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Comparative Micromorphological Study on the Pectoralis Muscle in the Flying and non-Flying Birds.

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ABSTRACT

The pectoralis major muscle of twenty adult female fowl and pigeon were examined using histological, histochemical and transmission electron microscope "TEM" methods. In addition to morphometric, histomorphometric, and enzyme activity determination methods were also performed. The histological examination showed that the pectoralis major muscle fibers of fowl run in different directions with few blood capillaries and more distinct cross striations. The opposite picture was found in pigeon. Histochemical stains showed more pronounced glycogen granules and myoglobin in pigeon muscle. The calcium granules were unequally distributed in both fowl and pigeon muscle. Ultrastructurally, the muscle fibers of this muscle in the fowl had different thickness and wide sarcomeres with thin Z-lines. The reverse structure was present in case of pigeon. Large numerous electronlucent lipid droplets were observed in pigeon muscle and not observed in fowl. Morphometric parameters and statistical analysis revealed no significant differences between sarcomere length in both birds but significant differences were obtained in the muscle fiber diameter. The histomorphometrical analysis of the white fibers of the pectoralis major muscles revealed highly significant differences between the fowl and pigeon and found significant differences between the red ones. Signifcant differences between the level of lipase and lactate dehydrogenase enzymes in this muscle of both birds were also recognized.

Keywords: Pectoralis muscle, Fowl, Pigeon, Ultrastructure and Histochemistry

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INTRODUCTION

Studies on the muscles were conducted in various species of birds **(George and Berger, 1966** and **Biewener, 2011)** in pigeon, **(Barnard et al., 1982)** in chicken, **(Jennifer et al., 2005)** in sparrow and **(Meyers and Stakebake, 2005)** in albatrosses. Scanty information was obtained in the available literatures regarding the comparative morphological and histochemical studies of the major flight muscles in domestic fowl, guinea fowl and pigeon.

Skeletal muscles were a heterogenous tissue that was characterized by multiple types of muscle fibers that affect diversified characteristics of particular muscles **(Bottinelli and Reggiani, 2000).**There were many factors that contribute to changes in the character of muscle fibers, these including: age **(Candek-Potokar et al., 1998),** physical activity **(Jurie et al., 1999** and **Karlssona et al., 1999),** gender **(Ozawa et al., 2000)**, and breed **(Ryu et al., 2008).**

The size and number of muscle fibers were factors that influence muscle function. It was well known that biophysical, histological and biochemical characteristics of muscle fibers play a key role of this subject **(Tumová and Teimouri, 2009)**.

Edwards et al. (1956) mentioned high frequency muscle (represented by flight muscle of hummingbird) are characterized by widely spaced, non-branching fibers of large diameter and short period. They showed little endoplasmic reticulum, and large quantities of large mitochondria. This structure was correlated with heavy vascularization, high oxidative activity, and dark color as compared with other muscles of the same species. In the vertebrates, low frequency muscle was characterized by relatively long period and few mitochondria.

MATERIALS AND METHODS

Collection of the samples

Twenty mature adult apparently healthy female fowl *"Galliformes"* and pigeons "*Columbiformes*" were obtained from poultry farm at Giza for light microscopy, histochemical stains, transmission electrone microscopy, morphometric, histomorphometric parameters and enzyme activity determination.

Light microscopy

The birds were slaughtered and the pectoralis major muscle "Breast" was dissected out immediately after death. Samples were fixed in 10% neutral buffered formalin, dehydrated in ascending grades of alcohol, cleared in benzene and methyl benzoate then embedded in paraffin wax. Paraffin blocks were sectioned at (3-5 μm thick) by rotatory microtome. Sections from each block were stained using the following methods:

Routine histological stains:

(1) Harris haematoxylin and Eosin (H&E) stain for general tissue structure.
(2)Masson's trichrome stain for demonstration of collagenous and muscle fibers**.**
(3) Gomori's reticulin method for demonstration of reticular fibers.
(4) Aldehyde fuchsin stain for demonstration of elastic fibres.
Histochemical stains:
(5) Best's carmine stain for demonstration of glycogen.
(6)Alcian blue pH 2.5 stain for demonstration of acidic mucopolycaccharides.
(7) Dunn-thompson method for demonstration of myoglobin.
(8) Von-kossa stain for demonstration of calcium.

The forementioned stains were conducted as outlined by **Pearse (1968), Druary and Wallington (1980)** and **Bancroft and Stevens (1996).**

Evaluation of histochemical observations:

Histochemically stained sections were examined using Leica Quin 500 analyser computer system (Leica Microsystems, Switzerland) in Faculty of Dentisty, Cairo university. The image analyser was calibrated automatically to convert the measurement units (pixels) produced by the image analyser program into actual micrometer units. Histochemical stains were measured as optical density and area percent in standard measuring frame in 5 fields from different slides in each group using magnification (X400) by light microscopy transferred to the monitor's screen. The areas showing the positive histochemical stains reaction were chosen for evaluation, regardless the intensity of the staining. These areas were masked by a blue binary colour to be measured by the computer system **(Figs.1&2).** Mean value and standard deviation were obtained for each specimen and statistically analysed.

Transmission Electron Microscopy (TEM)

Small tissues of pectoralis muscle from fowl and pigeon were fixed in paraformaldehyde-glutaraldehyde in phosphate buffer **(Karnovsky, 1965)**. Specimens were post fixed in 1% osmium tetraoxide for one hour, washed in 0.1 M phosphate buffer (pH 7.3), then dehydrated gradually in graded ethanol and embedded in open araldite mixture **(Mollenhaur, 1964)**. Semi-thin sections (1 µm thick) were cut, stained with toluidine blue **(Richardson et al., 1960)** and examined with light microscope**.** Ultrathin sections were cut and stained with uranyl acetate and lead citrate and examined under Transmission Electron Microscope TEM -a JEOL 1010 at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University.

Morphometric parameters to sarcomere length and muscle fiber diameter:

A core (2.54 cm) of muscle tissue was fixed in formal saline for 24 hours and blended using Lab blender at low speed for 30 seconds. A drop of the homogenate was placed over a glass slide, covered with cover slip and examined under a light microscope (Labomed, INC. U.S.A.) with X10 eye piece containing a calibrated micrometer. Sarcomere length and diameter of 21 muscle fibers of each bird were measured and the average of each measurement was expressed in microns **(Tuma et al., 1962) (Figs.3&4).** Mean value and standard deviation were obtained for each specimen and statistically analysed.

Histomorphometrical analysis:

Sections stained with H&E stain were used for histomorphometrical study. Five cross sections from white "broad" and red "narrow" fibers of fowl and pigeon pectoralis major muscle were measured by X40 eyepiece. A computerized microscopic image analyzer attached for full HD microscopic camera (Leica Microsystems, Germany) was used to determine the histomorphometric parameters using statistical analysis.

Enzyme activity determination:

It was performed at Animal Health Research Institute in El-Dokki.

Preparation of tissue homogenate:

0.2 gm from each muscle tissue sample was mechanically dissociated in 2ml phosphate buffer saline "PBS" using glass homogenizer. The cell lysate was centrifuged at 14000 xg for 20 minutes. The supernatant homogenate was stored for biochemical analysis. Enzyme activity determination included Lipase activity **(Moss et al., 1999)** and Lactate dehydrogenase "LDH": **(Vanderheiden et al., 1994).**

Statistical analysis:

Data related to the optical density, area percent, morphometric, histomorphometric parameters, and enzyme activity determination were presented as mean and standard deviation (SD) values. Student's t-test was used to determine statistical significance ($P \leq 0.05$) among the different groups. Calculations were performed using statistical package for social science (SPSS) software version 16 package for windows.

RESULTS

Light microscopy observations:

The pectoralis major muscle of fowl consists of bundles with fibers run in different directions while that of pigeon are regularely distributed (Figs.5&6). Moreover, in case of fowl these muscle fibers are branched, with distinct cross striations and peripheral, elongated flattened nuclei. Few blood capillaries are noticed between the fibers (Fig.7). The fibers of pigeon muscle have wide spaces in between, ill distinct cross striations and oval nuclei. These fibers are highly vascularized (Fig.8). In both fowl and pigeon, two types of muscle fibers are identified which differ in diameter; broad "white" fibers and narrow "red" ones. They show different distribution within the bundles. In case of fowl the broad fibers are randomly distributed within the bundles while in pigeon they are mostly peripherally situated (Figs.9&10), (Table. 1).

