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Research Article

Histomorphology of deep pectoral muscle in Chabro chicken

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Abstract

The present study was conducted on the deep pectoral muscle of 36 apparently healthy Chabro chicken (18 male and 18 female) which were divided into three age groups viz; Group I (6 weeks), Group II (8 weeks) and Group III (10 weeks). The deep pectoral muscle as any other muscle comprised of muscle fibers, blood vessels, nerve fibers, and connective tissue. The myofibers of deep pectoral muscle appeared rectangular, irregular or polyhedral in shape in cross section. The increase in number of myofiber per fascicle was highly significant between 6 to 8 weeks of age. The diameter of the muscle fascicles was always higher in the male chicken than the female. The muscular tissue area percent increased continuously with age. The connective tissue area percentage was higher in the female chicken than in male chicken of the same age groups, whereas, the diameter and cross-sectional area of muscle fiber and muscle fascicle was higher in male; indicative of stronger musculature of male birds.

Key words: Chabro chicken, Deep pectoral muscle, Histology, Histometry

Highlights

- The diameter, cross-sectional area of myofibers, fascicular diameter, cross-sectional area of fascicle and interfascicular distance of the muscle were more in male birds at all ages.
- The mean diameter, cross-sectional area of myofibers, number of myofibers per fascicle, fascicular diameter and area of fascicle of deep pectoral muscle increased with age in both sexes.
- Growth occurred in muscle by increase in number as well as size of muscle fiber.

INTRODUCTION

Demand for high quality cuts of meat to the consumers (Wats and Kennet, 1995) and the continued efforts made by breeders to produce superior quality birds necessitate continuing evaluation of the strain (Rahman, 2014). This study was aimed to determine the muscular tissue growth in Chabro chicken. It is a cross breed of Barred Plymouth Rock and Red Cornish bird (Yadav *et al*., 2018). The present study was done on histomorphology and histomorphometry of deep pectoral muscle in Chabro chicken to generate the base line data. Most of the companies producing high value meat production in poultry explain the features like microclimate, nutritional requirements, weight etc., but the results of present study on breast muscle of Chabro Chicken brings information about the textural quality of meat which depends on the amount of connective tissue and muscular tissue (Listrat *et al*., 2016). The breast muscles are considered as the white muscle which is very good for health as nutritional point of view (Keller, 2021). But the microanatomical features of these muscles are not recorded systematically. The deep pectoral muscle forms the major part of breast muscle, so it is an important component of white meat obtained from the breast region. Hence, the muscle was selected for the study. As there are certain diseases like wooden breasts or pale soft exudative (PSE) meat condition (Guarnieri *et al*., 2004) in which the muscle degeneration takes place and the muscle histology reveals it. So, the results of this study will help to compare the healthy muscle histology with diseased one.

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This study will also answer the curiosities among scientists and practitioners in poultry and food industries about how the meat cuts develop especially the muscle growth during rearing period by increase in size of muscle fiber, number of muscle fiber or amount of connective tissue. This study will also be helpful for the persons working in nutrition and livestock product technology fields to select the desired quality of meat as the muscular and connective tissue directly affects the meat quality.This study also revealed the comparative data of male and female birds so persons from poultry industry can go for the required sex as per the muscle quality.

MATERIALS AND METHODS

The present study was conducted on the deep pectoral muscle of 36 apparently healthy Chabro chicken (18 male and 18 female) which were divided into three age groups viz; Group I (6 weeks), Group II (8 weeks) and Group III (10 weeks). Twelve birds (6 males and 6 females) were randomly selected on 6^{th}, 8^{th} and 10^{th} week. Then the birds were sacrificed by standard scientific method (Lynch, 1988) after chloroform inhalation (painless killing). The breast region was dissected out meticulously. For histomorphological observations, thin pieces (0.5 cm^2) of tissue were fixed in 10% neutral buffered formalin. The fixed tissue was processed by routine paraffin embedding technique (Luna, 1968). The paraffin sections were cut at 5µm. Sections was stained in the various stains (Table 1) for the detailed histomorphological studies.

The detailed histomorphometrical study was conducted on stained slides. Following micrometrical parameters were recorded with the help of Leica DM 750 Computerized Image Analyzer as under:

- Diameter of myofiber was measured on cross sections of muscles. Two diameters were taken, one largest (MDI) and another was perpendicular to the previous one (MDII) and then the average of both the diameter was taken [(MDI+MDII)/2], Radu-Rusu *et al.* (2013).
- Cross sectional area of myofibers (CSM) was calculated by the formula-
  Cross sectional area = MDI x MDII x $\pi/4$ (π = 3.1416 or 22/7), Radu- Rusu *et al.* (2013).
- Diameter of fascicles was measured in a similar manner like that of myofiber.
- Cross sectional area of fascicle (CSF) was calculated by the formula as followed for the myofibers.
- Muscle tissue (MT) and connective tissue (CT) percentage was calculated by the formula- MT% = n x CSM x 100 / CSF
  CT% = 100 - MT%, Radu- Rusu *et al.* (2013)
- Length and width of nucleus, inter-myofiber distance, interfascicular distance was measured and number of myofibers per fascicle (n) was counted.

Statistical analyses: The data generated by micrometrical study was subjected to statistical analysis (Snedecor and Cochran, 1994).

