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# A Comparative Histological Study Of Protein And Calcium Content In Skeletal Muscles Of Green-Winged Teal And Domestic Duck

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***Abstract***
*The meat of poultry has been a source of beneficial protein and minerals. Thus, the aim of the current study was to identify the composition of protein and calcium in Green-Winged Teal and domestic ducks' skeletal muscles. Ten adult male green-winged teals and 10 adult male domestic ducks bought from commercial markets in Kut City were utilized for the study. The samples were taken from the chest and leg areas of Green-Winged Teal and domestic ducks. The samples were then prepared into histological sections, where two stains were applied: bromophenol blue to quantify total protein content and alizarin red to quantify the content of calcium. The study found that Green-Winged Teal skeletal muscles contained more protein and higher calcium deposition than domestic duck muscles. The difference in the composition of skeletal muscle between the samples is due to the difference in species, diet,and physical activity levels.*

***Keywords***: *alizarin red stain, green-winged teal, calcium, bromophenol blue stain, skeletal muscles.*

## 1-INTRODUCTION:

Ducks (Anatinae) are a diverse and ecologically important group of water birds with a global distribution across a range of environments (Johnsgard, 2010). Ducks play an important role in ecosystem functioning by participating in seed dispersal, nutrient cycling, and augmenting wetland biodiversity via migration and flexibility (Kaminski and Elmberg, 2014). Also, the prices of meat and eggs are usually too high for poor people to afford. Duck meat and egg production could tackle this problem to a great extent by being a cheap source of protein (Krainyk, A.,2018). The domestic duck, *Anas platyrhynchos*, is a widespread, common species,especially in the Northern Hemisphere. In most regions, it is an significant component of the regional diet (Agnieszka Sujak, 2019). Skeletal muscle fibers are large, multinucleated cells that enable voluntary movement and assist in keeping the body position. They are regulated by the somatic nervous system. system and are responsible for accurate movements (Yamakawa. et al., 2020). Muscle anatomy entails several important elements, including the neuromuscular junction, sarcomere, and cytoskeleton, all of which are important for its role (Brooks et al., 2023). Animals that have a high rate of protein synthesis and energy consumption have skeletal muscle, which is a large and metabolically active tissue. The principal components of muscle tissue are proteins, which also control different metabolic processes (Lana and Zolla, 2016) Muscles, which are crucial organs with an impressive ability to contract, account for over half of the body's total weight. Muscle tissue is classified based on differences in shape and function. (Krause, 2005). Vertebrates have three different types of muscle tissue: cardiac, skeletal, and smooth (Marieb and Hoehn, 2010).
According to Mukund and Subramaniam (2020), skeletal muscle is a well-organized tissue made up of several bundles of muscle fibres, or myofibers. Each myofiber represents a muscle cell, with the sarcomere serving as its fundamental cellular unit. Myofibrillar proteins are composed of structural, contractile, and regulatory proteins. Actin and myosin are contractile proteins forming thin and thick filaments; they regulate the contraction and relaxation of skeletal muscle (Hopkins, 2014). Muscle fibres are categorized. using several factors, including myoglobin concentration (colour), glycogen, fatigue, and other traitsIn mammals and birds, calcium is the most prevalent mineral in the body and is essential for the development and upkeep of bones and eggshells (de Matos 2008). The skeleton contains the majority of

the animal body's calcium (more than 99%) and phosphorus (the second most prevalent mineral in animals) (Veum, 2010). According to Zegyer et al. (2020), both calcium and phosphorus are essential for bone development and mineralisation. Many biological functions, such as metabolism, blood coagulation, enzyme activation, neuromuscular function, muscle contraction, cell adhesion, and intracellular signalling, depend on calcium (Proszkowiec-Weglarz and Angel, 2013).A critical macronutrient, calcium is essential for the growth, development, and quick repair of bones. The development of the skeletal system may be irreparably harmed by a calcium deficit, leading to serious skeletal and limb disorders. The availability of other minerals may be hampered by an overabundance of calcium, on the other hand (Bagheri *et al.,* 2022)

## 2.MATERIALS AND METHOD

### 2.1 Experimental design

The avian specimens utilized in this research were obtained from privately commercial in the city of Al-Kut. They consisted of 10 adult male Green-winged Teal and 10 adult male of Domestic duck. 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.

### 2.2 The Samples collection

The skeletal muscle samples (1 $cm^3$) were extracted from the (pectorals 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 weredetermined using the Mercuric Bromophenol Blue and Alizarin Red stains.

## 3- STATISTICAL METHODOLOGY

The slides were photographed using a Canon digital camera attached to a Mejia 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.

