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Research Article

Histomorphology of gastrocnemius muscle in Chabro chicken

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Abstract

The present study was conducted on the gastrocnemius muscle of 36 apparently healthy Chabro chicken, 18 males and 18 females, were divided into three age groups viz; Group I (6 weeks), Group II (8 weeks) and Group III (10 weeks). Anatomically the gastrocnemius muscle forms the drumstick part in poultry. In the inter myofiber space, large irregular shaped fibroblasts with one or more darkly stained heterochromatic nuclei and cytoplasmic projections extending around the muscle fibers, blood capillaries with nucleated RBCs and oval-shaped myosatellite cells with large spindle-shaped nucleus were observed. In female birds, the mean muscular tissue area percent increased continuously with age. In male birds, the mean fascicular diameter of muscle increased continuously with age.

Keywords: Chabro chicken, Gastrocnemius muscle, Inter myofiber space, Muscular tissue, Myosatellite cells

Highlights

- The diameter, cross-sectional area of myofibers, fascicular diameter, cross-sectional area of fascicle, number of myofibers per fascicle and inter fascicular distance of the muscle were more in male birds at all ages.
- The inter myofiber distance was more in female birds.
- The mean diameter, cross-sectional area of myofibers, fascicular diameter and area of fascicle of muscle increased with age in both sexes.

INTRODUCTION

According to APEDA (2019), poultry contributes 49.64% meat as against 4.61% of cattle, 18.85% of buffalo, 21.68% of small ruminants and 5.22% of other animals. Chicken meat has several advantages over red meat, especially its nutrient content. In the recent scenario, consumers have shown a strong interest in the overall nutritional values of food (Karakok *et al.*, 2010). Farmers and consumers have also shown interest in native or indigenous meat. Chabro chicken is a rural meat type bird. It is a crossbreed of Barred Plymouth Rock and Red Cornish bird (Yadav *et al.*, 2018). They are able to save themselves from their predators under free range and are adapted to all climatic zones of our country; hence, they are fit for backyard poultry farming. The textural quality of meat depends on the amount of connective tissue and muscular tissue, and the present study reveals it. The growth pattern of muscle (what is responsible for increase in size of muscle, i.e., increase in size of muscle fiber, number of muscle fiber or amount of connective) will also be revealed by the results of the present study, which always remains a matter of curiosity among scientists and practitioners in poultry and food industries during the rearing period. This study aimed to determine the muscular tissue growth in Chabro chicken and also reveal

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the comparative data of male and female birds, so persons from the poultry industry can select the required sex as per the muscle quality.

MATERIALS AND METHODS

The present study was conducted on the gastrocnemius muscle of 36 apparently healthy Chabro chickens, 18 males and 18 females, 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 the standard scientific method after chloroform inhalation (painless killing). The muscles of the leg region were dissected meticulously. For histomorphological observations, thin pieces (0.5 cm^3) 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 5µ in thickness. Sections were stained in various stains (Table 1) for detailed histomorphological studies.

A detailed histomorphometrical study was conducted on stained slides. Following micrometrical parameters were taken with the help of Leica DM 750 Computerized Image Analyzer. Diameter of myofiber was measured on the cross-section of histological slide of the muscle. Two diameters were taken, one largest (MDI) and another perpendicular to the previous one (MDII) and then the average of both the diameter was taken [(MDI+MDII)/2]. 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).

Diameter of fascicle: It was measured in cross sectional histological slide of muscle. Two diameters were taken, one the largest diameter of the fascicle (FDI) and another perpendicular to it (FDII) and then the average of both the diameter was taken [(FDI+FDII)/2] as the diameter of the fascicle. Cross sectional area of fascicle (CSF) was calculated by the formula:
Cross sectional area = FDI x FDII x $\pi/4$ (π = 3.1416 or 22/7).
Muscle tissue (MT) and connective tissue (CT) percentage were 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, and inter fascicular distance were measured, and number of myofibers per fascicle (n) was counted. The data generated by the micrometrical study were subjected to statistical analysis (Snedecor and Cochran, 1994) with the help of SPSS 20 software.

