

Research Article



Study the Functional Related Ultrastructural and Histomorphometrical Characteristics of Pectoralis Muscle of High-Flying Tippler Pigeons in Comparison to Low-Flying Fancy Pigeons

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Abstract | The flying muscles of birds exhibit the highest mass-specific metabolic rates among vertebrates, flapping flight significantly more energetically taxing than terrestrial movement due to the rapid sustained contractions required by the largest flight muscles for both lift and thrust. This study aimed to investigate the histological and ultrastructural characteristics of pectoralis muscles that influence functional flight ability among avian species. A study was undertaken on three pigeon breeds: high-flying tippler pigeons, moderate-flying red Iraqi pigeons, and low-flying fantail pigeons to examine the correlation between pectoralis muscle structure and high-flying ability. Nine pigeons each were categorized, and samples of the major pectoralis muscle were obtained for histomorphometrical assessments and transmission electron microscopy examination. The ultrastructural characterization revealed that the quantity of mitochondria in each breed exhibited considerable variation, as per the data. The mitochondrial density was 70, 48, and 32 per 800 μm^2 , and the average area of mitochondria was 4.51 ± 3.56 , 1.72 ± 4.56 , and $1.87 \pm 3.90 \mu\text{m}^2$ for high-flying tippler pigeons, moderate-flying red Iraqi pigeons, and low-flying fantail pigeons, respectively measured by Fiji image software. Tippler pigeons, with their distinct physical morphology and abundant mitochondria, demonstrated enhanced endurance and high-altitude flight capability. Fantail pigeons exhibited the lowest mitochondrial density, which is unsurprising considering their limited flight capabilities. The study indicates that the augmented mitochondria in the pectoralis muscle enhance the body's capacity to utilize oxygen and generate energy, essential for prolonged flights and endurance activities. These findings indicate that a significant physiological change affecting birds' flight capability is the number of mitochondria. They elucidate the achievements of elevated breeds, such as the Tippler pigeon, at significant heights.

Keywords | Major pectoralis muscle, Mitochondria, Electron microscope, Histology, Iraq red pigeons

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INTRODUCTION

Flight in birds requires extremely precise cellular and physiological adaptations since it is one of the most energy-intensive animal reactions (Suarez, 1992; Scott and Milsom, 2007). Because they supply the ATP required for extended flight, the number and efficiency

of mitochondria in muscular tissues especially in the pectoralis muscle are essential among these adaptations, as a largest flying muscle responsible for generating power (Mathieu-Costello, 1994; Guglielmo and Guglielmo, 2010; Zheng *et al.*, 2022). High mitochondrial density promotes better oxidative metabolism, due to the high percentage of oxidative enzymes that promote ATP

production through Krebs cycle and electron transport chain– ETC. which supports the prolonged energy requirements during prolonged airborne activity (Dawson *et al.*, 2020). Studies show that the mitochondrial density of birds varies significantly depending on their lifestyle, flying habits, and evolutionary factors (Weibel *et al.*, 1991; Stier *et al.*, 2022). Birds with strong flight abilities typically show greater mitochondrial volume density and oxidative enzymatic activity in contrast to ground-dwelling or ornamental species (Guglielmo and Guglielmo, 2010; Dawson *et al.*, 2020). In spite of behavioral differences, the peregrine falcon (*Falco peregrinus*) and the domestic pigeon (*Columba livia domestica*) exhibit remarkably similar muscular adaptations that support high-energy flight. Both species have highly advanced pectoralis muscles anchored to a pronounced keel, enabling powerful wing strokes (Miao *et al.*, 2025). Furthermore, their muscle fibers are rich in mitochondria, enabling effective aerobic metabolism necessary for sustained ATP production (Suarez, 1992; Mathieu-Costello, 1994). High mitochondrial density and oxidative muscle fiber specialization allow pigeons to endure long-distance flights, while peregrine falcons achieve intense, short bursts of speed during hunting dives. These cellular and structural adaptations highlight a convergent evolutionary approach to meet the extreme energetic demands of avian flight (Guglielmo and Guglielmo, 2010; Dawson *et al.*, 2020). In the current study, three pigeon breeds were selected for comparative analysis based on their distinct flight capabilities: the Pakistani tippler pigeon (*Columba livia domestica*) or high flying tippler pigeon, well-known for its exceptional endurance and high-altitude flight; the Iraqi Red pigeon, considered as moderate flying ability; and the Fantail pigeon, mainly an ornamental breed with limited flight capacity. Using transmission electron microscopy (TEM), significant variances in mitochondrial abundance within the pectoralis muscle tissues of these breeds were observed. This study aimed to investigate the association between the ultrastructure of the major pectoralis muscle, particularly mitochondrial density, and flight performance, which may be viewed as a physiological adaptive trait that underlies flight capability differences in pigeons.