## 3-RESULTS AND DISCUSSION:

### 3.1 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 Green-Winged Teal and Domestic duck. In Green-winged Teal 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(8.924,11.898),respectively, as revealed in surface plots in cross-sections of muscle samples (Figures B,1,2). In the Domestic duck , 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 ( 6.978, 5.006), respectively, as revealed in surface plots in cross-sections of muscle samples (Figures B, 3,4). The statistical analysis of histological results showed the Green-winged teal muscles contain the highest percentage compared to the Domestic duck skeletal muscles of protein ( chart 1). The statistical results indicate that the green-winged teal exhibited a higher overall protein content in both muscle regions compared to the domestic duck. This result was similar to previous studies by Santhi and Kalaikanna (2017). These results demonstrate a higher protein content in the teal, likely attributed to its wild nature

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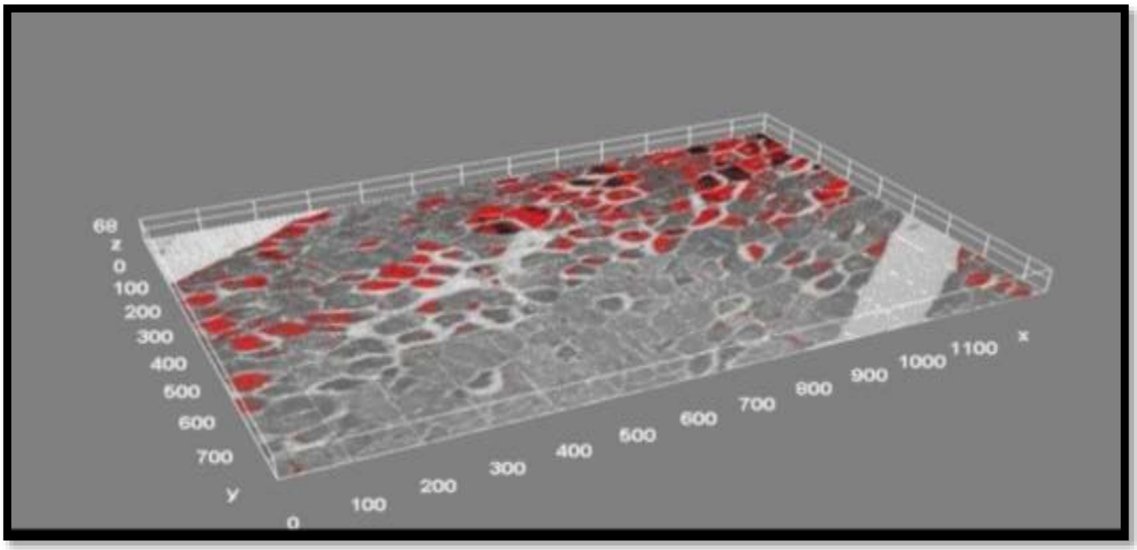
and increased muscular activity, in agreement with Biswas et al. (2019) and Guglielmo et al. (2002). The findings also agree with Tougan et al. (2013), who explained that the ability of muscle to perform depends on its fiber composition. Kiessling (1977) linked the activity and flight ability of Green-Winged Teal to their elevated aerobic metabolism, which contributes to enhanced protein development. Similarly, Guglielmo et al. (2002) reported higher protein levels in Green-winged Teal meat compared to Domestic duck, attributing it to higher physical activity and variation in muscle fiber types. elevated protein concentrations in migratory birds due to increased muscle enzyme activity.

**(Figure A,1):** Photomicrograph of cross cross-section in skeletal
Muscle fibers show the total protein chest area in Green-winged Teal (black arrow) contents appeared as dark-stained granules of all the myofibers of muscles. Bromophenol blue stain, 200X.




**(Figure B, 1)** Surface plot show of cross section in skeletal muscles fibers of the chest area in Green-winged teal.

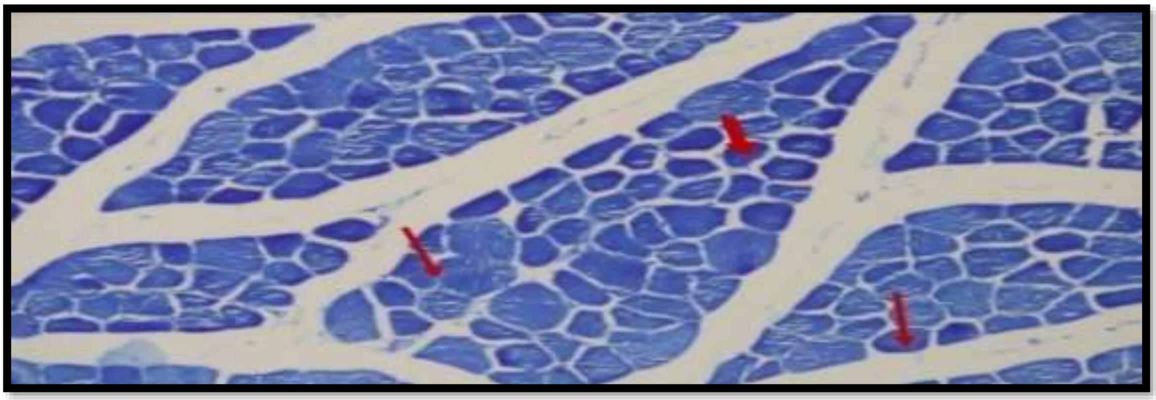


**Figure (A,2) :** Photomicrograph of cross section in leg skeletal muscles of Green-Winged Teal, show the total protein (red arrows) contents appeared as dark stained granules of all the myofibers of muscles Bromophenol blue stain, 200X .

