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Association of *CAST2*, *HSP90AA1*, *DNAJA1* and *HSPB1* genes with meat tenderness in Nellore cattle

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ABSTRACT

The objective of this study was to evaluate the association of expression of *CAPN1*, *CAPN2*, *CAST*, *HSP90AA1*, *DNAJA1* and *HSPB1* genes with meat tenderness in Nellore cattle. Three experimental groups were selected by shear force (SF): moderately tender (SF = 34.3 ± 5.8 N), moderately tough (SF = 56.8 ± 7.8 N), and very tough meat (SF = 80.4 ± 15 N). Gene expression was evaluated by real-time PCR. Expression of the *CAPN1*, *CAPN2*, *CAST* and *CAST1* genes did not differ between groups. Expression of the *CAST2* was up-regulated ($P < 0.05$) in the moderately tough and very tough meat groups. Down-regulation of the *HSP90AA1*, *DNAJA1* and *HSPB1* genes ($P < 0.05$) was observed in the moderately tender meat group. The present results suggest that meat tenderness in Nellore cattle does not directly depend on the expression of the *CAPN1* and *CAPN2* genes, but is associated with the expression of other genes such as *CAST2*, *HSP90AA1*, *DNAJA1* and *HSPB1*.

1. Introduction

The changes in consumers eating habits highlight the need for beef cattle farmers to provide tender meat in order to ensure the continuous growth of the industry (Liu et al., 2016). Biotechnological tools could be useful to identify potential biomarkers for the beef cattle breeding programs and selection of animals that permit the production of tender meat (Ouali et al., 2013). Genes belonging to the calpain complex (*CAPN1*, *CAPN2* and *CAST*) have been shown to play an important role in meat tenderness (Pinto et al., 2010; Guillemin, Jurie et al., 2011). The μ and m-calpain are calcium-dependent cysteine proteases that are involved in the decomposition of myofibrillar proteins and consequent meat tenderization (Goll, Thompson, Li, Wei, & Cong, 2003). Calpastatin and its four isoforms (*CAST*, *CAST1*, *CAST2*, *CAST3*, and *CAST4*), which are also controlled by calcium, act as endogenous inhibitors of the proteolytic activity of calpains (Cônsolo, Ferrari, Mesquita, Goulart, & Silva, 2016; Raynaud et al., 2005). However, meat tenderness is a complex trait that, in addition to calpains and its inhibitor calpastatin, involves an intrinsic network of structural proteins, proteases and heat shock proteins (HSPs), a fact hampering the understanding of their respective functions (Lana & Zolla, 2016). Thus, the gene and protein expression of HSPs may also be associated with meat tenderization (Baldassini et al., 2015; Ouali et al., 2013; Yang, Pandurangan, & Hwang, 2012).

Heat shock proteins (HSPs) are induced in response to heat stress. These proteins exert an antiapoptotic function and can also be activated to protect cellular proteins against denaturation (Kültz, 2003). HSPs are classified into subfamilies based on their molecular weight (kDa): Hsp90, Hsp70, Hsp40, and Hsp27. Some of these proteins act as chaperones during protein assembly, protein folding and unfolding, and refolding of damaged proteins (Marques et al., 2007).

Despite this knowledge, the role of HSPs in meat tenderness needs to be further investigated in order to introduce possible biomarkers that could contribute to the selection of quality meat-producing animals (Avilés, Martínez, Domenech, & Peña, 2015; Guillemin, Bonnet, Jurie, & Picard, 2011). In this respect, some studies have reported an association of the *HSP90AA1*, *DNAJA1* and *HSPB1* proteins with meat tenderness in *Bos taurus* cattle (Picard et al., 2010; Guillemin, Jurie

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et al., 2011; Guillemin, Bonnet et al., 2011). However, there are no studies evaluating the association of genes expression of the HSP family with meat tenderness in Nellore cattle. Therefore, the objective of this study was to analyze the association of the expression of calpain complex genes (*CAPN1*, *CAPN2* and *CAST*) and the expression of the genes encoding HSPs (*HSP90AA1*, *DNAJA1* and *HSPB1*) with meat tenderness in Nellore cattle.

2. Material and methods

2.1. Production of experimental animals and sample collection

A population of 90 contemporaneous uncastrated, male Nellore animals was used. The animals were from a single farm that uses a continuous pasture system and had a mean initial weight of 390 ± 37 kg and age of approximately 24 months. The finishing period in the Experimental Feedlot of Faculdade de Medicina Veterinária e Zootecnia (FMVZ), Unesp, Botucatu, SP, Brazil, lasted 95 days. The procedures were approved by the Ethics Committee on Animal Experimentation of FMVZ, Unesp, Botucatu/SP (Protocol No. 159/2014).

All animals were given the same diet, housing and management. The animals were treated with anthelmintics, weighed, and randomly allocated to 18 collective pens with 5 animals/pen before the beginning of the feedlot period. The animals received a diet twice a day consisting of sugarcane bagasse (9.4%), sunflower meal (69.24%), ground corn (17.93%), urea (0.64%), mineral supplement (2.17%), and Optigen (0.60%).

The animals were sent for slaughter at a final weight of approximately 550 ± 75 kg and 27 months of age and were slaughtered on the same batch of slaughter in accordance with guidelines for the Humane Slaughter of Cattle. During slaughter, the carcasses were properly identified and weighed and *Longissimus thoracis* muscle samples were collected and stored in RNAlater® solution (Ambion, ThermoFisher Scientific, USA) at −80 °C for the gene expression assays. Next, the carcasses were cooled for 24 h and 2.54 cm thick *Longissimus thoracis* muscle samples were collected between the 12th and 13th rib of the left half-carcass of each animal for shear force (SF) analysis.

