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Proteome changes of beef in Nellore cattle with different genotypes for tenderness

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ABSTRACT

In the present study, 155 Nellore cattle were genotyped for the CAPN4751 and UOGCAST polymorphisms and phenotyped for shear force (SF) at 1, 7 and 14 days aging. The effects of different genotypic combinations were evaluated on the *Longissimus* muscle proteomic profile using 2DE and mass spectrometry. A significant association was found between genotypes for UOGCAST and CAPN4751 and meat tenderness. The CC genotype for both markers was favorable for lesser shear force than TT. A total of 40 spots showed significant differential expression profiles ($P < 0.05$), of which eight had a main effect for the CAPN4751 marker, 11 for UOGCAST, two for both markers, and 19 had interactions between markers, including myosin (MYL1, MYL2, MYLPF and MYL6B), actin (ACTA1 and CAPZβ), troponin (TNNT1 and TNNT3) and heat shock proteins (HSPB6, HSPB1 and HSP70-2). The results demonstrated that UOGCAST and CAPN4751 genotypes led to variability on the expression of proteins that are involved in muscle metabolism, and consequently affect meat tenderness.

1. Introduction

The recent improvement of management techniques combined with genetic improvement has enabled a considerable productivity increase in several types of animal farms, including beef cattle farming. However, obtaining a quality end product that meets the requirements of an increasingly demanding market is still a challenge for the meat production chain. Although various studies have focused on discovering determinants of meat tenderness, which is one of the main attributes considered by the consumer, this issue remains a relevant topic and a challenge for the industry.

Tenderness is a complex phenotype with underlying mechanisms that have not been fully elucidated. The calpain/calpastatin system, which primarily comprises the proteins calpain-1, calpain-2 and calpastatin, is presumably one of the main factors associated with *post-mortem* meat tenderness. Calpain activity is related to myofibrillar protein degradation, and higher calpain-1 activity is associated with increased tenderness. Conversely, calpastatin has an inhibitory effect on calpain activity and therefore on the regulation of *post-mortem* proteolysis (Huff Lonergan, Zhang, & Lonergan, 2010; Lian, Wang, & Liu, 2013).

Several studies have identified single nucleotide polymorphism (SNP) markers in the genes that encode calpain-1 (CAPN1) and calpastatin (CAST) associated with meat tenderness in both *B. taurus* and *B. indicus* cattle (Avilés et al., 2015; Schenkel et al., 2006; White et al., 2005). In Brazil, some studies have found significant associations of calpain and calpastatin markers with the meat tenderness of Nellore cattle (Carvalho et al., 2017; Pinto et al., 2010). However, no studies have been reported evaluating at the same time the effect of SNPs in the calpain and calpastatin genes and the muscle protein expression of Nellore cattle.

Proteomics has emerged as a key research tool to complement genomics studies and further the understanding of complex biological processes. Several authors have discovered potential protein biomarkers of tenderness by characterizing the proteome of different muscles in various animal species as a function of differences in the tenderness level (Baldassini et al., 2015; Carvalho et al., 2014; Gagaoua, Terlouw, Boudjellal, & Picard, 2015; Lana & Zolla, 2016).

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Thus, the main objective of this study was to understand the biological mechanisms involved in meat tenderness through comparison of muscle protein profile among different genotypes for calpain and calpastatin genes in Nellore cattle.

2. Materials and methods

All procedures involving animals reported in this study were performed according to appropriate animal welfare standards and were authorized by the Ethics Committee of the School of Animal Science and Food Engineering, University of São Paulo (protocol number n.25042008).

2.1. Animals, housing

A total of 155 animals (80 steers and 75 young bulls), which progenies of eight purebred Nellore sires that originated from the Nellore beef herd of the University of Sao Paulo. Between weaning and the beginning of the study, the cattle were kept in a single group, grazed in *Brachiaria* spp. pastures and provided with free-choice mineral mixtures.

Throughout 2009 and 2010, Nellore bulls (n = 75, 523 ± 3.7 BW, 23-mo old) and steers (n = 80, 483 ± 32.4 BW, 23-mo old) were finished in feedlots receiving the same high-grain diets for all period (140 days). Cattle were allowed to adapt to the diet and feeding procedures for 21 days. During adaptation, corn silage was initially offered *ad libitum* as unique feed and gradually substituted by the experimental diet. After the adaptation period, the experimental diet (total mixed ration, 2.63 Mcal ME/kg and 14.6%CP in dry matter basis), was offered *ad libitum*, at 800 h so that 10% refusals were allowed.

2.2. Genotyping

Blood samples were collected in EDTA tubes from a jugular vein puncture at the beginning of the confinement period. Genomic DNA was isolated from whole blood according to the protocol reported by Olerup and Zetterquist (1992) and stored − 80 °C. Markers in the CAPN1 and CAST genes selected for this study were previously described by White et al. (2005) and Schenkel et al. (2006), respectively. The CAPN4751 SNP is a silent mutation located in intron 18 (nucleotide position 6545) of CAPN1 (GenBank accession No. AF248054, replaced by AH009246) on chromosome 29 that causes a substitution of cytosine with thymine (C/T). The CAST gene (GenBank accession No. AY008267) is located on chromosome 7. The UOGCAST SNP is a substitution of cytosine with guanine (C/G) located in intron 5 (nucleotide position 282). The primers used are shown in Table 1.