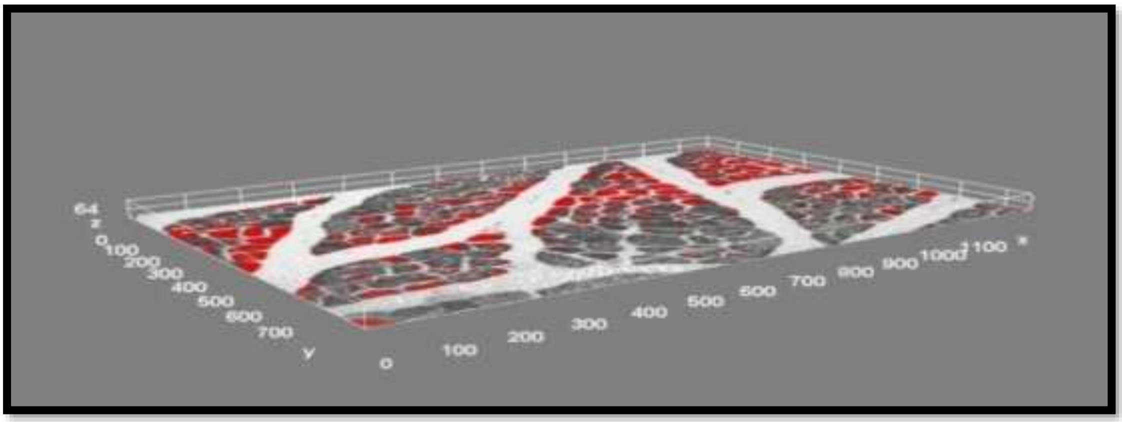



**Figure ( B,2):**Surface plot show of cross section in skeletal muscles fibers of the leg area in Green-winged Teal .

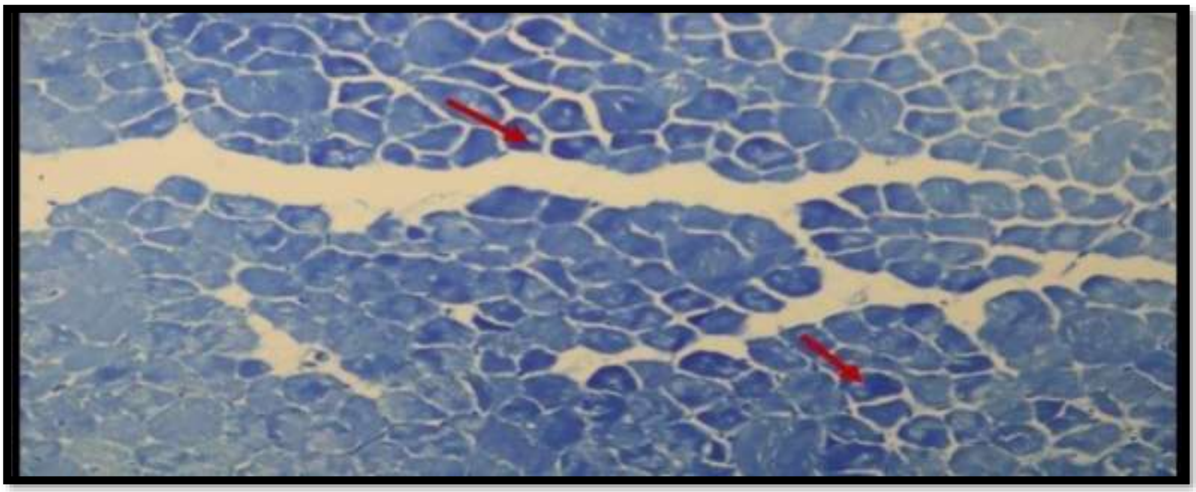
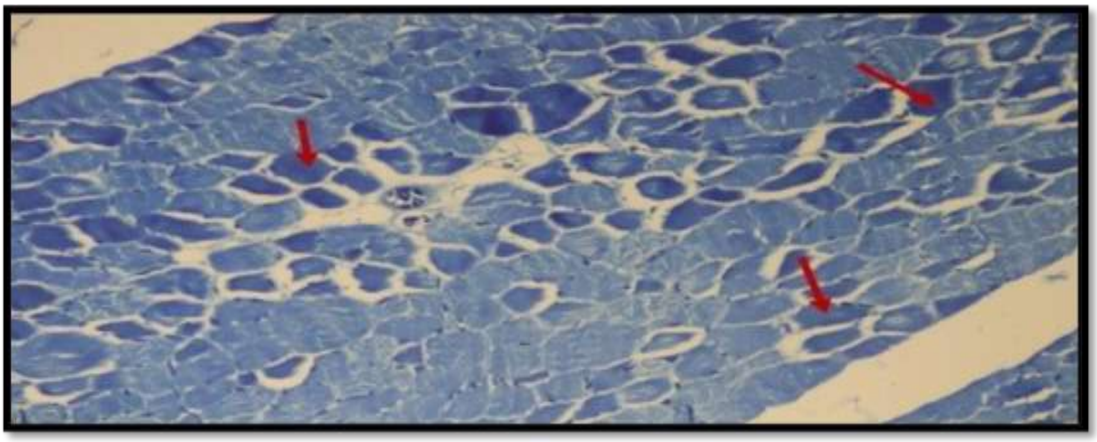


