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Comparative Histological Study of Calcification of the Heart Wall between Quails and Broiler Chickens

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Abstract

The histological study of the current work was completed to appear and comparison the general arrangement and distinguish the calcification in the heart wall of quails and broiler chickens. The study involved (20) healthy adult birds which collected from private commercial fields in the city of Al-Kut. The heart samples were excised and fixated with (10% formalin) and carried to follow the routine histological steps. Through the staining step, there are two dyes were used: the hematoxylin and eosin stain to view the general cardiac layer structures, and Alizarin Red stain to identify the deposition of calcium salts in the cardiac tissues. The histological findings showed appeared the heart wall consists of three major layers, and the histological arrangement of these layers was predominantly analogous for the two species of studied birds. The histochemical findings via an alizarin red dye revealed a profuse deposition of calcium, which was observed as calcification in the form of dark points within the myocardium in quail birds, while in broiler chickens, the calcification appeared as large dark foci. The statistical analysis demonstrated that the value of calcium accumulation in the cardiac muscle of quail was more than that of broiler chicken. This rise of myocardium calcification may be interrelated with the advancement of age, type and size of the bird, and the physiological movement of its heart muscle.

Keywords: : Myocardium, Calcification, Alizarin Red.

Introduction

Through forgoing years, entire poultry meat production and consumption have amplified rapidly and, in numerous regions of the world, single intake of fowl meat will continue to grow (1). About dietary features, fowl meat is a healthy, suitable the current customer demand for a little low-cholesterol grades of meat, low-fat meat with a high unsaturation degree of unsaturated fatty acids and low sodium amounts. In addition, chicken meat can be considered a functional diet, which is capable of providing the human body with vital constituents that have important and beneficial effects on health, such as most vitamins, antioxidant molecules, and the conjugated (isomeric) linoleic acid (2, 3).

Quail farming is emerging as a new kind of diversification in poultry farming, aimed at expanding the variety of flavour options and aggregate meat output to meet the growing demand for animal protein (4). Quail production holds a substantial position in poultry agriculture. Initially, this refers to the rate of advanced processes, which indicates to five times greater than comparable measures in broiler chickens and the earlier egg-laying starting stage (5). Quail meats as well as eggs are considered a source of high-quality foods due to their sensible diet and remarkable taste. As a consequence, there is a significant comprehensive demand for quail birds' products (6).

The muscle tissues are soft tissues created in animals and categorized as one of the vital tissues . Three kinds of muscle tissue in vertebrate animals can be known: cardiac, smooth, as well as skeletal muscle (7). The cardiac category is an involuntary muscle type existing only in the heart, which consists of the myocardium stratum, but it is similar in its arrangement to the skeletal category (8). The avian cardiovascular structure is vastly developed to billet the specialized desires of several species' abilities to swim, fly, and run (9, 10). The characteristic morphology, functions and mechanisms of the bird's heart allow for greatly effective blood circulation and oxygen supply (10).

In general, the heart structure of birds is very similar to that of the mammals, but it has a number of individualities (11, 12, 13). As in class Mammalia, the avian heart has four hollows or chambers (14). In the left ventricle, the muscular wall is 2-3 times thicker than the parallel in the right ventricle, and becomes thinner on the way to the apex (14). The pericardium consists of a hard fibrous membrane that encloses a tiny volume of liquid for lubrication. The minor thickness of the avian myocardial cells delivers a capacious surface area compared to mammalian myocardial

cells, which in turn allows for fast myocardial depolarization (10).

Calcium is necessary for several essential body processes, like the body metabolism, neuromuscular function, muscle contraction, as well as enzyme activation (15). Sufficient calcium intake is important for the healthy functioning of the heart, blood, neural cells, bones and muscles (16). Where higher levels of calcium can result in vascular and soft tissue calcification, as well as delay the growth of chickens (17). Cardiovascular (CV) calcification is an important topic in cardiology, principally because the calcium salt precipitate is attendant with main CV diseases, like atherosclerosis, heart valve diseases, and numerous hypertrophic cardiomyopathies (18). Calcifications can include arteries and parts of the heart and arteries, causing toughness and imbalances. Sometimes, this calcification event may arise in the myocardium and heart's envelop, which originates conduction weaknesses, heart fibrosis and inflammation (pericarditis) (19). This study object to compare the normal histological structure of the heart and cardiac muscle in quails and local chickens, and to inspect whether the cardiac muscles of both species contain calcium aggregates with special stains.