Table 1. Various histological methods used in the study

Sl. No.	Name of the stain	Method	Purpose
1.	Hematoxylin and Eosin method	Luna (1968)	For the study of general histoarchitecture of muscles
2.	Masson's Trichrome stain	Luna (1968)	For the study of collagen fibers
3.	Gordon and Sweet's stain Stevens (1979)	Bancroft and	For the study of reticular fibers
4.	Weigert's Resorcin-Fuchsin stain	Bancroft and Stevens (1979)	For the study of elastic fibers

RESULTS

The epimysium of deep pectoral muscle of Chabro chicken consisted of thick sheath of dense irregular connective tissue (Fig. 1). The outer layer comprised of circularly arranged connective tissue fibers, whereas in the deeper layer connective tissue fibers were arranged longitudinally. From the epimysium, connective tissue entered into muscle and divided it into groups or bundles of myofibers called muscle fascicles, these myofibers were variable in size and appearance. On the basis of that, three types of fiber were observed viz; large, intermediate and small (Fig. 2). The number of myofibers in each muscle fascicle was not constant. They also varied with age as well as with sex. As the age of Chabro chicken advanced the number of myofibers per fascicle increased in both the sex of Chabro chicken. The shapes of fascicles were triangular, leaf like, lanceolate or quadrangular (Fig. 3 and Fig. 4). Within the connective tissue of perimysium blood vessels, nerves, ganglion, vein and large amount of adipose tissue were present (Fig. 4). Few myofibers showed the typical flower petals arrangement in the fascicle (Fig. 5). There were 5-7 intermediate fibers present around one small fibers.

The thickness of connective tissue was increased with age in both the sexes of Chabro chicken. The connective tissue was observed thicker in female Chabro birds as compared to the male Chabro birds. The arteries present in the perimysium distinctly presented three layers. In tunica interna, the layer of elastic fibers was well formed. The middle muscular layer (tunica media) comprised of muscle, collagen reticular fiber and very fine elastic fibers and very few elastic fibers. The veins were thin walled. The large numbers of fibroblast cells were present with rounded large nucleus and cytoplasmic projections. The collagen fibers were predominantly present from 6 to 10 weeks of age. The relative amount of collagen was more in female chicken than in male birds at all ages. The elastic fibers were present in the form of fine fragmented threads in loose irregular connective tissue of perimysium at 6 weeks of age. The reticular fibers were present irregularly, intermingled with collagen fibers. The thickness of perimysium increased with the age. From the perimysium connective tissue extended further inside the fascicles into thin septa and surrounded the individual myofibers called endomysium. The endomysium was very thin layer of connective tissue present in the space between the adjacent myofibers. Within endomysium small blood vessels, nerves and very less amount of fat cells were observed (Fig. 5 and Fig. 7). The reticular fiber was closely attached to sarcolemma and formed the outline of myofibers, whereas, the collagen fibers were present at a considerable distance from myofibers. The single inconstant layer of fine elastic fiber was observed in the endomysium extending from fibroblast. The intermyofiber space decreased with increase in age of birds. This space was more in the muscle of female than in male birds at 6 weeks of age (Fig. 5 to Fig. 7). The fibroblasts present in the intermyofiber space as well as perimysial space showed cytoplasmic projections forming a network of connective tissue around each myofiber (Fig. 8 and Fig. 9).

The myofibers appeared rectangular, irregular or polyhedral in shape in cross section. Many nuclei were present towards the periphery of the cell. These nuclei were vesicular, small and stained lightly with hematoxylin. Some nuclei showed the chromatins and nucleolus (Fig. 8 and Fig. 9). On higher magnification the light and dark bands were observed forming the characteristic striations of skeletal muscle (Fig. 9). The sarcolemma was lined by thin layer of fine reticular fiber. The cytoplasm was eosinophilic and dark stained. It showed rough surface due to presence of cut surface of myofibrils (Fig. 10 and Fig. 11).

Close and external to the sarcolemma the myosatellite cells were observed. These cells had large, dense and dark stained spindle shaped nucleus. The myosatellite cells were more in 8 weeks and 10 weeks Chabro chicken. The large muscle fibers (myofibers) showed the nuclei deeper in the cytoplasm and the surface of these

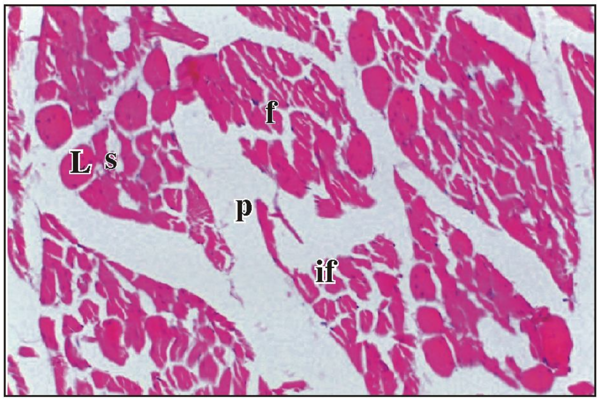

Fig. 1. Photomicrograph of 6 weeks old female Chabro chicken deep pectoral muscle showing perimysium (p), fascicle (f), large fiber (L), intermediate myofiber (if) and small myofiber (s). H & E X 200