MATERIALS AND METHODS

ETHICS STATEMENT

This study was carried out following the strictest standards regarding the main rules of care and the use of animals in research. The experiment was conducted according to the guidelines of the College of Veterinary Medicine, University of Diyala Animal Experimentation Ethics Committee (No. VM 154. October 2023. I and R) dated on 1/10/2023.

SAMPLE COLLECTION

Adult pigeons aged 7–8 months of three pigeon breeds the Pakistani tippler pigeon (*Columba livia domestica*), the Iraqi Red pigeon, and the Fantail pigeon were used in this comparative study. All birds were clinically healthy, and maintained under standardized housing and feeding circumstances to decrease external physiological variability (Al-Agele, 2021; Stier *et al.*, 2022; Nasser *et al.*, 2024). Muscle tissue samples were collected from the pectoralis major muscle, the primary flight muscle in birds and a basic site for studying aerobic metabolism and mitochondrial function (Weibel *et al.*, 1991; Hayat, 2000). Birds were euthanized following; a small biopsy (~1 cm³) from the central area of the pectoralis muscle was removed using sterile instruments (blade). Collected tissue samples were immediately immersed in a fixative solution containing 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C for 24 hours to preserve ultrastructural details, particularly mitochondrial morphology (Bozzola and Russell, 1999; Al-Agele *et al.*, 2019). Following primary fixation, samples were rinsed with phosphate buffer (0.1 M phosphate buffer), post-fixed with 1% osmium tetroxide, dehydrated through a graded ethanol series, and embedded in epoxy resin, according to standard protocols for transmission electron microscopy (Hayat, 2000). All sample handling and preparation procedures were conducted under sterile conditions to avoid contamination and ensure optimal preservation of cellular structures for subsequent quantitative TEM analysis (Alshammery and Mirhish, 2024).

STATISTICAL ANALYSES

All graphs were performed using Graph Pad software (Prism 6), and the results were presented as the mean ± SE using Excel software. The data that was collected by measuring the width of the fascicles as well as the data that was generated by measuring the diameter of mitochondria using Fiji image software as described by Al-Agele (2020, 2024); Kadhim *et al.* (2023); Karim and Al-Agele (2025) were analyzed by One-way ANOVA methods using Graph Pad software (Prism 8). The statistical analysis of significant differences was utilized for comparisons in all of the studies, and the threshold for significance was established at P < 0.05.

RESULTS

The pectoralis muscle signifies an energetic anatomical structure in birds, being chiefly responsible for producing the mechanical force required for flight. This muscle was particularly well-developed in species and breeds characterized by exceptional endurance and flight capability, such as the Tippler pigeon. In the present study, a histological contrast of the pectoralis muscle was done

using transmission electron microscopy (TEM) to assess and measure mitochondrial abundance across different pigeon breeds. Consistent with these results, our analysis revealed a significantly higher mitochondrial density in the pectoralis muscle fibers of the Tippler pigeon, provided that a clear cellular basis for its remarkable endurance and larger flight performance compared to less flight-capable breeds.

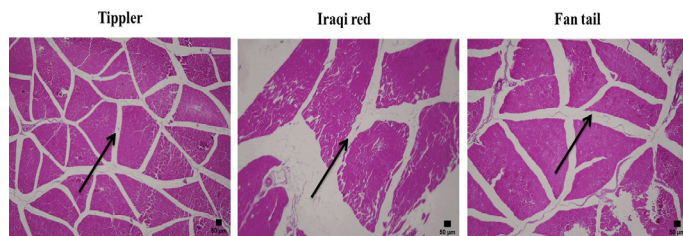


Figure 1: Photomicrographs (X4), (X10), (X40), showing the differences between the distances of (perimysial spaces) of pectoralis muscle tissue among different birds of study.

