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Histological Differences in Intramuscular Connective Tissues Composition Between Dark and Light Colored Muscles in Broiler Chickens

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Abstract: The properties of intramuscular connective tissue of meat determine the perceived meat structure and tenderness. The aim of this study was to study the histological properties of intramuscular connective tissues in *Quadriceps femoris* muscle (as red meat or dark colored muscle) and *Pectoralis superficialis* muscles (as white meat or light colored muscle) in the native chickens and the influence of sex on these properties. A total of 40 adult healthy native chickens of both sexes (20 female and 20 male) were used. After fixation in 10% buffered formalin solution, sections were prepared, using routine histological techniques. Tissue samples were stained by hematoxylin eosin for general observations and a variety of special techniques for determination of types of connective tissue fibers; (1) Van gieson's, (2) Verhoeff's, (3) Foot's method for reticulum. The conventional histological study revealed that the histology of intramuscular connective tissues was similar in the left and right sides of both dark and light colored muscles. Endomysium showed no histological difference between dark and light colored muscles in both sexes. Sex related differences were not observed for the all intramuscular connective tissues of light colored muscle. The perimysium and epimysium of leg muscle were affected by sex. Tenderness in the male broiler chickens was higher than the females. Light colored muscle than dark colored muscle was the most tender.

Key words: Broiler · Connective Tissues · Histological Differences · Muscle · Sex

INTRODUCTION

It has been shown that the properties of intramuscular connective tissue of meat determine the perceived meat structure and tenderness to some extent but the results were variable [1].

The intramuscular connective tissues in meat is in three hierarchical levels: epimysium is the layer surrounding the whole muscle, perimysium contained the large blood vessels and nerves of the muscle [2, 3], which surrounds bundles of muscle fibers and individual muscle fibers are surrounded by the endomysium [2, 4]. The role of intramuscular connective tissues in meat tenderness and eating quality of meat has been the ultimate goals in numerous studies [1, 5-8]. The most common intramuscular connective tissue research in meat science has been on beef [9, 10], but also in pigs [5, 11], turkeys [12] and some strains of chickens such as Silky [13], Rhode Island Red [6],White Leghorn [14], Laying hens [15], Red Cornish and New Hampshire [16, 17].

Connective tissue consists of proteins, complex polysaccharides and water as different mixtures depending on the type of tissue. In intramuscular connective tissues the main protein is collagen and another important protein is elastin [2, 3]. Also, reticular fibers are actually individual collagen fibrils (type III collagen) which form delicate networks around muscle fibers [18].

Rowe [19] demonstrated that collagen forms an organized network together with elastic fibers and Kerr *et al.* [20] reported that the collagen of fast growing animals is less matured than that of slow growing animals at the same slaughter weight. Nakamura *et al.* [16] showed that collagen content of breast muscles and leg muscles in broiler chickens is highest at the ages of two weeks and five weeks, respectively. In addition, collagen content of dark colored muscles (Red meat) was higher than in light colored muscles (White meat) in poultry and the increasing thickness of endomysium and perimysiun were related to increasing meat toughness.

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However, to the author's best knowledge, the histological properties of intramuscular connective tissues of the broiler chickens have not been studied previously. Thus, the present investigation was aimed at the investigation of the histological differences of intramuscular connective tissues between leg and breast muscles of the broiler chicken.

MATERIALS AND METHODS

A total of 40 adult clinically healthy Ross broiler chicks (56 days) from both sexes (20 female and 20 male) were obtained from the research farm of household bird's maintenance of College of Veterinary Medicine, Azad University of Shahrekord. 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 study, samples were taken from the middle parts of left and right *Quadriceps femoris* muscle (leg muscles as red meat or dark colored muscle) and *pectoralis superficialis* muscles (breast muscles as white meat or light colored muscle). The samples were immediately fixed in 10% buffered neutral formalin solution for 24-48 hours, dehydrated and embedded in paraffin blocks. Tissue sections (5 µm) were stained by hematoxylin eosin for general histological observations and types of fibres in the connective tissues: 1) Van gieson's, 2) Verhoeff's, 3) Foot's method for reticulum [21]. The tissue sections were documented in Olympus microscope, model BX50 and described histologically. Finally, histological differences of intramuscular connective tissues were evaluated between leg and breast muscles in both sexes.

RESULTS

No evident difference between the left and right sides of both dark and light colored muscles was observed in the histology of intramuscular connective tissues. There were not differences in histological structures of endomysium between *Quadriceps femoris* and *Pectoralis superficialis* muscles, so all connective tissue fibers of endomysium in both red and white meats were observed as mono layer in both sexes.