RESULTS

The muscle was wrapped in the sheath of dense irregular connective tissue, i.e., epimysium (0.05 µm to 0.21 µm thick in 10 weeks old birds), comprising of thick bundles of connective tissue fibers lined by layers of fibroblast cells (Fig. 1). The fibrous connective tissue of epimysium was predominated by thick bundles of collagen fibers intervened with large blood vessels and nerves running in different planes. The perimysium coursed inward deep into the muscle and divided it into fascicles of

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	Gomori's Reticulin stain or Gordon and Sweet's stain	Bancroft and Stevens (1977)	For the study of reticular fibers
4	Weigert's Resorcin-Fuchsin stain	Bancroft and Stevens (1977)	For the study of elastic fibers

various sizes. The primary fascicles further divided into smaller fascicles by secondary and tertiary branching of perimysium (Fig. 2). The perimysium was predominantly formed of reticular and collagen fibers with very few elastic fibers (Fig. 6 to 8). Adipose tissue was also abundantly distributed in the perimysium. Within the perimysium, arteries, veins and nerves were richly present. The number of collagen fibers in the perimysium of female birds appeared to be less than that of male birds. Most of the fascicles were bell-shaped or triangular-shaped; however, rounded, leaf-shaped, triangular and quadrangular-shaped fascicles were also observed (Fig. 1 and 2). Within the bounds of perimysium, the endomysium embraced individual myofibers of the muscle. The endomysium consisted of connective tissue fibers viz; collagen fiber was in greater proportion with reticular fiber (Fig. 6 to 8) and was overladen by the collagen fiber in advanced age. The elastic fiber was observed less in amount and lined only the sarcolemma. In the inter myofiber space, large irregular shaped fibroblasts with one or more darkly stained heterochromatic nuclei and cytoplasmic projections extending around the muscle fibers, blood capillaries with nucleated RBCs, nerves and oval shaped myosatellite cells with large spindle shaped nucleus were observed (Fig. 4 and 5). With the increase in age, connective tissue, especially collagen fibers, increased in epimysium, perimysium and endomysium.

In the cross section, the tendinous intersection was observed, which comprised of sheath of thick irregular dense connective tissue fibers, predominantly collagen fiber. The muscle was highly vascularized with a number of large and small blood vessels and capillaries in it. The muscle fibers were mainly intermediate type; however, few fascicles contained 1 to 2 large fibers. The fascicles near the tendon had 1 to 2 large fibers (Fig. 2 and 3). At 6 weeks of age, the muscle fibres were arranged in an organized manner within the fascicle. They were loosely packed in the fascicles near the tendon; however, the fascicles that were present away from the tendon or epimysium presented relatively more tightly packed myofibers. At 8 weeks, the primary, secondary and tertiary inter fascicular space became narrower. The primary fascicles divided further into secondary and tertiary fascicles, due to which the size of the fascicles became smaller. The number of fibers per primary fascicle increased with age. Most of the myofibers became rectangular or square shaped with rounded angles and of almost identical size. The thickness of epimysium increased with the age of the birds. The adipose tissue also increased in inter fascicular spaces. The large fibers were lightly eosinophilic, almost oval or rectangular (cross-section) in shape with rounded edges. Their nucleus was observed away from the sarcolemma deep into the sarcoplasm (Fig. 4). Their number per fascicle decreased with increase in the age of the birds. Its number was relatively more in fascicles located at the periphery. Their number reduced drastically in the fascicle located in the deeper part of the muscle. The large fibers were predominantly present in those fascicles, which occurred near the tendon (Fig.2). The approximate number of large fibers was 0 to 12, 0 to 8 and 0 to 9 per fascicle in the gastrocnemius muscle of male Chabro chicken at 6, 8 and 10 weeks of age respectively, whereas, in female Chabro chicken, their number was 0 to 12, 0 to 9 and 0 to 10 per fascicle at respective ages. In 10 weeks, old birds' muscle, the secondary and tertiary inter fascicular spaces became wider and more vascularized. The blood vessels encircled almost all the fascicles. All three types of muscle fibers were observed in the fascicle. Number of large fibers increased in the fascicle. However, the number of intermediate fibers remained maximum in each fascicle. The small fibers were minimum in number. Their number varied from 5 to 10 per fascicle. They were irregularly distributed in the fascicle. In some fascicles the myofibers were so tightly packed that the inter myofiber space was almost negligible.