**Figure (A,3) :** Photomicrograph of cross section in chest skeletal muscles of Domestic duck, show the occurrence of protein as dark stained granules of most the myofibers of muscles (red arrows) Bromophenol blue stain, 200 X.



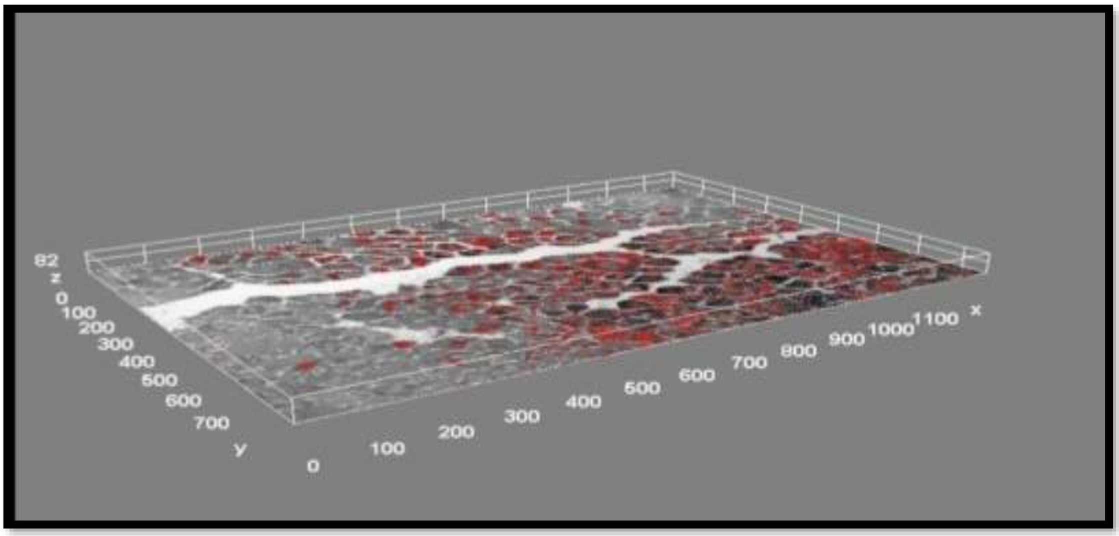



**Figure (B,3) :** Surface plot show of cross section in skeletal muscles fibers of the chest area in Domestic duck

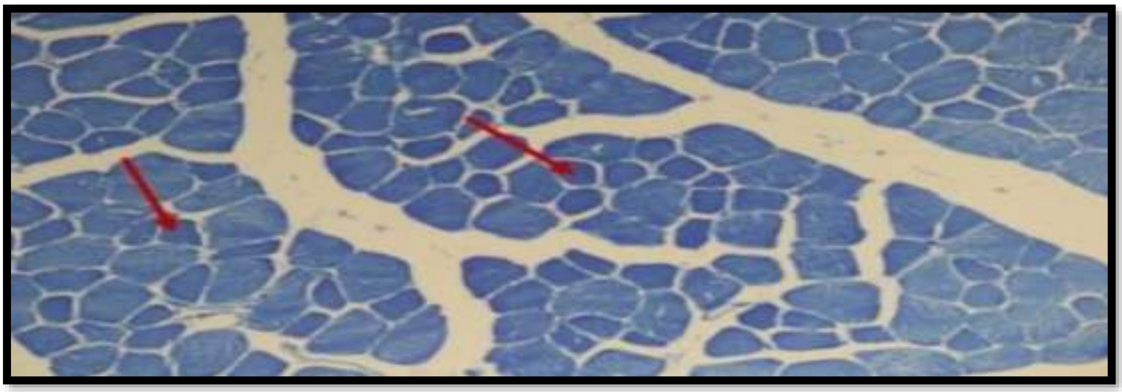


**Figure (A,4) :** Photomicrograph of cross section in leg skeletal muscles of Domestic duck, show the occurrence of protein as dark stained granules of most the myofibers of muscles(red arrows) .Bromophenol blue stain, 200 X

