RECOVERY FROM UNDERNUTRITION CHANGES CONNECTIVE TISSUE AND MUSCLE PROTEOLYTIC SYSTEM GENE EXPRESSION IN *BOS INDICUS* CULL COWS

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Abstract – The rate if structural remodeling of stromal and myofibrillar proteins are related to efficiency of muscle growth as well as meat quality, influencing collagen solubility and postmortem proteolytic rate. These characteristics may be limited in mature animals suchs as cull cows from *Bos Indicus* breed under extensive grazing systems. The question imposed would be whether differential growth rate during recovery from undernutrition is sufficient to change the remodeling process in the stromal proteins and the myofibrillar protelytic system on mature animals. In addition to the elucidation of the biological changes that occur in animals in undernutirion and realimentation situation, helps understanding the muscle remodeling process and its influence on protein structures and intracellular routes with the potential to chande the quality of the meat. The expression profiling based on m-number of readings obtained from the cDNA libraries ensured a broad screening of genes. There are presented 7 genes related with connective tissue and muscle proteolytic system. Enchanced transcription of genes like serpin, exostosin-like 1 and Sparc, related to changes during recovery from endernutirtion are indications of extracellular matrix remodeling of the stromal proteins.

Key Words – Connective tissue, Extracellular matrix, Protein metabolism.

I. INTRODUCTION

Although there have been advances in the understanding of the role of nutrition and growth rate to myofiber metabolism and other tissues deposition into the muscle, the impact of those on beef tenderness is still not fully described. The interaction of many factors, such as genetics and responses to the environment, that compose the tissues depositions pattern in response to nutritional management as well as the interrelations between those deposited tissues to define final tenderness may explain the difficulty of the task. Nonetheless, there has been an increasing body of studies showing changes in gene expression associated to muscle structural remodeling due to nutritional challenges [1, 2, 3]. Those changes are pointing to the possibility of modifying structure that are part of the muscle scaffold that is involved in meat tenderness, such as cytoskeletal and extracellular matrix structures. The proteinase systems, calcium signaling, intermediary metabolism, which may be somehow involved in meat tenderness, have also some of the genes regulated. Therefore, there are some insights of the changes that are taking place and may help to understandhow recovery affects muscle growth and characteristics in mature animals. The aim of this study was to verify the changes in gene expression at two growth rates after a period of undernutrition and to identify genes that are related to connective tissue remodeling in order to understand whether there is any indication that changes towards structural proteins had been related to meat tenderness.

II. MATERIALS AND METHODS

The experiment was held at the Brazilian National Beef Cattle Research Center/EMBRAPA with Nellore females, 5-16 years old. Sixteen cows with low body condition score (4.5 ± 0.3) were randomly assigned to control (CON) and high recovery gain (HRG; 1.2 kg of live weight gain) during the dry season. The cows were slaughtered at 0 (n=4; CON), 41 (n=6: HRG) and 103 (n=5: HRG) days of feedlot. The comparison was made between the

cows in CON and HRG groups. The diet was formulated based on ingredients nutritional values from NRC [4]. The *Longissimus dorsi* samples were collected immediately after slaughter and frozen in liquid nitrogen. They were kept frozen in nitrogen until the sequencing analysis could be performed.

The total RNA was analyzed by Bioanalyzer (Agilent) for evaluation of their integrity by RIN (RNA Integrity Number), and was used if RIN was equal or higher than 8, which is the recommended value. After this step, 2 µg of total RNA from each sample were purified and fragmented using the TruSeq RNA Sample Prep kit v2 to separate the mRNA from the total RNA, through poly-T tails adhered magnetic beads. After preparation, the samples were loaded in the cBot equipament (Illumina), which performs the sample clusterization in the flow cell by the addition of specific reagents (TruSeq TM SR Cluster kit v2 – cBot – HS) and capillary tubes that transfer samples and reagents to the flow cell (HiSeq cBot Manifold). After clusterization, the samples were loaded to the sequencer (HiScanSQ, Illumina). The sequencing was verified by the quality of reads by FASTQC (www.bioinformatics.bbsrc.ac.uk/projects/).