Histomorphology of gastrocnemius muscle in Chabro chicken

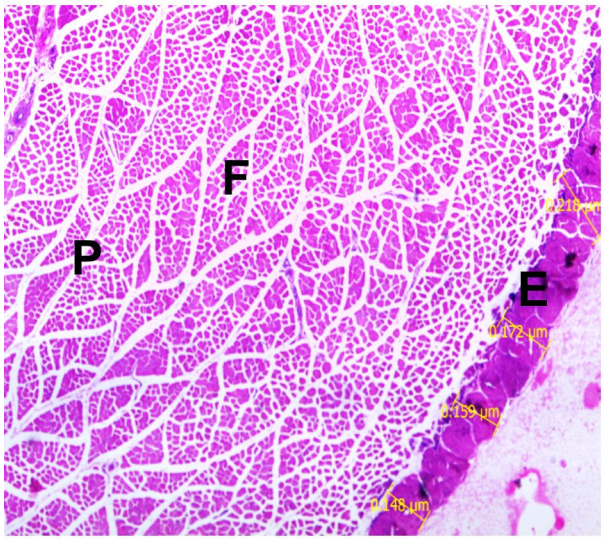

Fig. 1. Photomicrograph of gastrocnemius muscle of 10 weeks old male Chabro chicken showing epimysium(E), perimysium (P)and fascicle (F). H&E X 40

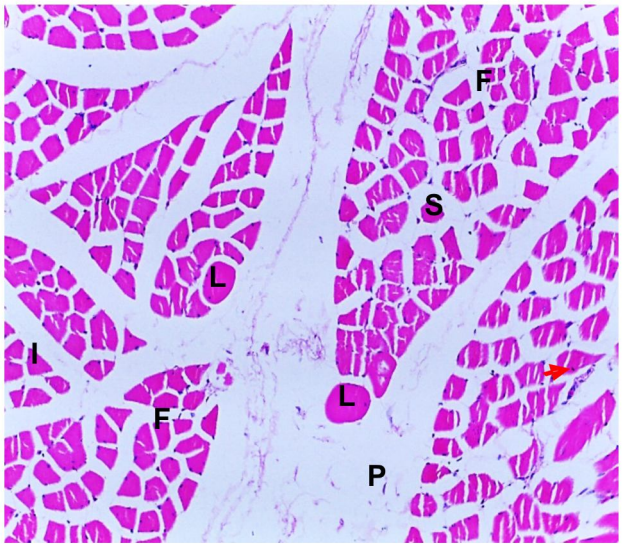

Fig. 2. Photomicrograph of gastrocnemius muscle of 6 weeks old female Chabro chicken showing large myofiber (L), intermediate myofiber (I), small myofiber (S), perimysium (P), fibroblast (arrow) and fascicle (F). H&E X 200

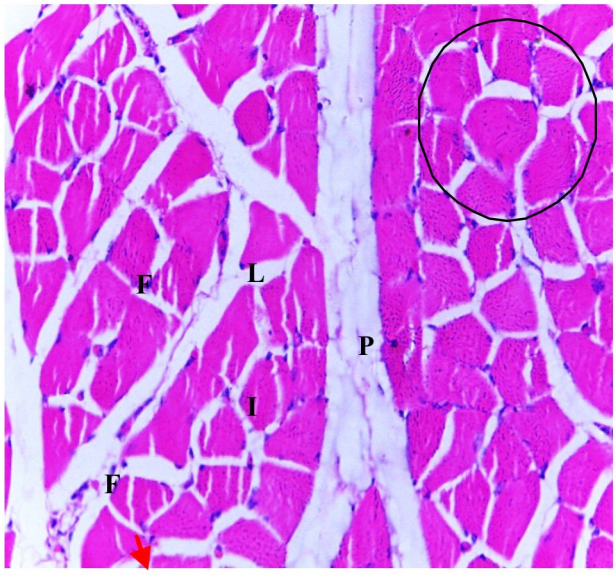

Fig. 3. Photomicrograph of gastrocnemius muscle of 6 weeks old female Chabro chicken showing large myofiber (L), perimysium (P), intermediate myofiber (I), fibroblasts (arrow), myofiber arrangement (circle) and fascicle (F). H & E X 400

