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Article

Effect Of Exposing Calf's Muscle (*Biceps Femoris*) To A Hydraulic Pressure On Some of Intrinsic Histological And Physiological Traits

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Abstract: Samples of Biceps Femoris muscle of Iraqi calves has been exposed to a hydraulic pressure and measuring some of physiological and histological changes. The experiment divided to five treatments, TRT cont. with no hydraulic pressure applied (0 bar), TRT2 with 50 bar, TRT3 with 100 bar, TRT4 with 200 bar and TRT5 with 300 bar hydraulic pressure applied. The studied physiological properties were (water holding capacity WHC, shear force SF, fragmentation index FI, myofibril fragmentation index MFI, protein solubility and myoglobin concentrations Mg. some sample from each treatment were tested microscopically under magnification of 600, 1000 and 1600X to find out the changes in muscle microstructure. All treatments had an obvious effect on muscle samples and the differences between means were significant ($p<0.05$). the histological profile of muscle differed significantly from each treatment to other with the increasing of applied pressure. At first (TRT2 50 bar) the muscle tissue structure had a slight deviation from TRT cont. while at the last treatment TRT5 (300 bar) the muscle microstructure torn up completely and the physiological tissue system had extremely disappeared.

Keywords: hydraulic pressure, muscle microstructure, WHC, shear force, FI, MFI, protein solubility, myoglobin concentrations, muscle histology

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1. Introduction

The histological and physiological changes which may occur in muscles profile may be a good method to study the factors that may lead to that changes whether pre or post mortem [1]. The most affective process that occur inside muscles after slaughter is the autolysis of the microstructure of them. This process will determine the whole future quality of the produced meat. The histological examination of muscles in the early periods after slaughter may enable the prediction of the quality in precis [2].

Skeletal muscle is consisting of many kinds of tissue, such as muscle fibers, connective tissue, adipose tissue, etc. This tissues are functionally cooperative with each other and the sum of these functions will reflex upon the physiological and histological whole profile mutually [3].

One of the most important and intrinsic phenomena that occur in muscle is the contraction-relaxation process. Which occurs naturally during animal life under the neural control or during arbitrary status through rigor-mortis process. Rigor mortis is the obvious status where the interaction between physiological functions and histological profile have appearance [3,4,5]. Rigor mortis related always with the aspect of tenderness the allusive nation that consider the output of all natural and unnatural operations that may happened pre or post animal mortem, so any treatment would interrupt or affect the normal processes will affect the degree of tenderness as well [5].

Under the light of all that the researchers in these fields of sciences used to manipulated one or more of the factors that effect on the rigor mortis or tenderness and study the effects even they were positive or negative by measuring the histological changes or testing the physiological properties or the interactions between the both [6].

Many kind of treatments had been designed to enhance the tenderness of meats via interacting with the autolysis process or interrupting it. Some of them were demanded upon mechanical aging [7] or traditional processes [8] or chemical or enzymatic treatments [9,10]. All kinds of these treatments have their benefits and limits, and all of them need to detected by one of the techniques that deal with these kind of works [11,12].

Our study is designed to interrupt the physiological normal process of rigor mortis in its earlier period by applying a hydraulic pressure obtained from a water pressing device on calves' muscle (biceps femoris BF) and study some of the physiological, histological and bacteriological changes in muscle microstructure.

2. Materials and Methods

2.1 Hydraulic pressure device: a hydraulic pressure generator has been made. The device was consisting of three main parts: the pressure pump which designed to generating a liquid (water) hydraulic pressure up to 400 bar (Kg/cm2), the applying pressure container this part designed to receive the pressure and conducting it to the muscle samples that were located inside the container cavity, the third part is the pressure gauge which was the part responsible for measuring the total applied pressure, all these three part were rested on a stainless steel platform.

2.2 Samples: four right leg (femoral) calf veal were purchased from the locally markets of Kerbala/ Iraq, exactly after slaughter. The muscles of biceps femoris BF had been extracted out manually by physical dissection. All BF muscles cut again to small pieces of approximately 50g to each one. The pieces were mixed together and divided randomly into five groups, each group has 20 piece of muscle samples. All samples kept in refrigerator inside plastic bags under cooling condition 4co for 30 minutes only, as a preparing for experiments.