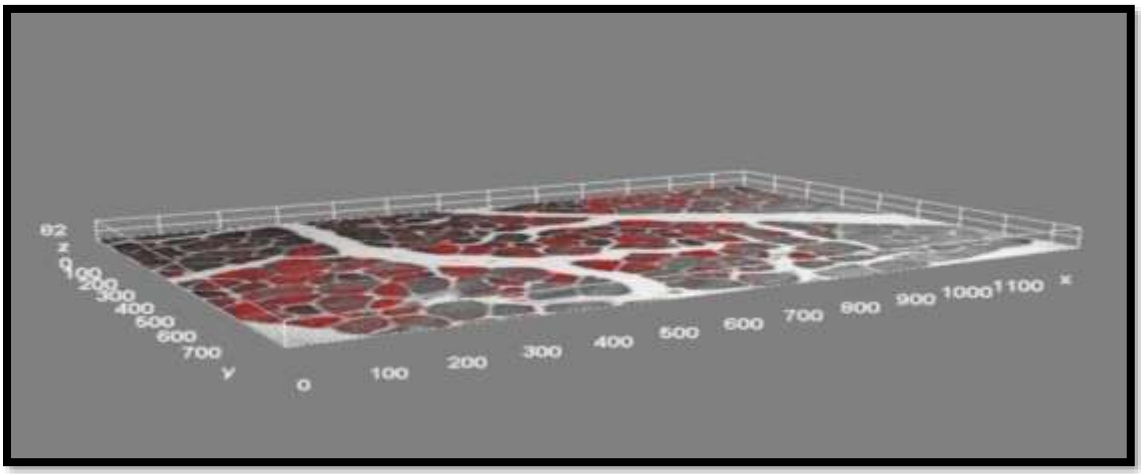
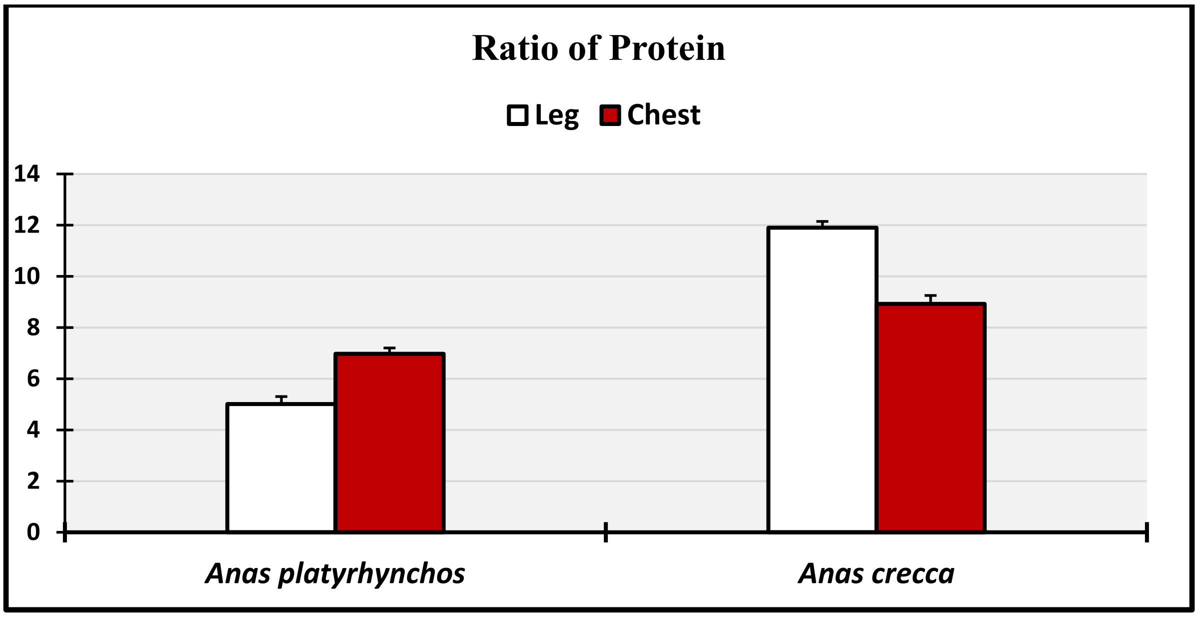



**Figure (B,4) :** Surface plot show of cross section in skeletal muscles fibers of the chest area in Domestic duck




**(Chart 1):** The ratio of the total protein content in skeletal muscle fibers of the leg and chest area in Domestic duck (Anas Platyrhynchos)

**3.2 Alizarin Red stain to calcium detecting**

The histochemical findings to determine the calcium content were performed both birds (Green-Winged Teal Domestic duck ) by using alizarin red stain.In Green-Winged Teal , 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,5). In Domestic duck, 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,6). The result of statistical analysis showed the quantity of calcium accumulation in Green-winged Teal muscle (7.065), which was more than the concentration in Domestic duck muscle(2.997), as revealed in surface plots in cross-sections of muscle samples(figures B, 5 , 6 )( chart 2).These differences suggest a physiological adaptation in the teal to sustain flight and movement, necessitating higher calcium deposition to support muscular and skeletal function. Zhang et al. (2018) emphasized that calcium levels are influenced by mobility, diet, and bone metabolism. Bombik et al. (2022) further supported that wild birds have higher tissue mineral concentrations due to their active lifestyle. The calcium content in muscles may be different in birds species according to reguiments of physiological movement. according to a study (Choi, et al. 2013) 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 (Genchev et al. 2008), 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. Thus, the observed variations in calcium distribution between species correspond with their locomotive demands, muscle structure, and habitat-driven physiologicaldifferences.