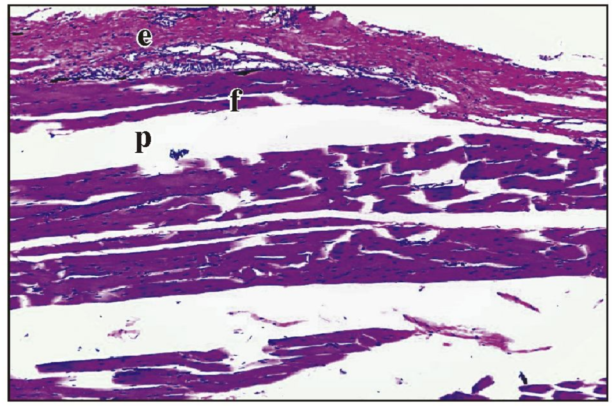

Fig. 2. Photomicrograph of 6 weeks old female Chabro chicken deep pectoral muscle showing epimysium (e), perimysium (p) and myofibers (f). H & E X 200

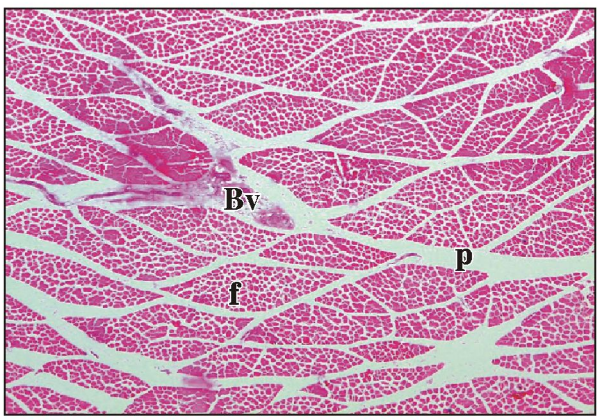

Fig. 3 . Photomicrograph of deep pectoral muscle of 6 weeks old female Chabro chicken showing interfascicular space (p), fascicle (f) and perimysial blood vessel (Bv). H & E X 40

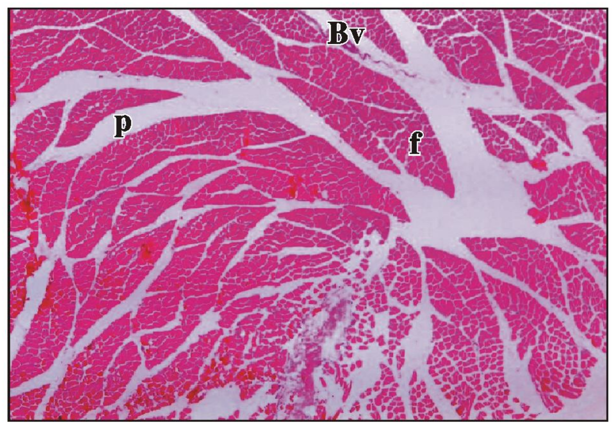

Fig. 4. Photomicrograph of deep pectoral muscle of 6 weeks old male Chabro chicken showing interfascicular space (p), fascicle (f) and perimysial blood vessel (Bv). H & E X 40

Fig. 5. Photomicrograph of deep pectoral muscle of female Chabro chicken showing the changes in the endomysium (arrow) with the increase in age (6 weeks), floral petal arrangement (circle) of myofibers. H & E X 400

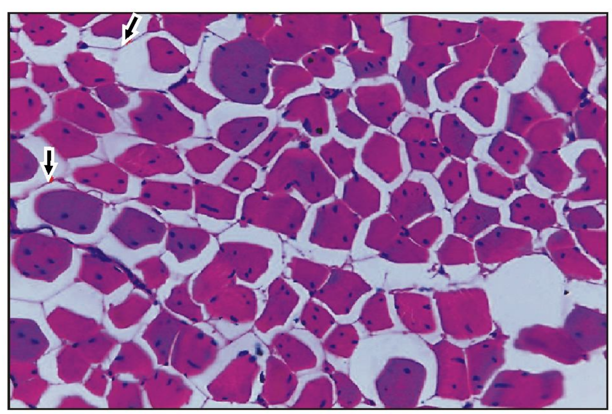

Fig. 6. Photomicrograph of deep pectoral muscle of female Chabro chicken showing the changes in the endomysium (arrow) with the increase in age (8 weeks). H & E X 400

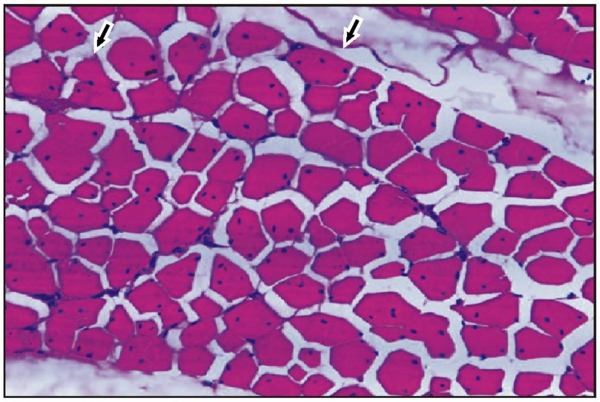

Fig. 7. Photomicrograph of deep pectoral muscle of female Chabro chicken showing the changes in the endomysium (arrow) with the increase in age (10 weeks). H & E X 400