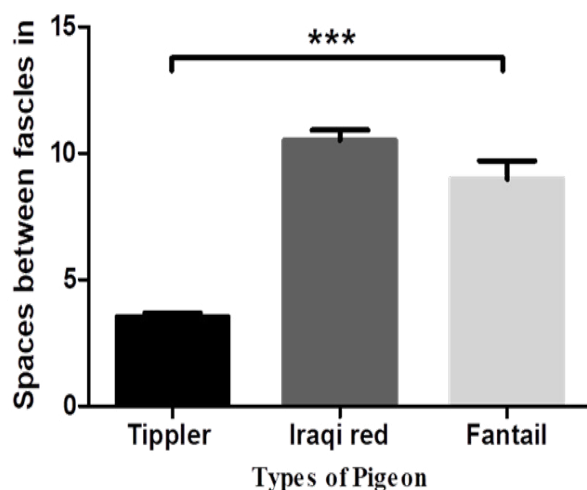


Figure 2: A graph represents the measured values of the spaces among the pectoral muscular tissue of the three studied birds.

ANATOMICAL FINDINGS

Pectoralis major muscle amongst the three studied pigeon breeds. In the Iraqi Red pigeon, a breed classified as a medium-flight performer, the average pectoral muscle length was (22.39 ± 0.75) mm, with a muscle thickness of (73.17 ± 0.02) mm. In contrast, the Tippler pigeon, known for its superior flight endurance, exhibited a substantially greater pectoral muscle length averaging (76.65 ± 0.85) mm, but with a thinner muscle thickness of (12.35 ± 0.25) mm. The Fantail pigeon, characterized by limited flight capability, showed an average pectoral muscle length of 18.01 ± 0.18 mm and a muscle thickness of (80.05 ± 0.98) mm. These measurements highlight the anatomical adaptations associated with flight performance, where longer and more efficient muscles, as seen in the Tippler pigeon, are linked to enhanced endurance flight capacity,

whereas thicker but shorter muscles, as observed in the Fantail pigeon, correspond to reduced flight ability.

HISTOLOGICAL FINDINGS

Upon investigation under a light microscope, the pectoralis muscle of the pigeons primarily responsible for generating the mechanical forces required for flight skeletal muscle tissue exhibits a highly organized structure with cylindrical cells and multiple peripheral nuclei. Supporting structures like endomysium, perimysium, and epimysium provide mechanical support and serve as conduits for blood vessels and nerve fibers. These bands, distinct from the bright I and dark A bands, contribute to the muscle's contractile properties. These structures ensure proper nourishment and innervation of the muscle tissue.

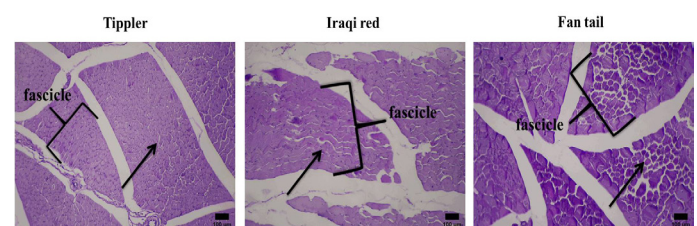


Figure 3: PAS and H and E-stained photomicrographs (X4), (X10), (X40) represent the pectoral muscles fascicles diameters for the three studied pigeons (black arrow).

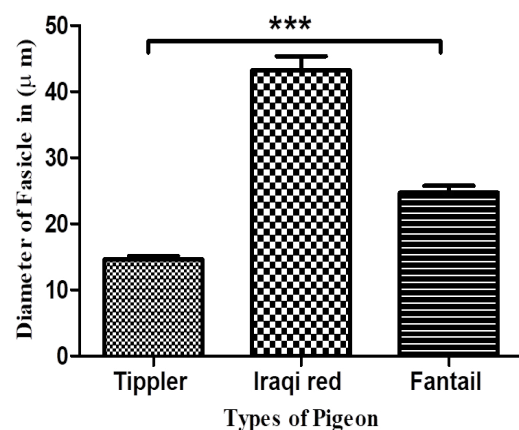


Figure 4: A graph represents the differences among measured values of the pectoral fascicles diameters between the three studied birds.

