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Expression of genes related to quality of *Longissimus dorsi* muscle meat in Nellore (*Bos indicus*) and Canchim (5/8 *Bos taurus* × 3/8 *Bos indicus*) cattle

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ABSTRACT

This study was performed to compare *CAPN1*, *CAPN2*, *CAST*, *TG*, *DGAT1* and *LEP* gene expressions and correlate them with meat quality traits in two genetic groups (Nellore and Canchim) in order to assess their expression profile and use their expression profile as genetic markers. We analyzed 30 young bulls (1 year old), 15 of each genetic group. Samples of the *Longissimus dorsi* muscle were collected for analysis of: total lipids (TL) and meat tenderness measured as Warner-Bratzler shear force (SF) and myofibrillar fragmentation (MFI) at day of slaughter and 7 days of aging. Gene expression profiles were obtained via RT-qPCR. TL and MFI showed differences between breeds, higher MFI in Canchim and higher TL in Nellore. Calpains showed no differential expression between groups, as did *DGAT1*, *TG*, and *LEP*. *CAST* was expressed more in the Nellore cattle. The only significant within-breed correlation (0.79) between gene expression and meat traits was found for *DGAT1* and MFI in Canchim breed. Although the number of animals used in this study was small, the results indicate that the increased expression of *CAST* in Nellore may reflect tougher meat, but the lack of correlations with the meat traits indicates it is not a promising genetic marker.

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1. Introduction

Variability among breeds regarding meat tenderness has been attributed to different levels of proteolytic enzymes found in animal muscles (Whipple et al., 1990), especially enzymes of the calpain system. Wheeler, Savell, Cross, Lunt, and Smith (1990) found a lower μ-calpain protein expression and higher calpastatin protein expression in Brahman (*Bos indicus*) when compared to Hereford (*Bos taurus*), which resulted in lower meat tenderness in *B. indicus* animals, because calpastatin is a calpain inhibitor.

The evolution of beef cattle breeding in Brazil in recent years has been marked by increased herd size, greater productivity and improved meat quality. The intensification of production systems is probably responsible for these developments, and wider use of feedlot finishing is an important element in this scenario. The adaption of this type of production reduces the time required for fattening and enhances meat commercial quality. However, the absolute predominance of Zebu in the composition of herds in Brazil contrasts with the need for animals that respond well to confinement, with rapid growth and backfat deposition (Silveira, Martins, & Arrigoni, 2004). The alternative is to use crosses of Zebu (*B. indicus*) cows, and to a lesser extent cows crossed with bulls of several European breeds and synthetic breeds. As a result, a wealth of genetic groups has been generated, leading to large meat quality variability, both in relation to tenderness and fat thickness and marbling. It is therefore important to understand the genetic factors that affect meat quality in Zebu cattle and its crosses, especially those responsible for the differences between this breed and Taurine cattle, to allow greater control over the quality of the beef produced in Brazilian feedlots (Luchiari Filho, 1998 and Vittori, Queiroz, Resende, Gesualdi Júnior, & Gesualdi, 2006).

Many authors have observed significant differences in tenderness meat between European cattle breeds and Zebu cattle breeds (O'connor et al., 1997; Shackelford, Koohmaraie, Miller, Crouse, & Reagan, 1991; Whipple et al., 1990). In general, as the percentage of *B. indicus* increases, the variability tends to decrease and tenderness tends to increase. The calpain system has been considered the main mechanism involved in meat tenderness (Koohmaraie, 1992, 1996; Wheeler, Cundiff, Koch, & Crouse, 1996). This system consists of two calcium dependent proteases, μ-calpain or calpain 1 (CAPN1) and m-calpain or calpain 2 (CAPN2), and a polypeptide whose function is to inhibit both calpain and calpastatin (CAST). Shackelford et al. (1991) indicated that a higher level of calpastatin was responsible

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for lower meat tenderness from animals with some proportion of Zebu in their genetic composition, while Wheeler, Cundiff, and Koch (1994) also found lower levels of calpain 1 in *B. indicus* cattle.

