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Histological properties of intramuscular connective tissues in native chickens and their relationship with meat tenderness

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Abstract

The most common connective tissue research in meat science has been conducted on the properties of intramuscular connective tissue of meat. The purpose of this study was to investigate histological properties of intramuscular connective tissues of left and right *Quadriceps femoris* muscle (as red meat) and *Pectoralis superficialis* muscles (as white meat) in the native chickens and the influence of sex on these properties. A total of 40 adult healthy native chickens (56 days) 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 and a variety of special techniques for determination of types of connective tissue fibers. The conventional histological study revealed that except the endomysium which was similar in both muscles, the other intramuscular connective tissues layers varied between leg and breast muscles and affected by sex. All the connective tissue fibers observed in the all intramuscular connective tissues of both red and white meats. Tenderness in the leg muscle was higher than the breast muscle in native chickens, as well as in males than females. Elastic fibers showed no histological difference not only among intramuscular connective tissues in both red and white muscles meats but also between males and females.

Key words: Histology, intramuscular connective tissues, meat, native chicken, tenderness

Introduction

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 (Bailey and Light, 1989; Lawrie, 1998). In addition, reticular fibers are actually individual collagen fibrils (type III collagen) which form delicate networks around muscle fibers (Dellmann, 1993). 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 (Bailey and Light, 1989; Lawrie, 1998) which surrounds bundles of muscle fibers , and individual muscle fibers are surrounded by the endomysium (Bailey and Light, 1989; Alberts *et al* 2002). The role of intramuscular connective tissues in meat tenderness and eating quality of meat has been the ultimate goals in numerous studies (Liu *et al* 1996; Totland *et al* 1988; Minvielle *et al* 2001; Correa *et al* 2006; Voutila, 2009).

Mobini (2013) demonstrated that the perimysium and epimysium of leg muscle were affected by sex and Kerr *et al* (2001) 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* (2004b) 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 coloured muscles (Red meat) was higher than in light coloured muscles (White meat) in poultry and the increasing thickness of endomysium and perimysium were related to increasing meat toughness. 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 (Voutila, 2009). The most common intramuscular connective tissue research in meat science has been on beef (Raes *et al* 2003; Li *et al* 2007), but also in pigs (Correa *et al* 2006; Therkildsen *et al* 2002), turkeys (Fernandez *et al* 2001) and some breeds of chickens such as Ross (Mobini, 2013), Silky (Nakamura *et al* 2003), Rhode Island Red (Liu *et al* 1996),White Leghorn (Sakakibara *et al* 2000), Laying hens (Coro *et al* 2000), Red Cornish and New Hampshire (Nakamura *et al* 2004b; Roy *et al* 2007). However, to the author's best knowledge, no research had been done on the histology of intramuscular connective tissues of the native chickens. The purpose of this study was therefore to investigate histological properties of intramuscular connective tissues of the native

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chickens and determine their variations between leg muscle (as Red meat) and breast muscles (as White meat) in both sexes.

Materials and Methods

A total of 40 adult healthy native chickens (56 days) of 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 euthanized. For histologic study, samples were taken from the middle parts of left and right *Quadriceps femoris* muscle (leg muscles as Red meat), and *Pectoralis superficialis* muscles (breast muscles as White meat). The samples were immediately fixed in 10% buffered neutral formalin solution for 24-48 hours, dehydrated and embedded in paraffin in routine manners. Tissue samples (5 µm) were stained by hematoxylin eosin for general histological examination, Masson's trichrome (for collagen fibers), Verhoeff's (for elastic fibers) and Gomori's method for reticulum (Kiernan 2008). Sections were observed under light microscope.

Results

No evident difference between the left and right sides of studied muscles was observed in the histology of intramuscular connective tissues.

In both *Quadriceps femoris* and *Pectoralis superficialis* muscles, endomysium which was composed of thin strands of loose connective tissue was consisted of blood capillaries (Figure 4), fibroblasts (Figure 4) and a layer of collagenous (Figure 1), elastic (Figure 2) and reticular fibers (Figure 3).