The collagen and reticular fibers are found between the muscle fibers and surround the blood vessels in both fowl and pigeon but the reticular fibers are more obvious in fowl than pigeon (Figs.11, 12, 13&14). On the other hand the elastic fibers are ill distinct in both birds.

Histochemical observation:

The glycogen granules are more prominent in the white fibers. In case of fowl these granules are few, dispersed and unequally distributed. On the contrary, they are more concentrated peripherally in case of pigeon. It shows the same optical density "O.D." but differs in the area percent "A.P." (Figs.15&16), (Table.2).

The myoglobin is more concentrated inside the red fibers in both birds. Moreover, it has higher density in the pigeon muscle fibers than in the fowl (Figs.17&18), (Table.2).

Both fowl and pigeon muscles show similar densities of the black calcium granules which are distributed inside the muscle fibers (Figs.19&20), (Table.2).The connective tissue of the muscle fibers show positive reaction for Alcian blue pH2.5 in both fowl and pigeon (Figs.21&22).

Transmission Electron Microscopy "TEM" observations:

Ultrastructurally the muscle fibrils of fowl are clear and have different thickness. They are widely separated due to increased amount of the sarcoplasm. The sarcomeres are wide with thin Z-lines**.** On the other hand the myofibrils of pigeon muscle are of regular thickness and very close to each other with little amount of the sarcoplasm. The sarcomeres are narrower than that of fowl with thicker Z-lines (Figs. 23&24).

The sarcolemma of the muscle fibers is more electron dense in fowl than pigeon and shows deep invaginations inside the sarcoplasm of fowl's muscle (Figs.25&26). The nucleus of fowl muscle is situated peripherally under the sarcolemma or between the myofibrils. It is ovoid, elongated with regular nuclear membrane and euchromatic with one or two nucleoli; some chromatin materials are condensed under the nuclear membrane (Figs.27&28). The nuclei of the muscle fibers of pigeon are oval with irregular nuclear membrane and more heterochromatin. Several chromatin materials are condensed under the nuclear membrane in addition to dispersed chromatin inside the nuclear matrix (Fig.29).

Singly arranged mitochondria are observed between the muscle fibrils of fowl (Fig.30). In the same time in pigeon, numerous mitochondria are arranged in several rows under the sarcolemma in contact with the sarcoplasmic reticulum and between the myofibrils. They have different sizes and shapes "spherical to oval" with characteristic complete elongated cristae which are sometimes bridged from side to side of the outer mitochondrial membrane. Some mitochondria are closely packed and connected or open with each other (Figs.31&32).

Glycogen granules are dispersed between the myofibrils and they are less electron dense in pigeon than in fowl (Figs.32&33). Several electronlucent lipid droplets of variable sizes are observed in the pigeon muscle between the myofibrils but not detected in the pectoralis muscle of fowl (Fig.34).

Morphometric and histomorphometric parameters:

Sarcomere length "SL"(µm)

No significant differences with icon statistics was found between SL mean of pectoralis major muscle of both fowl and pigeon as the P-value of the test reached 0.114 and it was more than the standard error 0.05 as shown in Table (1) and Histogram (1).

Muscle fiber diameter" MFD"(µm)

There was highly significant differences** with icon statistics between MFD mean of pectoralis major muscle of both fowl and pigeon as the P-value of the test reached 0.00527 and it was less than the standard error 0.05 as illustrated in Table (1) and Histogram (1).

White and red fibers diameter (µm)

Highly significant differences**with icon statistics was obtained between mean diameter of white fibers of pectoralis major muscle of both fowl and pigeon as the P-value of the test reached 0.003 and it was less than the standard error 0.05. However, there was significant differences* between red fibers of the same muscle as p-value reached 0.016 as in Table (1) and Histogram (1).

Evaluation of histochemical observations:

Optical density for glycogen:

There was no significant differences with icon statistics between mean of glycogen optical density of pectoralis major muscle of both fowl and pigeon as the P-value of the test reached 0.222 and it was more than the standard error 0.05 as in Table (2) and Histogram (2)

Area percent for glycogen:

Significant differences* with icon statistics was obtained between mean of glycogen area percent of pectoralis major muscle of both fowl and pigeon as the P-value of the test reached 0.006 and it was less than the standard error 0.05 as revealed in Table (2) and Histogram (2)

Optical density for myoglobin:

There was significant differences* with icon statistics between mean of myoglobin optical density of pectoralis major muscle of both fowl and pigeon as the P-value of the test reached 0.036 and it was less than the standard error 0.05 as revealed in Table (2) and Histogram (2).

Optical density for calcium:

The mean of the calcium optical density of pectoralis major muscle of both fowl and pigeon showed no significant differences with icon statistics as the P-value of the test reached 0.340 and it was more than the standard error 0.05 as in Table (2) and Histogram (2)

Enzyme activity determination:

Lipase (u/gm):

The results showed highly significant differences** with icon statistics between mean of lipase enzyme level in pectoralis major muscle of both fowl and pigeon as the P-value of the test reached 0.002 and it was less than the standard error 0.05 as revealed in Table (3) and Histogram (3).

Lactate dehydrogenase "LDH" (u/gm):

The pectoralis major muscle of both fowl and pigeon showed significant differences* with icon statistics between mean of LDH enzyme level as the P-value of the test reached 0.038 and it was less than the standard error 0.05 as revealed in Table (3) and Histogram (3).

Tables and histograms:

Table (1): Sarcomere length "SL", muscle fiber diameter" MFD" and white & red fibers diameter in µm (mean ± SE) of pectoralis major muscle in fowl and pigeon.

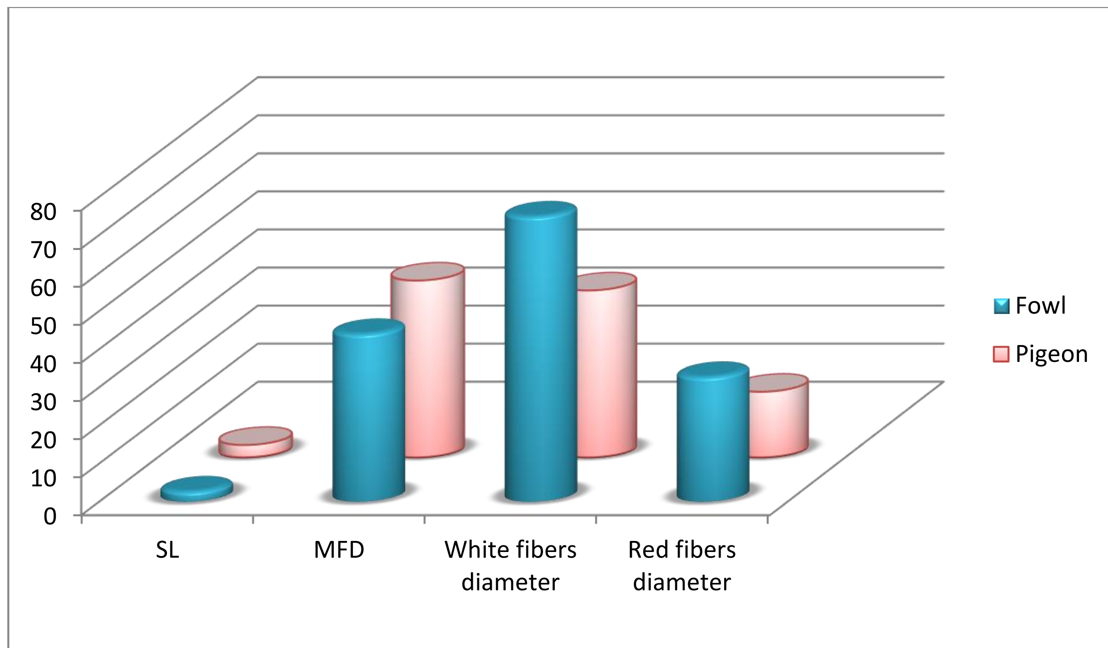
		Bird	Mean	SD	P-value
Morphometric	**SL**	Fowl	2.67	0.5	0.114
		Pigeon	3.23		
	MFD	Fowl	44	1	0.00527**
		Pigeon	46.4		
Histomorphometric	**White fibers**	Fowl	74.8700	5.84857	0.003**
		Pigeon	43.8000	9.14310	
	Red fibers	Fowl	32.7100	8.58133	0.016*
		Pigeon	17.1800	1.56828	

Table (2): Glycogen, myoglobin and calcium optical density "O.D." and glycogen area percent "A.P." (mean ± SE) of pectoralis major muscle in fowl and pigeon.