Materials and Methods

Animals and tissue preparation

Twenty adult birds (including 10 male quails, of age 45 days, and 10 male broiler chickens, of age 35 days) which collected from private commercial fields in the city of Al-Kut. The gross investigation was done, it was found that all altogether birds were in good health and they did not suffer from any illness. All birds were anaesthetized via chloroform mode, and then the heart was excised and samples were taken from different chambers of it. The samples were straightway fixed with (10% neutral buffered formalin) for (72 hours), then the specimens of the heart wall were washed using tap water for (4 hours). Then cardiac specimens were carried out to follow routine histological procedures: including "dehydration, clearing, infiltration, embedding and cutting". Through staining stapes there were two dyes were used: the hematoxylin and eosin stain (H&E) for displaying the complete cardiac muscle arrangements, in addition to Alizarin Red stain to identify the deposition of calcium salts in the cardiac tissues (20).

Methodology statistic

All the slides were inspected by the Mejia microscope equipped with different magnifications (4X, 10X, 40X and 100X) detached lens. The examined sections were captured via a Canon digital Photostat. The output pictures were inspected via a computerize program (Image J), which has a devoted feature for quantifying area, percentage area, and calculating objects.

Results

Histological result

The histological findings by using (H&E) showed appeared the heart wall of two species of studied birds consists of three chief layers: the inner stratum endocardium, the intermediate stratum myocardium and outside stratum epicardium. According to the organization of these layers from outside to inside the heart, the epicardium stratum consists of a thin layer of mesothelium as well

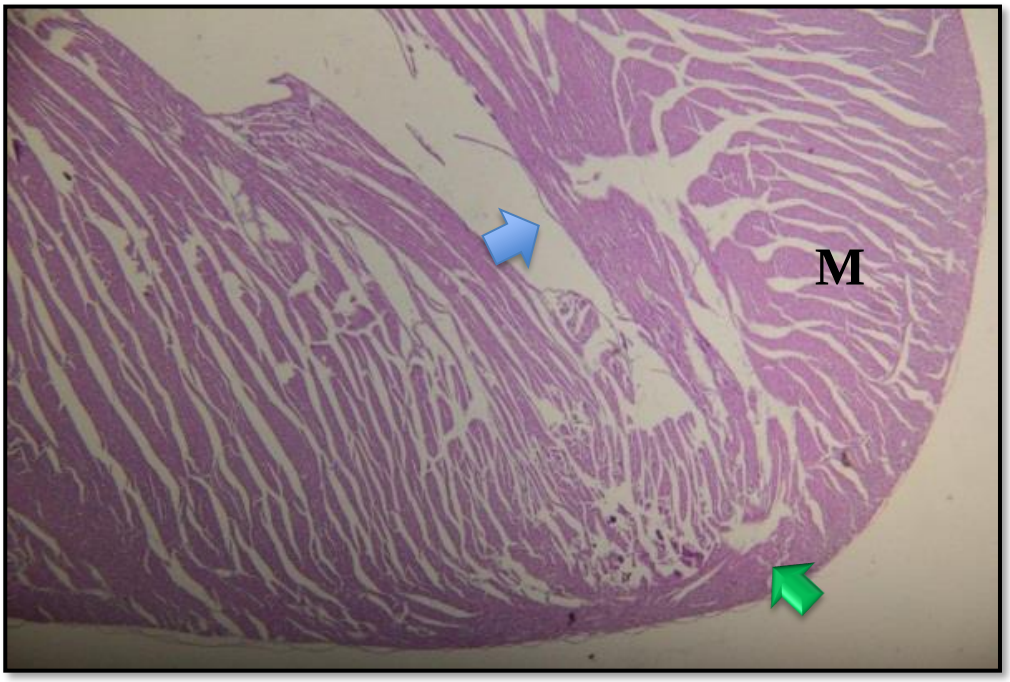
as a connective tissue layer. The epicardium stratum is relatively thin in contrast with the myocardium stratum (figures 1, 2). The myocardium stratum comprises heart muscles, composes the majority of the heart wall. It consists of striated cardiac muscle fibres (cardiomyocytes), which are characterized by branched muscle fibers. The myocyte comprises a single centrally located nucleus enclosed by a sarcolemma (Figure 3). Microscopic review findings showed, in the ventricle chamber, the thickness of the myocardium appears greater than atrium chamber (Figure 4). The histological inspection by routine stain (H&E) showed the endocardium stratum consisting of simple squamous epithelium (heart endothelium), which lines the heart's cavity (Figures 5, 6). This endothelial layer is continuous with the endothelial lining of the great blood vessels that attach to the heart.

