

## Comparative Histological Studies of Intramuscular Connective Tissues of Muscle Pectoralis Profundus from Native and Broiler Chickens

Behzad Mobini

Department of Anatomical Sciences, College of Veterinary Medicine,  
Islamic Azad University, Shahrekord Branch, Shahrekord - Iran

**Abstract:** The histological properties of intramuscular connective tissue of meat determine the perceived meat structure and tenderness. In order to find out the histological differences of the intramuscular connective tissues of muscle *Pectoralis profundus* of broiler and native chickens, 40 birds from both sexes (9-10 weeks) were used. Tissue samples were stained by hematoxylin eosin and a variety of special techniques for determination of types of connective tissue fibers. The results revealed that the histological structure of the intramuscular connective tissues of *M. Pectoralis profundus* in the native chicken was generally similar to those of Ross broiler except for the numbers of the all connective tissue layers in endomysium and perimysium. Histological differences were not found in the intramuscular connective tissues between the left and right *M. Pectoralis profundus* of both the strains of the chickens. All the connective tissue fibers observed in the all intramuscular connective tissues of *M. Pectoralis profundus* of both the broiler and native chickens. The number of collagenous layers of the epimysium was more in the broilers than those of the native chickens and in the females than the males. Tenderness was more in the native chickens than the Ross broilers. Also, tenderness might be more in males than the females in both the genotypes of the chickens. Sex related differences were observed only in the epimysium of *M. Pectoralis profundus* in both the genotypes of the chickens.

**Key words:** Comparative histology, • Connective tissues • *M. pectoralis profundus* • Chicken • Sex

### INTRODUCTION

Meat quality traits play an important role in purchase decisions [1]. Recent studies have demonstrated that the intramuscular connective tissues had major role in eating quality of meat [2, 3] and the properties of intramuscular connective tissue of meat determine the perceived meat structure and tenderness [4]. The intramuscular connective tissues in meat are in three hierarchical levels: epimysium, perimysium and endomysium [5, 6]. In intramuscular connective tissues the main protein is collagen and another important protein is elastin [7]. Also, reticular fibers are actually individual collagen fibrils (type III collagen) which form delicate networks around muscle fibers [5].

Some researchers have revealed the role of intramuscular connective tissues on meat quality and tenderness [2-4, 8-26]. Fanatico *et al.* [27] reported that slow growing chicken genotypes had a higher percentage of dark to white meat than conventional broilers and have been selected to produce dark meat rather than white meat. According to Kerr *et al.* [28] the collagen content of fast growing animals was less matured than that of slow growing animals at the same slaughter weight. Nakamura *et al.* [18] showed that collagen content of breast muscles in broiler chickens was highest at the ages of two weeks.

Voutila [4] reported that collagen content of leg muscles was higher than in breast muscles in poultry and the increasing thickness of endomysium and perimysium were related to increasing meat toughness. In addition,

Oshima *et al.* [21] showed that the variation of muscle collagen content was due to the variation in the state of development of perimysium. The most common intramuscular connective tissue research has been on beef [15, 29], pigs [30], turkeys [31] and some genotypes of chickens such as Laying hens [9], Silky [19], White Leghorn [24], Rhode Island Red [16], Red Cornish and New Hampshire [18, 23].

In Shahrekord, most of the farmers rear Ross broiler and native chickens (*Gallus domesticus*). These two chickens' genotypes reared in different conditions. The native chickens are scavenger in nature fed by green grasses, garden leftover, grains and seeds, in contrast, the broilers reared in well hygienic condition. Thus, the present investigation was aimed to find out the histological differences of intramuscular connective tissues of *M. Pectoralis profundus* between the Ross broiler and native chickens in both sexes.

