QUANTITATIVE PROTEOME ANALYSIS OF pH-RELATED CHANGES IN MUSCLE BOVINE

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Abstract – The pH is important factor to meat tenderness and changes in protein levels among different pH classes in the bovine *Longissimus dorsi* muscle can help to explain meat tenderness variation. Hence, the objective of this study was to investigate the changes in proteins in bovine muscle between different pH classes using multidimensional protein identification technology - MudPIT. Muscles from Nellore cattle were collected at 24 hours and carcass pH values were recorded at 1 and 24 hours *post mortem*. From the pH values at 24 hours *post mortem* 80 cattle were selected and divided in two different classes: low (5.4–5.6) and high (pH≥5.8) pH. Into each pH class ten animals was pooled to obtain four samples per group. The proteomic profile was analyzed by nanoACQUITY UPLC system coupled to the SYNAPT G2 MS system. ProteinLynx Global Server with Expression^{E} analysis identified a total of 1288 for high pH class and 1306 for low pH class. Five proteins were up-expressed significantly different level in High pH class versus Low pH class. The proteins glyceraldehyde 3-phosphate dehydrogenase, triose-phosphate isomerase, aspartate aminotransferase, malate dehydrogenase and myosin demonstrated to be related to muscle pH at 24 hours *post mortem*.

I. INTRODUCTION

Meat quality can be influenced by several factors [1,2], among them the ultimate pH. In general, the meat is considered to be good quality at an ultimate pH (pH_u) between 5.4 and 5.8, but detrimental effects on the color, tenderness, flavor and microbial stability can be noted at pH_u values higher than 5.8 [3]. Some works report tender meat when the carcasses have the highest pH_u values [4,5], whereas other studies report tender meat when the carcasses have the lowest pH_u values [6]. Another report demonstrates that less tender meat is found in the carcasses with pH_u values between 5.8 and 6.2 [7].

The basis of meat quality differences in relation pH can be explained by different proteolytic activity [7,8].

Proteomics studies based 2DE (two dimensional electrophoresis) has been performed to investigated the proteins involved in muscle to meat conversion [9]. However 2DE analysis have most severe limitation as proteins low abundance are not detected, limited dynamic range, low quantification and has limited solubility for hydrophobic and membrane proteins [10].

A new technology for quantitative proteomics as MudPIT (multidimensional protein identification technology) is being used to study of differential expression of proteins in complex biological samples as a rapid, reproducible and accurate quantification strategy [11].

Therefore the objective of this study was to investigate the changes in proteins in bovine muscle between different pH classes using MudPIT.

II. MATERIALS AND METHODS

Animals and sampling

Samples of *Longissimus dorsi* (LD) muscle were collected at 24 hours *post mortem* from 241 Nellore cattle reared on pasture and feedlot finished. The animals were slaughtered at an age of 24 ± 1.2 months and a live weight of 508 ± 39 kg. After collection, the samples were immediately stored at -80ºC for proteomic analysis.

The carcass pH values were recorded at 1 and 24 hours *postmortem*. The measurement was taken in the 12nd thoracic vertebrae in the *Longissimus dorsi* muscle on the left side from the carcass using a portable pH meter (pH 11 Economy Meter, Oakton instruments).

From the pH values at 24 hours *post mortem* 80 cattle were selected and divided in two different classes: low (pH 5.4-5.6) and high (pH≥5.8).

Extraction of muscle proteins

Sample preparation was performed according Bouley *et al.* [9]. Briefly, frozen muscle tissue from ten animals for each pH class was pooled to obtain four samples per group. The samples was homogenized in a lysis buffer containing 8 M Urea, 2 M Thiourea, 1% DTT, 2% CHAPS and 2% IPG buffer pH 4-7 and centrifuged at 10 000 x *g* for 30 min. at 4ºC. The supernatant was harvested and protein concentration determined (PlusOne 2-D Quant Kit; GE Healthcare).

LC-MS^E

The protein extract was digested with trypsin and 1 µl phosphorylase from rabbit was added as internal standard for each sample. The digested peptide mixture from each sample was loaded onto a nanoACQUITY UPLC system coupled to the SYNAPT G2 MS system (Waters, Manchester, UK). The chromatography step consisted of a two-dimensional nano-scale LC instrument that included a strong cation-exchange column and a C18 reversed-phase analytical column.
The mass spectra were acquired in the positive ion and V-mode. The TOF analyzer was calibrated with the MS/MS fragment ions of [Glu1]-fibrinopeptide B (GFP, 100 fmol/µl) injected once every 30s.
The analysis of spectra and identification of proteins in the database were performed with ProteinLynx Global Server v.2.5 (PLGS) searching into the UniProtKB/Swiss-Prot *Bos taurus taurus* and *Bos taurus indicus* database to which the sequence of phosphorylase was appended.