Histochemical results

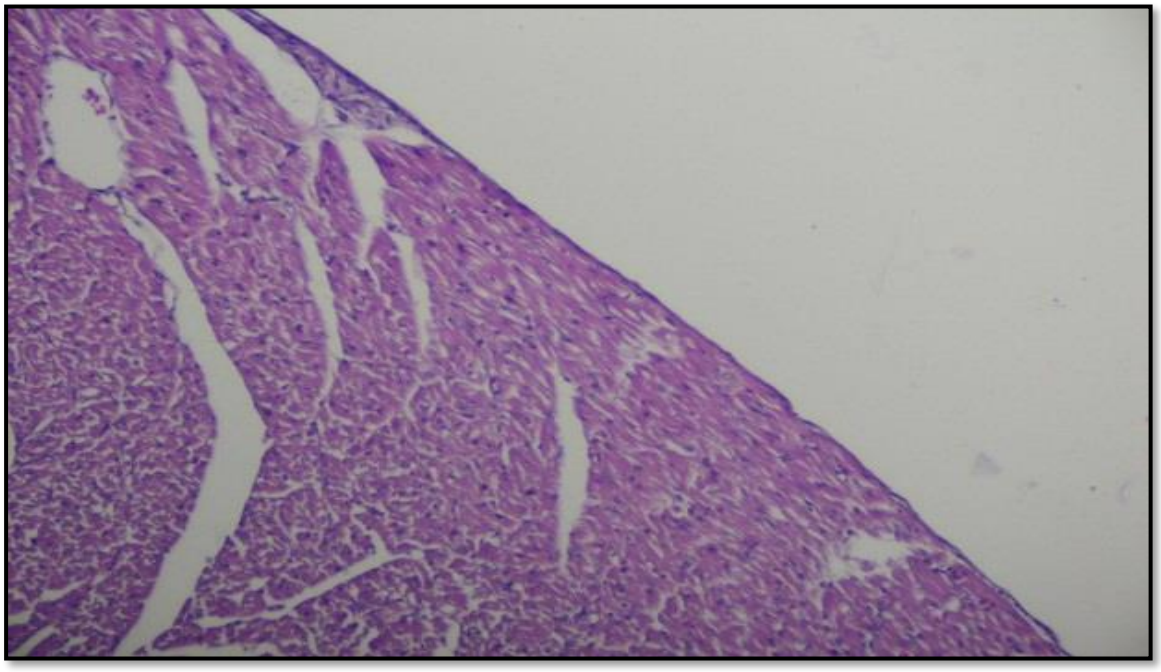
The histochemical results to define the calcium content were executed on both species of birds (quail and chicken) via an alizarin red dye. In the quail bird, the microscopic inspection of cardiac muscle samples revealed a profuse deposition of calcium, which was observed as calcification in the form of dark points scattered widely within the cardiac muscle bundles, as shown in (Figure 7). In chickens, the microscopic investigation of longitudinal sections of myocardium showed a high amount of calcification, which appeared as large dark foci spread in certain areas of cardiac muscle bundles, as revealed in (Figure 8). The end findings of statistical analysis demonstrated the amount of calcium accumulation in the cardiac muscle of quail (0.085/pixel), this value was more than the calcification quantity that was counted in the cardiac muscle fibers of broiler chicken muscle (0.016/pixel). These calcification data are revealed in surface plots in longitudinal sections of myocardium stratum of heart samples of both types of experimental birds (figures 9, 10) (chart 1). Analysis of these data reflects the accumulation of large amounts of calcium in the heart wall of quail compared to the low presence of this element in the heart wall of broiler chickens.

Discussion

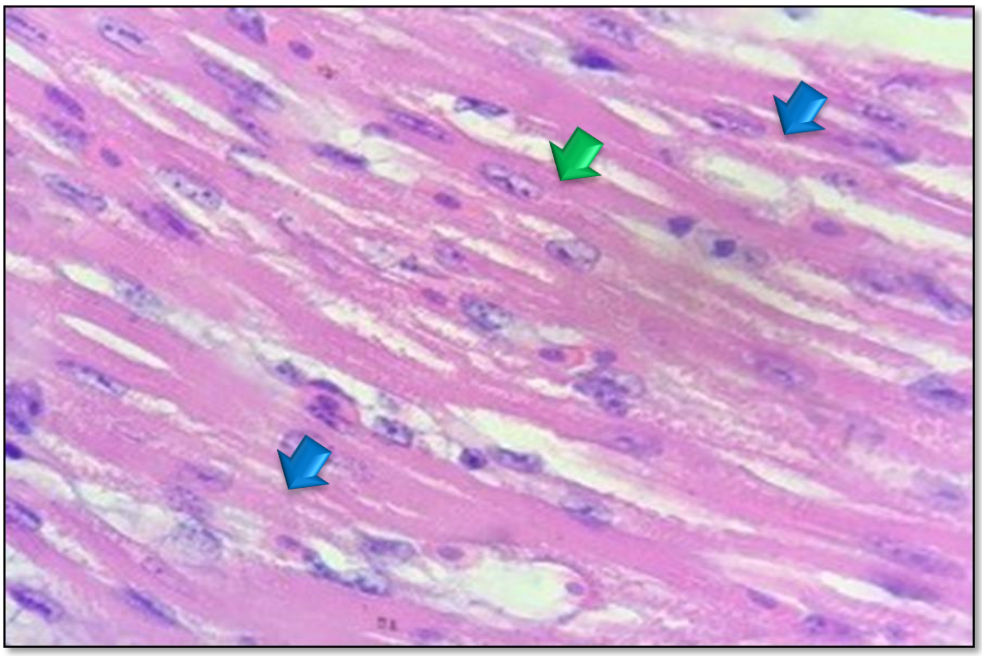
In the present study, the general description and histological structure of the heart wall of quail birds and broiler chickens, consisting of a layer of cardiac muscles supported by connective tissues, are lined by a layer of epithelial cells. These were comparable to that was seen by numerous authors (21, 22), they were described the histological configuration of the heart in other fowls. Regarding the accumulation and increase of calcium in soft tissues such as muscles and connective tissues, the heart wall calcification was described by some authors in different vertebral species, mice (23), bull (24), and human (25).



