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Research Article

HISTOMORPHOLOGY OF BICEP FEMORIS MUSCLE IN CHABRO CHICKEN

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ABSTRACT

The present study was conducted on the biceps femoris muscle of 36 apparently healthy Chabro chicken, 18 male and 18 female. Anatomically the biceps femoris muscle forms the part of the hip and thigh region in poultry. In Chabro chicken, the biceps femoris muscle comprised of muscle fibers, blood vessels, nerve fibers and connective tissue. The epimysium of biceps femoris muscle of Chabro chicken was composed predominantly of thick bundles of collagenous connective tissue fibers lined by a layer of fibroblast cells on its outer side.

Keywords: Biceps femoris, Chabro chicken, Epimysium, Muscle

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India has the largest livestock population in the world. Meat industry in India is however, still in growing stage. They are active, large in built, pugnacious in nature with contrast color. 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 from other animals. Chicken meat has several advantages over red meat, especially in their nutrient content. In the recent scenario, consumers have shown a strong interest in the overall nutritional values of food, and consumer has become more health conscious (Raut *et al.*, 2016). The farmers and consumers have also shown interest in native or indigenous meat. This study was aimed to determine the muscular tissue growth in Chabro chicken. Chabro chicken is a rural meat type bird. It is a cross breed 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, Singh and Pathak (2019) also stated its characteristic as hardiness, can tolerate harsh environmental conditions and poor husbandry practices without much loss in production. 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 be revealed by the results of the present study, which always remained matter of curiosity among scientists and practitioners in poultry and food industries during rearing period. This study also reveals the comparative data of male and female birds so that poultry industry can go for the selection of required sex as per the muscle quality.

MATERIALS AND METHODS

The present study was conducted on the biceps femoris muscle of 36 apparently healthy Chabro chicken, 18 male and 18 female, were divided into divided in to 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 6th, 8th and 10th week. Then the birds were sacrificed by standard scientific method after chloroform inhalation (painless killing). The leg 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 5µ and were stained in the various stains (Table 1) for the detailed histomorphological studies.

RESULTS AND DISCUSSION

The biceps femoris muscle was comprised of skeletal muscle tissue, blood and lymph vessels, nerve trunks and connective tissue. The muscle was wrapped in a thick sheath of dense irregular connective tissue i.e., epimysium (Fig. 1). It separated the biceps femoris muscle from the surrounding muscles and tissues and enclosed the group of muscular fascicles (Nickel *et al.*, 1977). The epimysium was composed predominantly of thick bundles of collagenous connective tissue fibers lined by a layer of fibroblast cells on its outer side. The dense irregularly arranged connective tissue fibers of epimysium were dispersed as interwoven thick bundles of collagen fibers, the collagen bundles moderately intervened numerous large blood vessels and nerves running in different planes similarly Mahon, (1999) in poultry reported that epimysium of both red and white muscles contained the larger blood vessels, neves, adipose tissue and all the connective tissue fibers (Fig. 1). Mobini (2015) stated that

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Table 1. Various histological methods used in the study

S.N.	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	Bancroft and Stevens (1979)	For the study of reticular fibers.
4.	Weigert's Resorcin-Fuchsin stain	Bancroft and Stevens (1979)	For the study of elastic fibers.

females had higher reticular and collagenous layers in epimysium (7-8 and 3-4 layers, respectively) than males (1-2 and 3) The thickness of epimysium ranged from 0.51 mm to 0.99 mm at 8 weeks of age. The sexual difference in thickness of collagen layer didn't notice. From the inner surface of epimysium, arose the connective tissue septae which passed inward deep in to the muscle tissue forming the perimysium, sexual difference was not observed in perimysium thickness. These latter encircled the group of true muscle fibers in to bundles called fascicles (Astruc, 2020). The perimysium was predominantly composed of reticular and collagen fibers with a few elastic fibers. Arteries, veins, nerves, ganglia, adipose tissue were also present within the perimysium (Dellmann and Brown, 1987; Lawrie, 1998; Listrat *et al.*, 2016) (Fig. 2). The amount of collagen fibers in the perimysium of female birds appeared to be more than that of the male birds of comparable age. The tendinous intersection; a thick sheath of dense irregular connective tissue was observed, which was comprised predominantly of collagen bundles (Fig. 5). Allison *et al.* (2011) stated that the perimysium of muscle was much sparser, variable and less defined as compared to the endomysium. The fascicles were mostly bell shaped although round, leaf, triangular and quadrangular shaped fascicle were also observed (Fig. 1). In the perimysium of biceps femoris muscle, the adipose tissue was abundantly present between two bundles of connective tissue fibers (Listrat *et al.*, 2016) (Figs. 6 & 7). From the perimysium the connective tissue entered inside the fascicle till it reached to and embraced the individual muscle fiber to form the endomysium. The endomysium of myofibers consisted of connective tissue comprising; collagen and reticular fiber (Eurell and Frappier, 2006; Mobini, 2015) (Figs. 5 & 6). The latter was over laden by the collagen fibers, as age advanced. The elastic fibers were present predominantly in the wall of blood vessels (Santos *et al.*, 2004). But in epimysium and perimysium, it was present in meager amount. It also lined the sarcolemma (Rodrigues and Junior, 2000). In the endomysium certain large irregular shaped fibroblast cells with dense and darkly stained heterochromatic nucleus were also seen (Eurell and Frappier, 2006). These fibroblast cells presented one to many nuclei and their cytoplasmic projections extended around the muscle fibers. The endomysium also contained blood vessels with nucleated RBCs and nerves in the 6 weeks old Chabro chicks. With increase in age, the amount of collagenous fiber increased along with other fibers in the epimysium, perimysium and endomysium. The sexual difference in endomysium was non-significant. In the intermyofiber space below the endomysium, certain myosatellite cells or muscle stem cells were also observed close to the sarcolemma. The myosatellite cells were characteristically ovoid in shape with a large spindle shaped nucleus.