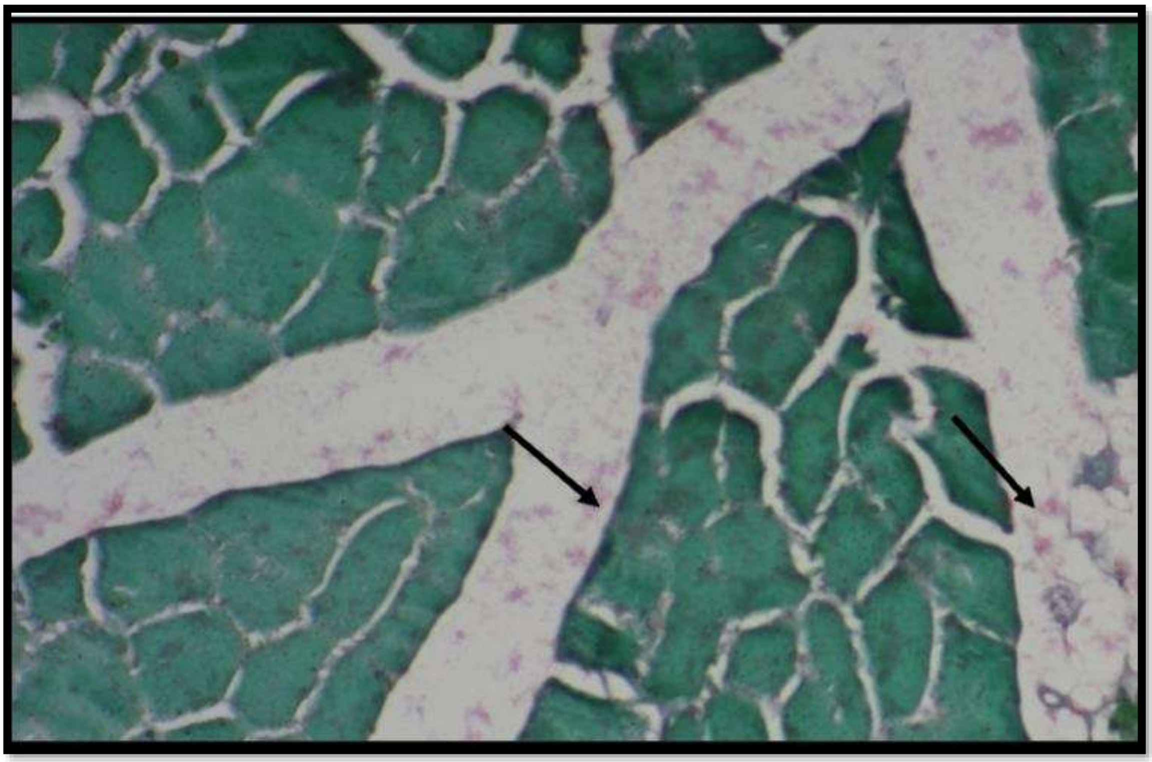
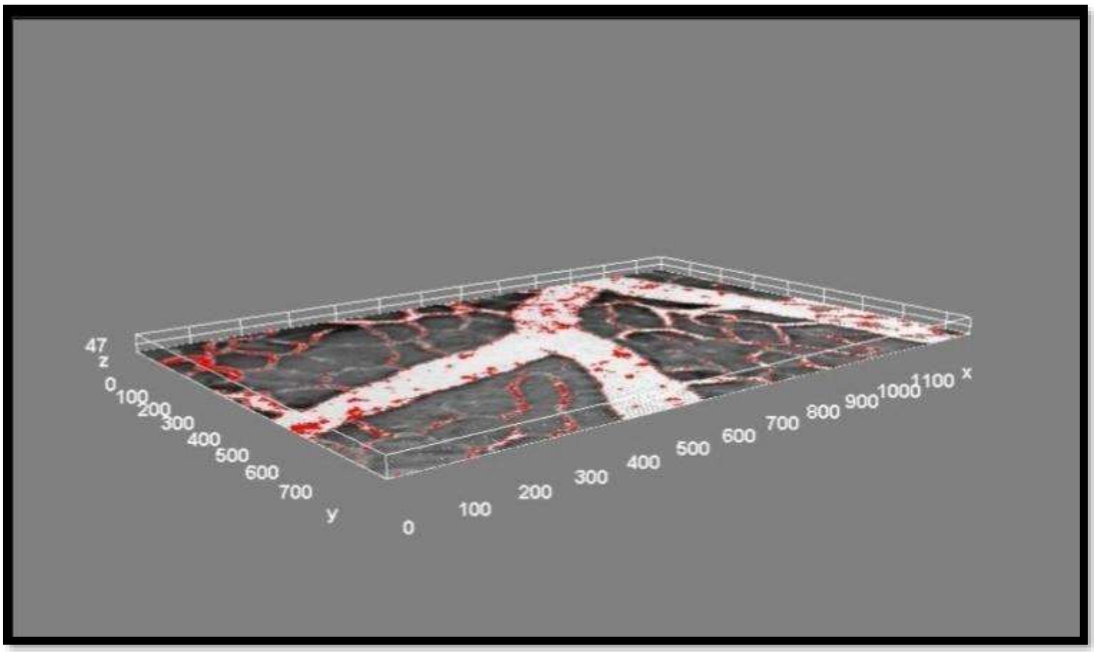