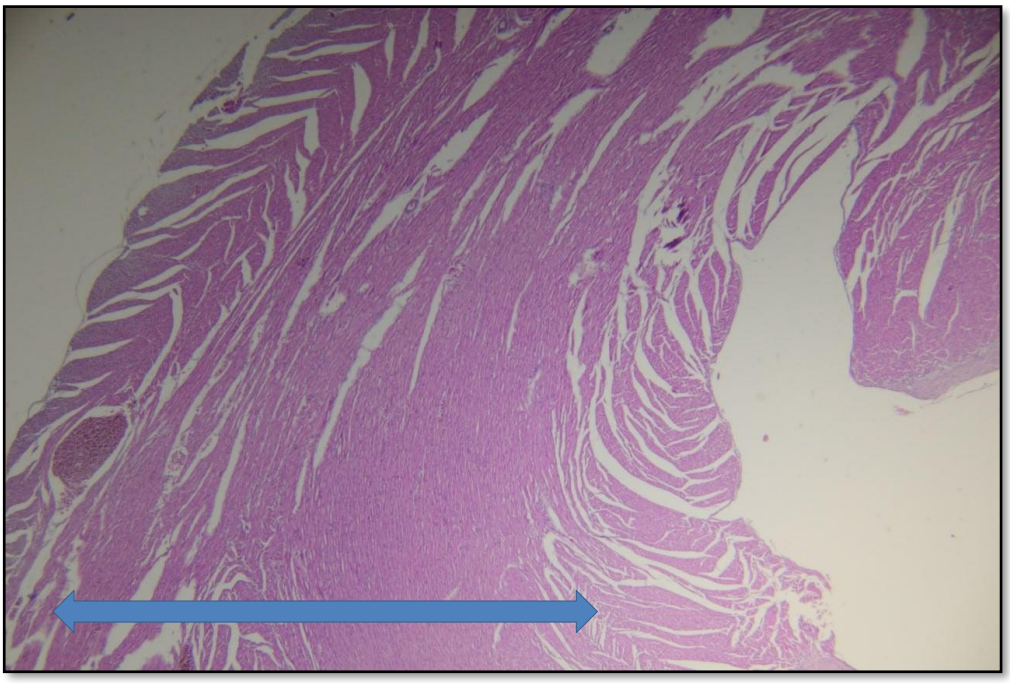
(Figure1): Section of heart wall of quail, viewing: Epicardium (green arrow), Myocardium (M), and epicardium (blue arrow) (H&E 40X).



