

Comparative Histological Analysis of Protein and Calcium Levels in Skeletal Muscles of Quail and Local Chickens

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ABSTRACT

Poultry meat is a vital source of high-quality protein and essential minerals, including calcium, making it a significant component of human nutrition. This study aimed to compare the protein and calcium content in the skeletal muscles of quail and local broiler chickens. A total of 10 male quails (45 days old) and 10 male broiler chickens (35 days old) were obtained from privately owned commercial farms in Al-Kut city. Muscle samples were collected from the chest and leg regions for analysis. Histological sections were prepared and stained with Mercuric Bromophenol Blue to quantify total protein and Alizarin Red to assess calcium accumulation in the muscle tissues. The results revealed that quail skeletal muscles had significantly higher protein content and calcium accumulation compared to chicken muscles. The differences observed in skeletal muscle composition between the two species can be attributed to variations in species-specific physiology, nutrition, and physical activity. These findings provide valuable insights into the nutritional quality of quail and chicken meat, emphasizing the potential of quail meat as a superior source of protein and calcium for dietary purposes.

Keywords: Alizarin Red Stain, Quail, Calcium, Bromophenol Blue Stain, Skeletal Muscles.

INTRODUCTION

The farming of poultry is a rapidly advancing sector of agriculture. Simultaneously, significant emphasis is placed on the cultivation of both meat and egg varieties of not just chickens but also quails. The quail breeding industry is a promising sector in agriculture, as this particular type of poultry possesses significant attributes that contribute to its profitability as well as productivity in agriculture techniques [1]. Firstly, this pertains to the rate of growth processes, which are five times faster than comparable measures in broiler chickens, while it is noteworthy that the commencement of egg laying occurs earlier. Furthermore, these birds' eggs and meat have a significant nutritional value [2]. Quails are hardy and adaptable birds that have a broad geographic range. These birds, being domesticated species, are well-suited for commercial breeding to produce meat and eggs under intense management conditions. The references cited are Egbeyale et al., 2013 [3] and Lukanov and Genchev, [4]. Their meat and eggs are widely recognized for their exceptional protein quality, high biological value, and minimal fat content. They are more flavorful than chicken, making them a preferred option for individuals with hypertension, stress issues, digestive disorders, stomach ulcers, liver difficulties, bronchitis, and depression [5]. Quail meat can be seen as a viable alternative to broiler meat. According to several studies, quail meat is rich in protein, polyunsaturated fatty acids, important trace minerals, and fat [6]. The muscles are a vital component of the body that possess a substantial ability to contract. They make up around 50% of the body's total weight. Muscle tissue is categorized based on differences in structure and function [7]. There are three types of muscle tissue: cardiac, skeletal, and smooth muscle in vertebrates [8]. Skeletal muscle is a metabolically active and significant tissue, distinguished by its rapid rate of energy metabolism and protein synthesis [9]. Skeletal muscles consist of many types of muscle fibers, each representing a muscle cell. The fundamental cellular unit of a muscle cell is the sarcomere [10]. The myofibrillar proteins are composed of contractile, structural, and regulatory proteins [11]. Calcium is the most abundant mineral in the body and

has a crucial role in the structure and maintenance of bones and eggshells in mammals and birds [12]. More than 99% of calcium in the animal body is mostly found in the skeleton, along with phosphorus, which is the second most abundant mineral in animals [13]. Both calcium and phosphorus play a crucial role in bone growth and mineralization [14]. Calcium is essential for various biological processes, including metabolism, blood coagulation, enzyme activation, neuromuscular function, muscle contraction, cell adhesion, and intracellular signaling [15]. Calcium is a vital macronutrient that plays a crucial role in the development, growth, and fast restoration of bones. Ca deficiency can cause irreversible harm to skeletal system development and result in severe skeletal and limb diseases. Conversely, an excess of Ca might have unfavorable effects by hindering the availability of other mineral [16].

MATERIALS AND METHODS

Experimental Design

The avian specimens utilized in this research were obtained from privately owned commercial fields located in the city of Al-Kut. They consisted of 10 male quails, aged 43–45 days, and 10 male broiler chickens, aged 34–35 days. Upon conducting a thorough examination, it was determined that all of the birds were in good health. Subsequently, all of the birds were euthanized by administering anesthesia with chloroform to induce death.