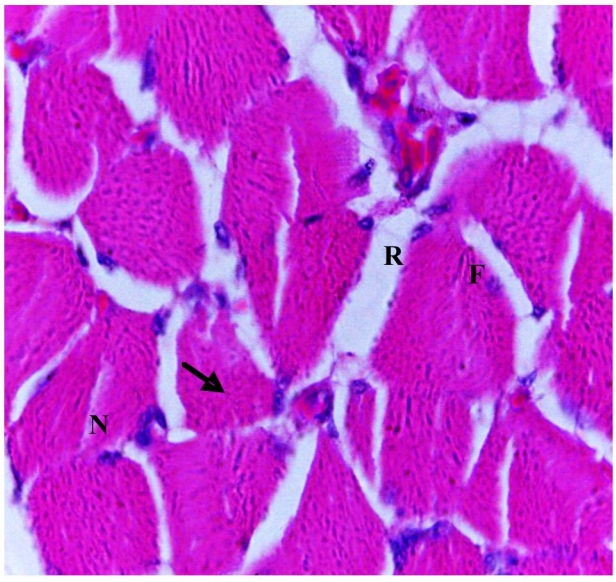

Fig. 4. Photomicrograph of gastrocnemius muscle of 6 weeks old female Chabro chicken showing capillaries with RBCs (R), myosatellite cells (arrow), fibroblast cells (F) and nucleus of myofiber (N). H & E X 1000

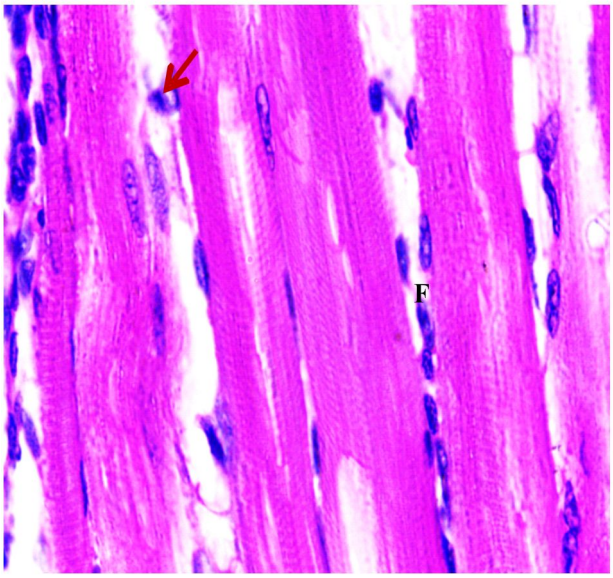

Fig. 5. Photomicrograph of gastrocnemius muscle of 6 weeks old male Chabro chicken showing nucleus of myofiber (N), fibroblast (F) and myosatellite cells (arrow). H&E X 1000

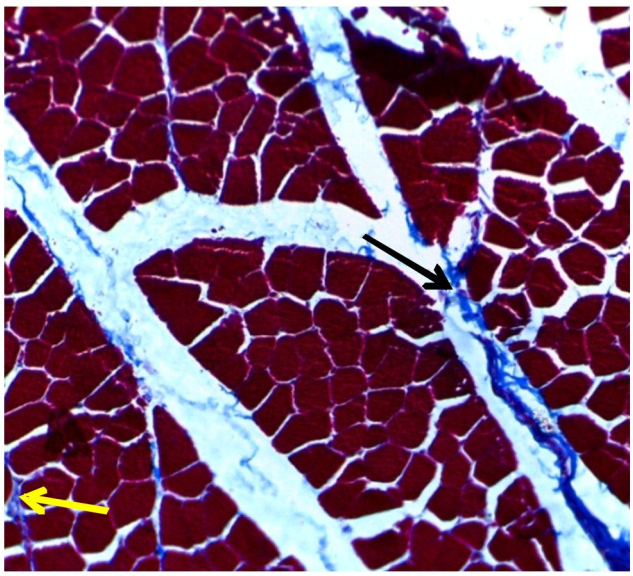

Fig. 6. Photomicrograph of gastrocnemius muscle of 8 weeks old female Chabro chicken showing collagen fibers (arrow) in perimysium and endomysium. Masson's trichrome stain X200

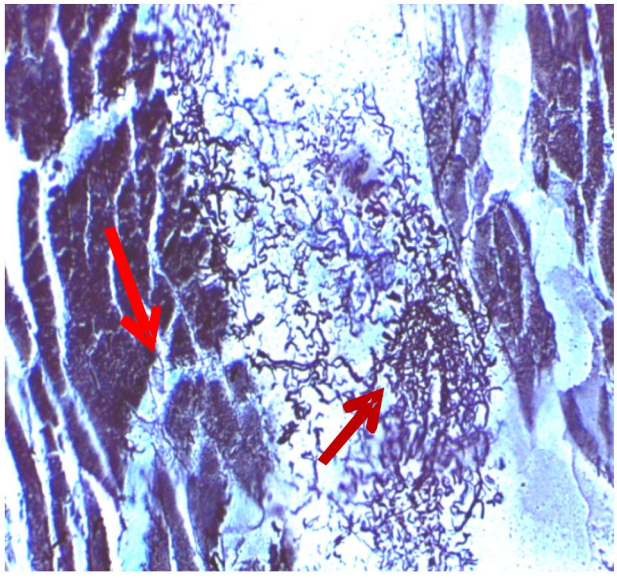

Fig. 7. Photomicrograph of gastrocnemius muscle of 6 weeks old female Chabro chicken showing reticular fibers (arrow) in endomysium and perimysium. Gordon & Sweet's stain X 200