In the cross sections, the muscle fibers appeared multinucleated polygonal or quadrangular in shape. As the age increased the angles of myofibers became more or less rounded. The multiple nuclei were located peripherally close to the sarcolemma. Some nuclei however, lay embedded within the cytoplasm. The nuclei were heterochromatic, vesicular, elongated oval in shape and lightly basophilic. Three types of muscle fibers could be identified in this muscle *viz*; large, medium and small as observed by Smith and Fletcher, (1988) in gastrocnemius muscle of Arbor Acre broilers and Listrat *et al.* (2016). The large fibers were almost oval or rectangular in shape with rounded edges, their cytoplasm was eosinophilic (Figs. 1 & 3). In these cells the nucleus was mostly observed embedded deep into the sarcoplasm and was located away from the sarcolemma. These large fibers occurred mostly near the angle or along the periphery of the fascicles. The approximate number of large fibers was 0 to 4, 0 to 3 and 0 to 2 per fascicle in the biceps femoris muscle of male Chabro chicken at 6, 8 and 10 weeks of age whereas, in female Chabro chicken, their number was 0 to 5, 0 to 3 and 0 to 2 per fascicle at respective ages. Hence the numbers of large fibers appeared depleting with increase in the age. The medium or intermediate size fibers were numerously present in the muscle. They stained darker than the large muscle fibers. Their nuclei were present along the periphery. These fibers were rectangular, irregular or polygonal in shape. The muscle fibers present in the deeper part of the fascicles showed a typical floral pattern. Here 5 to 7 intermediate fibers surrounded the single small muscle fiber in the form of flower petal pattern (Fig. 7). Each myofibers contained a rich number of myofibrils. This gave a rough appearance to the intermediate fibers, in the form of dark dots in the cross section of myofiber. Similar statements were given by Eurell and Frappier (2006), Choi