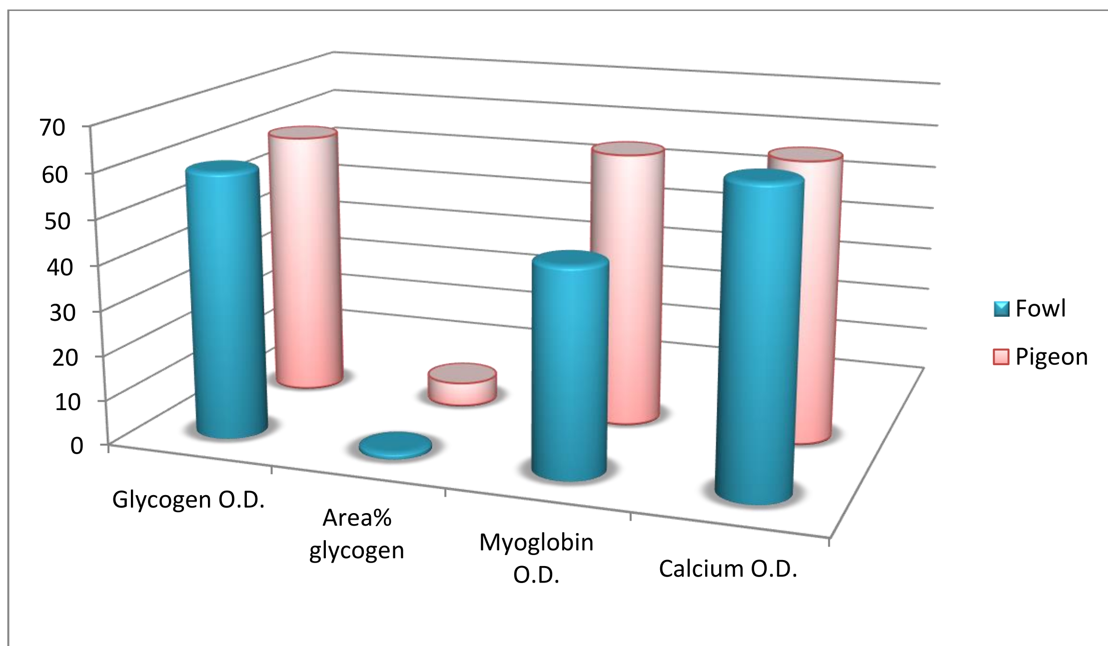
	Bird	Mean	SD	P_value
Glycogen O.D.	Fowl	59.333333	.5773503	0.222
	pigeon	59.833333	.1625833	
Glycogen A.P.	Fowl	1.33	.577	0.006*
	pigeon	5.33	1.155	
Myoglobin O.D.	Fowl	45.732500	8.0601794	0.036*
	pigeon	61.092500	8.0958606	
Calcium O.D.	Fowl	65.532500	4.8807675	0.340
	pigeon	62.395000	3.5937399	

Table (3): Lipase and LDH levels in u/gm (mean ± SE) of pectoralis major muscle in fowl and pigeon.

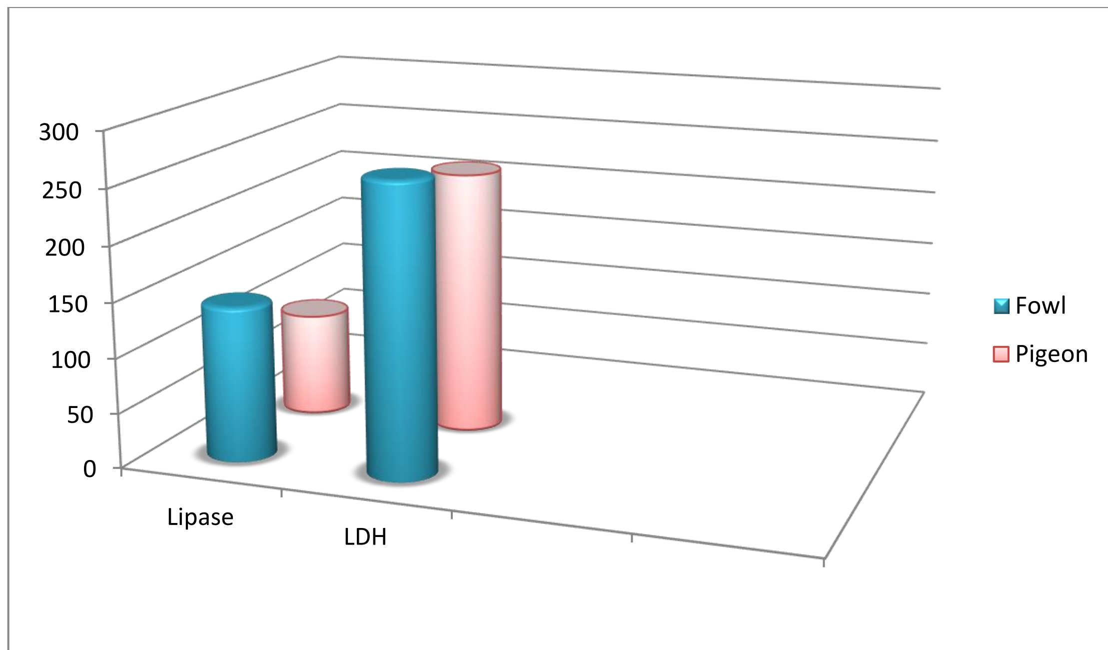
	Bird	Mean	SD	P-value
Lipase	Fowl	141.5	11.0	0.002**
	Pigeon	94	6.2	
LDH	Fowl	265	18.5	0.038*
	Pigeon	240	18.0	



Histogram (1): Morphometric and histomorphometric parameters (SL, MFD and white & red fibers diameter in "µm") of pectoralis major muscle of both fowl and



Histogram (2): Evaluation of histochemical observations of pectoralis major muscle of both fowl and pigeon.



Histogram (3): Enzyme activity determination "lipase and LDH" (u/gm) of pectoralis major muscle of both fowl and pigeon.