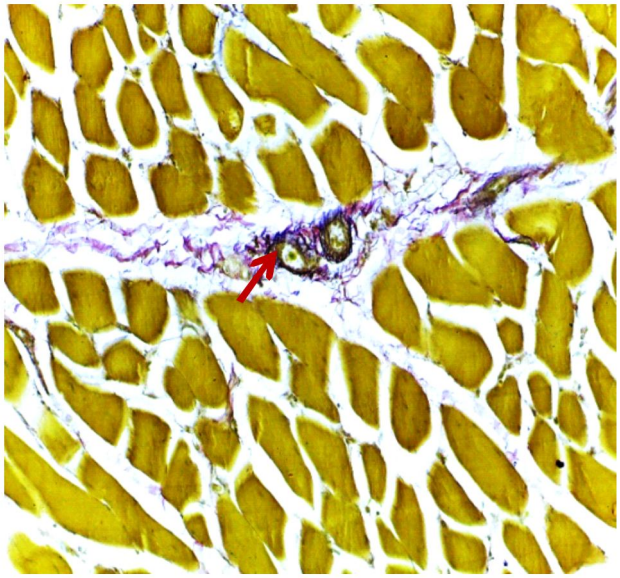

Fig. 8. Photomicrograph of gastrocnemius muscle of 6 weeks old female Chabro chicken showing elastic fibers (arrow) in the wall of blood vessel. Weigert's resorcin fuchsin stain X 400

Histomorphometry: The myofiber diameter and cross-sectional area of myofibers, length and width of the nucleus of myofibers, inter myofiber distance, number of myofibers per fascicle, fascicular diameter, cross sectional area of fascicle, inter fascicular distance, muscular tissue percentage and connective tissue percentage in the gastrocnemius muscle fascicles were recorded on male and female Chabro chicken of 6, 8 and 10weeks of age (Table 2, 3 & 4).

The diameter of myofibers was more in the male chicken than in the female at the same age. The sexual difference was statistically significant at all ages. The mean diameter of myofibers increased between 6 to 8 weeks and decreased between 8 to 10 weeks of age in male as well as in female chicken. This difference in

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

Parameters \ Groups	Group I (6 weeks)	Group II (8 weeks)	Group III (10weeks)
Diameter of muscle fiber (µm)	15.72±0.54^{c}	31.71±0.50^{b}	33.15±0.42^{a}
Cross-sectional area of muscle fiber (µm^2)	194±13.91^{c}	778±25.24^{b}	857±23.28^{a}
Length of nucleus of muscle fiber (µm)	5.46±0.36^{a}	5.41±0.11^{a}	6.41±1.220^{a}
Width of nucleus of muscle fiber (µm)	2.20±0.53^{a}	1.42±0.171^{a}	1.40±0.09^{a}
Inter myofiber distance (µm)	4.36±0.27^{a}	3.58±0.13^{a}	4.91±0.31^{a}
No. of myofiber per fascicle	96.67±7.96^{a}	105±1.52^{a}	103±4.71^{a}
Fascicular diameter (µm)	203±16.73^{b}	411.21±6.94^{a}	426.25±8.68^{a}
Cross-sectional area of fascicle (µm^2)	30935±4279^{b}	131561±4739^{a}	134632±6814^{a}
Inter fascicular distance (µm)	22.40±1.44^{a}	22.38±0.43^{a}	25.07±2.13^{a}
Muscular tissue area (%)	61.91±0.29^{b}	62.37±0.56^{b}	65.63±0.86^{a}
Connective tissue area (%)	38.09±0.29^{a}	37.63±0.56^{a}	34.37±0.86^{b}

