



Transcriptomic analysis identifies differentially expressed genes related to lipid metabolism in extreme animals for GEBV for iron content in the muscle Nelore steers¹

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Abstract: Iron is essential to several biological processes, such as oxygen transport and lipid metabolism. In this study, the RNA sequencing technology (RNAseq) was adopted to identify differentially expressed genes (DE) and metabolic pathways, as well as its relationship with meat quality parameters influenced by the variation in iron content (IC) in muscle. Samples of *Longissimus dorsi* (LD) muscle from Nelore cattle (n = 8), extreme genomic estimated breeding value (GEBV) for IC (four samples with high (HIL) and four low (LIL) GEBV) were used. Data analysis by Tuxedo Suite identified 49 annotated genes DE between the groups. The functional enrichment analysis identified important metabolic pathways associated with lipid metabolism, like stearate biosynthesis, fatty acid biosynthesis and palmitate biosynthesis, which participate in the gene *FASN*, *FABP4*, *SLC27A6* and *THRSP*. These results suggest biologic pathways influenced by iron and contribute information for the understanding of their participation in the process that affects muscle physiology in cattle.

Keywords: fatty acids, gene expression, iron metabolism, *longissimus dorsi*

Análise transcriptômica identifica genes diferencialmente expressos relacionados ao metabolismo de lipídeos em animais extremos para GEBV para conteúdo de ferro no músculo de novilhos Nelore

Resumo: O ferro é essencial a diversos processos biológicos, tais como transporte de oxigênio e metabolismo de lipídeos. No presente estudo, a tecnologia de sequenciamento de RNA (RNAseq) foi adotada para identificar genes diferencialmente expressos (DE) e vias metabólicas, bem como sua relação com parâmetros de qualidade da carne influenciados pela variação no conteúdo de ferro (IC) no músculo. Amostras do músculo *Longissimus dorsi* (LD) de animais Nelore (n = 8), extremos para valor genético genômico estimado (GEBV) para IC (quatro amostras com alto (HIL) e quatro com baixo (LIL) GEBV) foram utilizadas. A análise de dados pelo Tuxedo Suite identificou 49 genes anotados DE entre os grupos avaliados. A análise de enriquecimento funcional permitiu identificar vias metabólicas importantes associadas ao metabolismo de lipídeos, tais como biossíntese do estearato, biossíntese de ácidos graxos e biossíntese do palmitato, nas quais participam os genes *FASN*, *FABP4*, *SLC27A6* e *THRSP*. Esses resultados apontam rotas biológicas influenciadas pelo ferro e contribui com informações para o entendimento da sua participação em vias que afetam a fisiologia do músculo em bovinos.

Palavras-chave: ácidos graxos, expressão gênica, *longissimus dorsi*, metabolismo de ferro

Introduction

Essential micronutrient, iron (Fe) exhibits a central role in many biochemical functions which acts as an enzyme cofactor and/or structural component of proteins. Among the functions in which Fe participates are highlighted, for example, also the energy metabolism, lipid metabolism, binding and transport of oxygen. Furthermore, recent studies have demonstrated that Fe content in the muscle affects important parameters of meat quality in bovine, such as flavor and juiciness. Beef is an excellent source of iron, with respect to both amount and bioavailability, widely consumed worldwide (Duan et al., 201). Despite the importance for metabolism, the underlying genetic variation in iron homeostasis in muscle and its role in key biological pathways that affect meat quality remain poorly understood. Therefore, this study aimed to identify differentially expressed genes in the muscle *Longissimus dorsi*, as well as important biological pathways between extreme animals for GEBV for iron content in Nelore cattle.



Material and Methods

Nelore steers (n = 373), components of a half-sib families, were produced by the Brazilian Agricultural Research Corporation (EMBRAPA). The steers were sired by 34 unrelated Nelore sires which are representatives of the main genealogies of Nelore breed commercialized in Brazil. Animals were produced in feedlot system and slaughtered at an average age of 25 months. At slaughter, samples of LD muscle were collected from the region between the 11th and 13th ribs. The samples were used to measure the mineral content (iron and others) by inductively coupled plasma optical emission spectrometry (Vista Pro-CCD ICP-OES[®], Varian, Mulgrave, Austrália). Details about experimental population and determination of mineral concentration were described by Tizioto et al. (2015).

The variance components and GEBV of the present population were obtained by using a *Bayesian* approach, which was implemented in Gensel software, as described by Tizioto et al. (2015). The statistical model included the fixed effects contemporary groups, composed by birth (n = 4), feedlot location (n = 2) and breeding season (n = 2). The animal's age at slaughter was included as a covariate

Animals were classified regarding the GEBV for iron content (IC), then eight extremes animals were selected of which four high GEBV (HIL, High Iron Level) and four low GEBV (LIL, Low Iron Level). The total RNA of these eight selected animals was extracted from 100 mg of LD muscle by using TRIzol[®] (Life Technologies, Carlsbad, CA). The sequencing of total mRNA was performed adopting the technology Sequencing by Synthesis (Illumina), from 2 µg of total RNA using the TruSeq RNA Sample Preparation Kit[®] (Illumina, San Diego, CA), according to the protocol TruSeq RNA Sample Preparation kit v2 guide (Illumina). The clustering and sequencing were performed on the Illumina HiSeq 2500[®] (Illumina) to produce paired-end reads of 100 bp. Library preparation and sequencing were performed at ESALQ Genomic Center (Piracicaba, SP).

