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A protein co-expression network analysis in Nelore cattle revealing pathways associated with carcass traits (#498)

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Introduction

Producing animals with heavy carcass weight and a proper layer of fat is among the main objectives of the meat industry. Thus, growing studies have focused on carcass traits, such as ribeye area (REA) and backfat thickness (BFT) as indicators of the amount of muscle and fat in the carcass [1, 2]. Understanding the molecular mechanisms underlying these complex traits is crucial for contributing to breeding programs. In this context, the Weighted Correlation Network Analysis (WGCNA), allows us to explore the structure of genes and proteins within a co-expression network approach concerning the studied traits [3]. Therefore, our goal was to identify groups of co-expressed proteins associated with REA and BFT in a Nelore cattle population.

Methods

For this study, 160 Nelore steers were raised on pasture and finished in feedlots under identical nutritional and handling conditions. The animals were slaughtered at an average live weight of 452 kg and 24 months of age. At slaughter, samples were taken from the *Longissimus dorsi* (LD) muscle (12th-13th ribs) from each animal for proteomic analysis; and at 24 hours after slaughter, a sample of the LD muscle (12th-13th ribs) was used for REA (cm^2) and BFT (mm) measures. More details in Tizioto et al. [4].

Muscle protein extraction and sample preparation for mass spectrometry analysis were performed according to Poleti et al. [5]. The analysis of tryptic peptides was carried out using a nanoACQUITY UPLC 2D Technology system coupled to Synapt G2-S High Definition Mass Spectrometer (HDMS) (Waters, Manchester, UK). A total of 500 ng of protein digests was loaded on column for each of the 3 fractions (500 ng/fraction/load). MS data were acquired with Waters MassLynx v.4.1 software and processed and searched using ProteinLynx GlobalSERVER v2.5 (Waters, Manchester, UK). Protein identifications were obtained by searching against a Nelore transcriptome database built from RNA-sequencing data from LD muscle [5]. A maximum false discovery rate (FDR) was set to 4%. Label-free protein quantification values were generated based on the label-free Hi3 method. Data quality assessment was performed accordingly. Merged spectra were then normalized to sum of all intensities.

Proteins quantified in 80% of the samples were assigned for the co-expression network analysis, by using the R package WGCNA, with the step-by-step network construction and module detection [3]. To construct the weighted protein network, a β soft threshold of 10 ($R^2 = 0.80$) was selected, to which co-expression similarity is raised to calculate the adjacency matrix (AM). The AM was then transformed into a Topological Overlap Matrix (TOM), them its corresponding dissimilarity (1-TOM) was determined. Using a hierarchical clustering tree (dendrogram), we identified modules of highly co-expressed proteins. The modules were merged based on the dissimilarity of their eigengenes, the first principal component of each module, and named by colour. Module-trait associations were determined using a linear model fitted to analyze the association between the abundance profiles of the module eigengenes (MEs) and the phenotypes. Proteins of modules significantly (p-value ≤ 0.05) correlated with the phenotype were assigned for functional enrichment analysis. The enrichment analysis for KEGG Pathways (KP) and Biological Processes (BP) was performed using STRING v.11 (https://string-db.org/), with FDR ≤ 0.05.

Results

Based on the co-expression network approach, we identified eleven modules (Figure 1) of which the Blue and Green modules were significantly associated with REA (p-value ≤ 0.05). Besides, the Red module was significantly associated with BFT (p-value ≤ 0.05).

From the modules associated with REA, the enrichment analysis retrieved KEGG pathways (KP) and biological processes (BP) mainly related to muscle energy metabolism. We found 22 KP and 34 BP, highlighting the carbon metabolism (bta01200) and citrate cycle (bta00020). Regarding the Green module, we identify four KP and 23 BP, such as glycolysis/gluconeogenesis (bta00010), carbon metabolism (bta01200), and purine ribonucleotide biosynthetic process (GO:0009152). These are pathways with an essential role in the uptake and storage of energy for the body [6], supporting our findings. Calcium signaling pathway (bta04020) was overrepresented on Red module, which was associated with BFT. As reported by Greineisen et al. [7] lipid accumulation in muscle cells may lead a cytoskeletal remodeling altering calcium dynamics and resulting in signaling changes.

Notes

Conclusion

This study demonstrated that energy metabolism pathways and BP mostly influence REA; and, reveled the Calcium signaling pathway as a regulator of BFT. These results improve our knowledge of the molecular regulation of important carcass traits in bovine.

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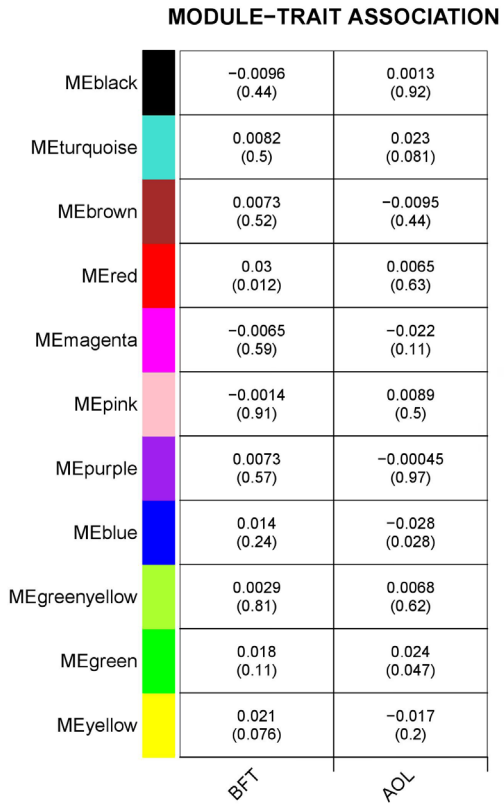
Notes



Module-trait associations

Figure 1. Module-trait associations between the module eingengenes (ME) and backfat thickness (BFT) or ribeye area (REA). Each row corresponds to a module eigengene, column to a trait. Each cell contains the coefficient from the linear model and, the *p*-values of the correlation (numbers within parentheses).

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