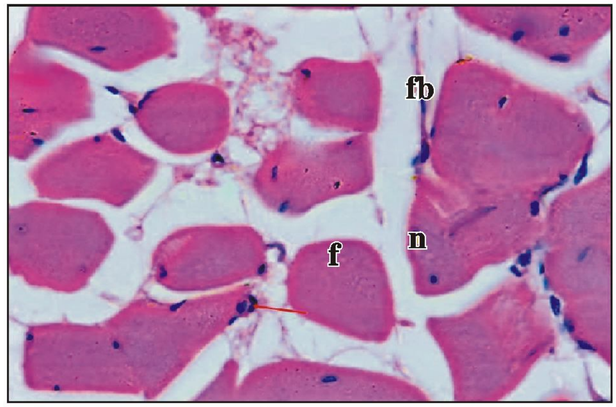

Fig. 8. Photomicrograph of deep pectoral muscle of 8 weeks old male Chabro chicken showing endomysium, myofiber (f), fibroblast (fb), nucleus of myofiber (n) and myosatellite cells (arrow). H & E X 1000

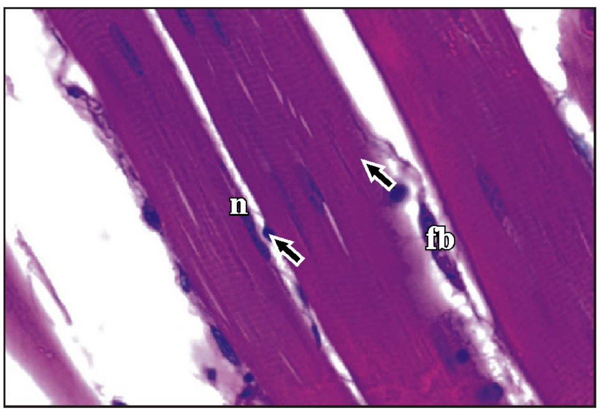

Fig. 9. Photomicrograph of longitudinal section of 10 weeks old female Chabro chicken of deep pectoral muscle showing nucleus of myofiber (n), myosatellite cells (arrow) fibroblast (fb) and striations in myofibers. H & E X 1000

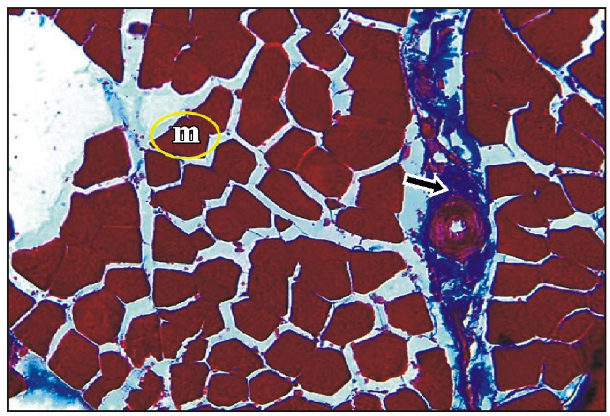

Fig. 10. Photomicrograph of deep pectoral muscle of 8 weeks old female Chabro chicken showing collagen fiber in perimysium and blood vessel (arrow). Rough surface of myofibers (m) is due to cut ends of myofibrils (circle). Masson's Trichrome stain X 400

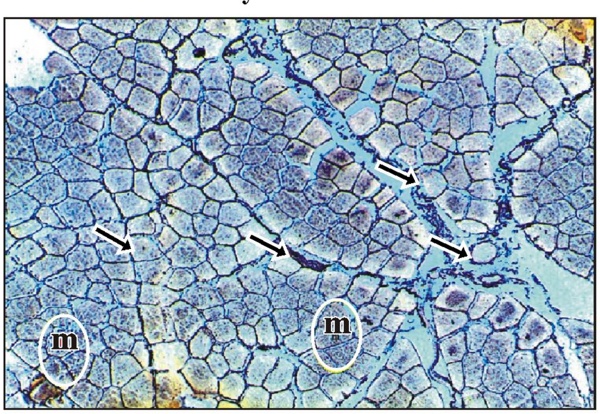

Fig. 11. Photomicrograph of deep pectoral muscle of 8 weeks old female Chabro chicken showing reticular fiber in endomysium, perimysium and blood vessels (arrow). Rough surface of myofibers (m) is due to cut ends of myofibrils (circle). Gordon & Sweet's stain X 200