The quality control of the reads was performed using the SeqClean software. Tuxedo Suite (Tophat v2.0.11 and Cufflinks v2.2.1 softwares) was used to map the reads to the reference genome bovine (*Bos taurus* UMD 3.1), to assembly and estimate transcripts abundance and to perform the analysis of differential expression. The transcripts abundance was accessed by the normalized number of Fragments Per Kilobase of exon per million fragments Mapped (FPKM).

Differentially expressed (DE) genes between the HIL and LIL groups were detected by maximum likelihood model. *Benjamini-Hochberg* methodology was adopted to perform correction for multiple tests (q value <0.05). For DE genes, the sign of the log₂ (*fold change*) was used to gene classification as up- or down-regulated (False Discovery Rate <0.05). In order to identify metabolic pathways over-represented and gene networks, DE genes functional categorization based on the Gene Ontology (GO) terms to biological processes were performed using the Ingenuity Pathways Analysis (IPA[®], QIAGEN, Redwood City; <http://www.ingenuity.com>) software.

Results and Discussion

Although the main iron homeostatic process are concentrated in liver, a previous study in this population showed that a genetic component influenced the iron content in LD muscle (Tizioto et al., 2015). The iron concentration average between the HIL and LIL groups were 5.9 mg/100 g and 0.76 mg/100 g, respectively. A total of 25,208 annotated genes were expressed in LD muscle, of which 49 DE genes were identified by Cuffdiff software (q < 0.05). In this context, 18 genes were classified as up-regulated and 31 genes as down-regulated in the LIL relative to the HIL groups. The lipid metabolism was the main biological process categorized by IPA[®] from the functional enrichment analysis. Furthermore, canonical metabolic pathways over-represented were identified: stearate biosynthesis in animals (P-value 0.0027), palmitate biosynthesis (P-value 0.00441) and fatty acid biosynthesis (P-value 0.00441).

Lipogenic genes, such as Cytochrome P450, family 4, subfamily B, polypeptide 1 (*CYP4B1*), Fatty acid binding protein 4, adipocyte (*FABP4*), Fatty acid synthase (*FASN*) and Thyroid hormone responsive (*THRSP*) were observed down-regulated in LIL group. Besides, the Component complemente 3 (*C3*), which encodes an adipokine with immune function was also down-regulated. On the other hand, Perilipin 5 (*PLIN5*) and Solute carrier family 27 (fatty acid transporter), member 6 (*SLC27A6*), related to the regulation of lipid deposition and transport of fatty acids, were observed up-regulated.

The *THRSP* gene encodes a nuclear protein that acts as lipogenic transcription factor regulating genes that encode enzymes necessary for the synthesis of long chain fatty acids, including *FASN* and *FABP4* genes identified as down-regulated in animals LIL group. In addition to nutritional factors, *THRSP* is responsive to thyroid hormone (*TH*) levels, whose synthesis is impaired in the case of low iron availability due to the reduction in the activity of thyroid peroxidase, which is heme-dependent (Zimmermann and Köhrle, 2002).

The *FASN* and *FABP4* genes encoding lipogenic enzymes are positively regulated by *TH* (Yao et al., 2014). The *FASN* is a key enzyme in the *de novo* fatty acid synthesis and catalyzes the synthesis of saturated long chain



fatty acids, such as myristate (C14:0), palmitate (C16:0) and stearate (C18:0). The *FABP4* gene is a member of a multi-gene family, which facilitate the transport of lipids to specific compartments in the cell, playing an important role in the uptake of free fatty acids, transport and metabolism. In this context, *THRSP*, *FASN* and *FABP4* genes have been suggested as functional candidate genes for deposition of intramuscular fat and marbling score in cattle, these parameters positively influence the juiciness, flavor, texture and tenderness of the meat (Wang et al., 2009).

According to Stangl and Kirchgessner (1998), iron availability affects the action of iron-dependent enzymes, and then various aspects of lipid metabolism. Lipogenic enzymes and ones belonging the citric acid cycle that are iron-dependent have a reduced activity under iron deficiency conditions. Besides the reduction in the activity of the enzymes fatty acid synthase (*FASN*), malic enzyme and other lipogenic enzymes, the authors above mentioned found a lower rate in the synthesis of lipids in mice iron-deficient.

Conclusions

This study showed biological pathways related to the iron content in the muscle, contributing with information for a better understanding of the molecular mechanisms in which iron is involved and the understanding of their participation in the process that affect the quality of meat. However, the effect of iron on the expression of these genes and biological pathways identified should be better investigated.

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