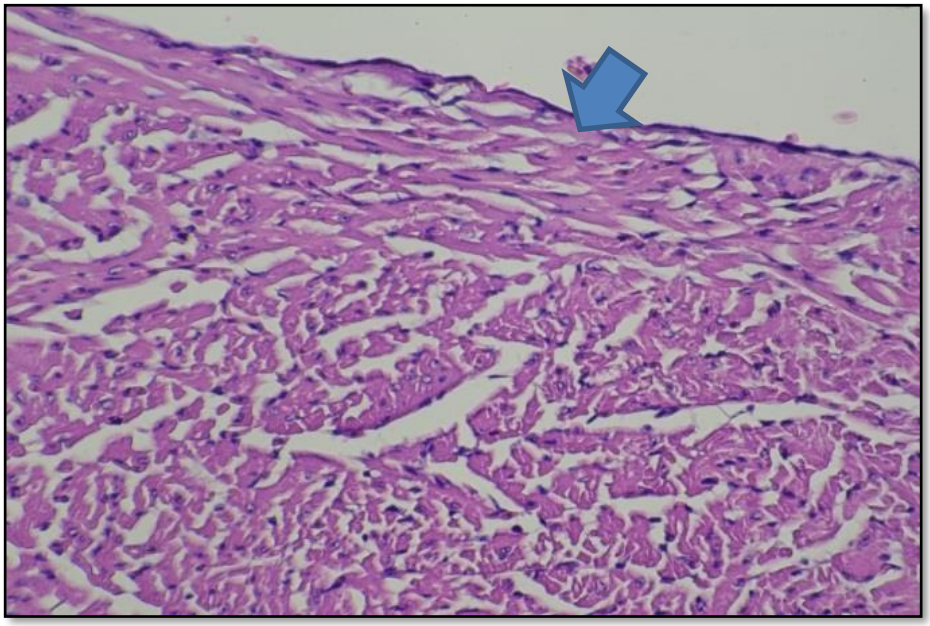
(Figure 2): Section of heart wall of quail, viewing: Epicardium (green arrow) (H&E 200 X).



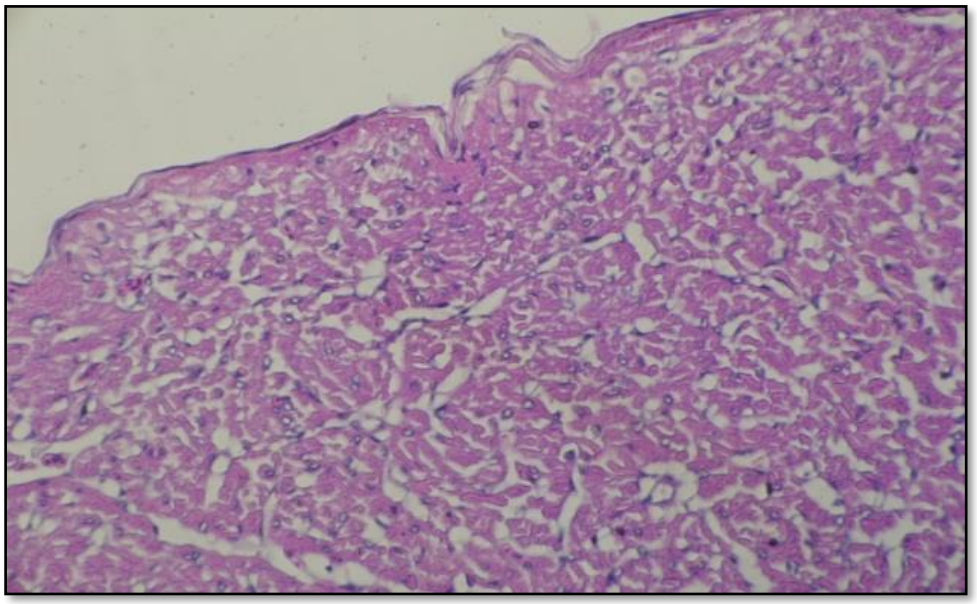
(Figure 3): Section of striated cardiac muscle fibers of quail, viewing: single centrally located nucleus (blue arrow), sarcolemma (green arrow) (H&E 400 X).



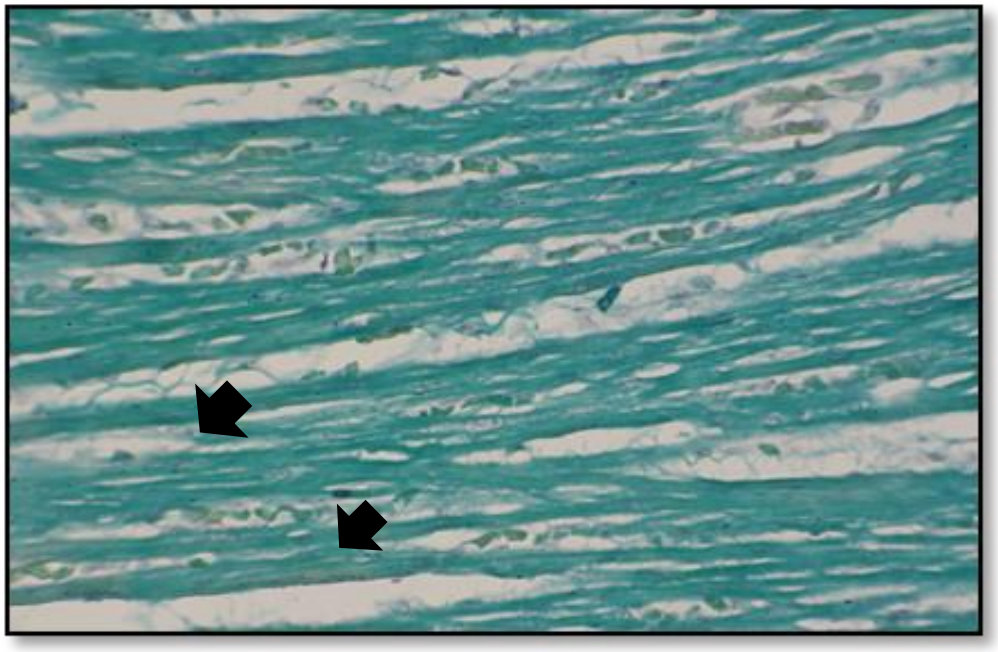
(Figure 4): Section of the heart wall of chicken, viewing the thickness of the myocardium (blue arrow) in the ventricle chamber (H&E 40X).