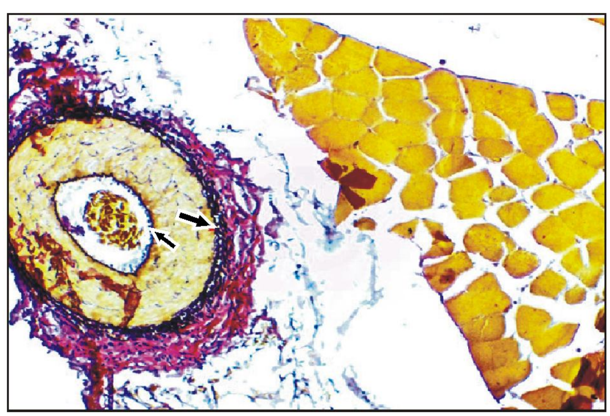

Fig. 12. Photomicrograph of deep pectoral muscle of 8 weeks old female Chabro chicken showing elastic fiber (arrow) in blood vessels. Weigert's resorcin fuchsin stain X 400

fibers was smooth. The shapes of these fibers were oval, round or rectangular with rounded angles. These fibers were light eosinophilic and present mostly at the periphery of the fascicles. The number of large fibers per fascicle were 2- 4 in male birds whereas, 1-7 in female birds. The intermediate fibers were rectangular, square, quadrangular and triangular in shape. They were strongly eosinophilic with rough surface due to presence of numerous myofibrils. The nuclei of intermediate fibers were located at the periphery of the cell. The small fibers were irregular in shape. Few intermediate fibers showed the typical flower petals arrangement in the fascicle. There were 5-7 intermediate fibers present around one small fibers. The tendinous intersection was observed in the cross section consisted of dense irregular connective tissue, predominantly collagen fibers.

On observation of longitudinal section, the myofibers were found in the form of several elongated parallel fibers. The multiple nuclei were observed at the periphery. The nuclei were vesicular and elongated oval in shape. The outer tunica externa of blood vessels comprised of collagen, reticular and elastic fibers (Fig. 10, Fig. 11 and Fig. 12).

Histomorphometry: The data on the myofiber diameter, cross-sectional area of myofibers, length of nucleus, width of nucleus, intermyofiber distance, number of myofibers per fascicle, fascicle diameter, area of cross section of fascicle, interfascicular distance, muscle tissue area percent and connective tissue area percent in the fascicles of deep pectoral muscle have been recorded and summarized in Table 2, Table 3 and Table 4.

The data indicated that, the diameter of myofibers of deep pectoral muscle was higher in the male than the female bird of the same age group. The sexual difference was strongly significant at each stage ($P \leq 0.01$). The mean diameter of myofibers increased with age in birds of both sexes and the increase was statistically significant among all age groups ($P \leq 0.01$). The cross-sectional area of myofibers in male was significantly ($P \leq 0.01$) higher than female of same age. The mean cross-sectional area of myofibers increased significantly and collinearly with age and this increase was statistically significant among different age groups ($P \leq 0.01$).

The length of nucleus measured in longitudinal histological section at higher magnification, it was more in female birds at 6 and 8 weeks but at 10 weeks of age it was more in male birds. The sexual difference was however, found to be statistically significant only at 8 weeks of age ($P \leq 0.01$). In the muscle of female birds, the length of nucleus increased

Table 2. Histomorphometrical parameters (Mean±SE) of deep pectoral muscle in female Chabro chicken

Groups / Parameters	Group I (6 weeks)	Group II (8 weeks)	Group III (10 weeks)
Diameter of myofiber (µm)	18.08 ± 0.39^{c}	31.52 ± 1.17^{b}	36.59 ± 0.44^{a}
Cross-sectional area of muscle fiber (µm^2)	254 ± 11.70^{c}	767 ± 66.42^{b}	1046 ± 24.87^{a}
Length of nucleus (µm)	5.94 ± 0.35^{a}	6.13 ± 0.18^{a}	4.98 ± 0.28^{b}
Width of nucleus (µm)	1.17 ± 0.04^{c}	1.59 ± 0.07^{a}	1.42 ± 0.08^{b}
Intermyofiber distance (µm)	5.40 ± 0.14^{a}	3.38 ± 0.11^{b}	3.36 ± 0.29^{b}
No. of myofiber per fascicle	82.25 ± 1.45^{b}	119 ± 12^{a}	104 ± 6.59^{a}
Fascicular diameter (µm)	228 ± 4.59^{c}	417 ± 12^{b}	476.96 ± 16.11^{a}
Cross-sectional area of fascicle (µm^2)	34524 ± 1104^{c}	124484 ± 7937^{b}	177622 ± 11334^{a}
Interfascicular distance (µm)	12.53 ± 0.37^{c}	34.41 ± 1.33^{a}	21.77 ± 0.91^{b}
Muscular tissue area %	62.09 ± 2.64^{a}	68.28 ± 3.75^{a}	61.17 ± 0.41^{a}
Connective tissue area %	37.91 ± 2.64^{a}	31.72 ± 3.75^{a}	38.83 ± 0.40^{a}

Table 3. Histomorphometrical parameters (Mean±SE) of deep pectoral muscle in male Chabro chicken

Parameters \ Groups	Group I (6 weeks)	Group II (8 weeks)	Group III (10 weeks)
Diameter of myofiber (µm)	19.85±0.29^{c}	34.94±0.774 ^{a}	39.41±0.45^{b}
Cross-sectional area of muscle fiber (µm^2)	306±8.79^{c}	958±44.24^{b}	1219.88±26.84^{a}
Length of nucleus (µm)	5.41±0.44^{a}	4.89±0.18^{a}	5.73±0.06 ^{a}
Width of nucleus (µm)	1.68±0.36^{a}	1.53±0.23^{a}	2.22±0.50^{a}
Intermyofiber distance (µm)	4.20±0.16^{b}	3.87±0.046^{b}	3.45±0.53^{a}
No. of myofiber per fascicle	95.31±4.55^{b}	106.22±2.17^{a}	138.33±4.05^{ab}
Fascicular diameter (µm)	250±4.67^{b}	461±13.50^{a}	572±12.47^{a}
Cross-sectional area of fascicle (µm^2)	46821±2393^{c}	162466±11343^{b}	254595±10955^{a}
Interfascicular distance (µm)	19.81±8.08^{b}	17.84±0.12^{b}	31.28±2.45^{a}
Muscular tissue area %	62.39±0.51^{a}	63.83±1.74^{a}	66.57±0.94^{a}
Connective tissue area %	37.61±0.51^{a}	36.17±1.74^{a}	33.43±0.94^{a}

n= 36; a, b, c superscripts showing the significant difference between groups; Means that do not have a superscript or share similar letter are non-significantly different