Several factors influence proteolytic activity. Very rapid cooling of the carcass is one of these, as the shortening of muscle fibers (cold shortening) leads to concealment of enzyme recognition sites, reducing the access to substrates. Thus, even if there is availability of the proteolytic enzyme, its action will be compromised and ideal proteolysis of muscle fiber components will not occur (Koohmaraie, 1996). Another important factor in muscle proteolysis is cold shortening, this can be prevented with correct quantity of backfat cover, that protects the carcass against rapid cooling under normal conditions, avoiding the occurrence of shrinkage of fibers (Lesser, 1993). Zebus are slower-growing animals and tend to deposit fat sooner when fed diets with the high energy content of feedlots (Rubiano et al., 2009), being less influenced by this process.

Studies indicate the diacylglycerol acyltransferase 1 (*DGAT1*) gene, which encodes the enzyme diacylglycerol acyltransferase 1, can be associated with milk fat (Grisart et al., 2004). However, conflicting results regarding its role in fat deposition in beef cattle have been reported (Casas et al., 2005; Pannier, Mullen, Hamill, Stapleton, & Sweeney, 2010; Thaller et al., 2003).

The gene that encodes thyroglobulin protein (*TG*), a glycoprotein synthesized in thyroid follicular cells that is a precursor molecule for the thyroid hormones, thyroxine and triiodothyronine. Some studies have shown associations of this gene with fat thickness (Casas et al., 2005), and with marbling score (Gan et al., 2008).

The gene encoding leptin (*LEP*), a cytokine secreted predominantly from adipose tissue, plays an important role in the regulation of body energy balance. Leptin is involved in food intake, energy balance, reproductive efficiency, fat deposition (Houseknecht, Baile, Matteri, & SPurlock, 1998; Lagonigro, Wiener, Pilla, Woolliams, & Williams, 2003; Pannier et al., 2009; Schenkel et al., 2005), and possibly formation of marbling fat (Taniguchi, Itoh, Yamada, & Sasaki, 2002).

The aim of this work was to study gene expression of proteins related to meat tenderness like *CAPN1*, *CAPN2* and *CAST* and intramuscular fat deposition *DGAT1*, *TG*, and *LEP* in Nellore (Zebu) and Canchim (3/8 Zebu × 5/8 Charolais) cattle, and to estimate the within-breed correlations of the gene expression profiles and the traits studied.

2. Material and methods

In this study we used 30 beef bulls, reared under creep feeding and weaned at seven months, with an average weight of 209.4 kg (± 23.3 kg). The animals were evenly divided between the two genetic groups, with 15 Nellore (Zebu) and 15 Canchim (3/8 Zebu × 5/8 Charolais).

2.1. Management, feeding and care of animals

After weaning, the animals were kept in experimental feedlot facilities at the School of Veterinary Medicine/UNESP-Botucatu. All animals were given the same diet (ad libitum), housing and management. They were weighed and subjected to a period of 21 days for diet adaptation.

The growth and fat deposition were monitored by ultrasound every weighing period (every 28 days). The diets had high quality nutrition, formulated according to the Cornell Net Carbohydrate and Protein System 5.0.26.

When the animals reached the pre-established slaughter weight of approximately 370 kg and finishing fat cover of at least 4 mm, they were submitted to the creation of a very early model, and were slaughtered in a commercial abattoir before mature.

2.2. Collection and processing of samples

The samples for RNA extraction, used in the gene expression analysis, were collected immediately after slaughter, taken from the *Longissimus dorsi* muscle in the region of the 11th and 13th rib of each animal, and immediately frozen in liquid nitrogen and subsequently kept at −80 °C in freezers. The samples used in the analysis of shear force (SF), myofibrillar fragmentation index (MFI) and total lipids (TL) were collected 24 h after carcass cooling in the same region of the *Longissimus dorsi* muscle and maintained at 4 °C. Half of the samples collected 24 h after carcass cooling was used to measure the shear force (SF0), myofibrillar fragmentation index (MFI0) and total lipids extraction without the influence of aging. The remainders of the samples were vacuum packed and kept at 4 °C for seven days (aging period) before analysis of shear force (SF7) and myofibrillar fragmentation index (MFI7).