(Figure 5): Section of heart wall of quail, showing the endocardium layer (blue arrow) (H&E. 400X).



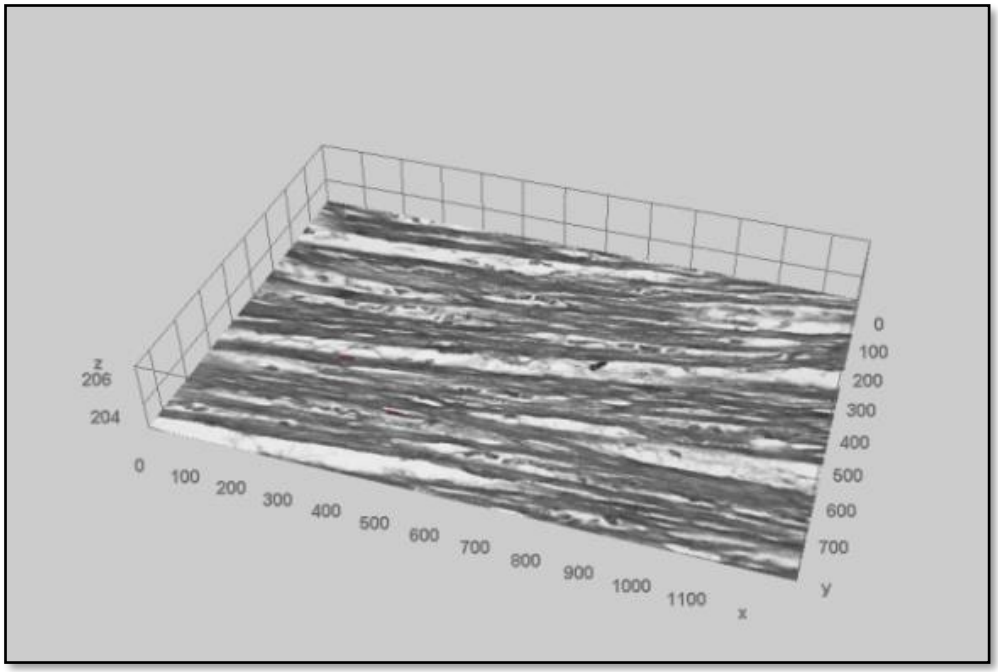
(Figure 6): Section of the heart wall of a chicken, showing the endocardium layer (blue arrow) (H&E.400 X).



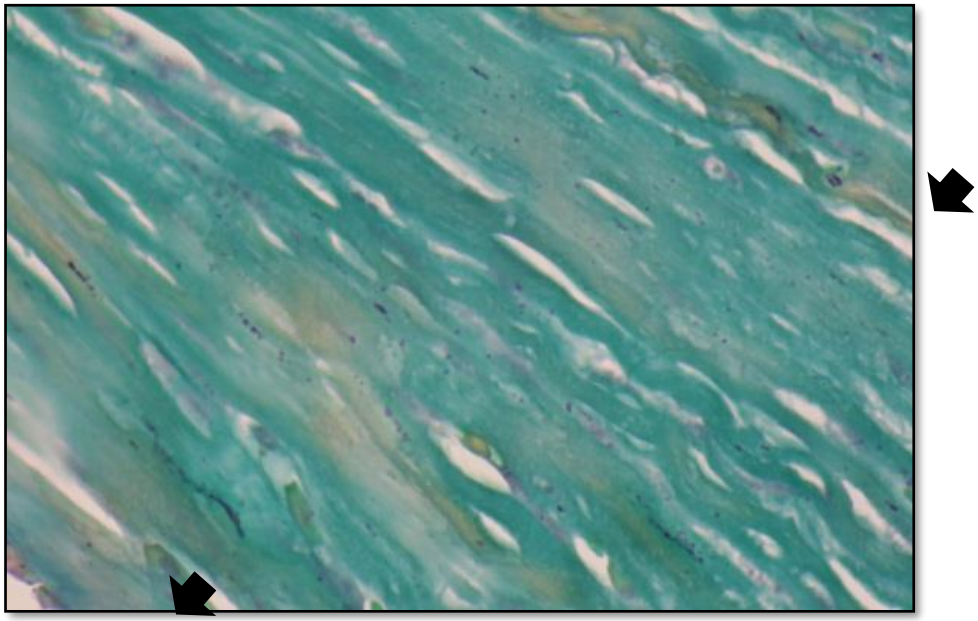
(Figure 7): Section of the heart wall of quail, showing the amount of calcium scattering (black arrows). (Alizarin Red stain, 400 X).