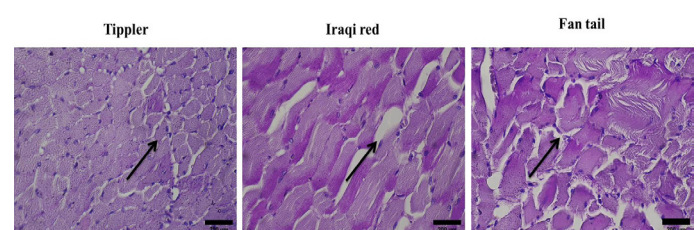


Figure 5: PAS and H and E-stained photomicrographs (X4), (X10), (X40), show the pigeons pectoralis muscle fascicles, muscle fibers, spaces between myofibrils (black arrows), white fibers scattered among red fibres mostly peripherally situated within the bundles and red fibers, nuclei.

Particularly, differences were observed among the three pigeon breeds under study. The Iraqi red pigeon exhibited the largest fascicle diameters, followed by the Fantail pigeon, while the Tippler pigeon showed the smallest fascicle diameters. Quantitative measurement of the perimysial spaces using image analysis software further revealed that the Iraqi Red pigeons had the largest perimysial space ($10.51 \pm 3.38 \mu\text{m}$), followed by the Fantail pigeons ($9.15 \pm 5.62 \mu\text{m}$), whereas the Tippler pigeons had the smallest perimysial space ($3.54 \pm 6.18 \mu\text{m}$). The relatively reduced perimysial space and smaller fascicle diameters observed in the Tippler pigeon suggest a denser muscle fiber arrangement, which may contribute to greater muscle mass and enhanced better mechanical efficiency. This muscular adaptation likely underpins the Tippler pigeon's superior flight endurance and greater resistance to fatigue compared to the other two breeds.

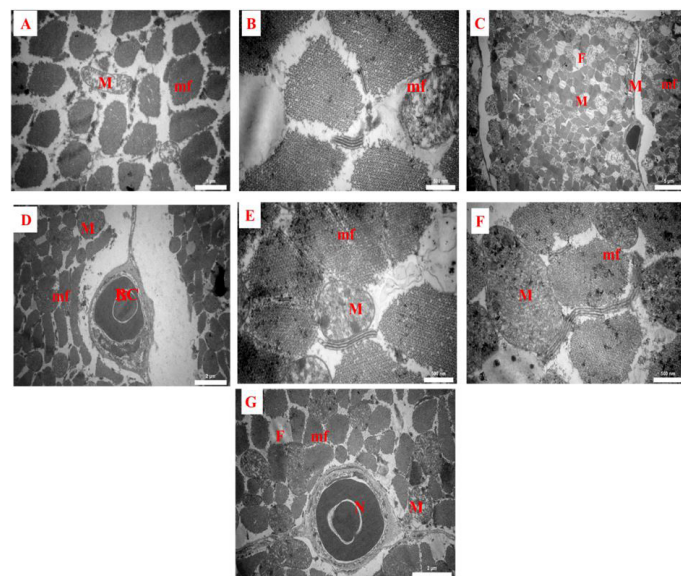


Figure 6: Transmission electron micrographs (TEMs) of pectoralis muscle in studied pigeons, (A) Elongated mitochondria (M) aligned with myofibrils (mf), (B) Mitochondrial internal structure showing cristae (arrows), (C) Cross-section of Tippler muscle with mitochondria (M), lipid droplets (F), and nucleus (N), (D) Mitochondrial aggregations near a blood capillary (BC), (E) Cristae (T) and microtubules between myofibrils (MF), (F) Large mitochondria (M) adjacent to myofibrils (MF), (G) Pericapillary mitochondrial clusters (M) and lipid droplets (F).

