MORPHOLOGICAL AND QUANTITATIVE CHANGES IN PROTEIN IN BROILER *MAJOR PECTORALIS* IN POST MORTEM.

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Abstract – To be able to obtain animal protein for food establishes we needs a complex network of operations for production, slaughter, boning, distribution, storage and preparation for human consumption. May be said also that the type of fiber, as well as the area where it is located, will interfere with the histochemical and biochemical properties of the meat, and therefore in their quality, the effect they suffer in the metabolism before and after slaughter. The polyacrylamide gel containing SDS is an effective method of separating the molecular components in protein blend. The assessment of changes in muscle fibers during the resolution process is fundamental to understanding the qualitative changes of the final meat product. After the resolution of *rigor mortis* and the transformation of muscle into meat, there is significant variation in morphological organization of the sarcoplasmic fibers, directly interrelated with varying densities of the larger protein fractions, and their losses on portions occurred during process.

I. INTRODUCTION

Of all beef cuts taken poultry, which has greater value is the chest (*Major pectoralis*), being marketed both *in nature* and in processed derivatives (5). In the constitution of animals, we can mention that about 30-40 % of body weight is skeletal muscle is composed of bundles of very long cells, and multinucleated cylindrical, with diameters ranging from 10 to 100μm, called skeletal muscle fibers (6). We can say that the type of fiber as well as the area where it is located, will be interfere with the histochemical and biochemical properties of the meat, and therefore in their quality, the effect they suffer in the metabolism before and after slaughter (12).

Characteristics like pH, color, water-holding capacity, cooking loss and tenderness of meat are consider important, and relate directly to the quality of the protein constituents of the meat product, showing the evolution of the physicochemical research of these products (4). The physicochemical quality of the meat is very similar between the most different species of farm animals, with only a few quirks (3). After the slaughter of animals, a series of biochemical and structural changes begin initiating a process called "conversion of muscle to meat". The biochemical and structural changes occur simultaneously and are dependent on *ante mortem* treatments, the slaughtering process and storage techniques of meat (10). With respect to poultry, electrophoretic techniques and electropherograms revealed that muscle protein having greater or lesser fractionation, resulting in muscle protein specific patterns for each species to be useful in identifying the muscle tissue of poultry, turkeys and ostriches (5).

There are a large number of variables that influence the electrophoretic technique in proteins; one of these is the animal species (1). The list of processes occurring in meat products, from slaughtering to treatment occurred in every process after slaughter; determine the quality of the final product (8).

In this sense, the protein loss measured by densitometry and electrophoresis and its relation to the assessment of changes in muscle fibers during the process of resolving the *post-mortem* is fundamental to understanding the qualitative changes of the final meat product.

II. MATERIALS AND METHODS

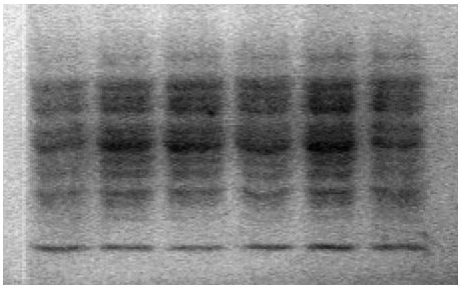
Six samples of pectoral muscle (*Major pectoralis*) from five poultries, in the same strain, sex and

weights that were used did not differ statistically. These animals were slaughtered in the laboratory, releasing CEUA - FMVZ - UNESP under No. 109/2012 of 21 May 2012, to not suffer the inherent common slaughter processes that influence biochemical muscle characteristics (such like as the process of scalding, plucking and cooling). Was retired couple aliquots of approximately 2 cm² as follows: 1 aliquot immediately after the bleeding of the animal and five more aliquots after each 30 min total of 2 hours and 30 minutes *post mortem* from each sample (T = 0, 30" - " which indicates time in minutes-, 60", 90", 120" and 150" for each bird, respectively). Samples for electrophoresis was frozen in liquid nitrogen at harvest, and for the morphological analysis, each aliquot was stored in a solution of 10% formaldehyde for fixation of the material and avoidance of changes that occur in nature. To determine the electrophoretic patterns of the samples, the electrophoresis Polyacrylamide Gel Native vertical alkaline (pH 8.3) in a discontinuous buffer system, containing sodium dodecyl sulfate (SDS-PAGE) (7) was used, with some modifications (9). Whereas the Native electrophoresis for the identification of protein fractions according to their relative mobility's, were employed patterns of muscle proteins of pure species studied, standardized by Figueira (5). For the analysis of Native gel electrophoresis, measures the relative mobility (Rf) for each protein band densitometry addition, the image obtained by the VDS hardware and software analysis on VDS, Pharmacia, manufactured by LG Electronics version were calculated 3.0. The results, after analysis of the statistical software StatPlus : mac AnalystSoft - statistical analysis program . 2009 version. For morphological analysis, after the step of fixation, the samples underwent dehydration and placed in intermediate substance (consisting of xylene) in a phase identified in the literature by bleaching. After preparation followed to step inclusion, who made with paraffin. For the step of microtome was used the rotary microtome Leica RM 2255, Leica Biosystems. Identified and prepared slides were stained with hematoxylin- eosin (HE), the most suitable dyes to highlight morphological structural features.

Blades at the ready, went to the stage of observation under optical microscope from Leica Microsystems DM2500/100W, in ascending order of objective so allowing a better description of the observed structures and their possible changes.