2.3 Treatments: five treatments had been conducted in the experiment:

- Control treatment TRT cont.: no hydraulic pressure has been applied. 0 bar.
- Treatment one TRT2: the muscle sample has been exposed to 50 bar hydraulic pressure for five minutes.
- Treatment two TRT3: the muscle sample has been exposed to 100 bar hydraulic pressure for five minutes.
- Treatment three TRT4: the muscle sample has been exposed to 200 bar hydraulic pressure for five minutes.
- Treatment four TRT5: the muscle sample has been exposed to 300 bar hydraulic pressure for five minutes.

2.4 Studied properties:

2.4.1 physiological properties:

- Water holding capacity WHC: which was conducted as what was mentioned by Dolatowski and Stasiak [13] with some modification.
- Shear force SF: which was by applying measured force over a standard blade and measuring the required strength [14].
- Fragmentation index FI: that was conducted as Davis et, al. [15].
- Myofibril fragmentation index MFI: as what was illustrated by Culler et. al. [16].

- Protein solubility PS: following the procedure of Dentlertog-Meischke et. al. [17] and Gomell et. al. [18] protein solubility was calculated.
- Myoglobin concentration MG: under the method that was illustrated by Zessin et, al. [19] the myoglobin concentration was measured.

2.4.2 histological changes (changes in microstructure of muscle samples): slices from each sample was taken for the farther histological studies. Each slice was kept in formalin 10% for 48 hours. Then dried and kept as a cubes of paraffin wax. A micro-slice was then being taken (5micrometer), stained with hematoxylin-eosin as a prepare to being studied under microscope with 600, 1000, 1600 x respectively [20].

2.5 Statistical model: CRD design was used to analyze data. Then Duncan test has been applied to compare among treatments.

3. Results

3.1 Physiological properties:

3.1.1 WHC: it can be seen from figure 1 that the values of WHC were different as a response to the changes in hydraulic pressure that been applied. The lowest value was from TRT cont. (0 bar) (9.21±0.3). all values differed from each other significantly ($p<0.05$) except TRT3 (100 bar) with TRT5 (300 bar) which recorded (23±0.4), (23±0.11) respectively. The highest value was belonging to TRT4 (200 bar) (31±0.43) with significant difference with all others.

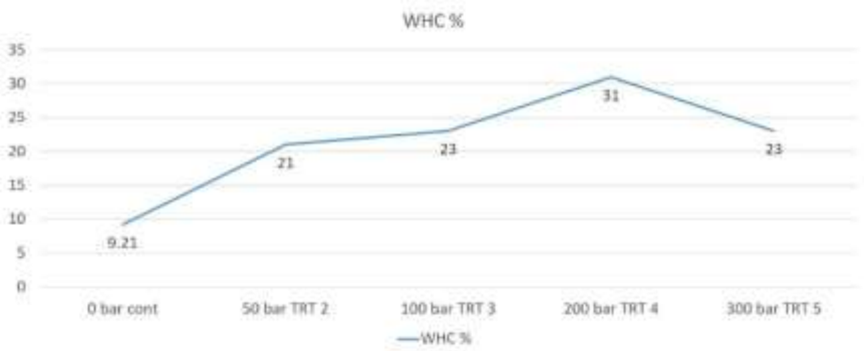


Figure 1: effect of hydraulic pressure on water holding capacity WHC in calf muscles

Water holding capacity is one of the most physiological properties, because it related directly with protein status and the surface area that afforded among its microstructure from side and the available chemical bond that may be Composed between protein structures and water molecules [21]. The changes in WHC values are a certain evidence on the changes in physiological properties, and these changes had their importance physiologically or nutritionally aspects. In general, the more WHC value the more enhanced meat. This results had the similarity with those of Bertram [22] how referred to the same explain about the bonds that may being occurred after the increasing of the surface area inside protein microstructure would occur. While the divergence that occurred in the last treatment TRT5 (300 bar) may be due to the exaggeration in protein structure breaking down that cause wide gabs inside protein structure in a manner that it's never being possible to the bonds to be composed between protein molecules and water molecules.