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

Parameters \ Groups	Group I (6 weeks)	Group II (8 weeks)	Group III (10 weeks)
Diameter of muscle fiber (µm)	17.45±0.39^{c}	39.56±0.45^{a}	36.30±0.49^{b}
Cross-sectional area of muscle fiber (µm^2)	238±10.55^{c}	1225±27.50^{a}	1032±45.49^{b}
Length of nucleus of muscle fiber (µm)	6.02±0.05^{a}	5.85±0.15^{a}	6.19±0.12^{a}
Width of nucleus of muscle fiber (µm)	1.08±0.02^{a}	1.87±0.86^{a}	1.34±0.03^{a}
Inter myofiber distance (µm)	3.90±0.14^{a}	3.57±0.05^{b}	5.47±0.63^{a}
No. of myofiber per fascicle	98.00±3.66^{b}	112±1.60^{a}	95.81±1.93^{b}
Fascicular diameter (µm)	250±8.84^{c}	510±7.85^{a}	448±11.13^{b}
Cross-sectional area of fascicle (µm^2)	43618±2530^{c}	203579±5629^{a}	156526±8259^{b}
Inter fascicular distance (µm)	43.89±10.59 ^{a}	21.82±0.25^{b}	14.88±0.19^{b}
Muscular tissue area (%)	53.87±0.35^{c}	68.30±0.89^{a}	62.94±0.62^{b}
Connective tissue area (%)	46.13±0.35^{a}	31.70±0.890 ^{c}	37.06±0.62^{b}

n= 36; Figures in parenthesis indicate range; 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. Comparison of histomorphometrical parameters (Mean±SE) of gastrocnemius muscle between male and female Chabro chicken of same age group

Groups / Parameters	Group I Female	Group I Male	Group II Female	Group II Male	GroupIII Female	Group III Male
Myofiber diameter	15.72^{A} ±0.54	17.45^{B} ±0.39	31.71^{A} ±0.50	39.56^{A} ±0.45	33.15^{A} ±0.42	36.30^{A} ±0.49
Cross-sectional area of myofiber	194^{B} ±13.91	238^{A} ±10.55	778^{B} ±25.24	1225^{A} ±27.50	857^{B} ±23.28	1032^{A} ±45.49
Length of nucleus	5.46^{B} ±0.36	6.02^{A} ±0.05	5.41^{B} ±0.11	5.85^{A} ±0.15	6.41^{A} ±1.220	6.19^{A} ±0.12
Width of nucleus	2.20^{A} ±0.53	1.08^{A} ±0.02	1.42^{A} ±0.171	1.87^{A} ±0.86	1.40^{A} ±0.09	1.34^{A} ±0.03
Inter myofiber distance	4.36^{A} ±0.27	3.90^{B} ±0.14	3.58^{A} ±0.13	3.57^{A} ±0.05	4.91^{A} ±0.31	5.47^{A} ±0.63
No. of myofiber per fascicle	96.67^{A} ±7.96	98.00^{A} ±3.66	105^{A} ±1.52	112^{A} ±1.60	103^{A} ±4.71	95.81^{A} ±1.93
Fascicular diameter	203^{A} ±16.73	250^{A} ±8.84	411.21^{A} ±6.94	510^{B} ±7.85	426.25^{A} ±8.68	448^{A} ±11.13
Cross-sectional area of fascicle	30935^{B} ±4279	43618^{A} ±2530	131561^{B} ±4739	203579^{A} ±5629	134632^{A} ±6814	156526^{A} ±8259
Inter fascicular distance	22.40^{B} ±1.44	43.89^{A} ±10.59	22.38^{A} ±0.43	21.82^{B} ±0.25	25.07^{A} ±2.13	14.88^{B} ±0.19
Muscular tissue area (%)	61.91^{A} ±0.29	53.87^{B} ±0.35	62.37^{A} ±0.56	68.30^{A} ±0.89	65.63^{A} ±0.86	62.94^{B} ±0.62
Connective tissue area (%)	38.09^{A} ±0.29	46.13^{A} ±0.35	37.63^{A} ±0.56	31.70^{B} ±0.89	34.37^{B} ±0.86	37.06^{A} ±0.62

n= 36; Figures in parenthesis indicate range; 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

the growth increment was also statistically significant among the birds of all age groups. The cross-sectional area of myofibers was significantly higher in the male chicken than the female at all ages. The mean cross-sectional area increased continuously with age in the birds of both sexes and was statistically significant among all age groups in both sexes. The length of nucleus of myofibers was more in male chicken than in female at 6 and 8 weeks of age but not at 10 weeks of age, where it was more in female birds than in male. The mean length of nucleus decreased between 6 to 8 weeks and increased between 8 to 10 weeks of age in both male and female birds. The width of nucleus of myofibers was higher in the female chicken than in the male at 6 and 10 weeks of age, but at 8 weeks of age, it was more in male chicken. In male Chabro chicken, the width of nucleus increased between 6 to 8 weeks of age but decreased between 8 to 10 weeks, whereas, in male chicken, it decreased continuously from 6 to 8 weeks and 8 to 10 weeks of age. However, the difference was statistically non-significant in the birds of both sexes.