III. RESULTS AND DISCUSSION

The results for soluble proteins of the *Major pectoralis* muscle, are represented by choosing only one of the gels performed (Figure 1), demonstrating selection of bands (Figure 2), which did not occur to the repetitiveness of images presented.



a b c d e f

Figure 1 - SDS-PAGE on 10% of Major pectoralis broilers from one of the samples, presenting related to time 0 (a) applications; 30" (b); 60" (c); " 90 (d); 120" (e) and 150" (f).

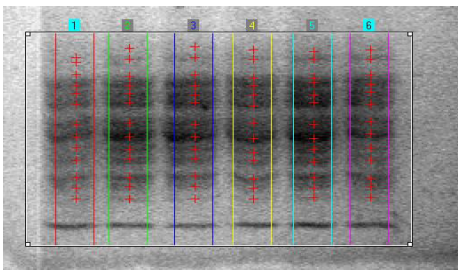


Figure 2 - Example of Selecting protein bands for analysis of Relative Mobility (Rf) and densitometry of one sample of chicken, with each selection on the time of collection, following.

Regarding the statistical evaluation, an ANOVA test (Test of variance) of a simple factor was performed, since the only variable would be sought densitometry, as a hypothesis, it could be changed in the present *post mortem*. This occurs because as we all samples come from poultries from the same lineage, and with similar weights and managements, the variation of Rf would not be taken into account, since only gene expression could significantly change their values. The data analyzed densitometry (calculated individually for each of the tested samples, and then performed simple average for each protein band, related to each time of collection) can be observed in Table 1.

Table 1: ANOVA data comparing mean and standard deviation of the protein fractions (average data, performed by an individual analysis of each gel).

c	Densitometry
	protein fractions %
r1	0,584±0,149
r2	1,373±0,171
r3	0,710±0,289
r4	3,714±0,555
r5	3,204±0,819
r6	4,521±0,678
r7	3,484±0,652
r8	8,025±1,360
r9	2,357±0,679
r10	2,204±0,406
r11	3,539±0,478
r12	2,400±0,350
r13	0,873±0,156
VC% (Medium)	20,23

With the analysis of the results was possible to evaluate the presentation of an electrophoretic patterns of the species, which will in meeting as described by Figueira (5), in all gels analyzed . The presentation of 13 protein bands in all stages of collection and statistical significance of the difference without relative mobilities corroborates the statement that the analyzes treated in the same species. For the analysis of images obtained from different gels produced was possible to emphasize the separation of protein fractions homogeneously. The varying only in the intensity of bands in different stages, can be concluded that there is a significant difference in the percentage incorporation of forming fractions of proteins sarcoplasmic, which also corroborates with the research conducted by Dierckx et al. (2) who researched the loss of protein fractions in post mortem , but in other species, the swine. In this sense, in terms of muscle metabolism (11), metabolic changes coexist in several different species of muscular forms, by default, only changing due to the individual's genotype, its organoleptic characteristics. With the ANOVA analysis, it was possible to see that change occurs in the densitometry in all protein fractions. Can also be evaluated using the same analysis that the various metabolic events that begin at the time of death of the animal until the end of these collections were more intense in the range that reflects the period between 60" and 90", where the visually poultries were more prominent macroscopic features of *rigor mortis*.

The result was been observed in blades of a gradual degeneration of the fibers, indicating a decrease in the structural organization of the fibers. Comparing the beginning, t=0 , showing the characteristic organization of sarcoplasmic fibers which characterized the material as muscle tissue, and the following processing sequence shown in t=0, t=30"and t=60" (figure 3)". The figure 4 shows t = 90", t=120" and t=150", when in which the appearance observed was a single smooth structure, no organization of fibers, which characterized the material as meat, being selected for each picture that best represents the moment of transformation, comparing all slides.

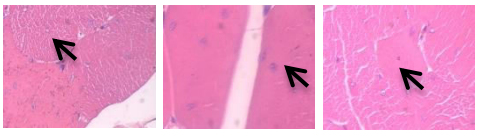


Figure 3 - Histological sections of *Major pectoralis*. Arrow indicates the organization of muscle fibers in processing.

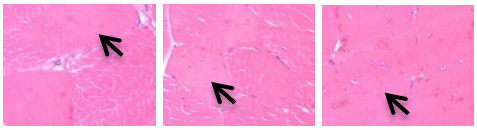


Figure 4 - Histological sections of *Major pectoralis*. Arrow indicates the organization of muscle fibers in processing.

The vital functions of the muscular system does not cease at death of the animal, and a series of biochemical and structural changes occur after slaughter, with the designation process of "conversion of muscle into meat" (10). Such modifications, accompanied by biochemical form could be observed in the present study where will sequentially noting the morphological changes (structural and organizational) in the investigated cells.

The results, when compared to papers made with meat from other species, has the same descriptive result, since only promote species change in the time factor in the process leads to obtain the resolution by the fact that the characteristics differ sarcoplasmic among themselves regarding the genetic characteristics differentiating species, but may also reflect the biochemical of sarcoplasmic proteins (5) behavior.

IV. CONCLUSION

It could be demonstrated that there is significant loss of protein fractions during the *post mortem* of poultries, especially as regards the density of the fractions present in the sarcoplasmic proteins. On the other hand, we could not demonstrate variations in relative mobilities of fractions of intra species form, which was found in the encounter with the literature. Morphological changes in *Major pectoralis* during the process of *rigor mortis*, was successfully obtained has been demonstrated descriptively changes occurring in several sequential time until his full resolution.

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