3.1.2 Shear force SF: the force that required to cut a sample of piece of PF muscle was illustrated in figure 2. This figure shows the gradually changes in required force with changes in the applied hydraulic pressure. The blade of warner-bratezler device required to a 5.88±0.05 Kg as an average to cut the samples of control treatment TRT cont. (0 bar). While it required only 3.3±0.04 Kg in treatment of 300 bar TRT5. All the values of other treatments distributed between these two values with significant differences except between the last two treatments TRT4 and TRT5 which recoded no significant differences between them.

The results in this study confirm the fact that there will be an alteration in protein microstructure when exposed to hydraulic pressure. And this fact came accorded to those of Swatland [23] and Lyon and lyon [24]. The decreasing in the value of the force that required to cut the muscle samples may be due to the changes that might occurred inside the muscle microstructure as an intrinsic alteration in physiological characteristics of muscles.

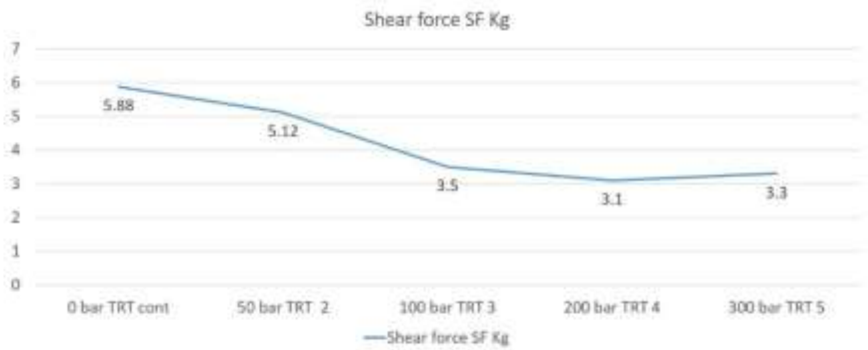


Figure 2: effect of hydraulic pressure on Shear force values in calf muscles

3.1.3 Fragmentation index FI: this property is one of the important one in muscle physiological studies. Because it measures the changes may occur in the muscle fiber level, so it may be close in this situation from the physiological aspects of the muscle as a physiological unit.

The values graduated from the highest one which belonged to control TRT cont. (0 bar) which was 290±0.6. which means that the lowest in meat tenderness as a related character to the physiological status of muscles, to the lowest value (150±0.4) that belonged to the last treatment of 300 bar TRT5, which considers the highest in tenderness if it been seen from meat technologies. All values differed from each other so significantly ($p<0.01$). it was clear from figure 3 that the value of FI in TRT3 (255±0.8) considered a midpoint between to physiological statuses, the muscle status before is completely in different manner in comparison with the values after it. This point may be considered as a physiological fatigue point when the response to the hydraulic pressure became at its peak. Davis et al. [25] linked between FI values and the degradation would be occurred in collagen fiber in muscles, and that's way it is related with tenderness in meat science and technologies. This results and ours came accorded to that of Cable [26] how mentioned to the physiological links between FI values and the status of connective tissues especially the collagen.

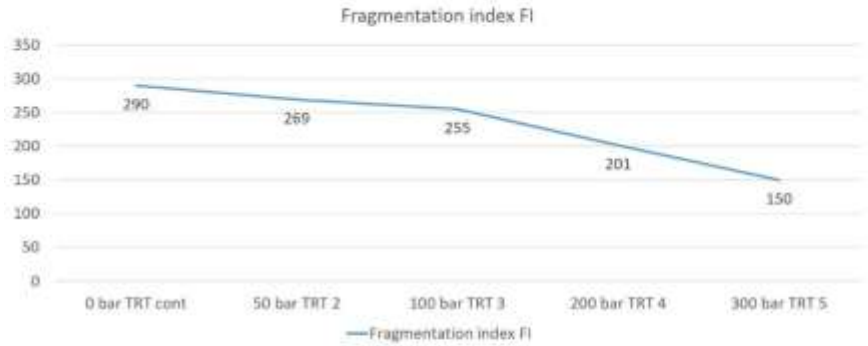


Figure 3: effect of hydraulic pressure on fragmentation index FI values in calf muscles

3.1.4 Myofibril fragmentation index MFI: this property is in the contrast to the previous one (FI), this means that the lowest value is the less in physiological changes.