Table 4. Sexual difference in histomorphometrical parameters (Mean±SE) of deep pectoral muscle of Chabro chicken

Parameters \ Groups	Group I Female	Group I Male	Group II Female	Group II Male	Group III Female	Group III Male
Myofiber diameter (µm)	18.08^{B} ±0.39	19.85^{A} ±0.29	31.52^{B} ±1.17	34.94^{A} ±0.77	36.59^{B} ±0.48	39.41^{A} ±0.44
Myofiber cross sectional area (µm^2)	254^{B} ±11.70	306^{A} ±8.78	767^{B} ±66.42	958^{A} ±44.24	1046^{B} ±24.87	1219^{A} ±26.84
Length of nucleus (µm)	5.94^{A} ±0.35	5.41^{A} ±0.44	6.13^{A} ±0.181	4.89^{B} ±0.18	4.98^{A} ±0.28	5.73^{A} ±0.05
Width of nucleus (µm)	1.17^{A} ±0.04	1.68^{A} ±0.36	1.59^{A} ±0.07	1.53^{A} ±0.23	1.42^{A} ±0.08	2.22^{A} ±0.50
Intermyofiber distance (µm)	5.40^{A}± 0.14	4.20^{B} ±0.16	3.38 ±0.11^{B}	3.87^{A} ±0.05	3.35^{B} ±0.29	3.45^{A} ±0.53
No. of myofiber per fascicle	82.25^{B} ±1.45	95.31^{A} ±4.55	119^{A} ±12.22	106^{A} ±2.16	104^{B} ±6.59	138^{A} ±4.05
Fascicular diameter (µm)	228^{B} ±4.60	250^{A} ±4.67	417^{B} ±12.76	461^{A} ±13.50	476^{A} ±16.10	572.13^{A} ±12.46
Cross-sectional area of fascicle (µm^2)	34524^{B} ±1104	46821^{A} ±2393	124484^{B} ±7937	162466^{A} ±11343	177622^{A} ±11334	254595^{B} ±10955
Interfascicular distance (µm)	12.53^{B}± 0.37	19.81^{A} ±8.08	34.41^{A} ±1.33	17.84^{B} ±0.12	21.77^{B} ±0.91	31.28^{A} ±2.45
Muscular tissue area %	62.09^{A} ±2.64	62.39^{A} ±0.51	68.28^{A} ±3.75	63.83^{A} ±1.74	61.17^{B} ±0.41	66.57^{A} ±0.94
Connective tissue area %	37.91^{A} ±2.64	37.61^{A} ±0.51	31.72^{A} ±3.75	36.17^{A} ±1.74	38.83^{A} ±0.41	33.43^{B} ±0.94

n= 36; A, B, C superscripts showing the significant difference between groups between 6 to 8 weeks of age but decreased significantly between 6 to 10 weeks and 8 to 10 weeks of age ($P\leq0.01$). In muscle of male chicken, the length of nucleus decreased between 6 to 8 weeks of age, but increased between 8 to 10 weeks of age. However, it was statistically non-significant. The data also indicated a non-significantly wider nucleus at 6 and 10 weeks, and a narrower nucleus in 8 weeks old male chicken muscle fiber than in the females of the same age. The width of muscle nucleus in female birds increased significantly ($P\leq0.01$) between 6 to 8 weeks, but decreased between 8 to 10 weeks. In contrast in male Chabro chicken the width of nucleus decreased between 6 to 8 weeks of age and increased between 8 to 10 weeks of age, although non-significant.

The mean intermyofiber distance was more in the female chicken than the male at 6 weeks of age but at 8 and 10 weeks of age it was more in male chicken than female. The difference between the two sexes was found to be statistically significant at all ages ($P\leq0.01$). The distance decreased at all ages but significantly between 6 to 8 weeks and 6 to 10 weeks in female, and between 6 to 10 weeks and 8 to 10 weeks in male chicken. But it was statistically non-significant between 8 to 10 weeks old male birds and between 6 to 8 weeks old female chicken. The mean number of myofiber per fascicle was higher in male birds of 6 and 10 weeks of age but at 8 weeks of age it was more in female. Further the difference in sex was statistically significant at 6 and 10 weeks ($P\leq0.01$) but not significant at 8 weeks age.