The Samples Collection

The skeletal muscle samples (1 cm³) were extracted from the (pectoralis major muscles) in the chest area and the (extensor iliotibialis anticus muscles) in the leg. The specimens were conserved in a 10% formalin solution for a duration of 72 hours. Afterwards, the specimens were washed with water for a duration of 2-3 hours and then underwent a series of histological procedures, including dehydration, clearing, infiltration, embedding, cutting, and staining. Protein

content and calcium content in paraffin slices were determined using the Mercuric Bromophenol Blue and Alizarin Red stains.

Statistical Methodology

The slides were photographed using a Canon digital camera attached to a Meija microscope, which has a 1/2 X photo converter and a 40X objective lens. The output images were analyzed on an Intel Core I3 computer using Video Test Morphology software (Russia), which has a specialized function for quantifying area, percentage area, and item counting.

Results AND Discussion

The Total Protein with Bromophenol Blue Stain

The muscles were subjected to histochemical analysis using mercuric bromophenol blue stain (BPB) to quantify the protein content in the muscle samples obtained from both quail and chicken. In quail birds, the microscopic investigation of cross-sections in muscle samples from the chest and leg areas showed a large quantity of protein contents that were seen as a dark blue area and spread throughout the muscle bundles of all samples, as shown in (Figures A,1,2). The statistical results obtained by using Image J revealed the value of protein contained in the chest and leg (116, 131), respectively, as revealed in surface plots in cross-sections of muscle samples (Figures B,1,2). In chicken birds, the microscopic investigation of

cross-sections in muscle samples from the chest and leg areas showed that the protein contents appear in the form of dark blue spots that vary and spread in the muscle bundles of all samples, as shown in (Figures A, 3,4). The statistical results obtained by using Image J revealed the value of protein contained in the chest and leg (105, 112), respectively, as revealed in surface plots in cross-sections of muscle samples (Figures B, 3,4). The statistical analysis of histological results showed the quail muscles contain the highest percentage of protein compared to the broiler chicken skeletal muscles (Figure 5). This result was similar to previous studies by Santhi and Kalaikanna, [17], who explained that quail meat contains a higher proportion of protein compared to chicken, which makes it the best and cheapest source for providing protein to the consumer.

The increase in protein content in quail meat may be a result of its activity and the different types of muscle fibers, as well as the increasing slaughter age. According to a previous study by Awan et al., [18], the ability of a muscle to perform a certain type of work depends on the type of fibers that make up it. As mentioned [19], the activity of the quail and its ability to fly and move over long distances are a result of the high aerobic metabolism in both types of muscles (the chest and leg), unlike chickens, which have little representation in the chest and more in the leg. To promote that, it was observed that there is not a clear difference in protein content between the chest and leg quail muscles, while there are evident differences in chicken muscles.

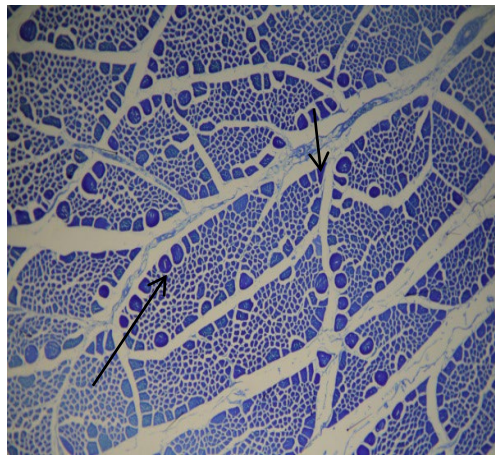


FIGURE 1(A): Photomicrograph of Skeletal Cross of Muscles of the Chest Area of Quail, Cross Section of Chest Muscle Shows the Total Protein (Black Arrows) Contents Appeared as Dark Stained Granules of All the Myofibers of Muscles. Bromophenol Blue Stain, 200 X.