TRANSMISSION ELECTRON MICROSCOPIC FINDINGS

By interpreting the relationship between flight performance levels and the amount of energy produced by cells within flight muscle tissue particularly the pectoralis major the findings from transmission electron microscopy have revealed detailed insights into the shape, size, and distribution of mitochondria (by enhancing their ability to produce ATP efficiently within the muscle cells).

These observations reflect the structural adaptations of mitochondria that correspond to the high energy demands required during flight.

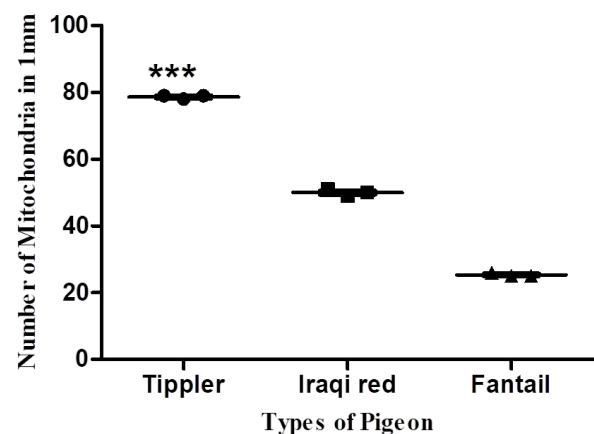


Figure 7: A graph which explains the differences among the numbers of mitochondria within pectoral muscle tissue for three birds.

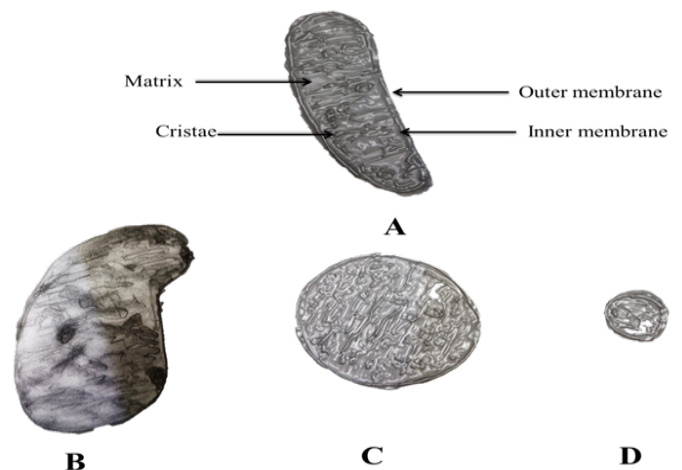


Figure 8: The diagram illustrates numerous forms of the mitochondria within the muscular tissue of the three studied birds.

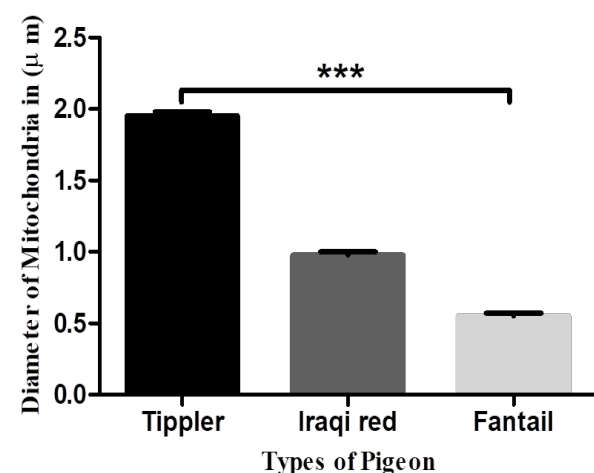


Figure 9: A graph show the differences among values of mitochondrial diameters of three studied birds.

