Relationship between hypothalamic transcriptome and feed efficiency in *Bos indicus* beef cattle

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Summary

Despite the importance of feed efficiency (FE) for improvement of productivity and reduction of environmental impact of livestock, the selection for that trait is difficult due to its high cost of measurement. Thus, the objective of this study was to investigate the hypothalamic transcriptomic profile of high and low FE bovines to uncover the molecular mechanisms responsible for the regulation of this trait. Hypothalamic transcriptomic data from nine animals from each FE group was analysed through differential expression and co-expression analysis. Eight differentially expressed genes and nine modules of co-expression were associated to FE. Modules of genes highly expressed in high FE group was mainly related to respiratory chain and ATP production in mitochondria while low FE group seems to present an hypothalamic innate immune response. These are preliminary results and further investigation are still ongoing.

Keywords: RNAseq, residual feed intake, immune response, satiety, Nellore cattle

Introduction

Feed efficiency (FE) has become a well-studied trait because it is related to some of the main current animal selection goals such as increase of productivity, reduction of the environmental impact of livestock and reduction of competition for grains with human nutrition (Hayes et al., 2013). Because it is expensive to measure individually, studies on FE focus on the identification of molecular markers in the DNA, which allow us to select superior animals, or more recently, in the identification of metabolic pathways and regulatory molecular elements of this phenotype. Some studies already evaluated differences in transcriptome profile of animals with high and low FE. Our group demonstrated that Nellore cattle with low FE presented altered lipid metabolism in the liver and increased periportal hepatic lesions associated with an inflammatory response composed mainly by mononuclear cells; some regulatory genes were also indicated (Alexandre et al., 2015). Although very interesting results were found by analysing the liver, a central organ of metabolism, FE is a complex trait and other mechanisms besides metabolism are pointed out as responsible for its regulation, such as ingestion, digestion, physical activity and thermoregulation (Herd & Arthur, 2009).

In this sense, the hypothalamus is an organ that connects the nervous to the endocrine system and regulates several important processes; e.g. response to stress, sexual activity, body temperature, hunger and thirst. Therefore, the objective of this study was to investigate the hypothalamic gene expression profile of high and low FE animals, through differential expression and co-expression modules of genes associated with this phenotype, to uncover the mechanisms responsible for the regulation of this trait.

Material and methods

A total of 98 animals were evaluated in a feeding trial and 9 individuals of each extreme of feed efficiency (high and low residual feed intake) had samples of hypothalamus collected at the time of slaughter. Details on the animals and experimental design can be found in Alexandre *et al.* (2015). RNA was extracted from those samples using AllPrep DNA/RNA/Protein Mini kit (QIAGEN, Crawley, UK) and was evaluated by 260/280 ratio in a spectrophotometer (NanoDrop 2000, ThermoScientific, USA) and RNA integrity number by gel capillary electrophoresis in Bioanalyzer 2100 (Agilent Technologies, Dublin, Ireland). The mRNA libraries were constructed using TruSeq™ RNA Sample Prep kit according to the manufacturer's guide and were sequenced in an Illumina HiSeq 2500 equipment in a single lane generating an average of 13 million reads per sample (100pb, pair-ended).

The quality of sequencing was evaluated through FastQC software and no additional filters were performed. The alignment of each sample with the bovine reference genome UMD3.1 was performed using STAR software, using default parameters and including the annotation file. Aligned reads were filtered using Samtools software to remove secondary alignments, PCR duplicates and poor quality alignments. Reads count for each gene was estimated using HTSeq software. A batch effect correction was performed on NOISeq software considering an unknown source of technical noise and genes with less than 1 CPM (counting per million) in more than 9 samples (half) were excluded. One sample was considered an outlier in a PCA and was excluded from downstream analysis. Then, the differential expression between the high and low FE groups was performed using EdgeR package in R environment. Genes were considered differentially expressed when Padj≤0.05.

Co-expression gene network (module) analysis was performed using the WGCNA (Weighted Correlation Network Analysis) package in R environment. For this analysis, only genes with more than 1CPM in all samples were maintained, eliminating from analysis values equal to zero. Samples were normalized by the TMM methodology (Trimmed Mean of M values) and log2 transformed. In addition, for computational reasons, only the 6,000 most connected genes were selected. Modules containing at least 30 genes were identified and assigned to colour names. To identify modules associated with FE (P <0.10), we calculated Pearson's correlation between the first principal component of the module (eigengene) and the residual feed intake (RFI) measure. Functional enrichment analysis was performed using the online platform WebGestalt (www.webgestalt.org), to determinate the biological significance of the modules correlated to FE. All genes expressed in hypothalamus were used as background and Benjamini & Hochberg (FDR) methodology was used for multiple tests correction. Terms with Padj<0.05 were considered significant.