Endomysium was composed of thin strands of loose connective tissue was consisted of blood capillaries, fibroblasts (Fig. 1) and a layer of elastic fiber (Fig. 2), reticular fiber (Fig. 3) and collagenous fibers in both leg and breast muscles (Fig. 4).

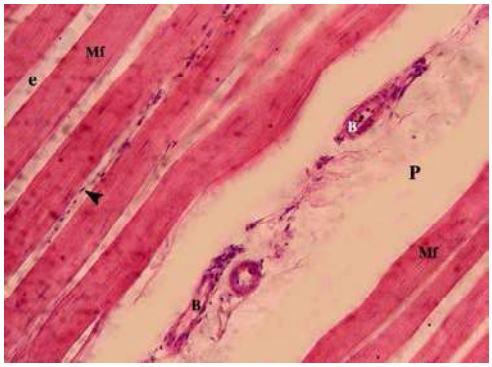

Fig. 1: Photomicrograph of the middle part of light colored muscles in the male Ross broilers, perimysium (P), endomysium (e), fibroblasts (arrowhead), blood vessels (B), muscle fibers (Mf). Hematoxylin eosin × 400

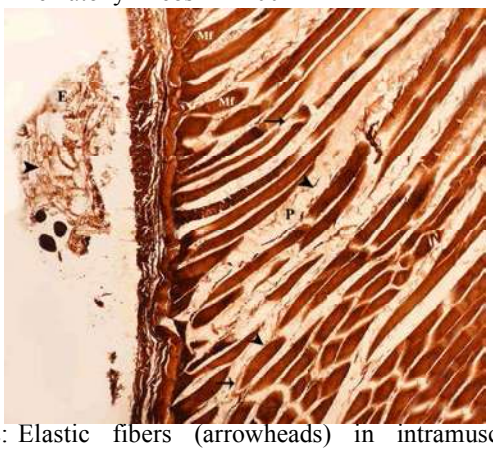

Fig. 2: Elastic fibers (arrowheads) in intramuscular connective tissues of dark colored muscle in male Ross broilers, epimysium (E), perimysium (P), endomysium (arrows), muscle fibers (Mf). Verhoeff's × 100

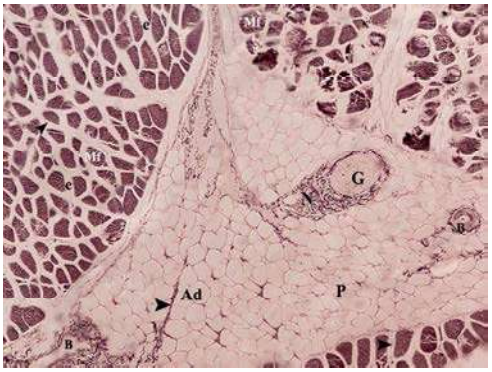

Fig. 3: Reticular fibers (arrowheads) in perimysium (P) and endomysium (e) of dark colored muscle in female Ross broilers, adipose tissues (Ad), muscle fibers (Mf), ganglion (G), nerve (N), blood vessels (B). Foot's method for reticulum × 100

Fig. 4: Collagenous fibers (arrowheads) in intramuscular connective tissues of dark colored muscle in female Ross broilers, epimysium (E), perimysium (P), endomysium (e), muscle fibers (Mf). Van giesson's × 100

In both dark and light colored muscles, perimysium was consisted of fibroblasts, large blood vessels (Fig. 1), ganglion, nerves, fat cells, reticular fibers (Fig. 3), elastic (Fig. 2) and collagenous fibers in both sexes (Fig. 4). Layer numbers of the all connective tissue fibers of perimysium were varied between dark and light colored muscles. Sex related differences were not observed for the layer numbers of all perimysial connective tissue fibers of breast muscle. The numbers of elastic layers in perimysium of *Pectoralis superficialis* muscle were seen as 3-4 layers in both sexes. Whereas, collagenous and reticular fibers were found as 2-3 and 1-2 layers, respectively.

In the present study, sex related differences are observed for the layer numbers of all perimysial connective tissue fibers of *Quadriceps femoris* muscle.

With the exception of elastic layers which in males (4-5 layers) were higher than females (3-4 layers), the layer numbers of collagen and reticular fibers of perimysium of *Quadriceps femoris* muscle in females (7-8 and 3-4 layers, respectively) were higher than males (4-5 and 2-3 layers, respectively).