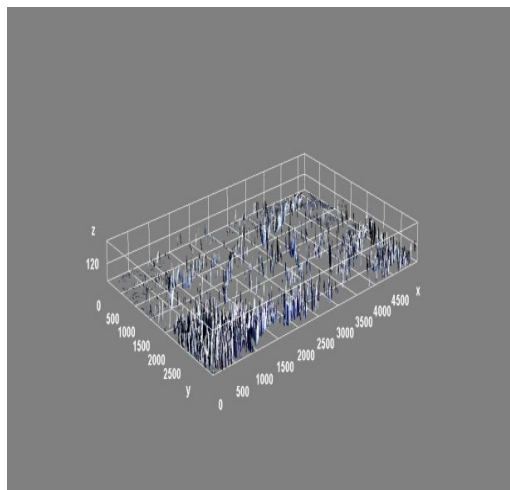


Figure 1(b): Surface Plot Show of Section in Skeletal Muscles Fibers of the Chest Area in Quail

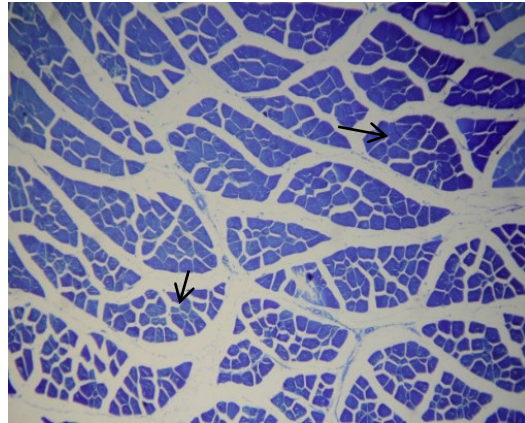


FIGURE 2(A): Photomicrograph of Skeletal Cross of Muscles of the Leg Area of Quail, Cross Section of Leg Muscle Shows the Total Protein (Black Arrows) Contents Appeared as Dark Stained Granules of all the Myofibers of Muscles. Bromophenol Blue Stain,200 X.

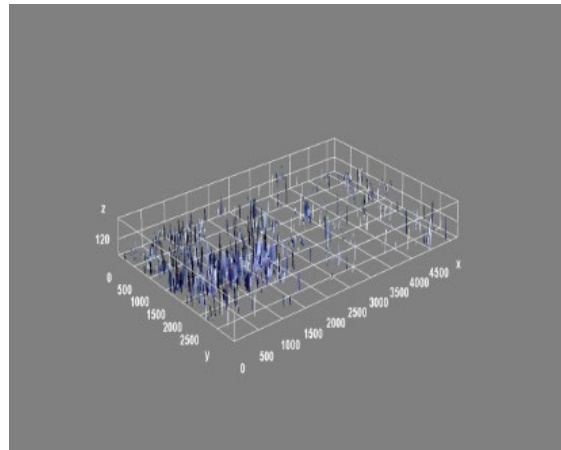


FIGURE 2(b): Surface Plot Show of Section in Skeletal Muscles Fibers of the Leg Area in Quail.

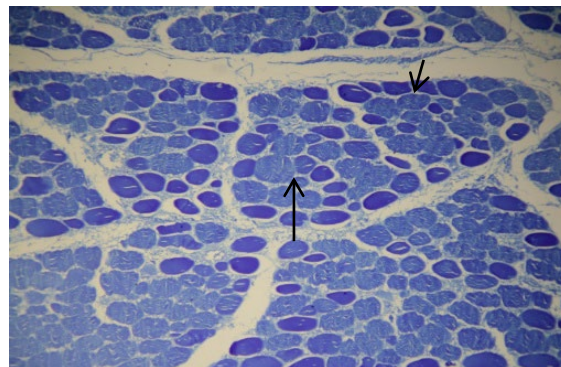


FIGURE 3(A): Photomicrograph of Skeletal Cross of Muscles of the Chest Area of Chicken, Cross Section of Chest Muscle Shows the Total Protein (Black Arrows) Contents Appeared as Dark Stained Granules of all the Myofibers of Muscles. Bromophenol Blue Stain,200 X.

