Proceedings, 10th World Congress of Genetics Applied to Livestock Production

# MyoD1 expression levels affect meat tenderness in Nellore beef cattle

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**ABSTRACT:** The MyoD1 gene play a key role in skeletal muscle differentiation through the regulation of muscle-specific genes expression. In this work, we have shown that MyoD1 gene expression can influence the Warner – Bratzler shear force (WBSF) measured on the Longissiums dorsi muscle after 7 and 14 days of meat aging in half-sib families of Nelore breed.. Lower MyoD1 expression levels were related to lower WBSF measures. The effect of MyoD1 gene expression on meat tenderness may emerge from its function as transcription factor of genes responsible for muscle development, composition and phenotype. Further analysis identifying the mechanisms which control the MyoD1 expression would be useful to elucidate the effect observed in this study.

**Keywords:** *Bos indicus*; qPCR; Shear force; Gene expression

## Introduction

Meat tenderness is one of the major concerns of the beef market (Veiseth et al., 2004). A study have shown that this trait is under control of many of quantitative trait loci (QTLs) and genes of small effects (Tizioto et al., 2013a).

Myogenic differentiation (MyoD) gene family encode for skeletal muscle-specific transcription factors and play key roles in growth and muscle development and composition (Bhuiyan et al., 2009). The Myogenic Differentiation 1 (MyoD1, Gene Bank ID: 281938), is mapped to BTA15, near a QTL already described for meat tenderness (Rexroad et al., 2001).

Physiological processes, as muscle development, depend on the expression of many genes acting together as a network. Functional genetics, which includes gene expression analyses, may provide opportunities for understanding the molecular processes related to meat quality. Considering both function and position of MyoD1 gene, it was elected in this study as a candidate for being involved in the control of meat tenderness through changes in gene expression.

A gene expression profiling approach was used to evaluate whether MyoD1 mRNA levels affect meat tenderness in half-sib families of Nelore beef cattle.

## Materials and Methods

**Animals and phenotypic data.** Nelore steers (36), offsprings of 18 sires, chosen to represent the main breeding lineages in Brazil, were used in this study. The half-sib families were produced by artificial insemination in commercial and purebred Nelore dams. Animals were raised and allocated to two feedlots, as previously described (Tizioto et al., 2013). After slaughter, three 2.5 cm thick steaks, corresponding to a cross section of the Longissimus dorsi muscle, were collected between the 11[a] and 13[a] ribs to perform the Warner-Bratzler shear force (WBSF) measurement as described by Tizioto et al., (2013).

RNA extraction and quantitative PCR experiment. Muscle samples collected at slaughter were immediately frozen in liquid nitrogen and kept at -80 ºC until RNA extraction. Total RNA was extracted from 50-100 mg of muscle tissue, quantified and quality checked and finally reverse transcribed to cDNA as described by Tizioto et al. (2013).

A mixed model methodoly approach was used to analyze whether the MyoD1 expression profile of 136 animals was related to their WBSF measurements, as described by (Tizioto et al., 2013b).

Different methods have been proposed for the selection of appropriate reference genes, including methods which take into account that the resulting variation among samples (Reverter et al.,2004; Reverter et al., 2005) might be due to environmental fixed effects (treatment, animal age, gender, breed, management, and genetic background), random effects (animal sample), their interactions and heterogeneous variances of reference genes. A general linear mixed model approach that considers the fixed and random effects originated from the experimental design was used.

Best linear unbiased predictor (BLUP) values were obtained for random sample effect, and these were used to obtain and adjust the quantification cycle (Ct) value (CtA). After the adjustment of the Ct values, a general linear mixed model that included effects of slaughterer, parentage, age and pH was applied to each meat aging time to verify the association of the MyoD1 gene expression level with meat tenderness.

## Results and discussion

The MyoD1 gene has been mapped to a QTL interval on BTA15 for meat tenderness, also affecting percentage of kidney, pelvic and heart fat and is considered as a potential candidate gene for meat quality and carcass traits (Rexroad et al., 2001).

In an attempt to understand the MyoD1 gene role in bovine meat tenderness, the influence of the expression level of this gene on the WBSF phenotypes was evaluated and it was observed that lower MyoD1 expression levels are related to more tender meat after 7 and 14 days of aging in this population of Nelore breed (P≤0.01) (Table 1).

**Table 1. Effect of the muscular expression level measured as threshold cycle (Ct) of *MYOD1* gene on the Warner-Bratzler shear force measured at different aging meat times in 136 Nelore steers.**

| WBSF | Estimated effect (Kg)±SE | P value |
|---|---|---|
| 7 | - 0.1766 ± 0.057 | 0.0029* |
| 14 | - 0.1274 ± 0.045 | 0.0069* |

* P≤0.01; WBSF= Warner-Bratzler shear force (measured at 7 days of aging= 7; measured at 14 days of aging= 14)

When the CtA qPCR values increased twice, the WBSF decreased 0.177 (±0.057) Kg and 0.127 (±0.046) Kg, which represents 2.18% and 2.39% of the phenotypic variance for WBSF7 and WBSF14, respectively. Therefore lower MyoD1 expression levels (higher Ct values) were related to more tender meat after seven days of aging in cold chamber.

The biological features of a specific phenotype are controlled by expression of different genes. The muscle tissue differentiation, aging and development are controlled by transcription factors called Myogenic Regulatory Factors (MRF), including MyoD (Calvo et al., 1999).

Much attention has been paid about the molecular mechanisms of activation and maintenance of skeletal muscle-specific gene transcriptions, including MyoD family. These transcription factors can turn a wide number of cell types into skeletal muscle and also control the coordinate changes in gene expression associated with myogenesis (Olson, 1993).

From the results obtained in this work it is possible to speculate that the MyoD1 gene can be associated with the biochemical process of rigor-mortis. However the effect of the MyoD1 gene in postmortem muscle aging is unclear.

The role of MRFs in maintaining muscle phenotype has been investigated. Previous studies in adult rats have shown that MyoD gene products are preferentially accumulated in the fast muscle fibers (Hughes et al., 1993; Kraus and Pette, 1997). Other studies suggest the contribution of MyoD to fast fiber type specification (Hughes et al., 1997 and Charge et al., 2008). Fiber type composition and sarcomere length of bovine muscles may have a role in determining meat quality and tenderness. (te Pas, Everts & Haagasman, 2004).

## Conclusion

The effects of MyoD1 gene observed in this work may reflect its function as activator of muscle-specific gene transcription, which determines muscle tissue development, growth, composition and aging. The changes of expression levels of MyoD1 gene may cause a cascading effect affecting many muscle-specific genes as α-actin (Moss et al.,1996), troponin I (Lin et al., 1991) and myosin light chain (Wentworth et al., 1991). The MyoD1 mRNA profile effect on meat tenderness showed in this work is new and could be useful for studies who integrated analysis of genotypic and expression data for association with complex traits and to identify novel genetic pathways and regulatory mutations involved in meat tenderness.

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