Statistical analyses

The data was submitted to ANOVA using PROC MIXED procedure of Statistical Analysis System (SAS), version 9.1.3, to test the effects of two pH levels. Due to the high number of proteins to be evaluated, it was applied Bonferroni correction to control the level of significance set at 5%.

III. RESULTS AND DISCUSSION

The total number of proteins identified was 1288 for high pH class and 1306 for low pH class (Figure 1). However, the number of common proteins, in all samples, between the pH classes was 87 proteins, which were submitted to statistical analysis.

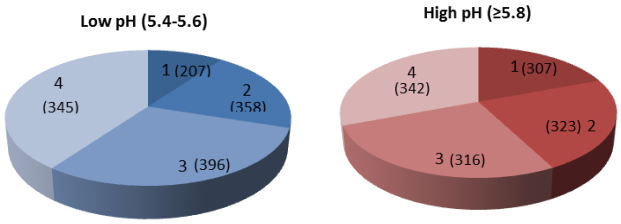

Figure 1. Number of proteins identified in each sample within each pH class.

We found 5 up-expressed proteins significantly different level in High pH class versus Low pH class (Figure 2). Two proteins found are glycolytic enzymes (glyceraldehyde 3-phosphate dehydrogenase and triose-phosphate isomerase), one is involved in transport (aspartate aminotransferase), one participate tricarboxylic acid cycle (malate dehydrogenase) and one is structural protein (myosin).

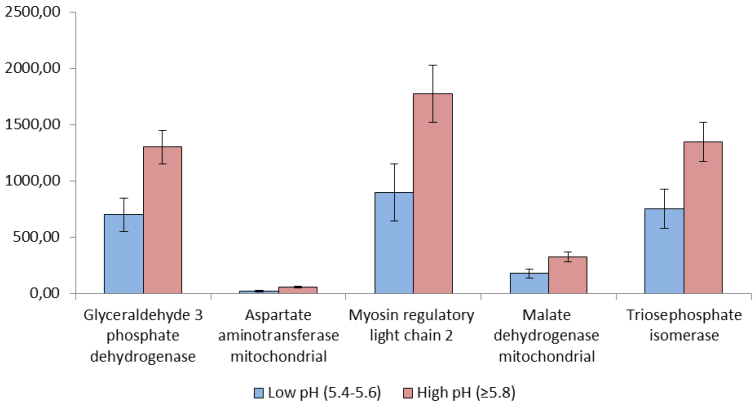

Figure 2. Differentially expressed proteins in bovine *Longissimus dorsi* muscle between two pH class (Low pH <5.8 and High pH >5.8).

The pH decline is a result of lactate production and accumulation within the muscle. The lactate production is resulted of the glycolytic pathway

under anaerobic conditions. Therefore, the enzymes that catalyze the glycolysis reactions affect the rate and extend of pH decline in *post mortem* muscle [12]. In this study, two enzymes that are involved in energy metabolism significantly increased in abundance in meat with high pH.

The aspartate aminotransferase (AST) is an important enzyme in amino acid metabolism and is known that elevation in AST levels is a specific indicator of skeletal muscle injury [13].

The malate dehydrogenase is involved in the TCA cycle. It's converts malate to oxaloacetate producing an equivalent amount of NADH [14]. The malate dehydrogenase was more abundant in the group high pH. Laville [14] found malate dehydrogenase overrepresented in the tough group in pig *Longissimus Lomborum*.

The myosin is considered to define the mechanism of meat tenderization. Therefore, a greater abundance of myosin in meat with high pH suggests less degradation and integrity of actomyosin rigor bond.

IV. CONCLUSION

In conclusion, the MudPIT was able to identify proteins that changed in bovine muscle between different pH. The results demonstrated that proteins involved in enzymatic reactions of the glycolysis and TCA cycle and therefore associated with energy production are related with the ultimate pH in *post mortem* muscle, as well as myosin and aspartate aminotransferase mitochondrial. Those proteins could explain the variation of some meat traits due different ultimate pH. The evaluation of peptides present only in one or another class of pH may help to understanding the biochemical changes involved in meat with different ionic hydrogen potential.

ACKNOWLEDGEMENTS

Authors thank São Paulo Research Foundation (Fundação de Amparo à Pesquisa do Estado de São Paulo-FAPESP) and National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq) by financial support.

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