Figure 1: Photomicrograph of the middle part of *Quadriceps femoris* muscle in the adult native chickens

Photomicrograph of the middle part of Quadriceps femoris muscle in the adult native chickens, Epimysium (E), perimysium (P), endomysium (arrowheads), fibroblasts (arrows), muscle fibers (Mf). Hematoxylin eosin × 400

Figure 2: Collagenous fibers (arrowheads) in intramuscular connective tissues of Quadriceps femoris muscle in the adult native chickens, fat cells

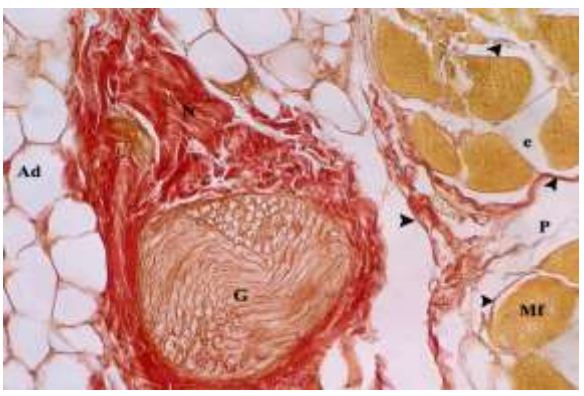

Figure 2: Collagenous fibers (arrowheads) in intramuscular connective tissues of Quadriceps femoris muscle in the adult native chickens, fat cells (Ad), ganglion (G), nerves (N), endomysium (e), perimysium (P), muscle fibers (Mf). Van giesson's × 400

Figure 3: Elastic fibers (arrowheads) in intramuscular connective tissues of *Pectoralis superficialis* muscle in the adult native chickens

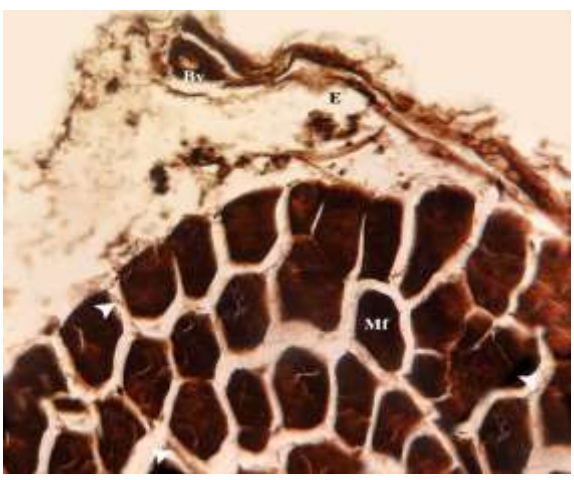

Figure 3: Elastic fibers (arrowheads) in intramuscular connective tissues of Pectoralis superficialis muscle in the adult native chickens, epimysium (E), blood vessels (Bv), muscle fibers (Mf). Verhoeff's × 400

Figure 4: Reticular fibers (arrowheads) in intramuscular connective tissues of Pectoralis superficialis muscle in the adult native chickens

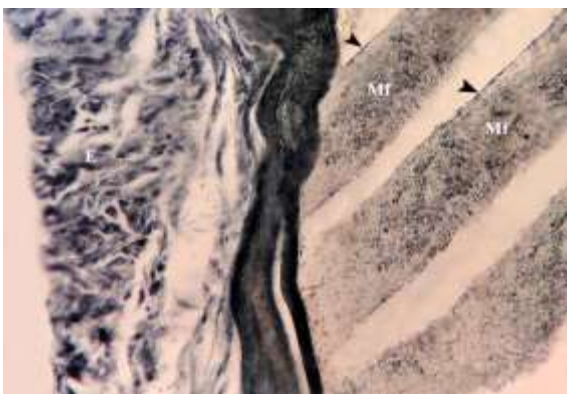

Figure 4: Reticular fibers (arrowheads) in intramuscular connective tissues of Pectoralis superficialis muscle in the adult native chickens, epimysium (E), muscle fibers (Mf). Gomori's method for reticulum × 400

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

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meats were observed as monolayer in both sexes.

Perimysium was consisted of large blood vessels (Figure 1), ganglion and nerves (Figure 1), fat cells (Figure 1), collagenous (Figure 1), elastic (Figure 2), reticular fibers (Figure 1), fibroblasts and other connective tissue cells in both muscle types.

With the exception of collagen, the layer numbers of other connective tissue fibers of perimysium were similar in leg and breast muscles.

