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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.

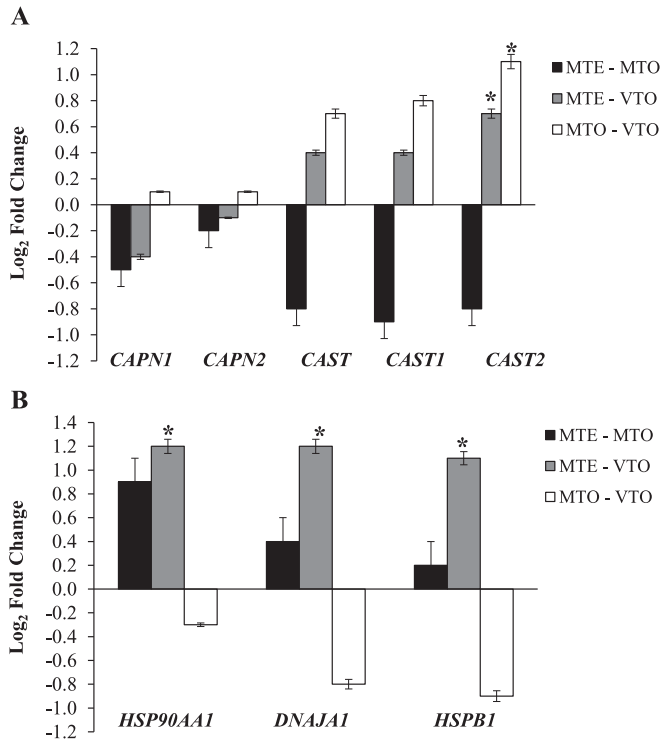

Fig. 1. Relative expression (Log_2 fold change) of genes in *Longissimus thoracis* muscle of Nellore animals belonging of groups moderately tender meat (MTE), moderately tough meat (MTO), very tough meat (VTO). A = calpain complex genes (*CAPN1*, *CAPN2*, *CAST*, *CAST1* and *CAST2*). B = heat shock protein genes (*HSP90AA1*, *DNAJA1* and *HSPB1*). *Significant difference ($P < 0.05$).

3. Result and discussion

Although an association between meat tenderness and calpain genes has been reported in cattle (Barendse, Harrison, Bunch, & Thomas, 2008), no significant difference in the expression of the *CAPN1* and *CAPN2* genes that encode μ- and m-calpain was observed between the different meat tenderness groups of Nellore animals (Fig. 1). Similar results have been reported by Smith, Thomas, Bidner, Paschal, and Franke (2009) who found no significant difference in the *CAPN1* genotype between Brahman animals with low SF (3.68 kg) and high SF (9.65 kg). Likewise, Casas et al. (2006) observed no effect of the *CAPN1* gene on meat tenderness in *Bos indicus* animals. However, in the same study, the authors found a positive effect of the *CAPN1* gene on meat tenderness in *Bos taurus* animals (Casas et al., 2006).

These results suggest that the expression levels of *CAPN1* and *CAPN2* genes do not exert a direct effect on meat tenderness in Nellore animals. According to genome-wide associations studies (GWAS), the Nellore breed (*Bos indicus*) possibly possesses different metabolic pathways related to meat quality, which have not been observed in *Bos taurus* breeds (Tizioto et al., 2013). Thus, the controversies between studies indicate inconsistencies regarding calpain mRNA activity and the phenotypic trait of meat tenderness in Nellore animals.

Regarding the calpastatin genes, the expression of the *CAST2* isoform was significantly up-regulated ($P < 0.05$) in the moderately tough and very tough meat groups when compared to the moderately tender meat group. On the other hand, expression of the *CAST* and *CAST1* genes did not differ significantly between the experimental groups (Fig. 1). This result corroborates the findings of Tang et al. (2010) who observed differences in SF values between pigs fed different diets, while no difference was found in the expression of the *CAST* gene. In contrast, in another study on pigs, Li et al. (2009) observed differences in the expression of *CAST* isoforms. In the study of Muroya et al. (2012), the expression of these isoforms also showed a distinct behavior in cattle and expressions levels were dependent on the type of muscle. Thus, the *CAST* gene isoforms apparently exhibit differential expression patterns in skeletal muscle, which possibly results in distinct inhibitory modulation of calpain and therefore may influence meat tenderness differently.