Epimysium which contained the larger blood vessels, nerves, adipose tissues and the all connective tissue fibers differed significantly between dark and light colored muscles (Fig. 2). Only in epimysium of *Quadriceps femoris*, numbers of the all connective tissue layers were affected by sex (Fig. 4) and females had higher epimysial collagen, elastic and reticular layers (25, 18-20 and 10 layers, respectively) than males (14-15, 10-12 and 2-3 layers, respectively). Sex related differences were not observed for the layer numbers of all epimysial connective tissue fibers of white muscle. The numbers of elastic, collagenous and reticular layers in epimysium of *Pectoralis superficialis* muscle were seen as 5-6, 4-5 and 3 layers, respectively.

DISCUSSION

In the present study, endomysium of both dark and light colored muscles was a thin connective tissue layer surrounding individual muscle fibers which is similar to previous findings [2, 4, 18].

Endomysial connective tissue was made up of adipose tissue, blood capillaries, fibroblasts, collagenous, elastic and reticular fibers. Roy *et al.* [17], Iwamoto *et al.* [22] and Nakamura *et al.* [23] reported only collagen fibers in endomysium of various muscles in differently bred chickens and Dellmann [18] reported collagen and reticular fibers in domestic animals. Jarvinen *et al.* [24] divided the endomysial collagen fibers into three separate compartments: i) collagen fibers located on the surface of the muscle fibers and mostly running longitudinally to the long axis of the muscle fibers, ii) collagen fibers connecting two adjacent muscle fibers and running perpendicular to the long axis of the muscle fibers and iii) collagen fibers running around the intramuscular capillaries and nerves.

In both dark and light colored muscles, endomysium exhibited similar structure which concords with the findings of Oshima *et al.* [25] in differently bred pigs.

The perimysium of both muscle types was the layer of intramuscular connective tissues surrounding the bundles of muscle fibers which again concords with Bailey and Light [2], Lawrie [3] and Dellmann [18]. Perimysium also contains the large blood vessels, fat cells, fibroblasts, ganglions, nerves, collagenous, elastic and reticular fibers. Bailey and Light [2] and Lawrie [3] reported only the large blood vessels and nerves in perimysium.

Nakamura *et al.* [13] showed that 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.

The dark colored muscle had higher perimysial collagen and reticular layer than that of the light colored muscle. The higher perimysial collagen layer of the dark colored muscle in comparison to the light colored muscle was an expected result. Some researchers reported that the leg muscles had thicker perimysial layers than the breast muscles and the perimysium was least developed in light colored muscles [26, 27]. Only Nakamura *et al.* [16] reported highest collagen contents in breast muscles from Red Cornish x New Hampshire chickens.

According to Mahon [28], in poultry, leg muscles grow fast initially, but then the breast muscles rapidly grow and mature actually later than the leg muscles. It is

well established that the increasing thickness of endomysium and perimysium relates to increasing meat toughness [1]. Thus, it could be concluded that tenderness in the light colored muscle was higher than the dark colored muscle in broiler chickens especially in males as compared to females. These differences might be due to the anatomical location and work load of the muscles [29] or differences between the breeds [1].

The epimysium of both dark and light colored muscles contained the larger blood vessels, nerves, adipose tissues and all the connective tissue fibers. This finding mirrors the results of Dellmann in domestic animals [18].

When comparing the epimysial collagen, elastic and reticular layers of the dark colored muscle to light colored muscle, it was again found that the *Pectoralis superficialis* muscle was tenderer than the *Quadriceps femoris* muscle in broiler chickens. Also when the epimysial layers of dark colored muscle in males were compared with those in females (Figs. 2, 4), it was determined that the leg muscle was tenderer in males than females. Tenderness in the light colored muscle showed no differences between males and females broiler chickens.

In the present study, epimysial elastic contents showed significant differences between dark and light colored muscles. Totland *et al.* [8] reported that the difference in tenderness in different muscles was related to the different elastic contents.

In conclusion, the histology of intramuscular connective tissues was similar in the left and right sides of both dark and light colored muscles. Sex related differences were not observed for the all intramuscular connective tissues of light colored muscle. Except endomysium, the other intramuscular connective tissues of dark colored muscle affected by sex and females had higher layers than males. Like other bird's, tenderness in the light colored muscle of Ross broiler chickens was higher than the dark colored muscle and also in males than females.

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