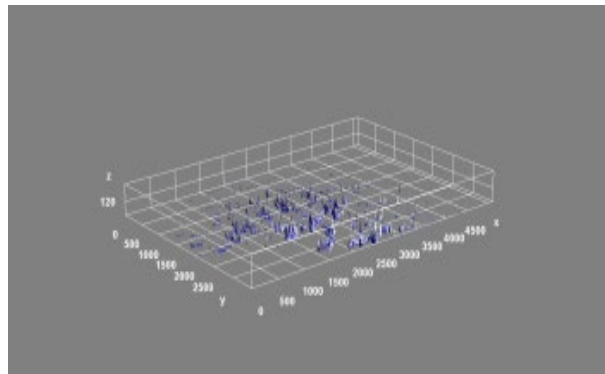


FIGURE 3(b): Surface Plot Show of Section in Skeletal Muscles Fibers of the Chest Area in Chicken.

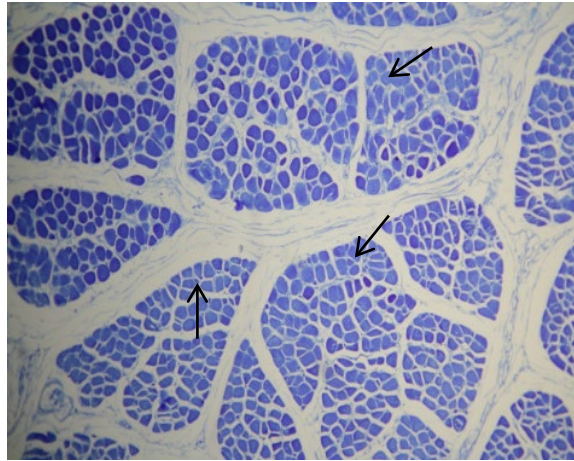


Figure 4(A): Photomicrograph of Skeletal Cross of Muscles of the Leg Area of Chicken, Cross Section of Leg Muscle Shows the Total Protein (Black Arrows) Contents Appeared as Dark Stained Granules of all the Myofibers of Muscles. Bromophenol Blue Stain, 200 X.

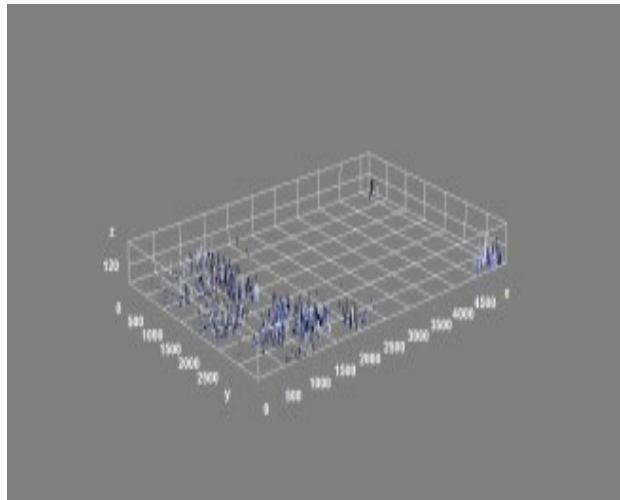


Figure 4(b): Surface Plot Show of Section in Skeletal Muscles Fibers of the Leg Area in Chicken.

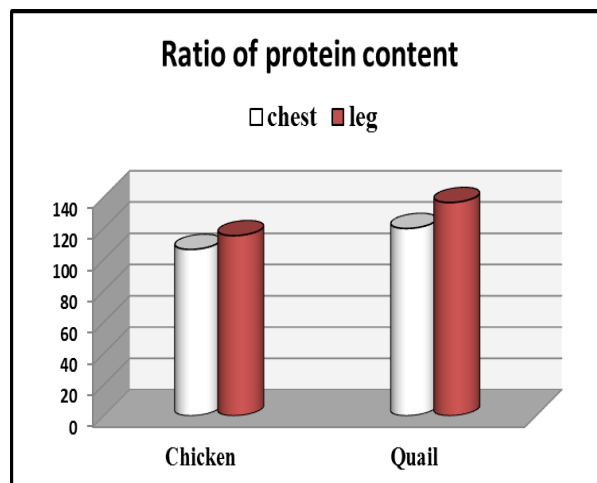


Figure 5: The Ratio of the Total Protein Content in Skeletal Muscle Fibers of the Chest and Leg Area in Quail.