The inter myofiber distance was higher in the male than in the female at 6 and 8 weeks of age, but at 10 weeks, it was more in female than the male. However, this sexual difference was statistically not significant at all ages. The mean inter myofiber distance of muscle decreased between 6 to 8 weeks and then increased between 8 to 10 weeks of age in the birds of both sexes. In female chicken, this difference was statistically non-significant

between I & II and II & III age groups, but in male, it was statistically significant between I to III and II to III age groups, while it was non-significant between I to II weeks.

The number of myofiber per fascicle of muscle was more in male at 6 and 8 weeks of age, but at 10 weeks of age, it was more in female chicken than in male of the same age. The sexual difference was found to be statistically significant only at 8 weeks of age and non-significant at 6 and 10 weeks. The number of myofibers per fascicle in gastrocnemius muscle increased between 6 to 8 weeks but decreased between 8 to 10 weeks of age in male as well as in female birds. Although the difference in the mean number of myofibers per fascicle was non-significant between all age groups of female chicken. In male chicken, the difference was statistically significant between 6 to 8 and 8 to 10 weeks of age but not between 6 to 10 weeks of age.

The fascicular diameter of the gastrocnemius muscle was higher in the female chicken than in the male of the same ages. The sexual difference was statistically significant at 6 and 8 weeks but not significant at 10 weeks of age. In female Chabro birds, the mean fascicular diameter of muscle increased between 6 to 8 weeks and decreased between 8 to 10 weeks of age, and the difference was statistically significant between all age groups. In male birds, the mean fascicular diameter of muscle increased continuously with age. However, the difference was statistically significant between 6 to 8 and 6 to 10 weeks and non-significant between 8 to 10 weeks of age.

The cross-sectional area of the fascicles of muscle was higher in the male chicken than in the female at all ages. The sexual difference was statistically significant at 6, 8 weeks and also at 10 weeks. The mean cross-sectional area of the fascicle of muscle increased with age in birds of both sexes. This increase was statistically significant in female between 6 to 8 and 6 to 10 weeks but not between 8 to 10 weeks of age, whereas, in male birds, the area increased significantly with age.

The inter fascicular distance of muscle was higher in the male chicken than that of female at 6 weeks, but at 8 and 10 weeks of age, it was higher in female than male. The sexual difference was found to be statistically significant at 6 weeks and 10 weeks but non-significant at 8 weeks of age. In male chickens, the mean inter fascicular distance of muscle continuously decreased between 6 to 8 and 8 to 10 weeks. However, the difference was statistically significant between group I and II and group I and III but not between group II and III. In male chicken, the inter fascicular distance increased gradually between 6 to 8 and 8 to 10 weeks, although insignificantly.

The muscular tissue area percentage value was significantly higher in the female than in the male at 6 and 10 weeks. The mean muscular tissue percentage in male chicken increased between 6 to 8 weeks but decreased significantly between 8 to 10 weeks. In female birds, the mean muscular tissue percentage increased continuously with age significantly between 6 to 10 and 8 to 10 weeks, but non-significantly between 6 to 8 weeks of age. The connective tissue area percentage was recorded higher in the female chicken muscle than in male at 6 and 10 weeks. The difference in the connective tissue area percentage of female and male birds was statistically significant at 6, 8 and 10 weeks. In female chicken, the mean connective tissue area percentage increased between 8 to 10 weeks of age significantly, but in male, it decreased continuously and significantly with age.