The region of interest was amplified using standard PCR techniques. The CAPN4751 and UOGCAST SNPs were genotyped by real-time PCR (ABI Prism® 7500 Sequence Detection System, Applied Biosystems). The PCR master mix contained 0.25 µL of Assay Mix® (Applied Biosystems), 5 µL of Taqman® Universal PCR Master Mix (Applied Biosystems) and 15 ng of DNA in a 10-µL total volume. The PCR cycling conditions were 95 °C (10 min) for 1 cycle, 92 °C (15 s) for 45 cycles, 60 °C (60 s) for 1 cycle and maintenance at 4 °C thereafter.

2.3. Slaughter and meat analysis

Animals were slaughtered in four groups containing approximately 24 animals each (12 of each gender). The carcasses were longitudinally divided in half, washed, weighed and chilled for 24 h at 0–2 °C.

Before and after chilling, the pH and temperature of *Longissimus thoracis* (LT) muscle was measured at 12th rib level, using a digital pHmeter (pH 11 Economy Meter, Oakton Instruments). Following, the right side of each carcass was ribbed between 12th and 13th ribs and three samples of the *LT* muscle were taken, vaccum packaged and aged for 1, 7 or 14 days. At the end of each aging period samples were analyzed for Warner-Bratzler shear force (WBSF) according AMSA (1995) and Poleti et al. (2015). The 2.5 cm tick steaks were weighted and cooked in an electric oven until reaching an internal temperature of 40 °C, when they were turned over and cooked until 71 °C. After cooking, steaks were cooled at room temperature and stored overnight at 4 °C. On the next day, 6 cores with a 1.25-cm diameter were removed parallel to the direction of muscle fibers. Finally, cores were sheared using the Warner–Bratzler equipment, and shear force values were expressed in Kg and converted to Newton (N). The cooking loss was determined by subtracting the difference between the weights of the steaks before and after roasting.

2.4. Proteomic analysis

2.4.1. Muscle samples

The 2-DE was conducted in the LT muscle samples collected 24 h *post-mortem* for each genotype group (CC, CG and GG for UOGCAST and CT and TT for CAPN4751). Six samples of 1 g of muscle tissue from each genotype group were mixed in glass plate. Then, 0.5 g of this mixture was collected and homogenized in 5 mL of 8 M urea, 2 M thiourea, 65 mM DTT, 2% CHAPS and protease inhibitors (Lametsch et al., 2003). The protein content was determined with the 2-D Quant Kit (GE Healthcare). The obtained protein extract was stored at − 80 °C prior to the 2-DE analysis.

2.4.2. Two-dimensional electrophoresis and image analysis

The 2-DE analysis was performed in triplicate for each sample. The first dimension was performed with the Ettan IPGphor (GE Healthcare) and IPG Strips of 13-cm pH 4–7 (GE Healthcare), loaded with 500 µg of protein in each one. Initially, 500 V was applied, followed by a gradient of 1000 to 8000 V and finishing with 8000 V and a 75 µA current limit per strip. The total product time *x* voltage applied was 17,403 V/h for each strip.

For the second dimension, the strips were equilibrated for 15 min in Equilibrium Buffer I under gentle agitation (6 M urea, 75 mM Tris-HCl (pH 8.8), 29.3% glycerol, 2% SDS, 0.002% bromophenol blue and 65 mM DTT) and for another 15 min in Equilibrium Buffer II (6 M urea, 75 mM Tris-HCl (pH 8.8), 29.3% glycerol, 2% SDS, 0.002% bromophenol blue and 197 mM iodoacetamide) under gentle agitation. The running buffer used was Laemmli 1 × concentrate for the anode, and 3 × concentrate for the cathode. A current of 15 mA/gel was used at an initial voltage of 100 V for 30 min; then, 600 V was applied for 3 h at a current of 50 mA/gel. After fixing the proteins for 30 min in 40% ethanol/10% acetic acid solution, the gels were stained with Coomassie G-250 colloidal stain (8% ammonium sulfate, 0.8% phosphoric acid,

Table 1
Sequences of the genotyping primers for Calpain-1 and Calpastatin genes markers.

Marker	Sequence	Probes
CAPN4751	F: TGGCATCCTCCCCTTGACT	CTGCGCCTCGGTTT
	R:CCCCCGTCACTTGACACA	CTGCGCCTCAGTTT
UOGCAST	F: GGAAGGAAGGAATTGCATTGTTTCA	CTTTGGGTAGAAAATT
	R:CACTTGTGTTTTATGTAGTCAATTGTGAGAA	TTGGGTACAAAATT