**Figure (A-5) :** Photomicrograph of cross section in leg skeletal muscles of Green-Winged Teal, show the a large amount in the distribution of calcium (black arrows) Alizarin Red stain, 400 X.

**Figure (B,5) :** Surface plot show of cross section in skeletal muscles fibers of the leg area in Green-winged Teal.



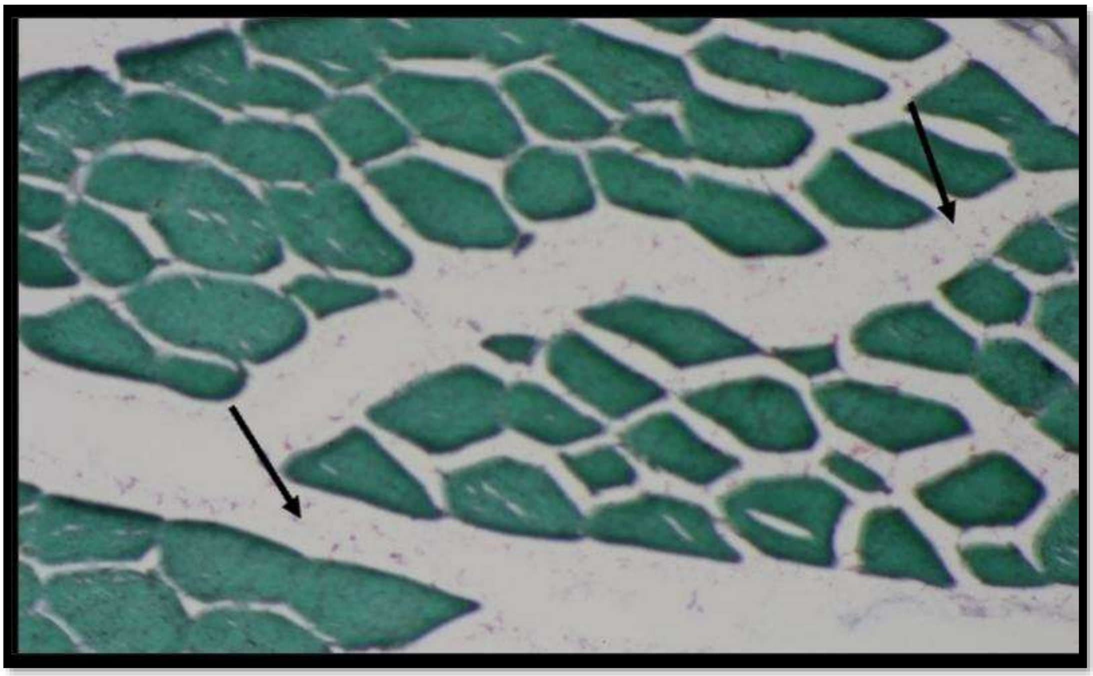


**Figure (A,6) :** Photomicrograph of cross section in leg skeletal muscles of Domestic duck, show a little amount in the distribution of calcium(black arrows).Alizarin Red stain, 400 X.