The mean number of myofiber per fascicle increased from 6 to 10 weeks of age but it showed a slump in female indicating slow growth rate between 8 to 10 weeks of age. In male birds, however, there was a continuous growth in the number of myofibers per fascicle with age. The increase was statistically significant between 6 to 8 weeks ($P\leq 0.05$) but non-significant between 8 to 10 weeks and 6 to 10 weeks of age. The increase in number of myofiber per fascicle was highly significant ($P\leq 0.01$) between 6 to 8 weeks and 6 to 10 weeks of age but not significant between 8 to 10 weeks of old female birds. The diameter of the muscle fascicles was always higher in the male chicken than the female among all three age groups. The sexual difference was statistically significant at 6 weeks and 8 weeks of age ($P\leq 0.05$), but not at 10 weeks of age. The fascicle diameter of muscle invariably increased with age in male as well as female chicken. In female chicken, the increase was highly significant between 6 to 8 weeks, 8 to 10 weeks and 6 to 10 weeks of age ($P\leq 0.01$). However, in male chicken, the increase was statistically significant between 6 to 8 weeks and 6 to 10 weeks of age ($P\leq 0.01$), but not statistically significant between 8 to 10 weeks of age.

The cross-sectional area of the muscle fascicle was found to be higher in the male than in the female chicken at all ages. The sexual difference in the cross-sectional area of the fascicle was statistically significant at 6 weeks (($P\leq0.01$), 8 weeks ($P\leq0.05$) and 10 weeks (($P\leq 0.01$) of age. The mean cross-sectional area of the fascicle increased collinearly with age in male as well as in female birds. This increase was statistically significant ($P\leq0.01$) among all three age groups. The inter fascicular distance of muscle was more in the males at 6 and 10 weeks of age, but at 8 weeks, it was more in female than in male. The sexual difference in interfascicular distance was found to be statistically significant at 6 weeks ($P\leq0.05$) and 8 weeks ($P\leq 0.01$) but non-significant at 10 weeks of age. In female Chabro chicken, interfascicular distance increased between 6 to 8 weeks of age but decreased between 8 to 10 weeks of age. The difference in the mean value of inter fascicular distance was found to be statistically significant ($P\leq 0.01$) among female of all three age groups. In male chicken the interfascicular distance decreased between 6 to 8 weeks but increased between 8 to 10 weeks. The difference was found to be statistically significant only between 8 to 10 weeks and 6 to 10 weeks ($P\leq 0.01$) of age and not between 6 to 8 weeks.

The muscular tissue area percentage was

slightly higher in the males of 6 and 10 weeks of age, but at 8 weeks it was reversed, in female it was more than in male. The sexual difference was also found to be statistically non-significant at all ages. In male Chabro chicken, the muscular tissue area percent increased continuously although insignificantly between 6 to 8 and 8 to 10 weeks of age. Similarly in the female chicken also the muscular tissue area increased between 6 to 8 weeks of age, and decreased between 8 to 10 weeks, but statistically insignificant.

The connective tissue area percentage was higher in the female chicken than in male chicken of the same age groups. The sexual difference was found to be statistically significant in the birds only at 6 weeks of age and non-significant at 8 and 10 weeks of age. In male chicken, mean connective tissue area percentage of muscle decreased with increasing age; however the difference was found to be statistically non-significant. In female Chabro chicken, although the connective tissue area percent decreased between 6 to 8 weeks of age and again increased between 8 to 10 weeks of age but the difference was found to be statistically non-significant.

DISCUSSION

Each muscle was wrapped in the epimysium (George and Berger, 1966; Copenhaver *et al*., 1971; Eurell and Frappier, 2006) comprised of thick bundles of connective tissue fibers, lined by a layer of fibroblast cells. The epimysium enclosed a number of groups of muscle fascicles and thus kept each muscle separated from the surrounding muscles and tissues as described by Copenhaver *et al*. (1971), Eurell and Frappier (2006) and Listrat *et al*. (2016). The connective tissue septa called, perimysium entered into the muscle and enclosed the groups of muscle fibers called fascicles, as stated earlier by George and Berger (1966) and Eurell and Frappier (2006).

The perimysium was predominantly composed of reticular and collagen fibers with very few elastic fibers. Artery, vein, nerve, ganglion and adipose tissue were observed within the perimysium (Copenhaver *et al*., 1971; Listrat *et al*., 2016). Allison *et al*. (2011) mentioned that collagen is the major structural protein in extra cellular matrix of skeletal muscle and it accounts for 1-10% of muscle mass dry weight. The amount of collagen fibers in the perimysium was more in female than in male Chabro birds of same age. In contrary to this, Mobini (2015) reported higher amount of collagen fibers in pectoralis superficialis muscle in male than female chickens of 56 days of age. Nakamura *et al.* (2003) classified perimysium into two different types: primary perimysium surrounding the muscle fiber bundles and secondary perimysium surrounding the muscle fiber bundles in larger amount.

The perimysium in Chabro chicken further divided and embraced almost each muscle fiber to form the endomysium as reported by George and Berger (1966), Nickel *et al*. (1977) and Mobini (2015). The endomysium comprised of collagen, reticular and very fine elastic fibers, along with the blood vessels and nerves as reported by George and Berger (1966) and Mobini (2015). But Copenhaver *et al*. (1971) and Eurell and Frappier (2006) described the endomysium as a layer of reticular fibers. With age, the amount of collagen predominantly and other fibers also increased in the epimysium, perimysium and endomysium (Bailey and Light, 1989; Fang *et al*., 1999; Eurell and Frappier, 2006; Das *et al*., 2010). The reticular fibers were closely attached with the sarcolemma as stated by Choi and Kim (2009) and Sharma and Sachan (2011).