Transmission electron microscopy (TEM) was employed to investigate the cytological differences between the red and white fibers of the pectoralis muscles across the three studied pigeon groups. red muscle fibers were readily distinguishable from white fibers by their smaller fiber diameter, higher myoglobin content, and the presence of large mitochondria and abundant lipid droplets interspersed among the myofibrils. Although substantial mitochondrial aggregations were observed near the subsarcolemmal region and adjacent to blood capillaries in both fiber types, the red fibers exhibited a significantly greater mitochondrial volume density. Moreover, within the red fibers, mitochondria were notably larger indicating an enhanced oxidative capacity. Additionally, numerous lipid droplets were prominently observed, particularly in the pectoralis muscle tissue of the Tippler pigeons. The abundant presence of these lipid stores suggests a greater capacity for fatty acid oxidation processes, supporting the Tippler's greater endurance and sustained flight capabilities compared to the Iraqi Red and Fantail pigeons. Enhanced lipid oxidation is well recognized as a crucial metabolic adaptation for prolonged flight activity at higher altitudes and over extended durations.

during flight **Figure 6**. Using the ultrathin sectioning technique for "TEM", several electron microscopists have reported detailed internal mitochondrial structures. A single-layered outer membrane was noted, exhibiting inward folds termed cristae that extended toward the mitochondrial center. Interestingly, a central region devoid of cristae was consistently observed along the length of many mitochondria, a feature that may be associated with specific metabolic or structural adaptations of the flight muscles.

One of the most significant findings emerging from the manual quantification of mitochondria within a defined counting area ($800 \mu\text{m}^2$) was the marked variation in both the number and size of mitochondria among the studied groups. These differences highlight potential adaptations in mitochondrial biogenesis and energy metabolism that may underlie the observed variations in flight endurance among the pigeon breeds. As in **Table 1**.

Table 1: The number of mitochondria detected within a consistent counting region of 800 in each of the birds' pectoral muscles.

Fantail	Iraqi red	Tippler	dimensions
32	48	70	Number
1.75 ± 6.71	1.37 ± 4.65	2.16 ± 6.23	diameter
1.87 ± 3.90	1.72 ± 4.56	4.51 ± 3.56	Area

Upon manual counting within a standardized area of $800 \mu\text{m}^2$ using transmission electron microscopy significant differences were noticed in both the quantity and size of mitochondria among the pectoralis muscle fibers of the studied pigeon breeds. The Tippler pigeon exhibited the highest mitochondrial density, with a significantly greater average count compared to the Iraqi red and Fantail pigeons ($P < 0.05$). Furthermore, the individual mitochondria in Tippler specimens were observed to be larger, with greater elongation and volume compared to those in the other groups.

In contrast, the Iraqi Red pigeons showed a moderate number of mitochondria with intermediate sizes, while the Fantail pigeons demonstrated the lowest mitochondrial density and smallest mitochondrial dimensions. The variability in mitochondrial number and size strongly correlates with the flight endurance capacity of the pigeons, suggesting that greater mitochondrial content and enlarged mitochondrial structures are adaptive features supporting enhanced oxidative metabolism essential for sustained flight. These findings are consistent with previous studies reporting that higher mitochondrial densities and larger mitochondrial morphologies are hallmarks of muscles specialized for prolonged aerobic performance.

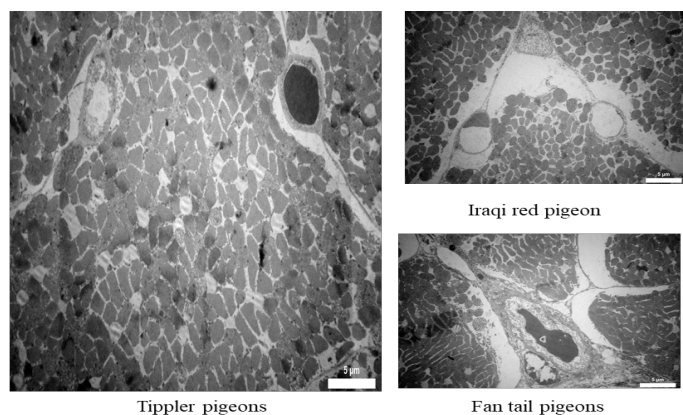


Figure 10: Transmission electron micrographs (TEMs), cross sections, show the comparison among architectural of mitochondrial distribution among the pectoral tissue of the studied birds.