Figure 4 exhibits the values in its range which began from the control treatment TRT cont. as a lowest value (57±0.02) to the highest one in the 300 bar treatment TRT5 (80±0.07), while all other values arranged between those two values respectively and significantly ($p<0.05$).

Many researches referred to the fact that the major part of skeletal muscles is the myofibril protein, which is responsible for transferring the movement that generated in muscles to the skeleton, and generating the movement from beginning by contraction-relaxation alternation process [27].

Physiologically, the condition of myofibril protein is a reflecting of the physiological history of the whole animal and an indicator to the total events that the animal been exposed to, like all environment condition and its genetics. All this writes its words intrinsically and gradually inside the myofibril protein status [28, 29].

The results in this study referred obviously to the response of myofibril protein to the changes in applied hydraulic pressure in a significant manner. This response appears as fragmentation and degradation in myofibril protein increase with the increment in hydraulic pressure. The results accorded with the results of Koohmaraie [30] and Wheeler et, al. [31] how mentioned that this status may become a good indicator to relating the factor that affecting myofibril protein with obtained meat tenderness.

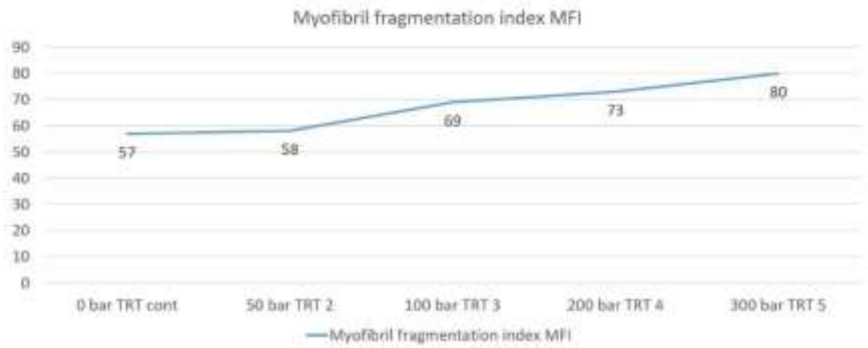


Figure 4: effect of hydraulic pressure on myofibril fragmentation index MFI values in calf muscles

3.1.5 Protein solubility: from the figure 5, it can be seen that the protein solubility increased with increasing applied hydraulic pressure. The lowest number in the TRT5 (300 bar) consider the highest soluble protein because the test measures the residual of insoluble protein. No significant differences were observed between control (0 bar) and TRT2 (50 bar), while all others treatments differed from each other significantly (p<0.05).

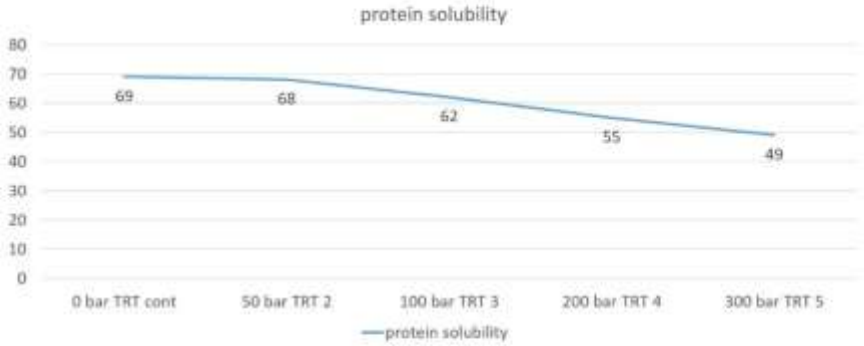


Figure 5: effect of hydraulic pressure on protein solubility values in calf muscles

Protein solubility is a situation deeper than MFI, because the myofibril fragments became finer and smaller in molecular weight, so they become more capable to being soluble [32]. Iwanowska et, al. [33] referred to the relation between animal physiological condition and myofibril protein solubility. The physiological situation demand upon age, sex, breed, etc. this physiological factor has a strong effect on this trait and may make the results different from condition to another [33]. The results in this study confirm that the myofibril protein solubility increase with the increasing of applied hydraulic pressure in very short time 95 minutes only) with any level on pressure in all treatments.