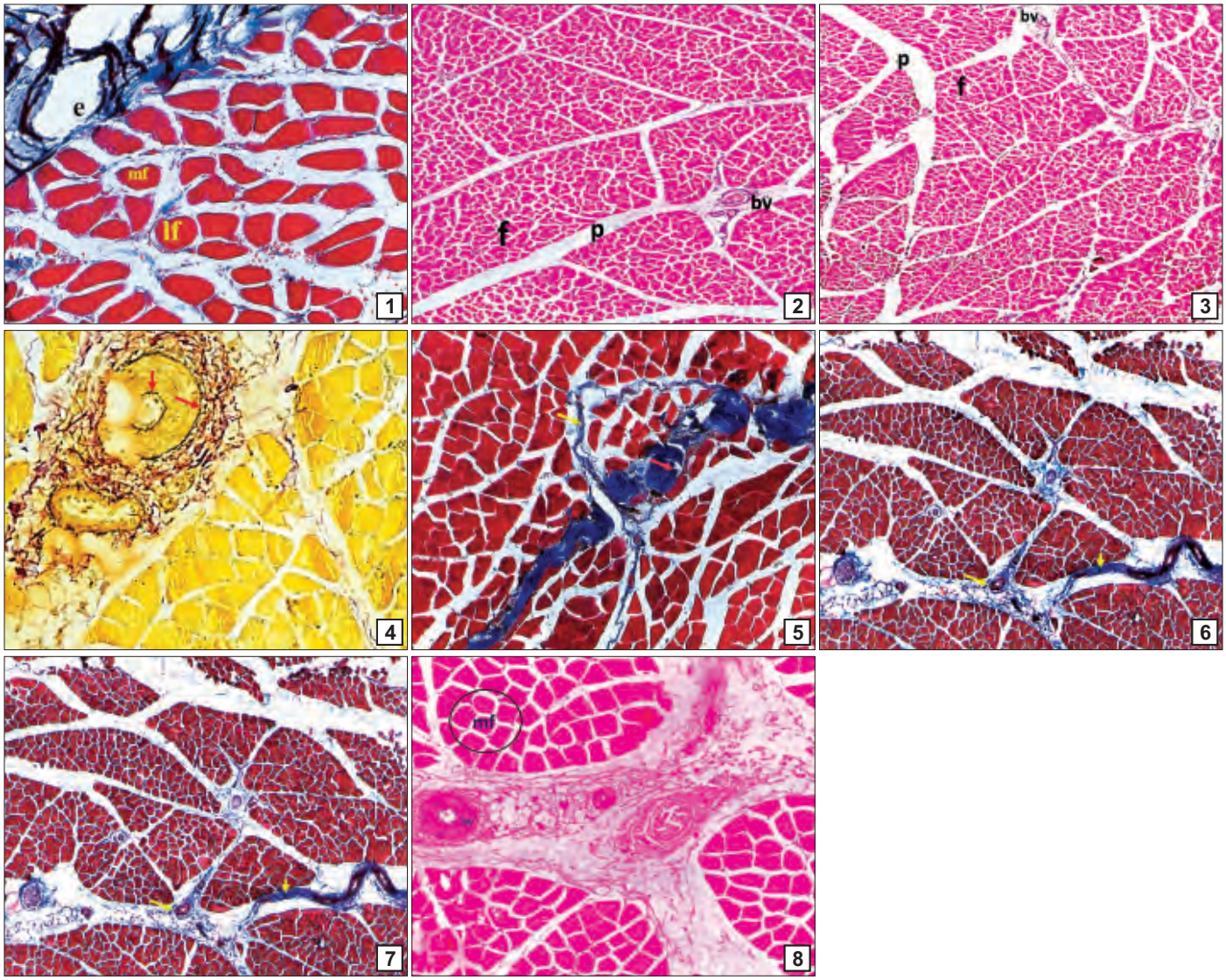

Fig. 1-8. **(1)** Photomicrograph of biceps femoris muscle of 6 weeks old female Chabro chicken showing epimysium(e), intermediate myofiber (mf) large myofiber (lf) Masson's trichrome stain X400; **(2)** Photomicrograph of biceps femoris muscle of 6 weeks old female Chabro chicken showing perimysium (p), fascicle (f) and perimysial blood vessel (bv). H & E X40; **(3)** Photomicrograph of biceps femoris muscle of 6 weeks old male Chabro chicken showing perimysium (p), fascicle (f) and perimysial blood vessel(bv). H & E X40; **(4)** Photomicrograph of biceps femoris muscle of 6 weeks old female Chabro chicken showing elastic fiber inblood vessel(arrow). Weigert's resorcin fuchsin stain X 400; **(5)** Photomicrograph of biceps femoris muscle of 6 week old female Chabro chicken showing collagen fiber (arrow) and tendinous intersection (t). Masson's trichrome stain X200; **(6)** Photomicrograph of biceps femoris muscle of 10 week old female Chabro chicken showing collagen fiber (arrow) Masson's trichrome stain X100; **(7)** Photomicrograph of biceps femoris muscle of 10 week old female Chabro chicken showing collagen fiber (arrow) Gordon & Sweet's stain X 200; **(8)** Photomicrograph of biceps femoris muscle of 6 weeks old female Chabro chicken showing myofiber (mf), blood vessels (bv) H&E X 200

and Kim (2008), Sharma and Sachan (2011). The small fibers were very less in number and scattered irregularly throughout the fascicles. These were mostly quadrangular in cross-sections with small vesicular nuclei. Their surface also appeared rough due to presence of the cut ends of myofibrils. In longitudinal section, the myofibers were found in the form of several elongated cylindrical structures arranged parallel to each other. The multiple nuclei were observed close to the periphery. The nuclei were elongated oval in shape. In the higher magnification the light and dark bands were observed imparting the characteristic striated pattern of the skeletal muscle.

CONCLUSION

The amount of collagen fibers in the perimysium of female birds appeared to be more than that of the male birds of comparable age. The endomysium of myofibers consisted of connective tissue comprising; collagen and reticular fiber. The large fibers were almost oval or rectangular in shape with rounded edges, their cytoplasm was eosinophilic. The muscle fibers present in the deeper part of the fascicles showed a typical floral pattern.

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