Correspondingly, observations revealed that mitochondria appeared to partially encircle some myofibrils within the muscle fibers. In red fibers, the mitochondria exhibited a thick matrix with densely packed lamellar cristae, characteristic of high oxidative capacity. Although both red and white fibers possessed a well-developed sarcoplasmic reticulum (SR), the white fibers demonstrated a more prominent and reticulated SR structure, suggesting differences in calcium handling and contraction kinetics between the fiber types. Numerous capillaries were also observed, widely distributed throughout the muscle tissue and often located adjacent to mitochondrial aggregations. This close association indicates an abundant and efficient blood supply, essential for sustaining high metabolic demands

Throughout this study, no mortalities have been recorded, and no abnormal behavioral traits have been discovered in any of the pigeon breeds. There are no abnormalities in the muscles during PM either.

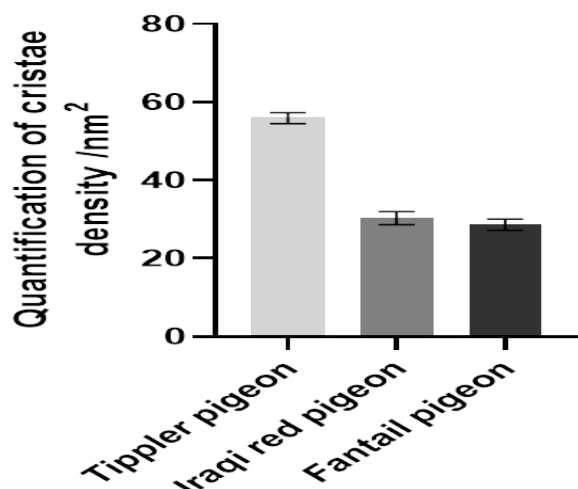


Figure 11: The graph illustrates the density of mitochondrial cristae in the pectoralis muscle of three examined bird species.

DISCUSSION

The current investigation revealed that's the pigeons pectoralis muscle was a powerful muscle that's accepted with the study of (Wada *et al.*, 1999) of pigeon skeletal muscle and (Herd and Arthur, 2009) in physiological basis for residual feed intake. The obtained results were corresponding to the anatomical origin of the pigeon pectoralis muscle aligns with previous findings, accepted with (Wada *et al.*, 1999) pigeon skeletal muscle and consistent with (Dial, 1992) in the wing muscles of the pigeon (*Columba livia*) and (Kuzmiak *et al.*, 2012) in skeletal muscle in pigeons, demonstrated its insertion on the ventral surface of the humerus which our results agree with this studies . pigeons exhibit a relatively large flight muscle mass, constituting around 21.2% of their body weight, along with darker muscle appearance and high densities of nuclei and mitochondria. These features correlate with the elevated metabolic rates, prolonged lifespans, persistent hyperglycemia (~15 mmol/L), and insulin resistance typically observed in flying birds. Approximately 40% of the pigeon pectoralis muscle volume of our results is occupied by non-contractile elements, including capillaries (10%) and mitochondria (30%) in which this results were agree with the study of (Powers and Jackson, 2008) in exercise-induced oxidative stress. These adaptations support the high metabolic demands associated with sustained flight especially in the high-flying Tippler pigeons, that's has been studied and our understandings about the adaptations in this types of pigeons for supporting them in their exceptional endurance capacity. The current