3.1.6 Myoglobin concentration: the applied hydraulic pressure had an obvious effect on myoglobin concentrations of muscle samples. These results had been shown in figure 6. The highest concentration was belonging to the control, it's the original concentration before any changes, which has the value (4.3±0.5) but just when a load applied in TRT2 (50 bar) the concentration decreased significantly (p<0.05) to (2.9±0.5). the decreasing in concentration continues with increasing of pressure until reaches (2.4±0.4) in TRT4 (200 bar). An obvious significant differences can be seen between the control and other treatments, but in general after TRT2 no significant differences would be observed. This may because the myoglobin pigment exposed to a kind of denaturation because of load applied or had a kind of lack in functions. (Figure 6)

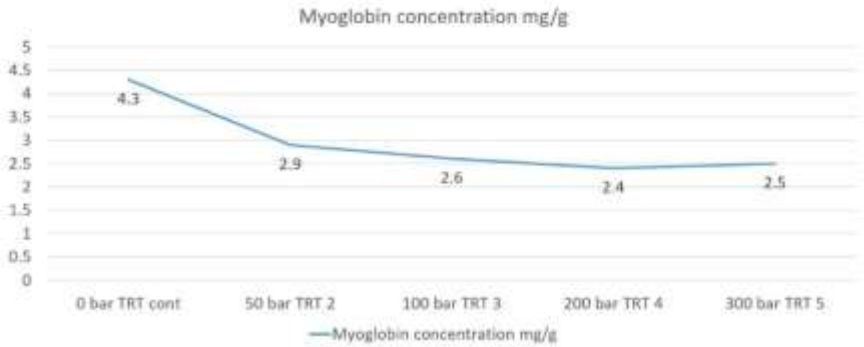


Figure 6: effect of hydraulic pressure on myoglobin concentration values in calf muscles

The physiological function of myoglobin is storing in muscles for aerobic oxidation in mitochondria, the muscle with low myoglobin concentrations tend to generating ATP from anaerobic function via oxidative metabolism [34], so, the high functioning muscle has high myoglobin concentration than the others. It can be seen from this study that the load applied has a negative effect on muscles appeared as denaturation in myoglobin pigment which would makes muscle lose its ATP metabolizing system. And the muscles have to possess more concentrations of myoglobin to compensating the loss [35,36]. It can be seen from Figure 7 that the color of sample changed very deep and the distinguished meat appearance which comes from intact myoglobin pigment disappeared immediately after applied 50 bar load (TRT2) and the new color continue up to the all next treatments. This results confirm the theory of compensation myoglobin concentration as an adaptation to environment pressure [34-36].

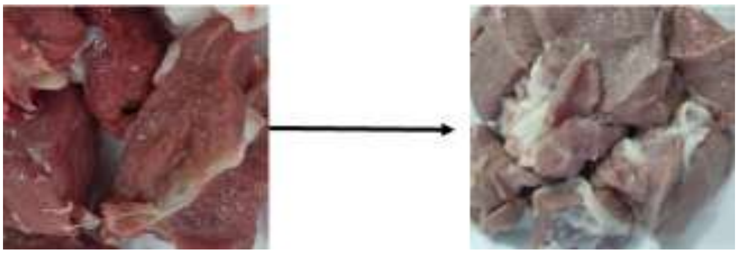


Figure 7: Changes in muscle samples color after applying 50 bar (TRT2) in five minutes only

3.2 Histological changes: studying the microstructure of muscle via histological method may offers a farther knowledge to understand the muscle properties in different situations directly as an eye vision [1]. Any changes in muscle after slaughter like proteolysis would have their reflection on the muscle microstructure and could been seen histologically under microscope as changes in muscle components (muscle fibers, connective tissues, lipids, etc) and the ratio among them [1,37].

In this study a histological analysis had bend conducted to a selected sample from each treatment and tested under microscope with different Magnification power (600X, 1000X, 1600X) to seeing the microstructure changes in muscle according with the changes in applied pressure. The selected pictures which had been shown are not insecurely all pictures obtains, but only some selected ones which have the clarity to been shown.

In Figure 8 exhibits a longitudinal section of a sample from TRT cont. with 0 bar (without pressure). The color of muscle (red) is still exists and appears. The sample looks coherent and no spaces could be seen between muscle fiber. The connective tissue could have been barely visible, absolutely no cracks among muscle components. In Figure 9 which exhibits a cross section of the same treatment (TRT cont.) under 1600X, the same coherence can be seen between muscle ingredients, cell nuclei can be seen also and two kinds of connective tissue (perimysium and endomysium). All vision reflected the compactness of intact muscle clearly.