## MATERIALS AND METHODS

A total of 40 adult clinically healthy chickens (9-10 weeks), 20 from Ross broiler and 20 from native chickens (*Gallus domesticus*) of both sexes (20 each sex) were obtained from the Research farm of household bird's maintenance of College of Veterinary Medicine, Azad University of Shahrekord. The *Gallus domesticus* were fed by green grasses, garden leftover, grains and seeds, in contrast, the Ross broilers were reared in a floor-pen house from hatch to 10 weeks of age and received feed and water *ad libitum*. The birds were deeply anesthetized by excess ether inhalation. The guidelines of the ethical committee of Shahrekord Azad University were strictly followed during the procedure. For histological studies, samples were taken from the middle parts of right and left sides of *Pectoralis profundus* muscle. The samples were immediately fixed in 10% buffered neutral formalin solution for 24-48 hours, dehydrated and embedded in paraffin wax. Tissue sections (6  $\mu$ m) were stained with hematoxylin eosin for general histological examination and a variety of special techniques for types of fibers in the intramuscular connective tissue: Masson's trichrome (for collagen fibers), Verhoeff's (for elastic fibers) and Gomori's method for reticulum [32]. The tissue sections were documented in Olympus microscope, model BX50 and described histologically. Finally, histological differences of intramuscular connective tissues of *M. Pectoralis profundus* and the influence of sex on these features were evaluated between Ross and *Gallus domesticus* chickens.

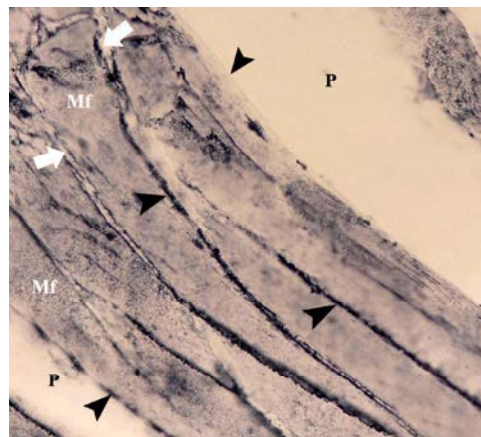


Fig. 1: Reticular fibers (arrowheads) in the perimysium (P) and endomysium (arrows) of *M. Pectoralis profundus* of the female Ross broilers, muscle fibers (Mf). Gomori's method for reticulum  $\times 400$

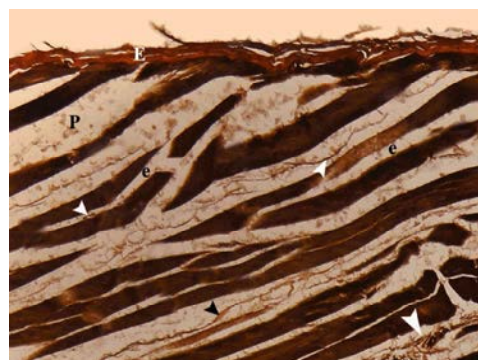


Fig. 2: Elastic fibers (arrowheads) in intramuscular connective tissues of the *M. Pectoralis profundus* of male Ross broilers, epimysium (E), perimysium (P), endomysium (e). Verhoeff's  $\times 100$

## RESULTS

In the present study, histological differences were not found in the intramuscular connective tissues between the left and right *M. Pectoralis profundus* of both the Ross broiler and native chickens. All the connective tissue fibers observed in the all intramuscular connective tissues of *M. Pectoralis profundus* of both the chickens' genotypes (Figs. 1-3).

Sex related differences were not observed for the all endomysial connective tissue layers of *M. Pectoralis profundus* of both the strains of the chickens. The conventional histological study revealed that the endomysium was composed of thin strands of loose connective tissue was consisted of blood capillaries, fibroblasts (Fig. 4) and a layer of

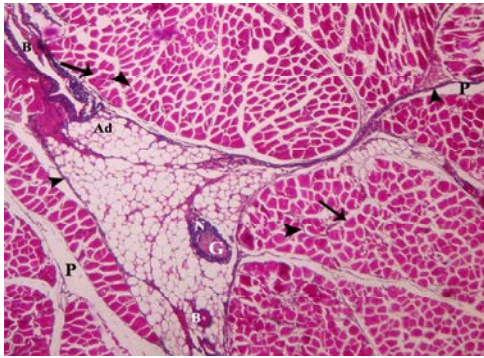


Fig. 3: Collagenous fibers (arrowheads) in the perimysium (P) and endomysium (arrows) of *M. Pectoralis profundus* of the female native chickens, adipose tissues (Ad), ganglion (G), nerve (N), blood vessels (B). Masson's trichrome  $\times 40$