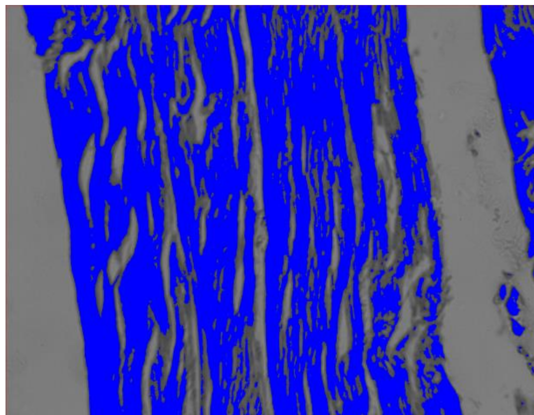


Fig.1: photomicrograph showing optical density (O.D.). X40

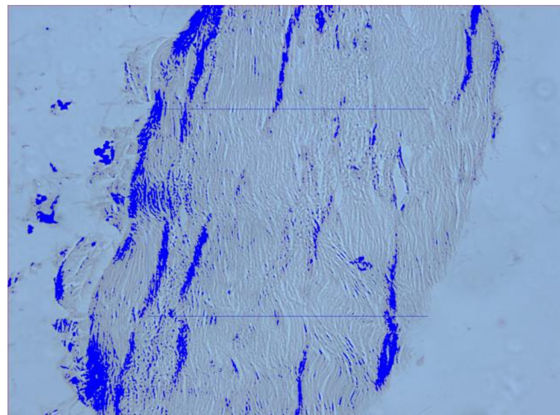


Fig.2: photomicrograph showing area percent (A.P.). X40

Fig.3: photomicrograph showing measurement of sarcomere length (SL). X10

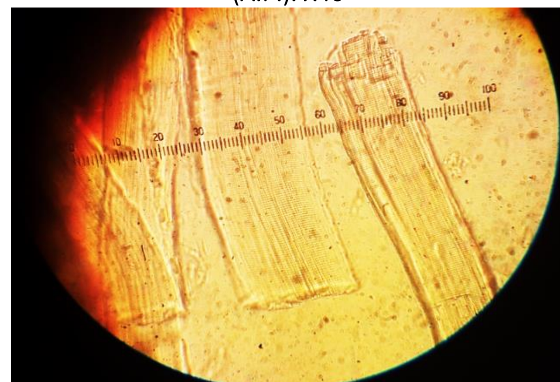


Fig.4: photomicrograph showing measurement of muscle fiber diameter (MFD). X10

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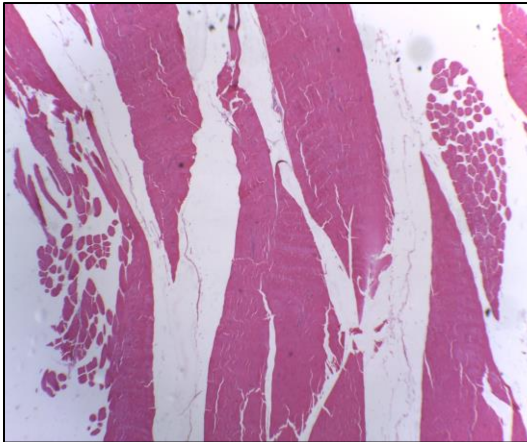

Fig.5: photomicrograph of longitudinal section of fowl's pectoralis muscle showing muscle bundles with fibers in different directions. H&E stain, X4

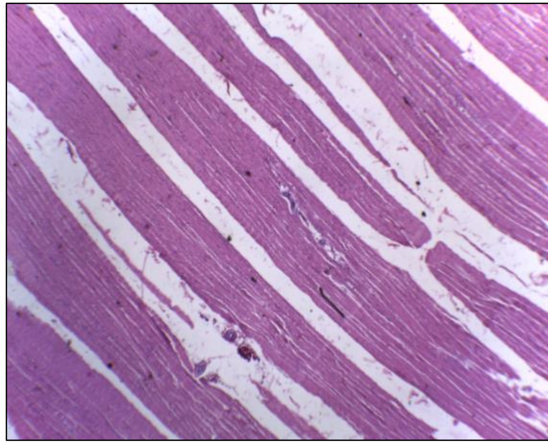

Fig.6: photomicrograph of longitudinal section of pigeon's pectoralis muscle showing bundles with regular muscle fibers. H&E stain, X4

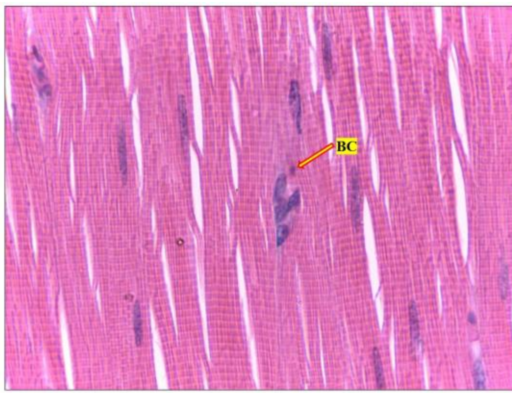

Fig.7: photomicrograph of longitudinal section of fowl's pectoralis muscle showing branching, prominent striations, flat elongated nuclei and few blood capillaries "BC". H&E stain, X100

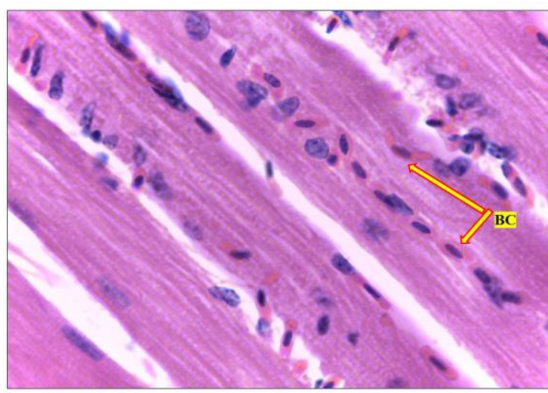

Fig.8: photomicrograph of longitudinal section of pigeon's pectoralis muscle showing wide spaces between muscle fibers, ill distinct striations, oval nuclei and many blood capillaries "BC". H&E stain, X100

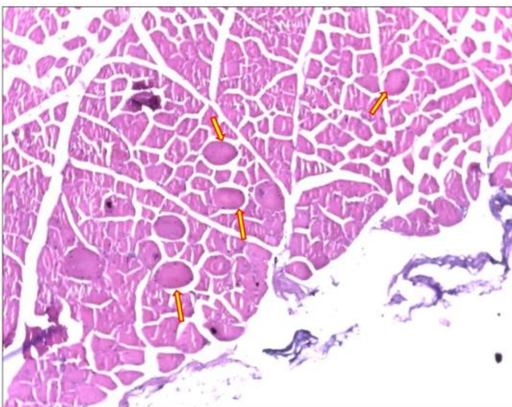

Fig.9: photomicrograph of cross section of fowl's pectoralis muscle showing white fibers "arrows" randomly distributed within the bundles and red fibers. H&E stain, X10

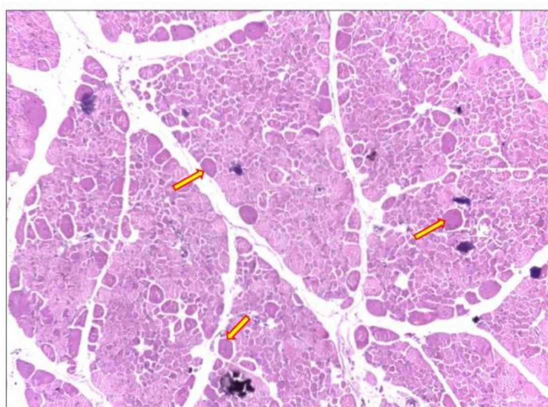

Fig.10: photomicrograph of cross section of pigeon's pectoralis muscle showing white fibers "arrows" mostly peripherally situated within the bundles and red fibers. H&E stain, X10

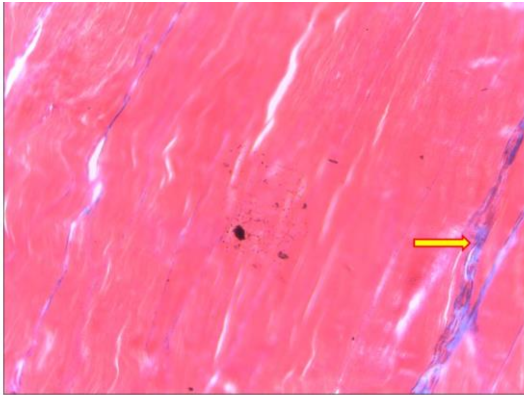

Fig.11: photomicrograph of longitudinal section of fowl's pectoralis muscle showing collagen fibers "arrow" between muscle fibers and around blood vessels. Masson's Trichrome stain, X40

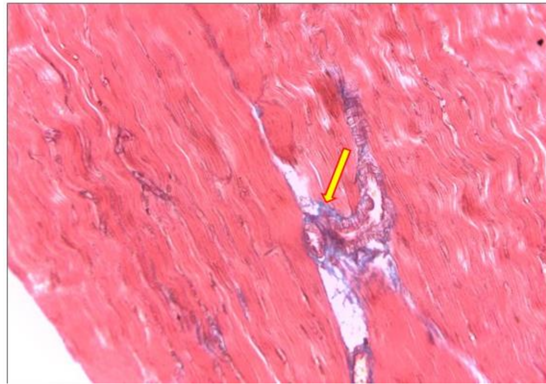

Fig.12: photomicrograph of longitudinal section of pigeon's pectoralis muscle showing collagen fibers "arrow" between muscle fibers and around blood vessels. Masson's Trichrome stain, X40