Results and discussion

An average of 13,309,272 sequence reads were generated and from those, 91% mapped uniquely to the bovine reference genome. A total of 13,738 genes passed quality control and were tested for differential expression. From eight differentially expressed genes (Padj≤0.5), five and three genes were up regulated in low and high FE animals, respectively (Table 1).

For co-expression analysis, 12,482 genes that passed quality control were reduced to the 6000 most connected genes, which resulted in 78 modules of co-expression. From those, nine modules presented correlation above |0.40| (P<0.10) with RFI, being three negatively and six positively correlated to the trait.

Table 1. Differentially expressed genes.

	logFC[1]	PValue	FDR[2]
ACTA1	-2.46	7.32E-09	1.01E-04
KRT18	2.38	2.26E-08	1.55E-04
CD163	2.25	4.42E-06	2,02E-02
PPM1J	-1.17	6.38E-06	2,19E-02
TAC3	3.06	1.11E-05	3,05E-02
HOMER2	-0.80	2.04E-05	4,67E-02
GPR52	2.66	2.81E-05	4,90E-02
ENSBTAG00000018481	1.34	2.85E-05	4,90E-02

[1]Log fold change, low/high feed efficiency
[2]False discovery rate, Padj≤0.05

Among the modules with higher expression levels in high FE animals (negatively correlated with RFI), two presented significant enriched GO terms. Module Bisque4 has 55 genes and correlation of -0.42 (P=0.09) with RFI, while module Lightgreen has 87 genes and correlation of -0.65 (P=0.005) with RFI. Both modules showed enrichment for mitochondrial inner membrane, but Bisque4 also is enriched for mitochondrial protein complex and oxidoreductase complex (P<0.05). Many of the genes, especially in Bisque4 module, are related to the respiratory chain and ATP production. One example is a subunit of pyruvate dehydrogenase complex, which catalyses the conversion of pyruvate to acetyl-CoA and CO2 and thereby links the glycolytic pathway to the tricarboxylic acid cycle. In fact, the literature shows that during satiety or positive energy balance, proopiomelanocortin-producing neurons (POMC) are more active, using glucose as main fuel (Gyengesi et al., 2012). Increased cellular activity (respiration) results in increased production of reactive oxygen species (ROS). The elevation of ROS levels contributes even more to the increase of POMC activity that act to suppress the ingestive behaviour (Gyengesi et al., 2012).

Considering modules with higher expression levels in low FE animals (positively correlated with RFI), only one presented significant enriched GO terms. Module Turquoise has 276 genes and correlation of 0.43 (P=0.08) with RFI. The main terms enriched in this module are innate immune response, positive regulation of cytokine production, G-protein coupled purinergic receptor signalling pathway and leukocyte migration and aggregation (P<0.05). This result can explain the up-regulation of gene CD163 in this group, which is exclusively expressed by monocytes and macrophages and induce local inflammation through the secretion of interleukin-6 and CSF1 (macrophage colony-stimulating factor 1). Moreover, gene KRT18, also up-regulated in low FE group, is likewise involved in IL-6 mediated barrier protection. Gene TAC3 and GPR52 are involved with signal transduction. Thus, one can suggest an immune response in hypothalamus of low FE individuals.

The hypothalamus is composed by highly heterogenic groups of neurons and resident glial cells (microglia, macrophages and astrocytes) that control metabolic homeostasis by regulating energy production and expenditure. A reactive response, characterized by glial reactivity, secretion of cytokines and increased level of inflammatory signals has been demonstrated to be associated with hypercaloric challenge and diet-induced obesity in humans and animals (Gyengesi et al., 2012). Moreover, in this scenario, POMC neurons showed reduced functionality (Gyengesi et al., 2012). In fact, animals from low FE group presented higher feed intake, higher visceral and subcutaneous fat deposition and higher serum cholesterol levels, aspects that resemble obesity (Castro Cabezas et al., 2001). Besides

that, studies of gene expression in different bovine tissue also showed the presence of immune response associated with low FE (Alexandre et al., 2015; Kern et al., 2016; Lindholm-Perry et al., 2016; Tizioto et al., 2017)

Conclusion

There are differences in hypothalamic transcriptome between high and low FE bovines, which can elucidate some of the molecular mechanisms responsible for the regulation of this trait and indicate interesting QTL for genomic prediction. The relationship between increased cellular activity and high FE, as well as the role of up-regulated genes in this group need to be further investigated. On the other hand, the relationship between inflammation and FE efficiency have been reported before in different tissues and lead to the possibility of a systemic investigation regarding this aspect.

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