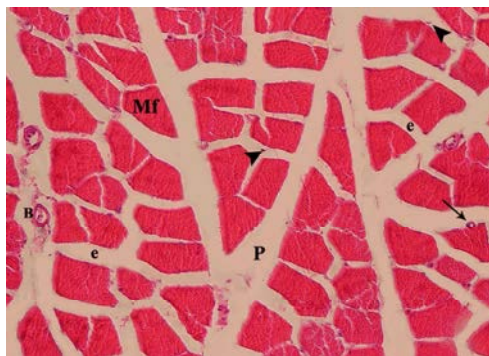


Fig. 4: Photomicrograph of the middle part of *M. Pectoralis profundus* of the male native chickens, perimysium (P), endomysium (e), fibroblasts (arrowheads), muscle fibers (Mf), blood vessels (B) and capillaries (arrow). Hematoxylin eosin  $\times 400$

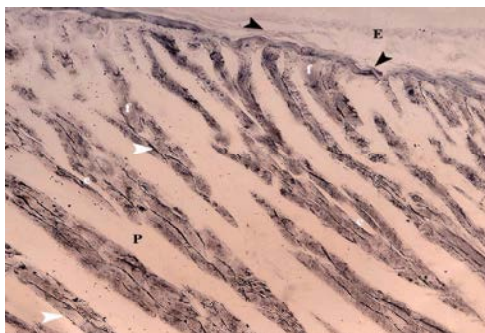


Fig. 5: Reticular fibers (arrowheads) in the epimysium (E), perimysium (P) and endomysium (e) of *M. Pectoralis profundus* of the male native broilers, muscle fibers (f). Gomori's method for reticulum  $\times 100$

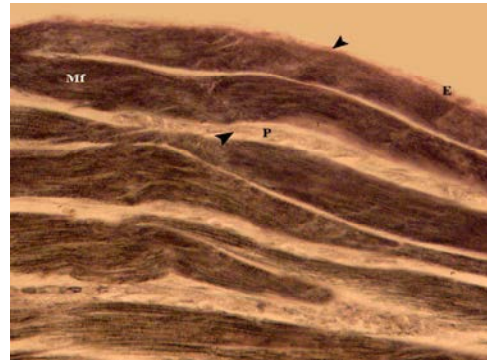


Fig. 6: Reticular fibers (arrowheads) in the epimysium (E) and perimysium (P) of *M. Pectoralis profundus* of the female native broilers, muscle fibers (Mf). Gomori's method for reticulum  $\times 400$

reticular fibers (Fig. 1), elastic fibers (Fig. 2) and collagenous fibers in both the genotypes of the chickens (Fig. 3).

Sex related differences were not also observed for the number of all connective tissue layers in the perimysium of both the broiler and native chickens. Perimysium was consisted of fibroblasts, large blood vessels ganglion, nerves, fat cells, collagen fibers (Fig. 3), reticular fibers (Fig. 1) and elastic fibers in both the Ross broiler and native chickens in the present study (Fig. 2).

In the native chickens, the numbers of the elastic, reticular and collagenous fibers in perimysium of *M. Pectoralis profundus* were 1, 2 and 2-3 layers, respectively and in contrast, their numbers in the *M. Pectoralis profundus* of broilers were respectively, 5-6, 1 and 3-4 layers.

In these two genotypes of chickens, epimysium of *M. Pectoralis profundus* was contained the larger blood vessels, nerves, adipose tissues and all the connective tissue fibers in both sexes. With the exception of elastic layers, the other epimysial connective tissue layers of *M. Pectoralis profundus* were affected by sex in the native chickens (Figs. 5, 6). In contrast, only reticular layers showed no sex related differences in the epimysium of *M. pectoralis profundus* of the broilers and the other epimysial connective tissue layers showed histological differences according to sex.