Alizarin Red Stain to Calcium Detecting

The histochemical findings to determine the calcium content were performed both birds (quail and chicken) by using alizarin red stain. In quail, the microscopic examination of cross-sections of muscle samples showed a high deposition of calcium, which appeared in the form of red points spread over large areas within the muscle bundles, as shown in (figure A,6). In chickens, the microscopic

examination of cross-sections of muscle samples showed that they contained a deposition of calcium, which appeared in the form of red points spread in a few areas within the muscle bundles, as shown in (figure A,7).

The result of statistical analysis showed the quantity of calcium accumulation in quail muscle (169) which was more than the concentration in broiler chicken muscle (140), as revealed in surface

plots in cross-sections of muscle samples (figures B, 6, 7) (figure 8). The result was identical to the previous study [20], which showed that the content of calcium in quail exceeds its amount in chicken. The calcium content in muscles may be different in birds species according to reguiments of physiological movement, according to a study [21] explained, a for volant species of birds, the major muscles of small

birds consist of fast-twitch oxido-glycolytic fibers. The greater proportion of these fibers indicates an evolutionary adaptation to generate and sustaining the relatively high contraction frequencies associated with flapping flight. On the other hand, it was mentioned [22], that the variations in mineral composition might be attributed to the age and species of the birds, as well as the nature of their food.

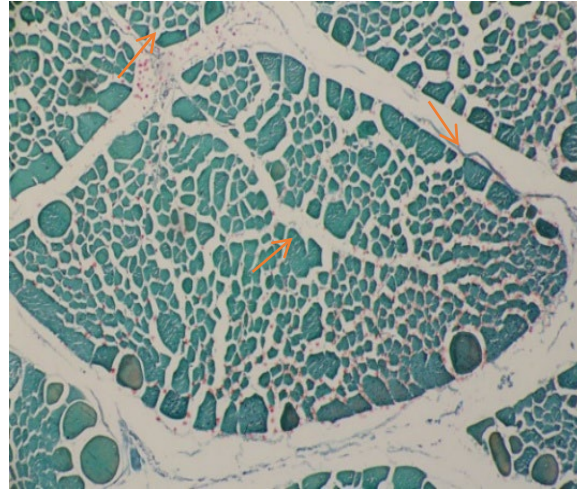


Figure 6(A): Photomicrograph of Skeletal Muscles of the Chest Area of Quail, Cross Section of the Chest Muscle Shows a Large Amount in the Distribution of Calcium (Red Arrow). Alizarin Red Stain.200X.

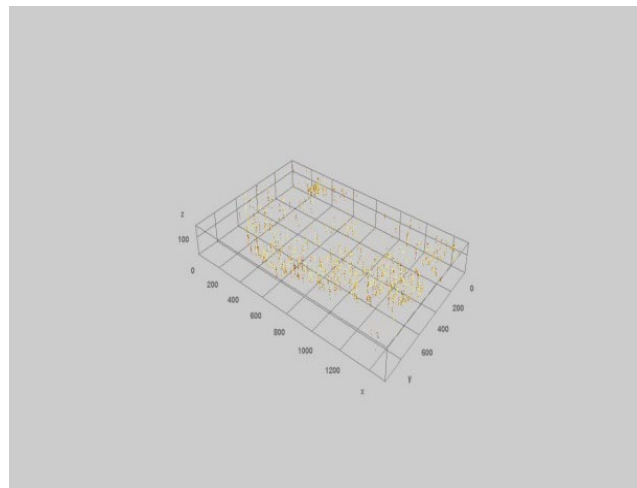


Figure 6(b): Surface Plot Show of Cross Section in Skeletal Muscles Fibers of Chest Area in Quail.