In perimysium of both dark and light coloured muscles, elastic fibers were seen as one layer, whereas the layer of reticular fibers was 1-2. Sex related differences were not observed for these fibers.

In the present study, sex related differences are observed for perimysial collagen layers. The numbers of perimysial collagen layers of *Pectoralis superficialis* muscle in males (7-8 layers) were higher than females (3-4 layers); whereas females (2-3 layers) had higher perimysial collagen layers in *Quadriceps femoris* muscle than males (2 layers).

Epimysium which contained the larger blood vessels, nerves, adipose tissues and all the connective tissue fibers differed significantly between leg and breast muscles and was also affected by sex. Only elastic fibers showed no significant differences according to sex and observed as one layer in both red and white examined meats.

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 layers, respectively), whereas reticular and collagenous layers in epimysium of *Pectoralis superficialis* muscle in males (4-5 and 9-10 layers, respectively) were higher than females (3-4 and 5-6 layers, respectively).

Discussion

The endomysium of both breast and leg muscles was a thin connective tissue layer surrounding individual muscle fibers, which is similar to previous findings (Bailey and Light, 1989; Dellmann, 1993; Alberts *et al* 2002). Endomysial connective tissue was made up of adipose tissue, blood capillaries, fibroblasts, collagenous, elastic and reticular fibers. Several authors (Iwamoto *et al* 2001; Nakamura *et al* 2004a; Roy *et al* 2007) reported only collagen fibers in endomysium of various muscles in differently bred chickens and Dellmann (1993) reported collagen and reticular fibers in domestic animals. Jarvinen *et al* (2002) 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 red and white meats endomysium exhibited similar structure which concords with the findings of Oshima *et al* (2009) in differently bred pigs.

In the present study, perimysium of both muscle types was the layer of intramuscular connective tissues surrounding the bundles of muscle fibers which again concords with the previous findings (Bailey and Light, 1989; Dellmann, 1993; Lawrie, 1998). It also contains the large blood vessels, fat cells, fibroblasts, ganglions, nerves, collagenous, elastic and reticular fibers. Bailey and Light (1989) and Lawrie (1998) reported only the large blood vessels and nerves in perimysium. Nakamura *et al* (2003) showed that perimysium could 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 breast muscle had higher perimysial collagen layer than that of the leg muscle in both sexes. The higher perimysial collagen layer of the breast muscle in comparison to the leg muscle was an unexpected result. Although, some researchers reported that the leg muscles had thicker perimysial layers than the breast muscles and the perimysium was least developed in light coloured muscles (Ono *et al* 1993; Papinaho *et al* 1996), only Nakamura *et al* (2004b) reported highest collagen contents in breast muscles from Red Cornish x New Hampshire chickens. In the present study, perimysial collagen layers of the breast muscle were higher than those of the leg muscle.

According to Mahon (1999), 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 (Voutila, 2009). Thus, it could be concluded that tenderness in the leg muscle was higher than the breast

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muscle in native chickens especially in males as compared to females. These differences might be due to the anatomical location and work load of the muscles (Zimmerman *et al* 1993) or differences between the breeds (Voutila, 2009).

The epimysium of both red and white muscles contained the larger blood vessels, nerves, adipose tissues and all the connective tissue fibers. This finding mirrors the results of Dellmann (1993) in domestic animals.

When comparing the epimysial collagen layers of the leg muscle to breast muscle, it was again found that the leg muscle was tenderer than the breast muscles in native chickens. Also when the epimysial collagen layers of both red and white muscles in males were compared with those in females, it was determined that the leg muscle was tenderer in males than females, while breast muscle was tenderer in females than males.

Totland *et al* (1988) reported that the difference in tenderness in different muscles was related to the different elastic contents, but in the present study, epimysial elastic contents showed no significant differences between leg and breast muscles and observed as one layer in both sexes.

Conclusion

In conclusion, all the connective tissue fibers observed in the all intramuscular connective tissues of both red and white muscles of the native chickens. With the exception of endomysium, the other intramuscular connective tissues layers varied between leg and breast muscles and affected by sex. Unlike other bird's species, tenderness in the leg muscle was higher than the breast muscle in native chickens, as well as in males than females. The difference in tenderness in different muscles was not related to the different elastic fibers of intramuscular connective tissues.

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