2.2. Warner-Bratzler shear force analysis and selection of the experimental groups

The meat samples were cooked in a pre-heated oven at 170 °C until they reached an internal temperature of 71 °C and cooled for 12 h at 1 °C. Half-inch cylinders were cut longitudinally from the center of the sample in the direction of the muscle fiber. The cylinders were cut with a Salter Warner-Bratzler Shear Force device with a capacity of 25 kg at a velocity of 20 cm/min. Eight measurements were obtained per sample (Wheeler, Koohmaraie, & Shackelford, 1995).

Based on the SF measurement of the 90 *Longissimus thoracis* muscle samples, three contrasting meat tenderness groups were selected: moderately tender meat (34.3 ± 5.8 N; $n = 6$), moderately tough meat (56.8 ± 7.8 N; $n = 7$), and very tough meat (80.4 ± 15 N; n = 7).

2.3. Real-time PCR analysis (RT-qPCR)

Total RNA was extracted from the three experimental groups by homogenization of 100 mg *Longissimus thoracis* muscle in 1 mL TRIzol® according to manufacturer instructions (Life Technologies Corporation, USA). The concentration, purity and integrity of the extracted total RNA were evaluated in a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, USA, 2007) and by agarose gel electrophoresis. Genomic DNA was eliminated using the DNA-se TURBO DNA-free™ Kit (Ambion, Thermo Fisher Scientific, USA) and the absence of genomic DNA was confirmed with the Qubit dsDNA Assay Kit (Invitrogen, USA). cDNA was synthesized using the High Capacity RNA-to-cDNA Kit according to manufacturer instructions (Life Technologies Corporation, USA). Eight probes and six primers were used in the RT-qPCR assays (Table 1). The stability of the reference genes was evaluated with GeNorm™ and NormFinder© softwares. The RT-qPCR assays were performed in duplicate using the QuantStudio 12 K Flex system (Applied Biosystems). For the probes, each sample of cDNA was amplified by TaqMan® Universal PCR Mater Mix (Thermo Fisher Scientific, USA, 2007) and for the primers the samples of cDNA were amplified by Fast SYBR® Green Master Mix (Thermo Fisher Scientific, USA, 2007) according to manufacturer instructions. The primers were designed though Primer Express 3.0.1. (Thermo Fisher Scientific, USA, 2007) and producted by Sinapse Biotecnologia Ltda.

Table 1
TaqMan® probes and primers used for the RT-qPCR assays.

Gene (GenBank ID)	TaqMan® probe	Manufacturer
CAPN1 (281664)	Bt03223357_m1	Thermo Fisher
CAPN2 (281662)	Bt03817738_m1	
HSPB1 (516099)	Bt03220563_m1	
HSP90AA1 (281832)	Bt03218068_g1	
DNAJA1 (528862)	Bt03219626_g1	
GAPDH (281181)	Bt03210915_g1	
TBP (516578)	Bt03241947_m1	
ACTB (280979)	Bt03279175_g1	
Gene (GenBank ID)	Primer sequence (5′ – 3′)	Manufacturer
CAST (281039)	F: ATGAGGAAACAGTCCCATCG	Sinapse
	R: GGGCTTGGGTTTTTCTTCAG	Biotecnologia
CAST 1	F: CACCCAGGAGCATGTCAGTA	
(NM_001030318.2)	R: ACTGCTCCCAAGGCTTGTT	
CAST 2 (NM_174003.2)	F: TGCAAGCTGGTGGTACAAGA	
	R: GAGAGCTGACTGCTCCCAAG	
EEF1A2 (515233)	F: GCAGCCATTGTGGAGATG	
	R: ACTTGCCCGCCTTCTGTG	
GAPDH (281181)	F: GAAATCGCCAATGCCAAC	
	R: GAGCCTTGTCTGCCTTCA	
RPL-19 (510615)	F: CCGTTCGACAGATAGCCGTAA	
	R: CGACCTTCACCATCTTGTCTCA	

F: forward; R: reverse.

2.4. Statistical analysis

The data were analyzed with the SAS statistical program (SAS Institute, Cary, NC, USA, 2011). The gene data obtained with the TaqMan® probe and primers were analyzed separately. For normalization of the data, the geometric mean of the cycle threshold (Ct) value obtained for three reference genes (*GAPDH*, *TBP* and *ACTB*) was used as suggested by Vandesompele et al. (2002). The relative expression of the target genes (*CAPN1*, *CAPN2*, *HSP90AA1*, *DNAJA1* and *HSPB1*) was calculated as proposed by Steibel, Poletto, Coussens, and Rosa (2009). The geometric mean of the Ct value of three other reference genes (*EEF1A2*, *GAPDH* and *RPL-19*) was obtained for normalization and relative expression analysis of the *CAST*, *CAST1* and *CAST2* genes. The following model was used for the quantification of relative gene expression:

$$Y_{gik} = G_{ig} + M_{gki} + D_{ik} + e_{igk}$$

where $Ygijk$ = Ct obtained with the software of the thermocycler for gene g (geometric mean of the reference genes and target gene), in well tr of the plate (technical replicate) for the sample of animal k of group i; Gig = effect of group i on the expression of gene g; Mgk = specific effect of gene g on meat tenderness of animal k; Dik = specific effect of the sample; $eigk$ = residual effect.