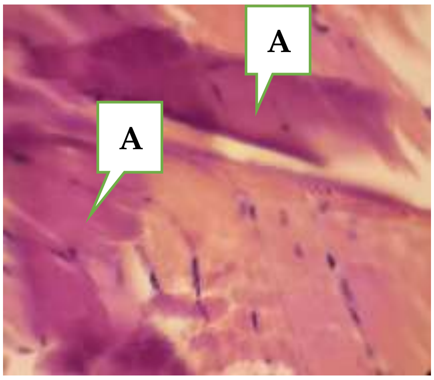


Figure 8: PF muscle sample 1000X
TRT cont. (0 bar)
longitudinal section

A: red, normal muscle color

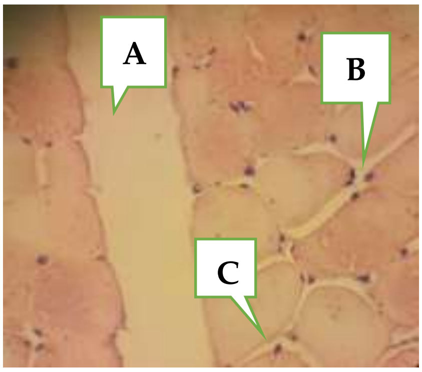


Figure 9: PF muscle sample 1600X
TRT cont. (0bar)
Cross-section
A: perimysium
B: endomysium
C: peripheral cell nucleus

Figure 10: PF muscle sample 1000X
TRT2 (50 bar)
longitudinal section
A: micro cracks
B: interaction of cracks with connective tissue appearance

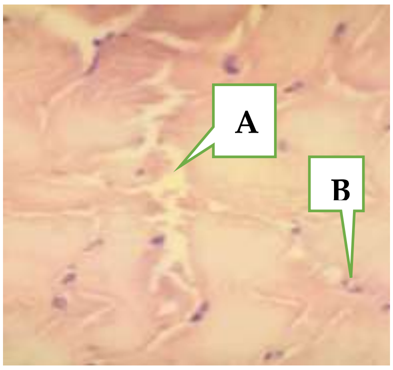


Figure 11: PF muscle sample 1600X
TRT2 (50 bar)
cross section
A: cracks
B: cell nucleus

In Figure 10 (longitudinal section 1000X) and Figure 11 (cross section 1600X) it could be seen the first micro cracks among the muscle components after being exposing to just 50 bar (TRT2). The redness of normal muscle slightly changed but the sample still considered red. The peripheral cell nuclei still appear clearly, but the borders that separate between muscle fibers and connective tissues became unclear or had some interaction with new cracks. The two picture clear the first obvious changes of microstructure muscle under moderate pressure.
Figure 12 and 13 (both longitudinal, 1000X) both exhibit a development in micro cracks with the increasing in exposed pressure which reach to 100 bar (TRT3), the muscle fibers begin to separate obviously against each other's and the connective tissue never been clear. Some of big cracks had been occurred in the muscle fibers themselves not only between them. Muscle color had not been appeared at all from now on. But cell's nuclei still appear in somehow. Muscle fibers appear individual in some part with no coherence between then at all. This treatment may consider the threshold of the intrinsic changes of muscles under hydraulic pressures.

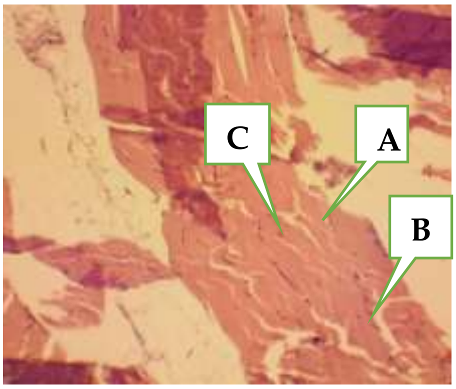


Figure 12: PF muscle sample 1000X
TRT3 (100 bar)
longitudinal section
A: micro cracks
B: big cracks
C: cell's nucleus