2.3. Shear force analysis

Longissimus dorsi samples, approximately 2.54 cm thick, not aged and aged for seven days, were subjected to shear force analysis, following the method described by Wheeler, Koohmaraie, and Shackelford (1995) as adapted by Hadlich (2007).

2.4. Myofibrillar fragmentation index (MFI) analysis

The determination of the myofibrillar fragmentation index (MFI) was based on the method proposed by Culler, Parrish, and Smith (1978).

2.5. Total lipids extraction

The determination of total lipids in subcutaneous fat-free samples was performed according to the protocol described by Bligh and Dyer (1959).

2.6. RNA extraction and reverse transcription

Total RNA extraction of skeletal muscle was performed using the TRIzol (Life Technologies, USA) protocol. Total RNA was eluted in distilled and autoclaved water, treated with diethylpyrocarbonate (Sigma — DEPC, 0.01%) and stored at −80 °C. To check the quality and quantity of total RNA, a NanoVue spectrophotometer was used (GE Healthcare Life Sciences, USA).

After quantification of the extracted RNA, the integrity of the material was analyzed. This process was accomplished through the presence of bands corresponding to 18S and 28S ribosomal RNAs after capillary electrophoresis (2100 Bioanalyzer, Agilent Technologies, USA). The RNA integrity was verified by calculating the RNA integrity number, with the mean value of all samples (Nellore and Canchim) being 8.0 ± 0.3 (range 1–10), indicating high-quality RNA and minimum degradation.

Total RNA was treated with the enzyme DNase to remove possible contaminating genomic DNA, as indicated by the protocol DNase I — Amplification Grade (Life Technologies, USA), and was then used for the reverse transcription reaction.

The reverse transcription reaction was performed using the High Capacity Archive cDNA kit (Life Technologies, USA) following the manufacturer's protocol. The specimens were stored at −20 °C.

2.6.1. Selection of reference genes

The stability of the reference genes was tested using the Assist v2.0 software (Life Technologies, USA), an algorithm to determine the most stable reference genes from a set of tested candidate reference genes in a sample panel given. Five reference genes were tested in *RT-qPCR* analysis 18S ribosomal RNA, β-actin,

glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), hypoxanthine-guanine fospforribosil transferase (*HPRT*) and TATA-box binding protein (*TBP*). Only three proved stable genes were used (*GAPDH*, *HPRT* and *TBP*). All RT-qPCR laboratory procedures were performed according to the MIQE guidelines (Bustin et al., 2009).

The RT reactions were performed in the qPCR-ABI 7300 platform according to the protocol of Life Technologies.

We used primers and hydrolysis probes (TaqMan® assays) for the *CAPN1*, *CAPN2*, *CAST*, *TG*, *LEP* and *TBP* genes (Table 1) expressed in *B. taurus* (Life Technologies, USA). For the *DGAT1*, *GAPDH* and *HPRT* genes, the primers and hydrolysis probes were designed based on the sequences available in the GenBank database of the National Center for Biotechnology Information (NCBI), using the Primer Express® 3.0 software (Life Technologies, USA) (Tables 1 and 2).

Taqman gene expression reactions were performed following the manufacturer's recommendations.

2.6.2. Determination of the parameters of the qPCR

The reactions were performed using the Real Time PCR system 7300 (Life Technologies, USA) under the following conditions: 50 °C for 2 min and 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The reactions were performed in triplicate for each sample, generating an average expression of each animal, to minimize technical errors. After the run, the melting curve was analyzed to confirm the specific amplification product of each qPCR gene.

