proteins, which are closely related to meat tenderness [10]. Calpastatin (**CAST**) inhibits μ- and m-calpain activity and, therefore, regulates *post mortem* proteolysis. Increased *post mortem,* CAST activity has been correlated with reduced meat tenderness. Schenkel et al. [9] associated of the CAST SNP with shear force across days of *post mortem* aging and genotype CC yielded beef that was tenderer than GG and CG had intermediate tenderness.

The interaction between *CAST* and maturation at 7, 14 and 21 days on Warner-Bratzler shear force (WBSF; kgf) was observed (Figure 1). Animals inheriting the all genotype for *CAST* marker

presented meat more tender from 7th to 21st days. However, animals inheriting the CC genotype for *CAST* produced meat more tender than the CG or the GG genotypes.

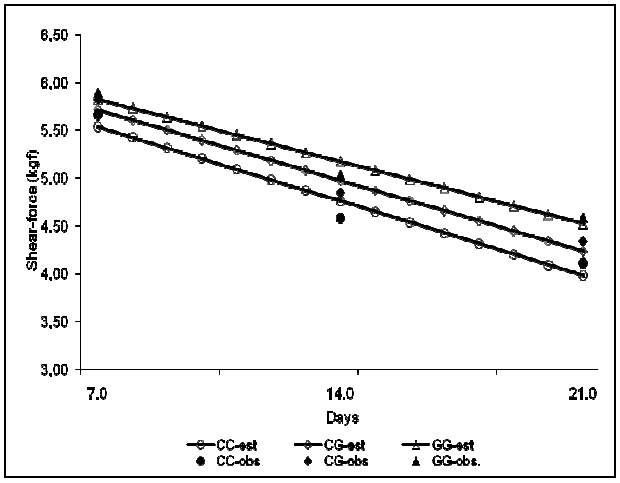

Figure 1. Interaction of *calpastatin* genotype and maturation at 7, 14 and 21 days on Warner-Bratzler shear force (WBSF; kgf).

Previous studies have associated of single nucleotide polymorphisms (SNP) developed at the *calpastatin* (*CAST*) and μ-*calpain* (*CAPN1*) genes with meat tenderness in three animal populations (*Brahman*, cycle 7 and 8 composite cattle *Bos indicus* with *Bos Taurus*) in MARC/Clay Center, Nebraska, USA [6]. Significant associations were verified for genotype and tenderness for both markers only for *Bos Taurus* and *Bos indicus* composite populations (cycle 7 and 8). In contrast, the reduced tenderness of the unfavorable genotype in *Bos indicus* did not reach significance because of the lack of CC homozygotes them, which reduced the ability to detect significant association [6]

IV. Conclusion

In conclusion, the results of allelic distribution and association between genotypes for CAST marker and time of maturation have shown the potential for the utilization of this marker in programmers of genetic improvement in the Nellore cattle. In this case, the marked-assisted selection (MAS) in the direction of the C allele may be improvement the tenderness of beef matured for 14 and 21 days in Nellore cattle.

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