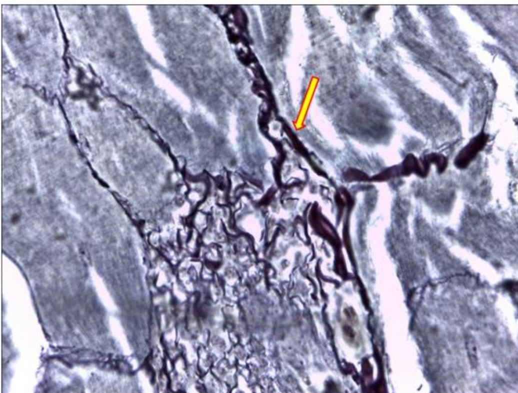

Fig.13: photomicrograph of longitudinal section of fowl's pectoralis muscle showing reticular fibers "arrow" between muscle fibers and around blood vessels. Gomori's reticulin stain, X100

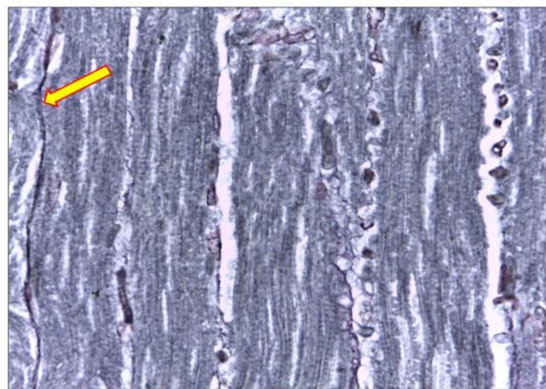

Fig.14: photomicrograph of longitudinal section of pigeon's pectoralis muscle showing reticular fibers "arrow" between muscle fibers. Gomori's reticulin stain, X100

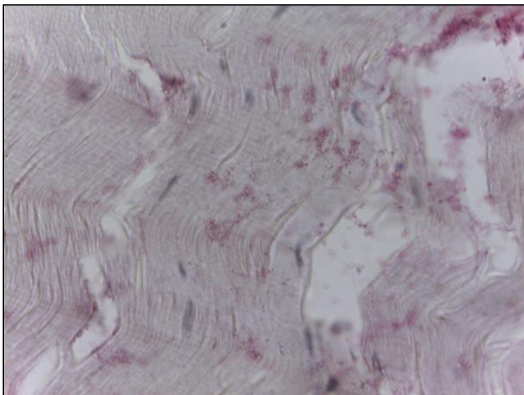

Fig.15: photomicrograph of longitudinal section of fowl's pectoralis muscle showing few dispersed glycogen granules unequally distributed inside muscle. Best's carmine stain, X100

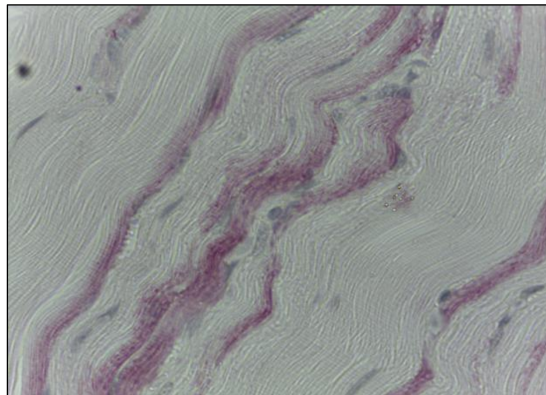

Fig.16: photomicrograph of longitudinal section of pigeon's pectoralis muscle showing glycogen granules concentrated peripherally inside muscle fibers. Best's carmine stain, X100

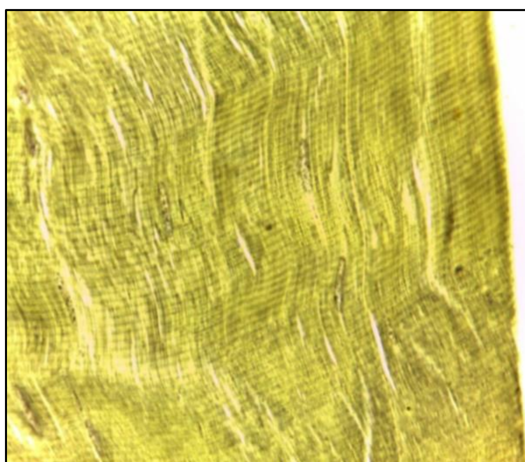

Fig.17: photomicrograph of longitudinal section of fowl's pectoralis muscle showing low density myoglobin inside red fibers. Dunn thompson methode, X100

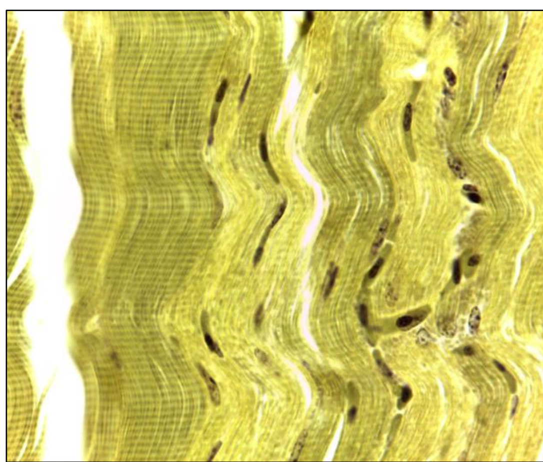

Fig.18: photomicrograph of longitudinal section of pigeon's pectoralis muscle showing high density myoglobin inside red fibers. Dunn thompson methode, X100

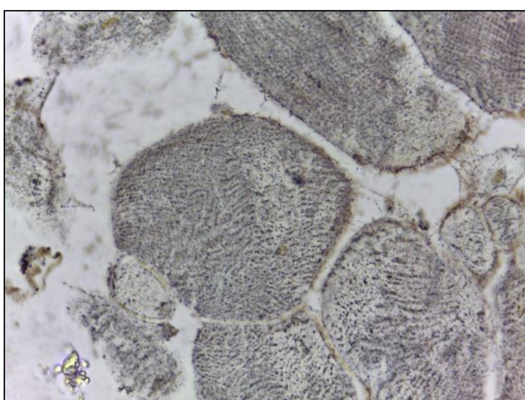

Fig.19: photomicrograph of cross section of fowl's pectoralis muscle showing Ca granules distributed inside muscle. Von kossa stain, X100

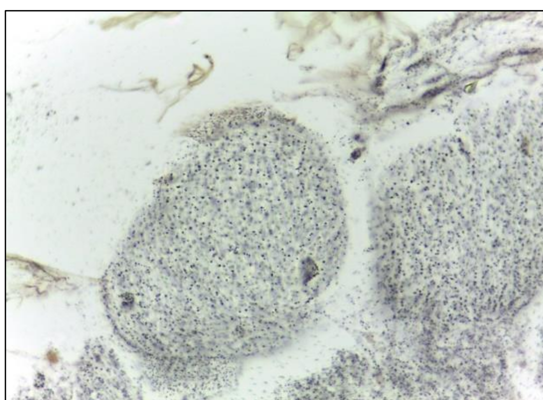

Fig.20: photomicrograph of cross section of pigeon's pectoralis muscle showing Ca granules distributed inside muscle. Von kossa stain, X100

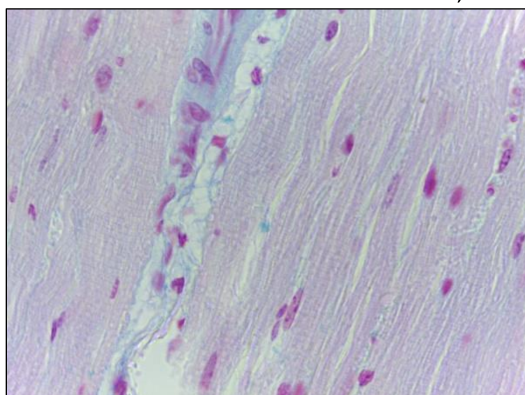

Fig.21: photomicrograph of longitudinal section of fowl's pectoralis muscle showing positive reaction in the connective tissue. Alcian blue pH2.5, X100