The results were presented as fold changes relative to a Canchim animal, using the method described by Livak and Schmittgen (2001). All mathematical procedures were performed by using the Data Assist v2.0 program (Life Technologies, USA).

2.7. Statistical analysis

All statistical analyses were performed using the Statistical Analysis System V.9.1 program (SAS). The data for total lipids were subjected to analysis of variance using the GLM procedure. The fixed effect of genetic group and the residual were the only effects in the model. For comparison of gene expression data the same model was applied to qPCR relative quantification. For SF and MFI data analyses, the model included, along with the genetic group, the effects of aging time (zero or seven days), interaction between genetic group and aging time, animal within genetic group (used as error term for genetic group), and residual. Snedecor's F-test was used to access the significance of the effects. Pearson's correlation was used to analyze the association between gene expression data and meat traits within genetic group. For all statistical tests, the Bonferroni method was applied to control the level of significance in the experiment.

Table 1
Access number of custom and sequences TaqMan® assays (Life Technologies, USA) used for the analysis of gene expression by qPCR.

Gene	Assay sequence
CAPN1	BSF03223357_m1
CAPN2	BSF03817738_m1
CAST	BSF03252008_m1
TG	BSF03211731_m1
LEP	BSF03211909_m1
TBP	BSF03241947_m1
	Access number
DGAT1	EE_00668544.1
GAPDH	NM_001034034.1
HPRT	NM_001034035.1

CAPN1: μ-calpain, *CAPN2*: m-calpain, *CAST*: Calpastatin, *TG*: Thyroglobulin, *LEP*: Leptin, *TBP*: TATA binding box protein, *DGAT1*: Diacylglycerol acyltransferase 1, *GAPDH*: Glyceraldehyde 3-phosphate dehydrogenase, *HPRT*: Hypoxanthine-guanine fospforribosil transferase.

Table 2
Primers and hydrolysis probe sequence synthesized for RT-qPCR.

Gene	Sequence	Fluorescence
DGAT1	*Primer F*: CATGCTCTGGAAACCCTACAGAT	
	Primer R: TGCACAGCACTTTATTGACACATT	
	Probe: TCGCCCAAGGGTC	FAM
GAPDH	*Primer F*: TCCACCCACGGCAAGTTC	
	Primer R: TGACGAGCTTCCCGTTCTCT	
	Probe: CGGCACAGTCAAGG	FAM
HPRT	*Primer F*: TGGCGTCCCAGTGAAATCA	
	Primer R: CAGCTGGCCACAGAACAAGA	
	Probe: CAGTGACATGATCCAATG	FAM

DGAT1: Diacylglycerol acyltransferase 1, *GAPDH*: Glyceraldehyde 3-phosphate dehydrogenase, *HPRT*: Hypoxanthine-guanine fospforribosil transferase.

3. Results and discussion

3.1. Meat quality

3.1.1. Tenderness

Two measures indicative of meat tenderness were evaluated in this study, shear force (SF) and myofibrillar fragmentation index (MFI). The shear force values were lower for the Canchim (3.8 kg) animals than for the Nellore (4.2 kg) animals, and were also lower for both breeds after seven days of aging (3.7 kg) in relation to day zero (4.3 kg). There were significant differences between groups ($P<0.05$) and between days ($P<0.05$), but the differences between breeds decreased between day zero and day seven. However, the interaction was not significant, indicating that the aging process occurs similarly in both genetic groups.

The results of the MFI showed higher values in Canchim cattle (65.5) than in Nellore cattle (54.7), indicating more tender meat of this breed ($P<0.05$) (MFI is a parameter that has no unit). The values after seven days were greater than those at day zero ($P<0.01$). These results agree with Olson, Parrish, and STromer (1976), who established that the MFI increases with increasing days postmortem. Just in the SF analysis, although there was an apparent decrease in the differences between the two genetic groups between day zero and day seven, the interaction was not significant. In terms of tenderness values, the two measures (SF and MFI) indicate a similar trend, tender meat from Canchim cattle and a similar maturation process to tenderize meat in the two breeds. Before the maturation period, the meat of Nellore cattle was less tender than that of Canchim cattle, a result that did not change after seven days.