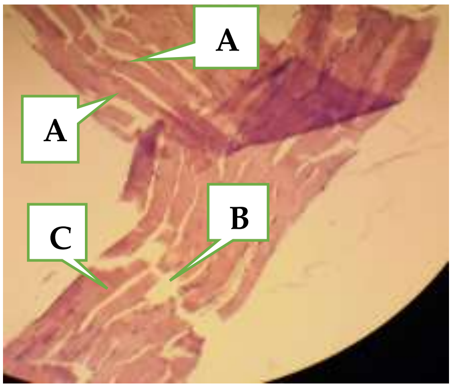
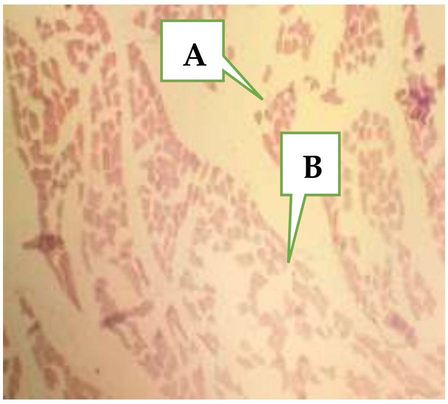


Figure 13: PF muscle sample 1000X
TRT3 (100 bar)
longitudinal section
A: individual muscle fibers
b: big cracks in the muscle fibers themselves (intrinsic changes).
C: cracks between muscle fibers

The changes in muscle samples after this points were so obvious. The microstructure of the muscle ultimately changed. Figure 15 exhibits a cross section of a sample exposed to 200 bar hydraulic pressure (TRT4) the muscle fibers appeared absolutely separated and the normal muscle color almost ultimately disappeared. No appearance to the connective tissue but the muscle fibers still exhibit a kind of aggregation but not in clear bundles. The cell nucleus not clear too.

Exposing muscle samples to an extremely high pressure in treatment 5 (TRT5 300bar) had the very intrinsic changed ever. Figure 16 showed that changed occurred in the muscle microstructure. It may be never called muscle at all. Individual denaturized muscle fibers exist under microscope without any kind of physiological system. The muscle tissue under this kind of pressure loss any tissue properties may described.

From the side of meat technology, the muscle under pressure of the treatments of 100 bar and 200 bar may have an enhancement in its properties particularly its tenderness. While the muscle under the last pressure 300 bar would become out of benefits because of the extremist changed had been occurred. So it may become obvious the interaction between physiological properties and histological profile of muscle tissue.



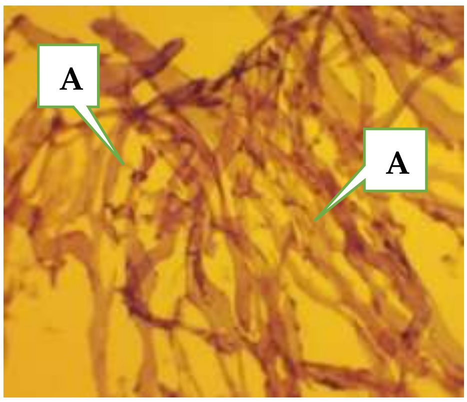


Figure 15: PF muscle sample 600X
TRT4 (200 bar)
cross section

A: muscle fibers aggregations
B: individuals muscle fibers

Figure 16: PF muscle sample 1600X
TRT5 (300 bar)
longitudinal section

torn muscle fibers with no physiological system
chaos manner

4. Conclusion

Applying hydraulic pressure on muscle sample has an obvious effect on many of physiological and histological properties. Physiologically, the protein properties have been changed. This changes appear as a deviation in some criteria like WHC and protein solubility and so on. This deviation may have a good or a bad effect on muscle, and the effect would be considered good or bad according to the aspect of view, it may be good if we look to the matter from the meat technologies view or it be bad if we look from the muscle function Point of view.

That deviation in physiological properties always accompanied with deviation in histological profile. The microstructure of muscle tissue would suffer of a dramatically changes in its components and relation among them. Muscle fibers may lose gradually its internal matrix and muscle tissue will lose its physiological functions. And all these changes may have a good enhancement of meat properties if we look to that from meat science point of view. Except the final treatment with 300 bar pressure, it could have been seen a kind of physiological system within the components while this will be diminished at the last.

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