In the *postmortem* process, calpastatin acts as an endogenous inhibitor of the proteolytic activity of calpains (Cônsolo et al., 2016; Raynaud et al., 2005). The present results suggest that the expression of *CAST2* isoform is negatively involved in meat tenderness in Nellore cattle. However, the lack of association between *CAPN1* and *CAPN2* gene expression levels and meat tenderness shows the complexity of the mechanisms that occur during the tenderization phase. Thus, some aspects of the effect or association of the expression of calpain system gene with meat tenderness in Nellore animals suggest that, in addition to this complex, tenderness depends on other metabolic pathways that involve different genes.

The *HSP90AA1* and *DNAJA1* genes, which encode Hsp90-alpha and Hsp40, respectively, were down-regulated in the moderately tender meat group compared to the very tough meat group ($P < 0.05$) (Fig. 1). These results suggest that the expression of *HSP90AA1* and *DNAJA1* is negatively associated with meat tenderness in Nellore animals.

The results of this study corroborate the findings of Bernard et al. (2007) who identified the *DNAJA1* gene in *Longissimus thoracis* muscle of Charolais cattle, with lower expression in samples of high-quality meat (tenderness, flavor and juiciness). The *DNAJA1* gene encodes Hsp40, a co-chaperone of Hsp70. The Hsp40/Hsp70 complex possesses antiapoptotic activity and is involved in the preservation of cellular functions and in the repair of damaged protein structures (Arrigo, 2005; Picard et al., 2010; Guillemin, Jurie et al., 2011). This specific function of Hsp40 is probably responsible for the negative association of *DNAJA1* gene expression with meat tenderness in Nellore cattle observed in this study.

The *HSPB1* gene expression levels, which encodes Hsp27, was also down-regulated in the moderately tender meat group compared to the very tough meat group ($P < 0.05$), demonstrating a negative association of its expression with meat tenderness. The present results corroborate the recent findings of Baldassini et al. (2015) who observed a trend towards lower expression of Hsp27 protein in Nellore animals with tender meat. According to Picard et al. (2016), knock-out of the *HSPB1* gene results in a reduction of α-actin. This myofibrillar protein is considered one of the targets of *postmortem* proteolysis.

Kim et al. (2008) observed an increase in Hsp27 protein expression in native Korean cattle. In that study, higher levels of Hsp27 protein expression were correlated with high SF values, suggesting that the negative regulation of Hsp27 may increase the degradation of muscle proteins such as actin and myosin and consequently improve meat tenderness. Thus, although the function of HSPs during the *postmortem* period is not fully understood, these proteins appear to interact with the calpain complex and caspases and could therefore negatively influence the tenderness of beef (Lana & Zolla, 2016).

According to the results of this study, meat tenderness in Nellore cattle does not directly depend on the expression of the *CAPN1* and *CAPN2* genes, as widely reported in studies involving other cattle breeds. Meat tenderness in this breed seems to depend on the expression and modulation of other genes such as the calpastatin isoform (*CAST2*) and HSPs (*HSP90AA1*, *DNAJA1* and *HSPB1*). Evidence in the literature suggests that the proteolytic activity of μ-calpain is influenced by the inhibitory regulation of HSPs according to the pH of meat. In the study of Lomiwes, Farouk, Frost, Dobbie, and Young (2013), beef with an intermediate pH (5.8–6.19) exhibited lower proteolytic activity of μ-calpain and cathepsin B, which may have coincided with the increase in HSP expression. Therefore, a more in-depth study of gene expression and of the relationship and interaction of genes of the calpain complex, HSPs and myofibrillar proteins is needed to aid the understanding of

meat tenderness and to permit the identification of biomarkers and application of this information in Nellore breeding programs.

Finally, despite the confirmation of the association of *CAST2* gene polymorphisms with meat tenderness in Nellore cattle (Enriquez-Valencia et al., 2017), this is the first study to demonstrate an association of the expression of *CAST2* and HSP (*HSP90AA1*, *DNAJA1* and *HSPB1*) genes with meat tenderness in this breed.

