EFFECT OF ULTIMATE pH IN BOVINE MUSCLE BY QUANTITATIVE PROTEOME ANALYSIS

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Abstract – Proteomics studies has being performed to explain meat traits variation and some studies were performed using pooling samples. 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- from individual samples. 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* 241 cattle were selected and divided in two different classes: low (pH<5.8) and high (pH≥5.8) pH. Into each pH class six animals were used to obtain six individual samples. 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 609 for high pH class and 607 for low pH class. Five proteins were expressed significantly different level in High pH class versus Low pH class. Glutathione Transferase, muscle fiber development, Peptidyl-prolyl cis-trans isomerase, Malate dehydrogenase and, Cytochrome b-c1 complex subunit 2. The bovine muscle proteins abundance identified in pH classes were different from individual samples compared to pooling samples.

Key Words – DFD beef, Protein Profile, Meat Quality.

I. INTRODUCTION

Proteomics studies based 2DE (two dimensional electrophoresis) has been performed to investigated the proteins involved in muscle to meat conversion [1]. However 2DE analysis have some limitation as proteins low abundance are not detected, limited dynamic range, low quantification and has limited solubility for hydrophobic and membrane proteins [2]. A technology for quantitative proteomics as MudPIT (multidimensional protein identification technology) is being used to study of differential expression of proteins in complex biological samples. This technology has advantages as rapid, reproducible and accurate quantification strategy [3]. In 2014, we showed results of this technology based on pooling samples of two or three animals for each biological replication [4].

The objective of this study was to investigate the changes in proteins in bovine muscle between different pH classes using MudPIT, with individual samples.

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 finished on feedlot. The animals were slaughtered witch 24,11 ± 1.21 months and a live weight of 507,71 ± 39,17 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 between the 11th and the 13th 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* cattle were selected and divided in two different classes: low (pH <5.8) and high (pH≥5.8).

Extraction of muscle proteins

Sample preparation was performed according Bouley *et al.* [1]. Briefly, frozen muscle tissue

from six animals for each pH class was used to obtain six biological replications. 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* and *Bos indicus* database to which the sequence of phosphorylase was appended.

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 609 for high pH class and 607 for low pH class. The number of common proteins, in all samples, between the pH classes was 131 proteins, which were submitted to statistical analysis. Poleti et al [4] identified 1,288 for high pH class and 1,306 for low pH class, in pooling samples, however, the number of common proteins, in all samples, between the pH classes were 87 proteins.

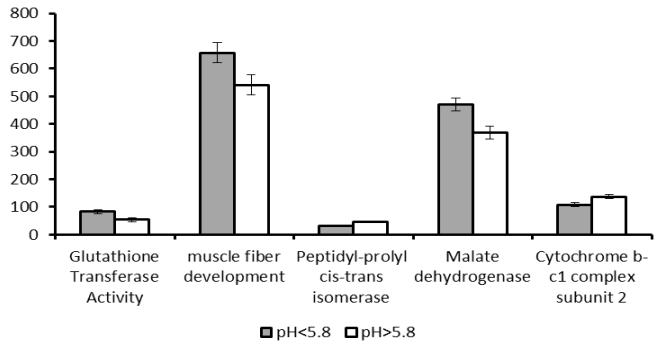
We found five expressed proteins significantly different level in High pH class versus Low pH class (Figure 1). The proteins found are: Glutathione Transferase Activity; muscle fiber development (Uncharacterized protein); Peptidyl-prolyl cis-trans isomerase; Malate dehydrogenase; and, Cytochrome b-c1 complex subunit 2. Poleti et al. [4] identified five up-expressed proteins significantly (glyceraldehyde 3-phosphate dehydrogenase, triose-phosphate isomerase, aspartate aminotransferase, malate dehydrogenase and myosin) in pooling samples.

The Glutathione Transferase Activity (GTA) is involved in catalysis of the reaction: R-X+glutathione=H-X+R-S-glutathione, where R may be an aliphatic, aromatic or heterocyclic group and X may be a sulfate, nitrile or halide group. Sayd et al. [5] identified GTA overrepresented in light group (on the basis of extreme *L** values of meat) using a differential proteome analysis (2DE), on the sarcoplasmic proteins of pig *Semimembranosus* muscles wich 36h postmortem.

The muscle fiber development (Uncharacterized protein) has been associated in the process whose specific outcome is the progression of the muscle fiber over time, from its formation to the mature structure. In skeletal muscle, fibers are formed by the maturation of myotubes.

The Peptidyl-prolyl cis-trans isomerase keeps in an inactive conformation TGFBR1, the TGF-beta type I serine/threonine kinase receptor, preventing TGF-beta receptor activation in absence of ligand.

Figure 1. Differentially expressed proteins in bovine *Longissimus dorsi* muscle between two pH class.(Low pH <5.8 and High pH ≥5.8)



The Peptidyl-prolyl cis-trans isomerase may modulate the RYR1 calcium channel activity. This protein folding was identified by Chase et al. [6] in alkaline proteome of bovine skeletal muscle by using protocol to characterize proteins over the entire range of pH 7–11 by 2DE methodology.

The malate dehydrogenase is involved in the TCA cycle and it's converts malate to oxaloacetate producing an equivalent amount of NADH [7]. The malate dehydrogenase was less abundant in the group high pH in individual samples and overrepresented in to high pH from pooling samples [4]. Laville [7] found malate dehydrogenase overrepresented in the tough group in pig *Longissimus Lomborum* muscle.

The cytochrome b-c1 complex subunit 2 has important functions. This protein involved in the transport of electrons, a process by which electrons are transported through a series of reactions from the reductant, or electron donor, to the oxidant, or electron acceptor, with concomitant energy conversion. This protein is involved in respiratory chain too. In aerobic respiration electrons are transferred from metabolites to molecular oxygen through a series of redox reactions mediated by an electron transport chain. The resulting free energy is used for the formation of ATP and NAD.

IV. CONCLUSION

The bovine muscle proteins abundance identified in pH classes were different from individual samples compared to pooling samples. Some proteins on individual samples are not detected in pools, except for malate dehydrogenase. We suggest a pilot experiment before principal experiment to evaluate the employing the pooling approach.

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