(Figure 8): Section of heart wall of chicken, showing the large amount of calcium scattering (black arrows), (Alizarin Red stain, 400 X).

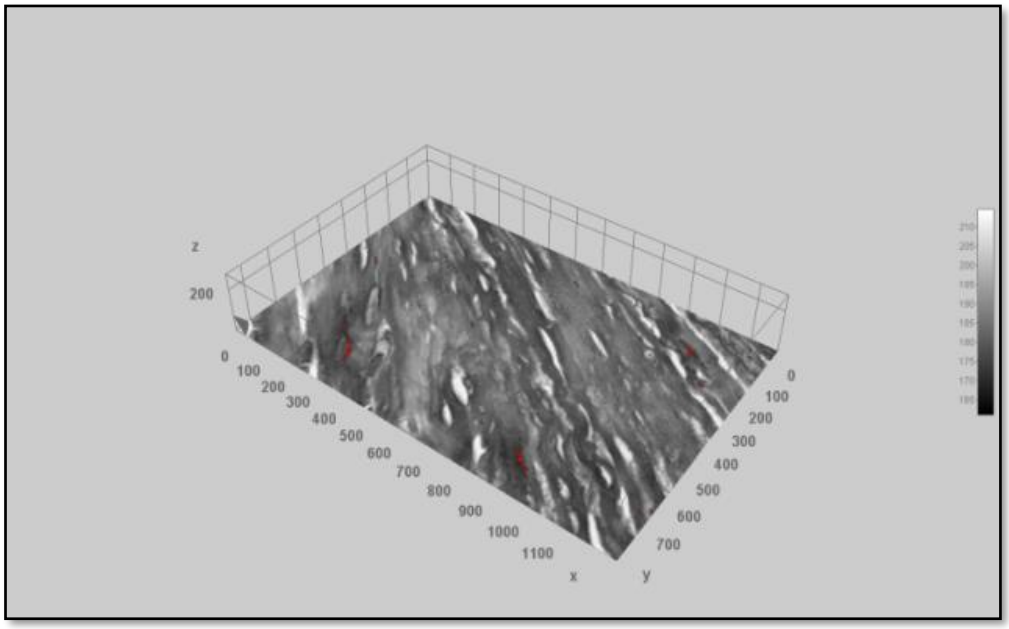
(Figure 9.A): Section of heart wall of chicken, showing the amount of calcification scattering (black arrows), (Alizarin Red stain, 1000 X).



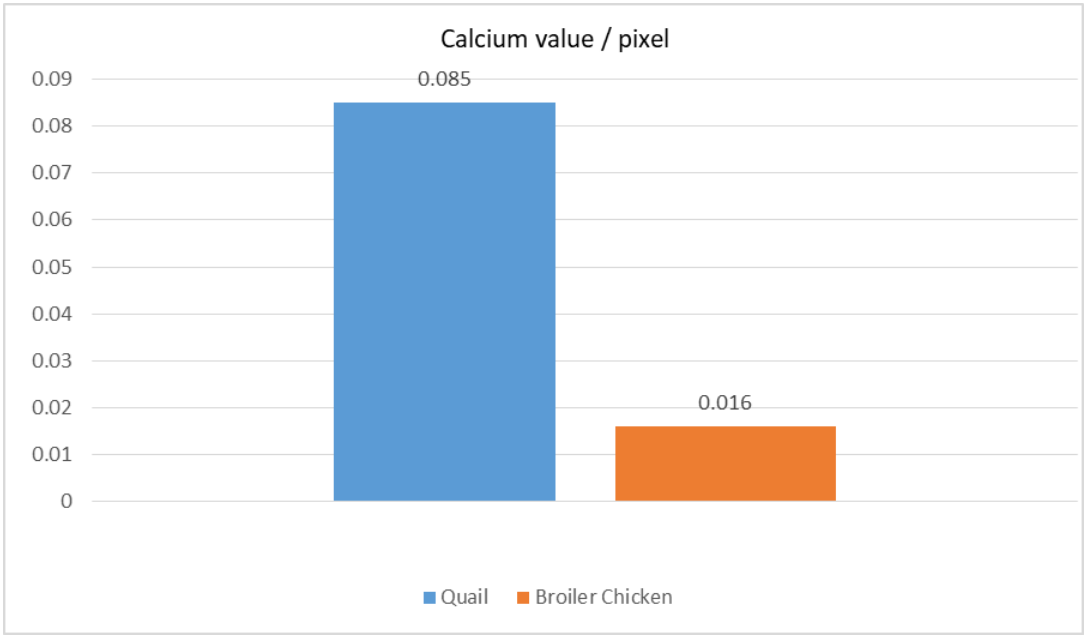
(Figure 9.B): Numerical surface plot viewing the percentage of calcification for the heart wall of the chicken.