4. Conclusion

Meat tenderness in Nellore cattle apparently does not depend directly on the expression of the *CAPN1* and *CAPN2* genes, but is rather associated with the expression and modulation of other genes such as *CAST2* and genes encoding some members of the HSP family (*HSP90AA1*, DNAJA1, and *HSPB1*).

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References

Arrigo, A. P. (2005). In search of the molecular mechanism by which small stress proteins counteract apoptosis during cellular differentiation. *Journal of Cellular Biochemistry, 94*, 241–246.

Avilés, C., Martínez, A. L., Domenech, V., & Peña, F. (2015). Effect of feeding system and breed on growth performance, and carcass and meat quality traits in two continental beef breeds. *Meat Science, 107*, 94–103.

Baldassini, W. A., Braga, C., Chardulo, L. A., Vasconcelos, J., Malheiros, J., Albuquerque, L., & de Padilha, P. M. (2015). Bioanalytical methods for the metalloproteomics study of bovine Longissimus thoracis muscle tissue with different grades of meat tenderness in the Nellore breed (*Bos indicus*). *Meat Science, 169*, 65–72.

Barendse, W., Harrison, B. E., Bunch, R. J., & Thomas, M. B. (2008). Zebu and composite breeds of cattle. *BMC Genetics, 9*, 1–8.

Bernard, C., Cassar-Malek, I., Le Cunff, M., Dubroeucq, H., Renand, G., & Hocquette, J.-F. (2007). New indicators of beef sensory quality revealed by expression of specific genes. *Journal of Agricultural and Food Chemistry, 55*, 5229–5237.

Casas, E., White, S. N., Wheeler, T. L., Shackelford, S. D., Koohmaraie, M., Riley, D. G., & Smith, T. P. L. (2006). Effects of calpastatin and μ-calpain markers in beef cattle on tenderness traits. *Journal of Animal Science, 84*, 520–525.

Cônsolo, N., Ferrari, V., Mesquita, L., Goulart, R., & Silva, L. (2016). Zilpaterol hydrochloride improves beef yield, changes palatability traits, and increases calpain-calpastatin gene expression in Nellore heifers. *Meat Science, 121*, 375–381.

Enriquez-Valencia, C. E., Pereira, L. G., Moraes, J., Augusto, J. S., Albuquerque, L., Oliveira, H., & Curi, R. (2017). Effect of the g. 98535683 A > G SNP in the CAST gene on meat traits of Nellore beef cattle (*Bos indicus*) and their crosses with *Bos taurus*. *Meat Science, 123*, 64–66.

Goll, D. E., Thompson, V. F., Li, H., Wei, W., & Cong, J. (2003). The calpain system. *Physiological Reviews, 83*, 731–801.

Guillemin, N., Bonnet, M., Jurie, C., & Picard, B. (2011). Functional analysis of beef tenderness. *Journal of Proteomics, 75*, 352–365.

Guillemin, N., Jurie, C., Cassar-Malek, I., Hocquette, J., Renand, G., & Picard, B. (2011). Variations in the abundance of 24 protein biomarkers of beef tenderness according to muscle and animal type. *Animal, 5*, 885–894.

Kim, N., Cho, S., Lee, S., Park, H., Lee, C., Cho, Y., & Park, E. (2008). Proteins in Longissimus muscle of Korean native cattle and their relationship to meat quality. *Meat Science, 80*, 1068–1073.

Kültz, D. (2003). Review evolution of the cellular stress proteome: from monophyletic origin to ubiquitous function. *The Journal of Experimental Biology, 206*, 3119–3124.

Lana, A., & Zolla, L. (2016). Proteolysis in meat tenderization from the point of view of each single protein: A proteomic perspective. *Journal of Proteomics, 147*, 85–97.

Li, Z., Cao, B., Zhao, B., Yang, X., Fan, M. Z., & Yang, J. (2009). Decreased expression of calpain and calpastatin mRNA during development is highly correlated with muscle protein accumulation in neonatal pigs. *Comparative Biochemistry and Physiology, Part A, 152*, 498–503.