The results here are in agreement with those reported in many studies, where *B. taurus* and their crosses produced more tender meat than pure *B. indicus* animals, even after aging (Crouse, Cundiff, Koch, Koohmaraie, & Seideman, 1989; Wheeler et al., 1996). However, they do not agree with the findings of Hadlich (2007). Studying meat tenderness of purebred *B. indicus* and *B. taurus* cattle, they found no difference in meat tenderness between groups after aging. These show that the aging process is efficient in reducing the difference between tenderness of *B. indicus* and *B. taurus* meat.

Based on studies of meat quality and aging in Nellore and Taurine breeds, already cited in this paper, we expected the pure Nellore cattle to present lower MFI and higher SF than the cross-bred Canchim animals, both in the meat with and without aging, since these have only 3/8 Zebu genes in their composition. Nevertheless, the MFI values (MFI0 and MFI7) were greater than 60 and the SF was below 4.5 kg, indicating tender meat in Nellore (Culler et al., 1978; Mckeith, 1985 and Shackelford et al., 1991), even before the aging process.

Although significant, the difference between the MFI and SF parameters was small, which can be explained by the production system. According to Hadlich (2007), although animals differ in their growth,

they can have similar meat quality with appropriate handling and similar feeding conditions from birth.

3.1.2. Indices of total lipids

With respect to total lipids, there were differences between the breeds (P<0.05). The Nellore animals had more deposition of marbling fat (1.3%) than the Canchim animals (0.9%). The higher rate of lipids in the *Longissimus dorsi* muscle of the Nellore animals indicates greater deposition of marbling fat, as noted in the literature (Fortes et al., 2009). Thus, Nellore cattle have better marbling fat deposition characteristics than the Charolais (Taurus Continental) and Canchim breeds, which were selected to have higher muscle deposition (Zadra, 2005).

3.1.3. Gene expression

3.1.3.1. Real-time polymerase chain reaction after reverse transcription (RT-qPCR). The defaults for carrying out reactions via qPCR allowed checking the linearity and efficiency of the reaction from the slope of the standard curve generated by the 7300 SDS System software (Life Technologies, USA) for each of the genes analyzed. Normalization by the correction factor (generated by the expression of the three reference genes) is the most accepted method to prevent these disparities (Vandesompele et al., 2002).

The normalization of target genes by gene reference data was done for each group. Gene expression of calpains showed no differences (P>0.05) for *CAPN1* or *CAPN2* between the Nellore and Canchim genetic groups (P>0.05) (Fig. 1). The results corroborate with Ferraz's (2009) work, who compared the expression of *CAPN2* in Nellore (*B. indicus*) and Angus (*B. taurus*), finding no difference.

The results reveal that the slightly lower tenderness of meat from Zebu animals is probably not a result of lower expression of genes encoding proteases (*CAPN1* and *CAPN2*), but rather is due to the increased expression of *CAST*, an inhibitor of *CAPN*s, whose expression was higher in the Nellore animals (P<0.05). This assumption is reinforced by the results obtained by Whipple et al. (1990), who in analyzing the activity of enzymes encoded by these genes for Taurine and Zebu cattle found higher enzymatic activity of calpastatin in Zebu animals, but no differences in activity of the enzyme calpain in the two genetic groups.

According to Geesink and Koohmaraie (1999) and Geesink, Kuchay, Chishti, and Koohmaraie (2006), the increased level of calpastatin activity results in reduction of calpain-mediated proteolysis and consequently decreases meat tenderness. The increase in enzyme activity associated with this process works by increasing the amount of enzyme that is synthesized. Therefore, the higher the expression of the gene encoding the enzyme, the greater its activity will be. Other factors are also involved in intracellular control of enzyme activity, but were not addressed in this work.