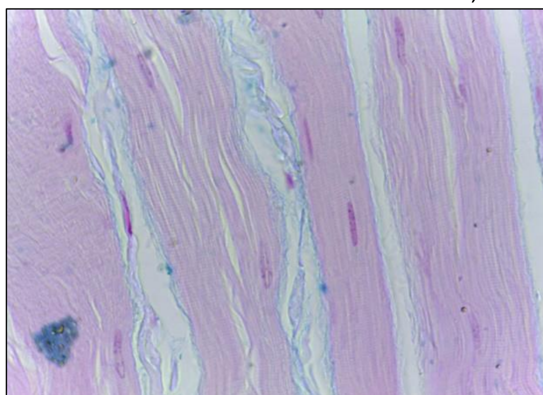

Fig.22: photomicrograph of longitudinal section of pigeon's pectoralis muscle showing positive reaction in the connective tissue. Alcian blue pH2.5, X100

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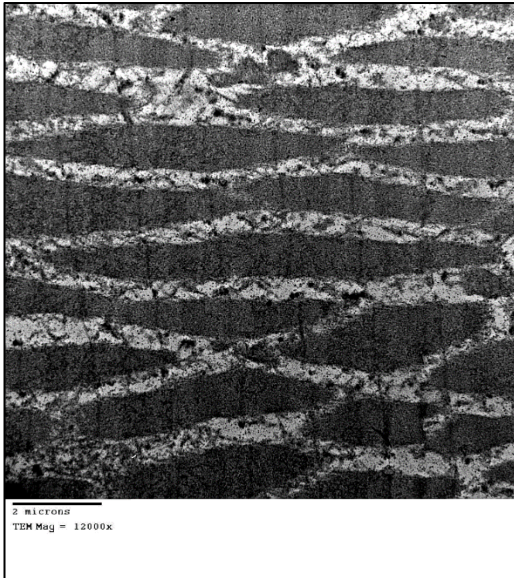

Fig.23: Transmission electron micrograph of pectoralis muscle of fowl showing wide sarcomeres and thin Z-lines. X12000

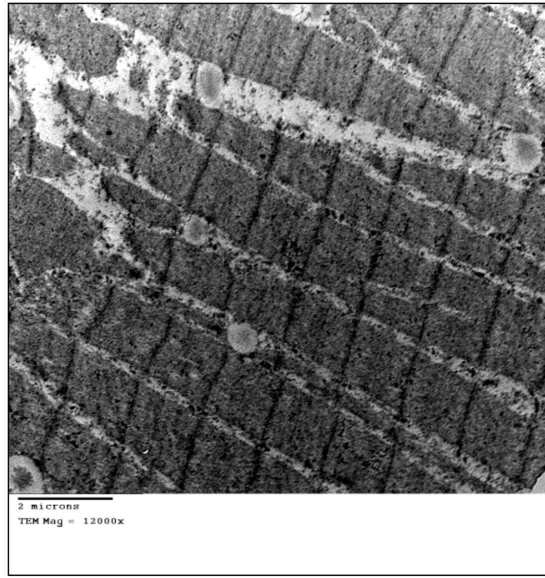

Fig.24: Transmission electron micrograph of pectoralis muscle of pigeon showing narrow sarcomeres and thick Z-lines. X12000

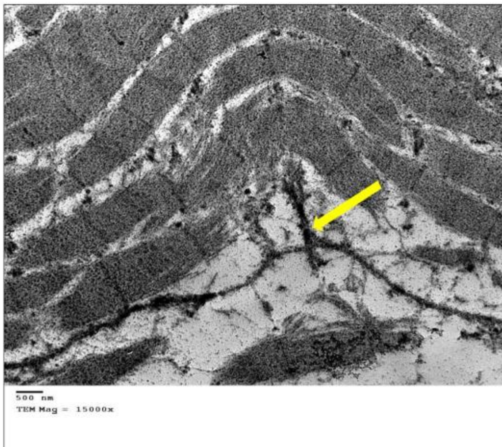

Fig.25: Transmission electron micrograph of pectoralis muscle of fowl showing invagination of sarcolemma "arrow". X15000

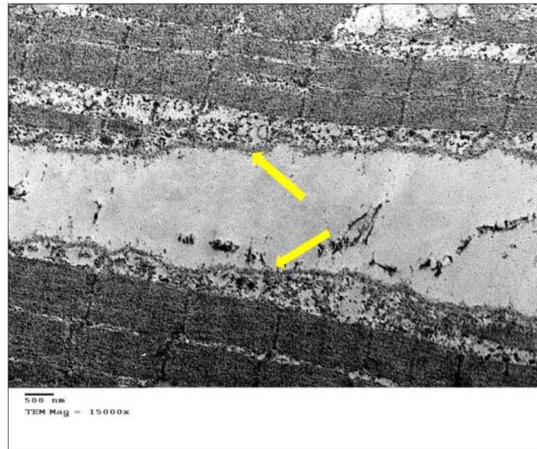

Fig.26: Transmission electron micrograph of pectoralis muscle of pigeon showing thin sarcolemma "arrows". X15000

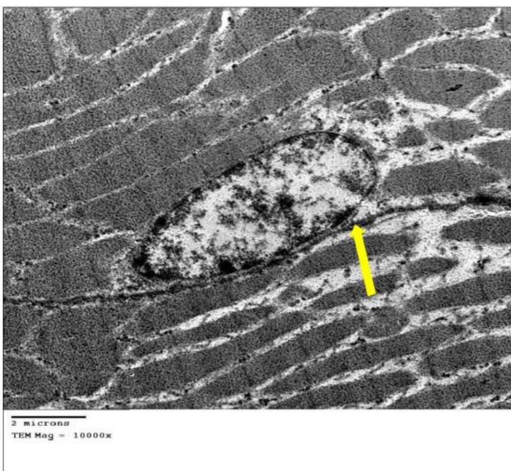

Fig.27: Transmission electron micrograph of pectoralis muscle of fowl showing elongated euchromatic nucleus under sarcolemma "arrow". X15000

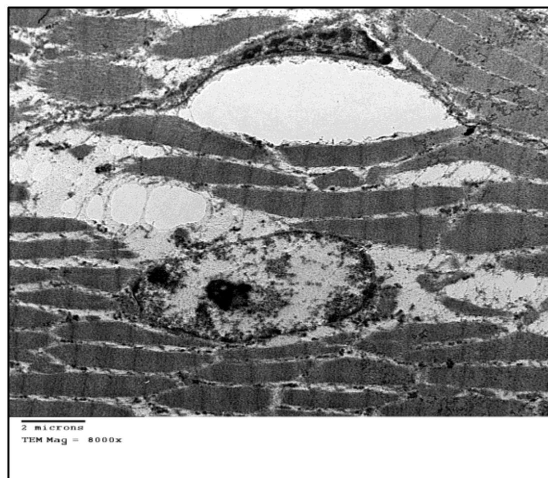

Fig.28: Transmission electron micrograph of pectoralis muscle of fowl showing nucleus between the myofibrils. X8000

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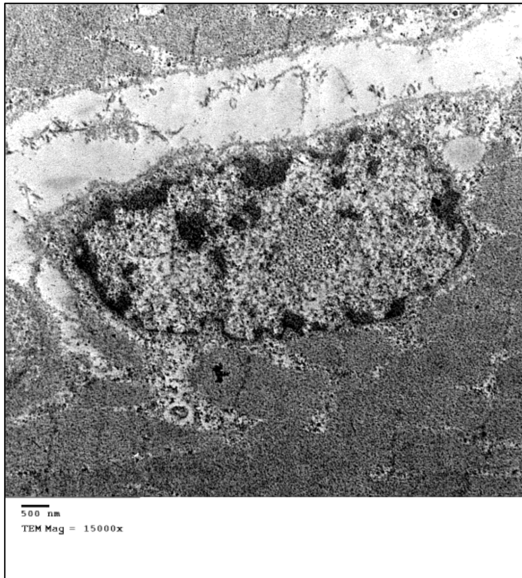

Fig.29: Transmission electron micrograph of pectoralis muscle of pigeon showing ovoid heterochromatic nucleus. X15000