The programs TopHat v.2.0.5 and Bowtie v. 0.12.8 [5, 6], were used to map the reads in relation to *Bos taurus* genome (UMD_3.1, NCBI project 32899). For each sample was generated a file with the alignment in relation to the reference genome.

The reads count of the mapped genes was obtained through HTSEq-count (http://www-huber.embl.de/users/anders/HTSeq/doc/count.html?highlight=count). Data normalization and analysis of differential expression were done by the DESeq procedure of the R program (version 2.15.0), followed by the correction by Benjamini- Hochberg multiple tests [7] for the p values obtained.

III. RESULTS AND DISCUSSION

From the 463 significative differentially expressed genes, we will discuss the role of the genes that might be involved on major pathways that could be involved on tissue connectiveness and proteolytic system. There are some interesting genes involved in connective tissue remodeling as an inflammatory response in the recovery group, such as Immunoglobulin superfamily member precursor, which could be a concerted response that depends largely on macrophage secretory and physiological function [8]. The SPARC protein is also related to tissue injury or high turnover rate with important role within the extracellular matrix [9]. Another indication of connective tissue remodeling and growth, now related to increased transcription of genes of the extracellular matrix proteins, is the up-regulation of Collagen type IV subunits 1, 2 and 3 transcripts as well as genes that are involved in the collagen biosynthesis as Serpin. There were also some transcripts that may be related to proteoglycans synthesis that were up-regulated, such as Exostosin-like 1. The increase of collagen type IV in connective tissue of cows and their mature collagen molecules may be expected, since that change is observed in the basement membrane during aging in other animal model [10]. Changes occur at the extracellular matrix but they may be limited to minor components related to connective tissue scaffold. Collagen type IV transcripts would represent this limited connective tissue remodeling on mature animals, where great amount of advanced glycation end products exert a feedback signal to reduce other types of collagen synthesis as well as matrix metalloproteinases initiated collagen degradation [11]. The Proteasome subunit was up-regulated in control animals. Those genes are involved in higher muscle protein degradation (catabolism) which is related to muscle mass waste. The accelerated proteolysis via proteasome-ubiquitin pathway has been recognized as the principal cause of muscle atrophy in a number of catabolic conditions [12]. The proteolytic system related with the calpain was more expressed in the high recovery gain after undernutrition and it can be related with improvements in the meat quality. The calpain degrades a number of endogenous proteins, among which the myofibrillar proteins, cytoskeletal hormone receptors and protein kinases, and to regulate its own proteolysis [13].

Table 1. Muscle genes in Nellore Cull Cows during recovery from undernutrition in feedlot

Gene	Identification	Function	Log2 Fold change	Padj<
SERPH_BOVIN	Serpin H1	Collagen biosynthesis	1.82	0.001
IGSF4	Imunoglobulin superfamily member 4 precursor	inflammatory response	1.34	0.025
A6QR03	Exostosin like 1	Proteoglycan synthesis	2.41	0.001
CO4A1_BOVIN	Collagen alpha-1 (IV) chain	Extracellular matrix	1.51	0.001
CO4A2_BOVIN	Collagen alpha-2 (IV) chain	Extracellular matrix	1.64	0.001
CO4A3_BOVIN	Collagen alpha-3 (IV) chain	Extracellular matrix	1.03	0.004
SPRC_BOVIN	SPARC	ECM remodeling	2.86	0.001
PSMA7	Proteasome subunit alpha type 7	Protein catabolism	-0.84*	0.034
CAPN-6	Calpain	Proteolytic system	1.15	0.001

* upregulated genes in control samples

IV. CONCLUSION

The muscle tissue from mature cows have their gene expression changed during recovery from undernutrition in a way that points to remodeling of the stromal proteins. Those changes in the extracellular matrix structural scaffold related to perymisium contributions to meat texture may be very limited.

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