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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.

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,