DISCUSSION

Each individual muscle sheathed in a thick layer of dense irregular connective tissue, the epimysium, which separated each muscle from the surrounding muscles and tissues, and enclosed the group of fascicles (Nickel *et al.*, 1977). The dense irregularly arranged connective tissue fibers of epimysium were dispersed as interwoven thick bundles of collagen fibers; the collagen bundles moderately intervened by numerous large blood

vessels running in different planes (Mahon, 1999). Mobini (2015) stated that females had higher reticular and collagenous layers in epimysium (7-8 and 3-4 layers, respectively) of quadriceps femoris muscle than males (1-2 and 3). From the inner surface of epimysium, the connective tissue septae entered inward deep into the muscle tissue encircling each fascicle forming the perimysium in Chabro birds (Astruc, 2020). The perimysium was predominantly composed of collagen fibers with reticular fibers and very few elastic fibers. Arteries, veins, nerves, ganglia, and adipose tissue traversed through the perimysium (Copenhaver *et al*., 1971; Dellmann and Brown, 1987; Lawrie, 1998; Listrat *et al.*, 2016). The number of collagen fibers in the perimysium of female birds appeared to be more than that of the male birds of comparable age (Mobini, 2015); the adipose tissue was abundantly present between two bundles of connective tissue fibers (Listrat *et al.*, 2016). The endomysium of myofibers consisted of connective tissue comprising of collagen and reticular fibers (George and Berger, 1966; Copenhaver *et al.*, 1971; Eurell and Frappier, 2006; Mobini, 2015). The elastic fibers were present predominately in the wall of blood vessels (Santos *et al.*, 2004). It also lined the sarcolemma (Rodrigues and Junior, 2000). Allison and Richard (2011) stated that the perimysium of muscle was much sparser, more variable and less defined as compared to the endomysium. These fascicles vary in shape and may be rounded, triangular, quadrangular, rectangular or lanceolate (Astruc, 2020).

The nuclei were heterochromatic, vasicular, elongated oval in shape and lightly hematoxiophillic (Eurell and Frappier, 2006). In the muscles of leg region, three types of muscle fibers were identified on the basis of size and histomorphology. They are large myofiber, intermediate myofiber and small myofiber as observed by Smith and Fletcher (1988) in gastrocnemius muscle of Arbor Acre broilers and Listrat *et al.* (2016). The cut surface of myofibers appeared rough due to presence of the cut ends of myofibrils. Similar statements were given by Eurell and Frappier (2006), Choi and Kim (2008) and Singh and Sachan (2011). Papinaho *et al*. (1996) measured the diameter of muscle fiber of gastrocnemius (60.45 µm) muscle in broiler chicken. Tumova and Teimouri (2009) reported larger muscle fiber in intact male chicken than in female or castrated male. Whereas, Radu-Rusu *et al.* (2013) reported thicker fibers in female Cobb 500 birds than in male. Koomkrong *et al.* (2015) also recorded the diameter of muscle fiber of biceps femoris muscle in Arbor acres broilers (45.10 µm) and Thai native chickens (35.30 µm) and stated that it increased with age in both the birds. Remignon *et al.* (1994) also documented the same. Koomkrong *et al.* (2015) also recorded the cross-sectional area of biceps femoris muscle in Arbor acres broilers (2555.00 μm^2) and Thai native chicken (1681.50 μm^2) and stated that it increased with age in both the birds.

The inter myofiber distance increased between 8 to 10 weeks of age in both sexes of birds (Koomkrong *et al.*, 2015). Burke and Henry (1998), in 16 days old embryos, reported that female chicken embryo had lower number of total fibers in gastrocnemius muscle than males. Scheuermann (2003) reported that in commercial broiler chicken, males had 16% greater muscle fiber diameter than females. Tumova and Teimouri (2009) reported a greater number of muscle fibers in male chicken than in female. Radu-Rusu *et al.* (2013) reported a greater number of fibers in male than female in Cobb 500 broilers (513.84 myofibers/mm^2 in female and 810.15 myofibers/mm^2 in male). In female Chabro birds, the number of myofiber increased between 6 to 8 (Dransfield and Sosnicki, 1999). The fascicular diameter in female birds increased continuously with age (Astruc, 2020). Aberle and Stewart (1983) and Dransfield and Sosnicki (1999) in chicken also reported that the muscle fiber cross-sectional area increased with age in chicken.

The diameter, cross-sectional area of myofibers, fascicular diameter, cross-sectional

area of fascicle, number of myofibers per fascicle and inter fascicular distance of the muscle were more in male birds at all ages. The inter myofiber distance was more in female birds. The mean diameter, cross-sectional area of myofibers, fascicular diameter and area of fascicle of muscle increased with age in both sexes.

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Author's contribution: YP: The main author who did the research during the PhD, and the present manuscript is a part of her PhD research work; AP: Suggested the work and guided in the research as major advisor; MMF and AP: Give their expertise suggestions during writing and lab work; AP: Did all the statistical analysis; AV: Contributed in staining and photography with his expert suggestions during present research work.

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