Liu, J., Fu, R., Liu, R., Zhao, G., Zheng, M., Cui, H., & Wen, J. (2016). Protein profiles for muscle development and intramuscular fat accumulation at different post-hatching ages in chickens. *Plosone, 11*, 1–19.

Lomiwes, D., Farouk, M. M., Frost, D. A., Dobbie, P. M., & Young, O. A. (2013). Small heat shock proteins and toughness in intermediate pH u beef. *Meat Science, 95*, 472–479.

Marques, C., Guo, W., Pereira, P., Taylor, A., Patterson, C., Paul, C., & Shang, F. (2007). *NIH Public Access, 20*, 741–743.

Muroya, S., Neath, K., Nakajima, I., OE, M., Shibata, M., Ojima, K., & Chikuni, K. (2012). Differences in mRNA expression of calpains, calpastatin isoforms and calpain/calpastatin ratios among bovine skeletal muscles. *Science Journal, 83*, 252–259.

Ouali, A., Gagaoua, M., Boudida, Y., Becila, S., Boudjellal, A., Herrera-mendez, C. H., & Sentandreu, M. A. (2013). Biomarkers of meat tenderness: Present knowledge and perspectives in regards to our current understanding of the mechanisms involved. *Meat Science, 95*, 854–870.

Picard, B., Berri, C., Lefaucheur, L., Molette, C., Sayd, T., & Terlouw, C. (2010). Skeletal muscle proteomics in livestock production. *Briefings in Functional Genomics & Proteomics, 9*, 259–278.

Picard, B., Kammoun, M., Gagaoua, M., Barboiron, C., Meunier, B., Chambon, C., & Cassar-malek, I. (2016). Calcium homeostasis and muscle energy metabolism are modified in HspB1-null mice. *Proteomes, 4*, 1–21.

Pinto, L. F. B., Ferraz, J. B. S., Meirelles, F. V., Eler, J. P., Rezende, F. M., Carvalho, M. E., ... Silva, R. C. G. (2010). Association of SNPs on CAPN1 and CAST genes with tenderness in Nellore cattle. *Genetics and Molecular Research, 9*, 1431–1442.

Raynaud, P., Gillard, M., Parr, T., Bardsley, R., Amarger, V., & Levéziel, H. (2005). Correlation between bovine calpastatin mRNA transcripts and protein isoforms. *Archives of Biochemistry and Biophysics, 440*, 46–53.

Smith, T., Thomas, M. G., Bidner, T. D., Paschal, J. C., & Franke, D. E. (2009). Single nucleotide polymorphisms in Brahman steers and their association with carcass and tenderness traits. *Genetics and Molecular Research, 8*, 39–46.

Steibel, J. P., Poletto, R., Coussens, P. M., & Rosa, G. J. M. (2009). A powerful and flexible linear mixed model framework for the analysis of relative quantification RT-PCR data. *Genomics, 94*, 146–152.

Tang, R., Yu, B., Zhang, K., Guo, X., Tian, G., Huang, Z., & Chen, D. (2010). Effects of nutritional level on pork quality and gene expression of l -calpain and calpastatin in muscle of finishing pigs. *Meat Science, 85*, 768–771.

Tizioto, P. C., Decker, J. E., Taylor, J. F., Schnabel, R. D., Mudadu, M. A., Silva, F. L., & Regitano, L. C. (2013). Genome scan for meat quality traits in Nellore beef cattle. *Physiological Genomics, 45*, 1012–1020.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., & Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology, 3*, 1–12.

Wheeler, T. L., Koohmaraie, M., & Shackelford, S. D. (1995). *Standardized Warner-Bratzler shear force procedures for meat tenderness measurement.* Clay Center, NE: Roman L. Hruska U.S. MARC. Agricultural Research Service, USDA.

Yang, Y. B., Pandurangan, M., & Hwang, I. (2012). Changes in proteolytic enzymes mRNAs and proteins relevant for meat quality during myogenesis and hypoxia of primary bovine satellite cells. *In Vitro Cellular and Developmental Biology - Animal, 48*, 359–368.