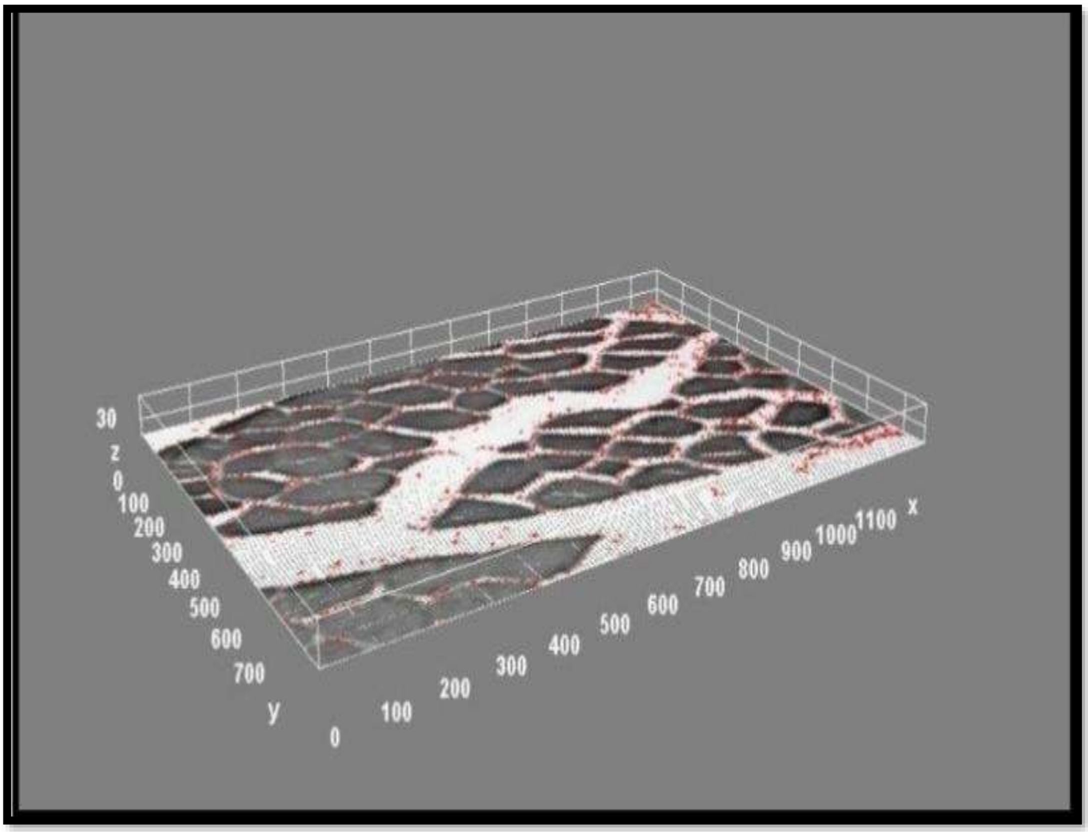
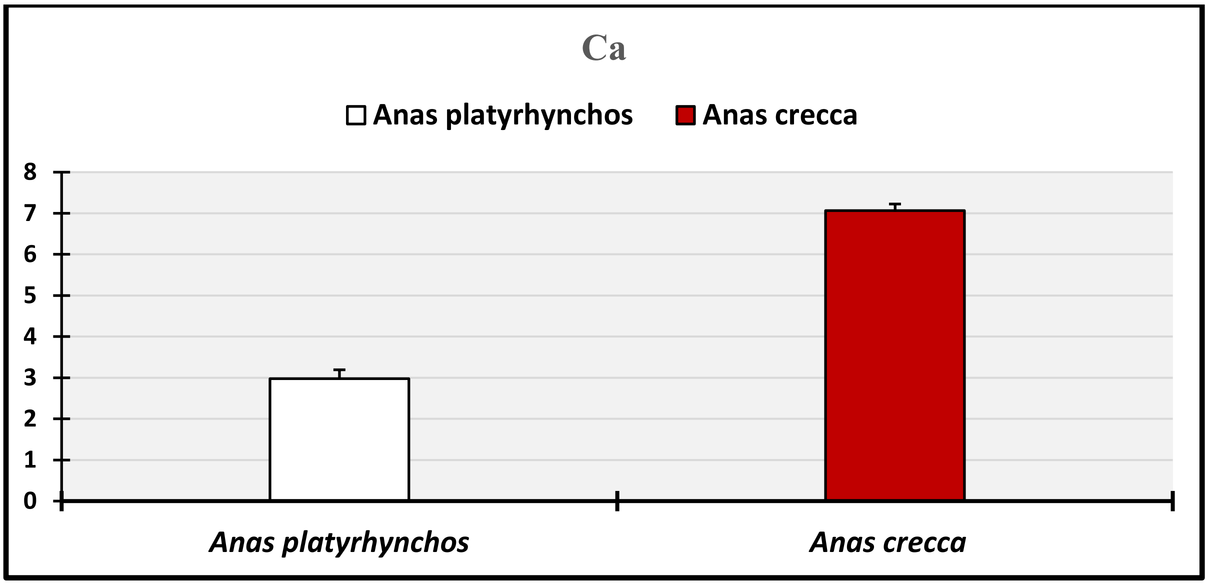



**Figure (B,6) :** Surface plot show of cross section in skeletal muscles fibers of the leg area in Domestic duck .




**(Chart 2):** The ratio of the calcium content in skeletal muscle fibers of the leg area in Green-winged Teal ( Anas crecca ) and Domestic Duck (Anas platyrhynchos)

## 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 Green-Winged Teal contain large amounts of protein compared to Domestic duck 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 Green-Winged Teal was greater than that of Domestic duck. Bird species' mineral composition varies based on their physiological movements, age, species, and the type of food they consume.

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