(Figure 10.A): Section of heart wall of quail, viewing the large amount of calcification scattering (black arrows), (Alizarin Red stain, 1000 X).



(Figure 10.B): Numerical surface plot viewing the percentage of calcification for the heart wall of quail.



(Chart 1): The ratio of calcium accumulation in cardiac muscle tissue of quail and broiler chicken.

The appearance of calcium deposition in all cardiac muscle samples of two model species of birds in the present work was similar to that mentioned by the previous author in skeletal muscle in quail and chicken birds, which revealed that the calcium content muscles of quail birds was more than their content in the meat of chicken birds (26). This increase in myocardium layer calcification was probably related to the advancement of age, which is often accompaniments by physiological disturbance in the level of blood calcium. Calcium plays a vital role in muscle function in everything living organisms, and most importantly, those birds, and is directly associated with muscle type and the environment of its movement. On the other hand, the higher calcium content in the cardiac muscle of quail compared to broiler chickens is because quail is a small-sized bird that originally lives in the wild, and the physiology of its heart function is generally different and faster to meet the survival and adaptation needs of this bird, compared to the living and farming conditions of broiler chickens. The variances of muscular calcium content may be dissimilar in bird species according to the systemization of muscular physiological movement (27). The preceding authors reported that the variations in mineral composition may be credited to the type of species and age of birds, as well as the kind of their food (28).

Conclusion

We conclude from studying the level of calcium accumulation in the muscular layer of the heart wall in two different types of birds that the high level of calcium in the cardiac muscle tissue is mainly due to ageing in both types. In addition, this level is associated with the species of bird, the size of the bird, and the physiological pattern of its heart movement, which enables it to adapt to the appropriate environment.

Conflict of Interest

The Author declares there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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دراسة نسيجية مقارنة لتكلس جدار القلب بين طائرالسمان والدجاج اللاحم

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الخلاصة

تم إجراء الدراسة النسيجية الحالية لمقارنة الترتيب العام وتمييز تجمع الكالسيوم في جدار القلب لطائري السمان والدجاج اللاحم. شملت الدراسة (٢٠) طيرًا بالغًا سليمًا، تم جمعها من الحقول التجارية الخاصة في مدينة الكوت. تم استئصال عينات القلب وتثبيتها بالفورمالين (١٠٪)، ثم اجريت عليها الخطوات الروتينية الخاصة بالتقنية النسيجية. خلال خطوة التلوين، تم استخدام صبغتين، هما صبغة الهيماتوكسيلين وإلايوسين لإظهار التركيب العام لطبقات القلب، وصبغة ألاليزارين الأحمرلتحديد ترسيب أملاح الكالسيوم في انسجة جدارالقلب. أظهرت النتائج النسيجية أن جدار القلب يتكون من ثلاث طبقات رئيسية، وكان الترتيب النسيجي لهذه الطبقات متشابهة إلى حد كبير في نوعي الطيور المدروسة. أظهرت النتائج النسيجية الكيميائية باستخدام صبغة الأليزارين الاحمر ترسيباً غزيراً للكالسيوم، حيث لوحظ على شكل نقاط داكنة داخل عضلة القلب في طيور السمان، بينما ظهرت التكلسات في الدجاج اللاحم على شكل بؤر داكنة كبيرة. أظهرت التحليلات الإحصائية أن نسبة تراكم الكالسيوم في عضلة قلب طائر السمان أعلى مما هي عليه في الدجاج اللاحم. يمكن ان يرتبط هذا الارتفاع في تكلس عضلة القلب بتقدم العمر، ونوع وحجم الطائر، والحركة الفسيولوجية لعضلة قلبه.

الكلمات المفتاحية: عضلة القلب، التكلس، صبغة الأليزارين ا