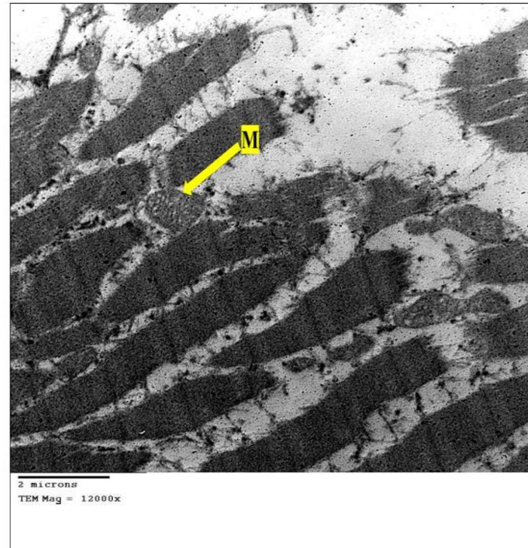

Fig.30: Transmission electron micrograph of pectoralis muscle of fowl showing single mitochondria "M" between the myofibrils. X12000

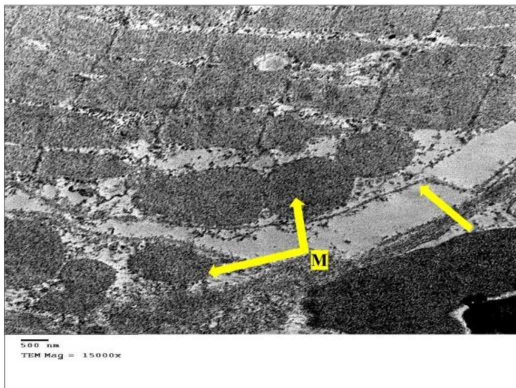

Fig.31: Transmission electron micrograph of pectoralis muscle of pigeon showing rows of mitochondria "M" under the sarcolemma "arrow". X15000

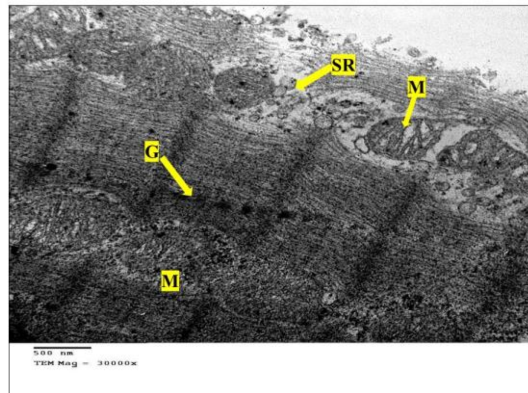

Fig.32: Transmission electron micrograph of pectoralis muscle of pigeon showing numerous mitochondria "M" in contact with sarcoplasmic reticulum "SR" between fibrils with complete cristae and glycogen granules "G". X30000

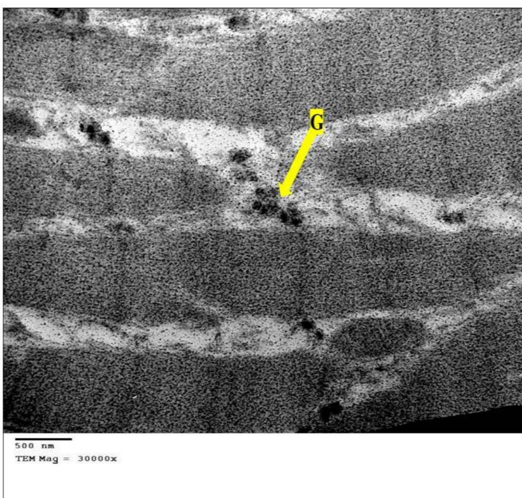

Fig.33: Transmission electron micrograph of pectoralis muscle of fowl showing dispersed and

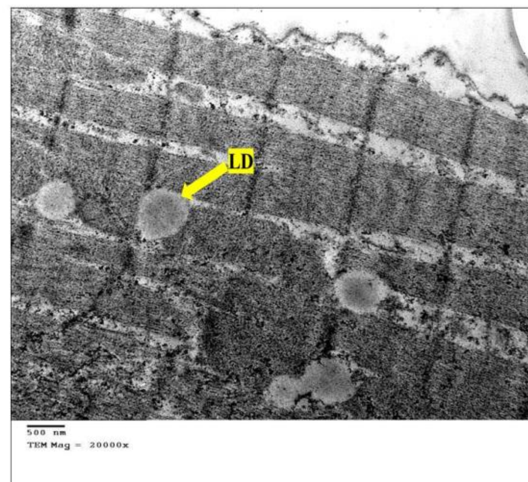

Fig.34: Transmission electron micrograph of pectoralis muscle of pigeon showing lipid droplets

more electron dense glycogen granules "G". X30000

"LD". X20000

DISCUSSION

The current study showed that the pectoralis major muscle of fowl consists of bundles run in different directions and are formed of branching fibers, while that of pigeon are regularely distributed and not branched**.** The muscle fibers in case of the fowl are characterized by distinct cross striations, peripheral, elongated flattened nuclei and few blood capillaries in between. On the other hand, the fibers of pigeon showed ill distinct cross striations, wide spaces, oval nuclei and more blood capillaries**.** This is similar to the description of **(Danmaigoro, 2016)** who mentioned that, the pectoralis muscle of the domestic fowl had thick fibers with smaller and few capillaries. The same author added that, the fiber type of fowl's muscle are typically of fast twitch types while in pigeon, slow twitch fibers are abundant with a few fast twitch fibers scattered throughout the muscle bundle.

The present study clearified the random distribution of the red "slow" and white "fast" fibers in the pectoralis muscle of fowl and pigeon, yet the white fibers are mostly peripherally situated in case of pigeon.

George and Naik (1959) reported the presence of broad fibers in the pectoralis muscle of wild pigeon which were mainly concentrated toward the periphery of the bundle.

George and Berger (1966) described two types of fibers in the pectoralis muscle of the pigeon which they called type 1 and type II. They showed that the type 1 fiber was best suited for aerobic metabolism and the type II for anaerobic metabolism.

The pigeon may have prone to have flight muscles composed of homogeneous slow twitch fibers. These fibers are primarily responsible for movement requiring a low degree of force over a prolong period of time in order to support the demand for an excellent sustained flight. On the other hand non-flying birds such as domestic fowl and guinea fowl have a high proportion of fast twitch fibers with few slow twitch fibers in their muscles. These fibers provide muscle movement that supports a large amount of force over a short period of time **(Wada et al., 1999** and **Danmaigoro, 2016)**

The increase in the thickness of white muscle fibers has a positive impact on tenderness but a negative impact on juiciness of meat. In case of red fibers, this dependency is opposite **(Cameron et al., 1998** and **Migdal et al., 2005).**

In small birds, three general types of twitch fibers have been recognized by **Kenneth et al. (2009)** as components of avian musculature; slow oxidative fibers, fast glycolytic fibers and fast oxidative glycolytic fibers which may be due to species and breeds variations.

George and Naik (1958a, 1958b) have shown that the pectoralis major muscle of the pigeon consists of two distinct types of fibers, a broad glycogen-loaded white variety with few or no mitochondria and a narrow fat-loaded red variety having a large number of mitochondria in them.

The myoglobin in the present investigation found that was unequally distributed inside the red muscle fiber in both fowl and pigeon. Moreover it was more concentrated in pigeon muscle. The mean value optical density was 45.73 in fowl and 61.09 in pigeon this result agree with these records that myoglobin and hemoglobin levels were lowest in the glycolytic muscles, Pectoralis superficialis, and highest in the oxidative muscle and the heart. The mixed type (glycolytic-oxidative) differed considerably with respect to their myoglobin level, in accordance with the difference in muscle type **(Crow and Stockdale, 1986** in avian**), (Sams and Jankey, 1990** in broilers**)** and **Kranen et al., 1998** who added that the myoglobin content in the pectoralis superficialis muscle of chicken was below the detection level .

Hemoglobin and myoglobin are important factors determining meat quality. These factors affect the color of meat and can cause undesirable discoloration when they exudate from muscle tissue or extravasate from the circulatory system **(Griffiths and Nairn, 1984)**.

Regarding the calcium granules, the present work showed its presence in similar densities in the muscle fibers of both fowl and pigeon. None of the available literatures recorded this subject. The differences in the fine structure of striated muscles are related to specific functions **(Haggqvist, 1956).**

The myofibrils of the fowl pectoralis major muscle in this study were clear and had different thickness. The distances between fibrils were wide due to increase amount of sarcoplasm. The sarcomeres appeared wide with thin Z-lines but the light and dark bands are not clear. This results agreed with the records of **(Macnaughtan, 1974)** who added that there was a prominent M line.