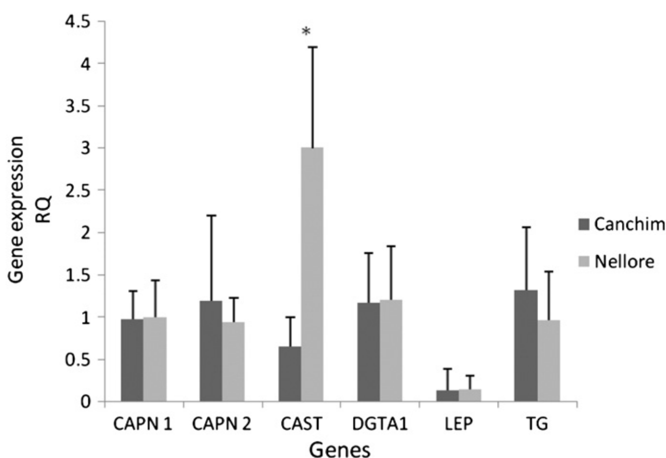

Fig. 1. Gene expression in different genetic groups: Nellore and Canchim.

Several other authors have related increased enzymatic activity of calpastatin to higher SF values in the muscle of *B. indicus* animals compared with *B. taurus* (Ibrahim et al., 2008; Morgan, Wheeler, Koohmaraie, Savell, & Crouse, 1993; O'Connor et al., 1997; Pringle, Williams, Lamb, Johnson, & West, 1997; Shackelford et al., 1991; Wheeler et al., 1990; Whipple et al., 1990). However, the quantification of messenger RNAs (mRNA) involved in the process has not been widely studied, so further investigation in this respect is fundamental to provide information on the process of the calpain–calpastatin proteolytic system.

The relationships between MFI and lower levels of *CAST* expression reported in the literature are consistent with the results observed in this work, where the MFI parameter was lower in the Nellore animals and the *CAST* gene expression was higher. However, gene expression alone does not explain the small difference in MFI between the breeds, suggesting that this influence can be lower in young animals because they have high protein turnover and thus do not require large amounts of proteolytic enzymes to produce tender meat.

Given the results of gene expression, shear force and MFI, we can affirm that the meat of Nellore cattle is less tender than that of Canchim animals, although it is still within the acceptable standards of tenderness. These animals, because they are young or less mature, have benefited from a favorable cell environment where there is high protein turnover, high concentrations of muscle glycogen and consequent reduced pH, which makes the meat tenderer.

Analysis of the expression of genes related to fat deposition showed no differential expression between breeds (P>0.05). This equality of gene expression can be the result of similar development of the breeds in the feedlot period, promoting the deposition of body tissues in the same period. Thus the Canchim animals, which naturally have little marbling fat deposition because of their genetic makeup (5/8 Charolais–*B. taurus* continental) (Nardon, Razook, & Sampaio, 2001), showed the expected expression of genes related to fat deposition. In contrast, the Nellore animals likely have higher expression, since their meat showed higher amounts of total lipids (marbling fat), as found in this study. These results indicate that other factors mediate the process of gene transcription for effective translation into protein, and possibly there are other genes involved in the formation of fat. The results could be more helpful if the expression of genes for fat deposition had been measured in adipocytes and muscle tissue, but these analyses were not performed in this study. However, one should look at the results carefully, since the analysis was performed on small numbers of animals.