In the broilers, reticular fiber in epimysium of *M. Pectoralis profundus* was observed as mono layer. In contrast, the epimysial elastic fiber of the native chickens was seen as mono layer.

The number of collagenous layer in the epimysium was more in the female's broilers and native chickens (12-13 and 7-8 layers, respectively) than their males (4-5 and 3-4 layers, respectively).

In the native chickens, the number of reticular fibers in epimysium of *M. Pectoralis profundus* was more in the males (4-5 layers) than those of the females (2-3 layers) (Figs. 5, 6).

The male's broilers had more elastic layers in epimysium (14-15 layers) than the females (8-9 layers) in the present study.

## DISCUSSION

In the present study, endomysium of *M. Pectoralis profundus* of both the Ross broiler and native chickens was a thin connective tissue layer surrounding individual muscle fibers which is similar to previous findings [5, 6, 8]. Endomysium exhibited similar structure in both the genotypes of the chickens and was made up of fibroblasts, blood capillaries, adipose tissue, elastic, reticular and collagen fibers which is in agreement with the results of a previous study [21].

Although, several authors reported only collagen fibers in endomysium of various muscles in differently bred chickens [13, 17, 23] and Dellmann [5] reported collagen and reticular fibers in domestic animals, but in the present study, all the connective tissue fibers observed in the all intramuscular connective tissues of *M. Pectoralis profundus* of both the chickens' genotypes. According to Jarvinen *et al.* [14] the endomysial collagen fibers can be divided into three separate compartments: 1) collagen fibers located on the surface of the muscle fibers and mostly running longitudinally to the long axis of the muscle fibers, 2) collagen fibers connecting two adjacent muscle fibers and running perpendicular to the long axis of the muscle fibers and 3) collagen fibers running around the intramuscular capillaries and nerves.

The perimysium of *M. Pectoralis profundus* was the layer of intramuscular connective tissues surrounding the bundles of muscle fibers in both the Ross broiler and native chickens which again concords with the previous findings [5, 7, 8]. In the present study, it contained the fibroblasts, large blood vessels, ganglions, adipose tissue, nerves, elastic, reticular and collagen fibers in both the strains of the chickens, but Lawrie [7] and Bailey and Light [8] reported only the large blood vessels and nerves in perimysium.

According to Nakamura *et al.* [19] the perimysium can be divided into two different types: primary perimysium surrounding the muscle fiber bundles and secondary perimysium surrounding the muscle fiber bundles in larger scale.

In the present study, the numbers of the elastic and collagenous layers in perimysium was more in broiler in comparison to the native chickens. Voutila [4] reported that the increasing thickness of perimysium relates to increasing meat toughness. Thus, it could be concluded that tenderness of *M. Pectoralis profundus* was more in the native chickens than the Ross broilers.

The histological structure of epimysium in both the genotypes of the chickens was similar and consisted of the larger blood vessels, nerves, adipose tissues and all the connective tissue fibers which mirror the results of Dellmann [5].

In the present study, the numbers of collagen layers in epimysium was more in the broiler than those of the native chickens, which again indicates that the tenderness of *M. Pectoralis profundus* was more in the native chickens than the broilers. Furthermore, females had more collagen layers in epimysium than the males in the both the strains of the chickens. Thus, it might be concluded that the *M. Pectoralis profundus* of males was more tender than the females in both the genotypes of the chickens.

In the native chickens, elastic layers showed no sex related differences in the epimysium of *M. pectoralis profundus* but in the broilers, the numbers of elastic layers in epimysium was more in the males than those of the females. Totland *et al.* [25] reported that the difference in tenderness was related to the different elastic contents.

## CONCLUSION

In summation, the histological structures of the intramuscular connective tissues of *M. Pectoralis profundus* in the Ross broiler was generally similar to those of native chicken except for the numbers of the all connective tissue layers (especially collagen) in endomysium and perimysium. Tenderness of *M. Pectoralis profundus* was more in the native chickens than the Ross broilers and also in males than the females in both the genotypes of the chickens. Sex related differences were not observed for the numbers of the all connective tissue layers in endomysium and perimysium of *M. Pectoralis profundus* of both the strains of the chickens.

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