The current investigation mentioned that the Z-lines were thin in fowl but thick in case of pigeon muscle. **(Edwards et al., 1956)** stated that the most constant feature of striated muscle was the Z line. Which might be in phase across the fiber, might line up stepwise, giving a helical effect.

The present study found that the sarcolemma of the pectoralis muscle fibers in fowl is more electron dense than that of pigeon. It shows deep invaginations inside the fibrils opposite to the Z-line which agreed with the results of **(Edwards et al., 1956).**

The nuclei of fowl muscle fibers are elongated with regular nuclear membrane and euchromatic while in pigeon, they are oval with irregular nuclear membrane and more heterochromatin**.** These results confirm the records of **(Edwards et al., 1956)**.

The mitochondria of the fowl pectoralis muscle in the present study were few and found singly between the muscle fibrils**.** On the contrary in case of pigeon, numerous mitochondria were arranged in several rows between the myofibrils and under the sarcolemma with different shapes and sizes. Similar results were mentioned by **(Edwards et al., 1956)** who observed numerous mitochondria between the fibrils. They also recorded that the mitochondria were frequently concentrated just beneath the inner layer of the sarcolemma than between the fibril bundles.

The muscle fibers were of two distinct types, those with a high content of mitochondria and those in which they were very sparse. In the former type the mitochondria were mostly disposed in long parallel rows between columns of myofibrils **Kitiyakara and Harman (1953)** and **Allan (1956).**

Suarez et al., 1991 observed mitochondria in hummingbird flight muscles were clustered beneath the sarcolemmal membrane adjacent to capillaries to a greater extent than in mammalian muscles.

In the current result the mitochondria of pigeon characterized by presence of complete elongat

ed cristae in agreement with **Harman, 1955** and **Weinreb and Harman, 1955** found no evidence for the existence of enveloping membranes in pigeon breast muscle mitochondria.

Edwards et al., 1956 explained this result as the mitochondria vary in size, form, internal structure, and distribution from one muscle to the next, and also with the activity of a given muscle. Large, rounded, or chunky mitochondria were found in the flight muscles of the hummingbird.

Some mitochondria in the present work are closely packed and connected or open with each other. This results accepted with the description of **Biesele, 1955** in the humming birds pectoralis muscle.

In the present study the redistribution of mitochondria towards the cell membrane and closer to capillaries should reduce intracellular diffusion distances. Its importance for improving oxygen transport is emphasized by previous human studies showing that increases in aerobic performance after exercise training are associated with a preferential proliferation of subsarcolemmal mitochondria **(Hoppeler et al., 1985).**

High capillary densities and high myoglobin contents, might allow higher rates of flux of oxygen and substrates to working muscles of humming birds than in mammals. In addition, it had been suggested that a high degree of clustering of subsarcolemmal mitochondria adjacent to capillaries as is seen in hummingbird flight muscles makes possible higher rates of oxygen flux than would be possible if mitochondria were uniformly distributed within muscle fibers **(Suarez et al., 1991).**

The sarcoplasmic reticulum was hardly recognized in the current investigation especially in the muscle of the fowl in agreement with **Edwards et al., 1956** who showed in many preparations neither a longitudinal reticulum nor a Grundmembran is visible.

The present study revealed that cytoplasmic deposits of glycogen granules were noticed between the myofibrils of the white fibers. They were dispersed and more electron dense in fowl which agreed with the results of **(Bendall, 1960)** who noticed in the fowl pectoralis muscle single particles are concentrated between the myofibrils. The same author was of opinion of that the amount of glycogen varied depend on antemortem conditions. However in pigeon muscle fibers, the glycogen granules were more condenced in high quantities peripherally between the myofibrils.

No lipid droplets could be observed in fowl pectoralis muscle while several electronlucent ones were seen between myofibrils in pigeon which disagreed with **(Macnaughtan, 1974)** who recorded rare lipid droplets and not necessarily associated with mitochondria.

The mean sarcomere length in fowl pectoralis muscle was 2.67 µm while in pigeon was 3.23 µm with no significant differences between them. However, the mean muscle fiber diameter in fowl was 44 µm but in pigeon was 46.4 µm with significant differences between them. This result similar to the findings of **(Khoshoii et al., 2013)** who found the mean muscle fiber diameters in pectoralis superficialis of chickens ranged from 29-52.5 µm, whereas in other broilers ranged from 31-39 µm. On the other hand, the diameter of breast muscle fibers in the group of Hubbard JA 957 birds reached 75.61 µm, whereas in the group of Silkies chickens it was more than two times smaller (33.23 µm) **(Lukasiewicz et al., 2013).**

Muscle fibers from fast growing lines of chickens have larger fiber diameters than slow growing lines and larger fiber diameters are often associated with an increased number of giant fibers **(Essen-Gustavsson, 1993)** and **(Dransfield and Sosnicki, 1999).** Increased diameter and length of muscle fibers may be due to intensive selection **(Brocka et al., 1998** and **Guernec et al., 2003)** and changes appearing in the size and shape of muscle fibers **(Bogucka and Kapelanski, 2004)**. A higher number of fibers with small and medium diameters improves meat quality **(Choi and Kim, 2008).**

The mean concentration of lactate dehydrogenase enzyme in pectoralis muscle of fowl in the present study was 265 u/gm but in pigeon 240 u/gm with significant difference. The mean of lipase in fowl muscle was 141.5 u/gm but in pigeon was 94 u/gm with significant difference between them. The records of **(Bass et al., 1969)** was that lactate dehydrogenase level in white fast pectoralis major muscle of chicken was 1325u/g while in red slow fibers of pigeon pectoralis muscle was 600u/g. Moreover, **Crabtree and Newsholme (1972)** recorded this enzyme level in fowl pectoralis 870.0 but in Pigeon 314.0 µmol/min per g fresh wt. at 25ºc.

The red fibers of pigeon breast muscle, due to their remarkably well developed enzyme systems, play a major role in effecting the sustained contractions of the muscle. In white fibers, on the other hand, the oxidative processes are not developed or developed only to a negligible extent, in that the dehydrogenase activity in these fibers, as shown by histochemical method, is negligible or nil **(George and Scaria, 1958b).**

The red muscle "type I" is rich in succinic dehydrogenase, fat and lipase. The white muscle "type II" is rich in glycogen, poor in fat and its related enzymes **(Ogata, 1958; Dubowitz and Pearse, 1960, 1961; Jinnai, 1960; Backett, 1962; Engel, 1962; Stein and Padykula, 1962; Henneman and Olson, 1965; Nishiyama, 1965; Beatty et al., 1966; Edgerton, 1968** and **Edgerton and Simpson, 1969).** These findings agreed with **George and Scaria (1958a)** who histochemically demonstrated higher lipase activity in the red narrow fibers. The Krebs' cycle enzymes, too, seem to be localized in the narrow fibers **(George and Scaria, 1958b).**

White (fast) muscle is characterized by high capacities of glycogenolysis, glycolysis and lactate fermentation, whereas the capacities of glucose phosphorylation, citric acid cycle and fatty acid oxidation are low. Red (slow) muscles, heart and smooth muscle show inverse characteristics **(Bass et al., 1969).**

The pectoralis muscle contained only fast-twitch-glycolytic (FG) fibres as their SDHase activity was uniformly low and all fibres reacted strongly for myosin ATPase and GPase **(Ashmore and Doerr, 1971a** in chick and **Macnaughtan, 1974** in fowl**)**. It can be assumed that SDHase activity is directly related to mitochondrial content;thus the sparsity of these organelles in the pectoralis conforms to the uniform population of FG fibres.

George and Scaria (1958) in histochemical study of dehydrogenases (succinic, malic, lactic, and glycerophosphate dehydrogenases) in pectoralis major muscle of the pigeon could not detect the presence of any of these dehydrogenases in the broad white fibers.

George and Scaria (1958) and **George et al. (1958)** observed that the concentration of the dehydrogenases "oxidative enzymes" has a relationship with the color and the mitochondrial content of the individual muscle fibers in the sense that the red narrow fibers possess a high concentration of these enzymes and mitochondria, in sharp contrast to the broad white fibers.

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