3.2. Correlations between meat quality and gene expression

None of the correlations between the traits measured in the animals and the gene expression in the Nellore cattle (Table 3) were significant after applying correction by the Bonferroni method, which ensures maintenance of the error rate of statistical tests. The lack of correlation between measurements of tenderness and gene expression of *CAPNs* and *CAST* was not expected, since a greater amount of *CAST* has been associated with lower tenderness of meat after the aging period (Geesink et al., 2006). Moreover, calpain acts to increase tenderness, so one could expect animals with higher *CAPNs* expression to have more tender meat, although the action of calpain is only significant 150 h after the onset of rigor mortis. Possible explanations for the results found in this study are the fact that the animals were young and therefore had higher expression of enzymes involved in the process of protein turnover, including calpain and calpastatin. The correlation between the expression of *CAPN*s and *CAST* in this breed is greater than 0.63 (data not shown), which seems to indicate that the more calpastatin available, the more calpain also is available in the muscle. Thus, some interference between the "antagonistic" expressions of these genes may have affected the results. Moreover, other factors

Table 3
Correlation between parameters of meat quality and gene expression in Nellore animals.

	CAPN1	*CAPN2*	*CAST*	*DGAT1*	*LEP*	*TG*
SF0	−0.38	−0.35	0.32	0.02	−0.09	0.17
SF7	−0.29	−0.18	−0.54	0.29	0.07	−0.48
MFI0	0.42	0.13	0.32	0.39	−0.08	0.25
MFI7	0.45	0.45	0.26	0.13	0.52	0.17
TL	0.042	−0.19	0.003	0.22	0.04	0.21

CAPN1: μ-calpain, *CAPN2*: m-calpain, *CAST*: Calpastatin, *DGAT1*: Diacylglycerol acyltransferase 1, *LEP*: Leptin, *TG*: Thyroglobulin.

Table 4
Correlation between parameters of meat quality and gene expression in Canchim animals.

	CAPN1	*CAPN2*	*CAST*	*DGAT1*	*LEP*	*TG*
SF0	0.08	0.07	−0.26	−0.35	0.27	0.14
SF7	−0.19	−0.17	−0.08	−0.19	0.24	−0.04
MFI0	0.06	0.32	0.34	0.79[a]	−0.24	−0.48
MFI7	−0.19	0.19	0.03	0.31	−0.07	−0.04
TL	0.04	0.01	−0.47	−0.25	0.11	0.27

CAPN1: μ-calpain, *CAPN2*: m-calpain, *CAST*: Calpastatin, *DGAT1*: Diacylglycerol acyltransferase 1, *LEP*: Leptin, *TG*: Thyroglobulin. Higher values: correlation coefficient (r); values below: significance (P).
[a] Significant correlation ($P<0.05$).

inherent to young animals may have acted to decrease the variability in meat tenderness. The present results do not agree with those of Ferraz (2009), who studied the gene expression of *CAST* in young Nellore and Angus cattle and found a negative correlation with shear force.

Schenkel et al. (2005) studied polymorphisms in the gene *LEP* in Taurine and Zebu cattle and found no association with shear force or MFI. Although their results are not directly comparable with those of the present study, they point in the same direction as those found here.

There was a positive correlation of MFI0 ($r=0.79$ $P<0.05$) in the Canchim animals with the expression of the *DGAT1* gene (Table 4). Curi et al. (2010) reported the influence of *DGAT1* gene polymorphism on the fat layer thickness in cattle, which could lead to greater protection of the meat against the effects of rapid cooling and thus mean greater meat tenderness. However, that work could not establish a direct relationship between tenderness and polymorphism. In the present study, we did not measure the thickness of the fat layer, but rather only the TL muscle tissue, which was not significantly correlated with the expression of *DGAT1*.

4. Conclusions

Although the number of animals used in this study was small, and so the results must be taken with care, they indicate that gene expression cannot be used as a genetic marker for screening individuals with tender meat, since no evidence was found indicating that variations in gene expression were accompanied by variations in meat tenderness within breeds, even though there was, as expected, higher expression of the gene encoding the enzyme for calpastatin in Nellore (*B. indicus*) animals than in Canchim (5/8 *B. taurus* × 3/8 *B. indicus*) ones. Compared to Canchim animals, the Nellore animals had higher percentages of marbling fat. However, perhaps due to the small sample size, we could not find differences between breeds with respect to the expression of the genes under study or establish a cause and effect relationship between the expression of these genes and the proportion of lipids in the muscle tissue.

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