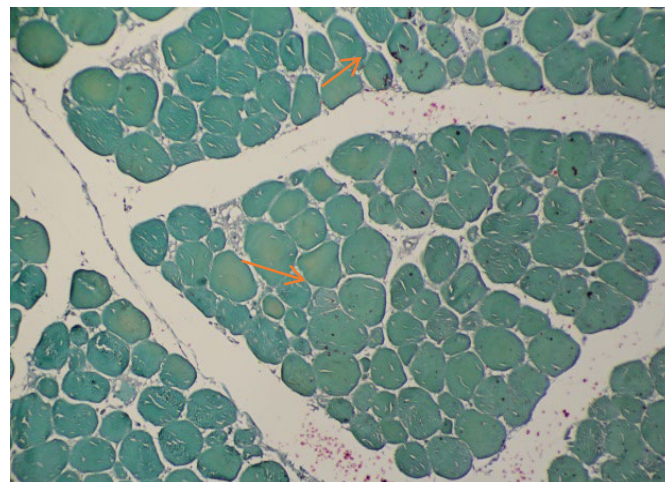


Figure 7(A): Photomicrograph of Skeletal Muscles of the Chest Area of Chicken, Cross Section of the Chest Muscle Shows a Large Amount in the Distribution of Calcium (Red Arrow). Alizarin Red Stain.200X.

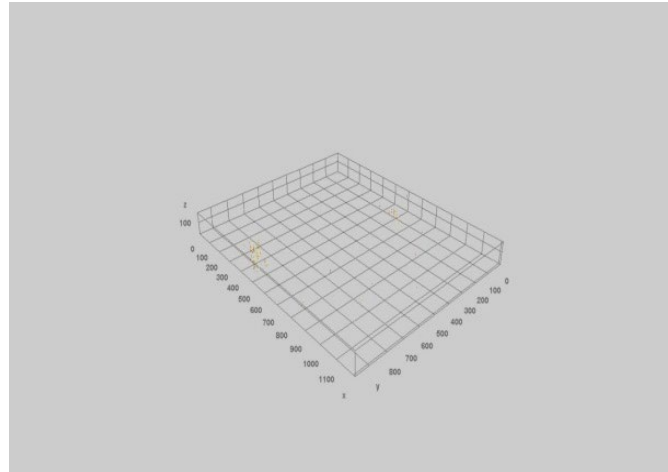


FIGURE 7(b): Surface Plot Show of Cross Section in Skeletal Muscles Fibers of Chest Area in Chicken.

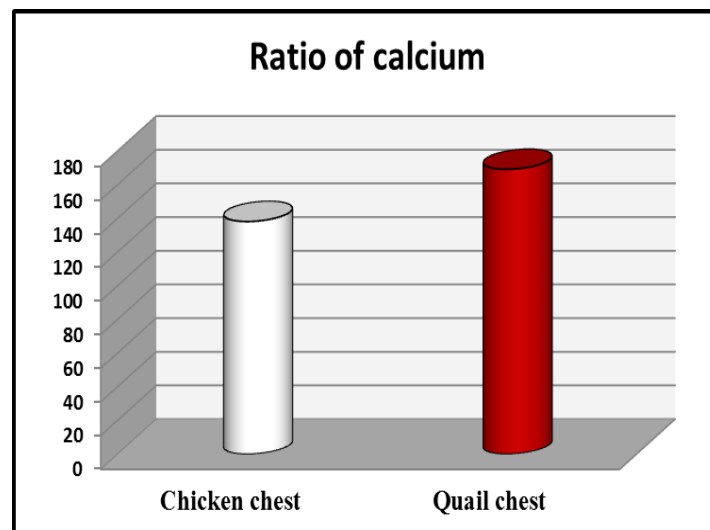


FIGURE 8: The Ratio of the Calcium Content in Skeletal Muscle Fibers of the Chest Area in Quail and Chicken.

Conclusion

In the first part of the study, the skeletal muscles of both types of birds showed a different protein content after examining tissue samples treated with Mercuric bromophenol blue stain. The results showed that the skeletal muscles of quail contain large amounts of protein compared to chicken muscles. This difference was caused by

breed, type of muscle fibers, and physical activity. Secondly, this section used Alizarin red stain to detect the calcium content in the skeletal muscles. After examining the tissue samples, the results showed that the accumulation of calcium in the skeletal muscles of quail was greater than that of chickens. Bird species' mineral composition varies based on their physiological movements, age, species, and the type of food they consume.

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