results achieved that there is a significant differences in the spaces between the muscular fascicles (perimysial spaces) of the three studied birds and there is significant variations within the fascicle's diameters, quantitative measurement of the perimysial spaces using image analysis software further revealed that the Iraqi Red pigeons had the largest perimysial space ($10.51 \pm 3.38 \mu\text{m}$), followed by the Fantail pigeons ($9.15 \pm 5.62 \mu\text{m}$), whereas the Tippler pigeons had the smallest perimysial space ($3.54 \pm 6.18 \mu\text{m}$), while the Iraqi Red pigeon exhibited the largest fascicle diameters, followed by the Fantail pigeon, while the Tippler pigeon showed the smallest fascicle diameters. The relatively reduced perimysial space and smaller fascicle diameters observed in the Tippler pigeon suggest a denser muscle fiber arrangement, which may contribute to greater muscle mass and enhanced mechanical efficiency. Mitochondria play an essential role in cellular energy metabolism, responsible for producing over 90% of cellular ATP and consuming a corresponding proportion of oxygen, according to (Rhodes *et al.*, 2024) in the mitochondrial remodeling in response to environmental challenges, our findings accepted this study and revealed that's the pectoralis muscle of the three species of birds and under transmission electron microscope investigation explained that's there is significant differences in the number and size of the mitochondria. Differences in mitochondrial size and density are key criteria for distinguishing muscle fiber types. According to (Anderson and Neuffer, 2006) and we will agree with him, Type I fibers generally contain smaller and fewer mitochondria with less densely packed cristae, while Type II fibers exhibit numerous, densely packed, irregularly arranged cristae. Recent studies indicate that major changes in mitochondrial genes was key to how bats developed the ability to fly, accounting for about 23% of the genetic changes related to this ability (Shen *et al.*, 2023). This substantiates the concept that flight serves as a significant natural selection pressure, promoting the enhancement of mitochondrial efficiency to increase energy generation necessary for vigorous activity. These alterations signify a crucial physiological adaptation process that enhances metabolic efficiency to fulfill the requirements of prolonged flight, consistent with analogous observations in avian species (Mito *et al.*, 2022). Consequently, flying may be seen as a crucial evolutionary element that enhances mitochondrial cellular activities, hence augmenting locomotor ability and survival.

The presence of lipid droplets behind mitochondria in Type II fibers suggests that lipid metabolism plays an important role in their energy supply. Structurally, myofibrils within Type II fibers are slightly bigger in cross-sectional region than those in Type I fibers and are scattered with extensive membrane systems and mitochondria (Rasmussen *et al.*, 2004). TEMs results indicates that's the highly flying

AUTHOR'S CONTRIBUTION

Yamamah Adnan Kamila: Writing original draft; Ramzi Al-Agele: supervision, conception, validation, writing, reviewing the manuscript.

ETHICAL APPROVAL

This study has been approved by the General Animal Use and Care Committee, College of Veterinary Medicine, University of Diyala, Diyala Governorate, Iraq.

FINANCIAL SUPPORT

This project was not supported by grants.

GENERATIVE AI OR AI-ASSISTED TECHNOLOGY STATEMENT

The author(s) declare that no Genrative AI was used in the creation of this manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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Tippler pigeons have the greater proportions of the number and size of the distributed mitochondria which enhance more energy production comparing with the Iraqi red pigeons which have moderate values and the lowest one which is the Fantail pigeons. These findings reveals that's there is strong correlation among the cytological organelles especially mitochondria and the flying levels of birds.

CONCLUSIONS AND RECOMMENDATIONS

This study demonstrates that mitochondrial size and density in the pectoralis muscle are critical determinants of flight performance in pigeons. Tippler pigeons, known for their stamina and long flight, have the largest mitochondrial density and size, enabling effective aerobic metabolism. Fantail pigeons, on the other hand, have the lowest mitochondrial density and smaller organelles, indicating a lower energy route. The current study emphasizes the necessity of understanding pigeon mitochondria and muscle fibers to comprehend how birds use energy for flight and how their muscles have evolved to meet diverse demands. The results highlight how the way mitochondria are spread out and their shape in birds' flight muscles is important, showing a close link between muscle structure and how birds fly. These physical and structural differences help us understand how birds use energy, how their muscles are specialized, and how evolution has shaped their ability to move in different ways. Future studies should address the analysis of gene expression of mitochondrial factors such as PGC-1 α , NRF1, and TFAM in the pectoral muscles of different pigeon species using standardized molecular techniques. We also recommend measuring cellular respiration using high-resolution techniques to estimate mitochondrial oxygen consumption and replicate the conditions at high altitude.

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We appreciate the assistance provided by my institution at the University of Diyala.

NOVELTY STATEMENT

This study provided significant insights into biological science and researchers, demonstrating that mitochondrial morphology and organization influence muscle fiber functionality, elucidating the flight endurance of avian species due to cellular variances. The study looked at mitochondrial density as a crucial cellular factor which assists to explain how the muscles adapt for more effectively flight endurance.

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