The satellite cells were oval in shape with a large spindle shaped nucleus, considered as precursor or immature muscle cells as mentioned by Eurell and Frappier (2006). The muscle fascicles were of different shapes in the deep pectoral muscles of Chabro birds, like triangular or leaf shaped as reported by Astruc (2020). In Chabro chicken the muscle fibers of deep pectoral muscle were multinucleated and rectangular, polyhedral or irregular in shape (Eurell and Frappier, 2006; Tumova and

Teimouri, 2009). The multiple nuclei were generally located peripherally in close proximity of the sarcolemma. Copenhaver *et al*. (1971), Eurell and Frappier (2006) and Choi and Kim (2009) also reported similar features in skeletal muscles. The large, medium (intermediate) and small muscle fibers were also discussed by Smith and Fletcher (1988), Listrat *et al*. (2016) and Hena *et al.* (2017) in pectoral muscle of Arbor Acre broilers. The intermediate fibers stained darker than the large muscle fibers as reported earlier by George and Berger (1966). Their nuclei were present at the periphery of the cells as stated by Copenhaver *et al*. (1971) and Eurell and Frappier (2006).

Each muscle fiber contained bundles of myofibrils manifest itself in the form of dark dots on the cross section of myofibers as described by Eurell and Frappier (2006). This gave a rough granular appearance to the cytoplasmic matrix of the intermediate fibers as reported by Copenhaver *et al*. (1971) and Eurell and Frappier (2006). George and Berger (1966) in domestic fowl (64 µm), Copenhaver *et al*. (1971) in skeletal muscles of animals (10-100 µm), Papinaho *et al*. (1996) in broiler chicken (60 µm), Eurell and Frappier (2006) in skeletal muscles of animals (10-110 µm), Tumova and Teimouri (2009) in broiler chicken (60 µm), Khoshoii *et al*. (2013) in chickens (29.0 to 52.5 µm) and in other broilers (31.0 to 39.0 µm), Lukasiewicz *et al.* (2013) in Hubbard JA 957 (75.61 µm) and in Silkies (33.23 µm), Koomkrong *et al*. (2015) in Arbor acres broilers (52.31 µm) and Thai native chickens (33.31 µm) and Wagih *et al.* (2017) in fowl (44 µm) and in pigeon (46.4 µm) also reported the diameter of muscle fiber. Radu-Rusu *et al*. (2013) studied the dimensional features of the muscular fibers in Cobb 500 strain of chicken and stated that the mean myocytes diameter in the deep pectoral muscles was 41.11±0.56 µm in cockerels and 36.51±0.45 µm in pullets. They stated that the muscular fibers were thicker in males (41.11±0.56 µm) than females (36.51±0.45µm). Scheuermann (2003) reported in commercial broiler chicken that males had 16% greater muscle fiber diameter than females.

In Chabro birds, the diameter of myofibers of muscle increased significantly with age in the male as well as in female birds, as reported by Li *et al*. (2020) in meat type birds. Koomkrong *et al*. (2015) in Arbor acres broilers (2860.50 μm^2) and Thai native chickens (1531.80 μm^2) recorded the cross-sectional area of myofibers. The mean cross-sectional area of myofibers of muscle was more in male than in female Chabro chicken. In contrary to this Burke and Henry (1998) observed higher cross-sectional area in female than male in 20 days old chick embryo. This may be due to excessive development of muscles after birth in male bird as compared to females of Chabro birds. The cross-sectional area increased with age in Chabro birds of both sexes as stated by Aberle and Stewart (1983) in chicken and Li *et al*. (2020) in meat type birds. Dransfield and Sosnicki (1999) also recorded the myofiber area in slow (2755 μm^2) and rapid (1946 μm^2) growing chicken and stated that the muscle fiber cross-sectional area increased with age of chicken. Scheuermann (2003) in commercial strain crosses (SC) broilers and Tumova and Teimouri (2009) in broiler chicken reported higher number of fibers in male than female. The mean muscular tissue area percent of deep pectoral muscle was more in males at 6 and 10 weeks but at 8 weeks it was more in female birds. Radu-Rusu *et al*. (2013) also reported higher muscular tissue in male (65.22%) than female (62.56%) pectoral muscle. In male Chabro birds, the muscular tissue area percent of muscle increased collinearly with age.

The diameter, cross-sectional area of myofibers, number of myofibers per fascicle, fascicular diameter, cross-sectional area of fascicle and interfascicular distance of deep pectoral muscle was more in male birds at all ages. But the intermyofiber distance was more in female birds. The mean diameter, cross-sectional area of myofibers, number of myofibers per fascicle, fascicular diameter and area of fascicle of deep pectoral muscle increased with age in both sexes. Hence the growth in muscle occurred by increase in number as well as size of muscle fiber. The connective tissue percent was higher in female birds than male at 6 weeks and 8 weeks of age.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: YP: Done the research during the PhD and the present manuscript is the part of her PhD research work; AP (2nd Author): Suggested the work and guided in the research as major advisor; MMF, AP (4th Author) : Give their expertise suggestions during writing and lab work; AP (Last Author): Performed statistical analysis.

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