


RESEARCH ARTICLE

How Light and Stocking Density Affect the Morphometric and Mechanical Traits of Quail Tibiotarsus?

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How to cite this article?

Yıldırım İG, Sevil Kilimci F, Khan K, Türker Yavaş F, Koç Yıldırım E, Raza S: How Light and Stocking Density Affect the Morphometric and Mechanical Traits of Quail Tibiotarsus? *Kafkas Univ Vet Fak Derg*, 2025 (Article in Press). DOI: 10.9775/kvfd.2025.34495

Article ID: KVFD-2025-34495

Received: 20.05.2025

Accepted: 13.09.2025

Published Online: 19.09.2025

Abstract

Quails (*Coturnix coturnix japonica*) are a significant migratory bird species, widely recognised for their rapid growth and efficient production of meat and eggs, making them an important component of the poultry industry. Despite their economic importance, the impact of environmental factors, particularly light and stocking density, on their skeletal health remains an area requiring further investigation. This study addresses this gap by examining how these factors influence the morphometric and mechanical properties of quail long bones. This study aimed to investigate the effects of different light colors and stocking densities on the morphometric and mechanical properties of long bones in Japanese quails. The experiment utilised three different light colors (white, blue, and green) and two different stocking densities (100 cm²/animal and 200 cm²/animal). A total of 120 Japanese quails (72 male, 48 female) were used to evaluate morphometric (length, diameter) and mechanical (bending strength, stiffness) properties. It was determined that light color significantly affected bone stiffness but had no marked effect on bone strength and elastic modulus. Furthermore, it was observed that the morphometric and mechanical properties of female quail bones were higher than those of males. These findings highlight the impact of light on quail bone health and open new avenues for future research. Stocking density was found to have no significant effect on bone properties.

Keywords: Biomechanics, Bone, Light-emitting diodes, Quail, Tibiotarsus

INTRODUCTION

The quail (*Coturnix coturnix*) is a migratory avian species with a wide distribution across Eurasia and Africa. It is also known for its unique taste characteristics in meat and eggs, rapid reproductive activity, and short-term capital recovery.

Despite a wide market for quail meat and eggs, quail management, including lighting requirements, housing density, and other welfare variables, is still not well-developed at extensive or intensive production levels ^[1].

Some researchers indicated that lighting is one of the most important environmental factors affecting poultry performance and physical activity ^[2]. Light plays a

significant role in growth, skeletal development, welfare, and reproductive performance. Rozenboim et al.^[3] suggested that green light increases body weight, but blue and green light-emitting diodes (LEDs) combination was found much more effective than green. Studies have shown a positive correlation between specific light intensities and broiler activity, increasing pressure and load on bones, and supporting bone development ^[4,5]. Light can also cause changes in some skeletal-related metabolic pathways, such as calcium and phosphorus metabolism ^[6]. Appropriate photoperiods have increased body weight, cortical bone formation, and bone mineralisation ^[7]. The tibiotarsus length and weight of broiler chickens were positively affected by intermittent lighting ^[8].



Cage characteristics and conditions are also important environmental factors affecting animal production traits. High housing density has been shown to worsen skeletal problems and progressively reduce walking ability [9,10].

Bone exhibits viscoelastic properties, meaning that its mechanical characteristics are directly influenced by its density, porosity, and micro-architecture [11]. In mechanical testing, the slope of the elastic region on the force-displacement curve represents the extrinsic stiffness or rigidity of the bone structure, while the elastic modulus quantifies the intrinsic stiffness of the bone material itself. The maximum stress the bone can withstand before failure is termed its ultimate strength, a parameter independent of its size and shape. However, the force required to break the bone-referred to as the breaking load or fracture force-does vary with bone size, distinguishing it from intrinsic strength [12]. Light can also cause changes in some skeletal- repeated metabolic pathways such as calcium and phosphorus metabolism [6].

Since light affects the metabolic structure of bone tissue, studies in this field evaluate both intrinsic and morphometric properties of bone tissue. In light of all this information, the study aimed to investigate the morphometric and mechanical effects of three different light colours on quail long bones in two animal groups with different housing densities.

MATERIALS AND METHODS

Ethical Statement

This study was approved by the Animal Experiments Local Ethics Committee of Aydın Adnan Menderes University, under the number 64583101/2023/29.

Animals and Experimental Design

The bones used in the study were obtained from a completed study at the Poultry Research Unit of the Veterinary Faculty of Aydın Adnan Menderes University.

In the study, the right tibiotarsus bones of a total of 120 quails (*Coturnix coturnix japonica*), aged 42 days, with an

average weight of 226 ± 30.19 g female and 228 ± 28.84 g male (mean \pm SD), including eight females and 12 males in each group, were used (Table 1).

The quails used in the research were raised at the Poultry Research Unit of the Veterinary Faculty of Aydın Adnan Menderes University for 42 days. The chicks were randomly selected on the first day of hatching and divided into groups. From the first day, they were separated into rooms with three different lighting applications, where light colour, temperature, and humidity values were controlled. The quails were fed ad libitum with feed containing 2910 kcal/kg ME and 24% CP during the growing period of 0-14 days, and 2900 kcal/kg ME and 22% CP during the development period of 15-42 days (NRC, 1994). Fresh water was provided daily ad libitum through a nipple drinker system. The quails were housed in four-tier brooding cages, each tier measuring 25x45x90 cm and equipped with heaters, feeders, and drinkers that remained constant in location and number throughout the trial. A continuous lighting program of 24 h of light - 0 h of darkness was applied to all groups throughout the study. The feeders and drinkers in the compartments were checked twice daily during the research period. Lighting was provided with LED bulbs emitting white, green, and blue light. Adjustable thermostatic automatic heaters were used in each compartment of the cages to maintain the desired ambient temperature. Humidity levels were also maintained at $60 \pm 5\%$ throughout the trial.

Different 9 W LED bulbs (CT-4277 CATA, Türkiye) emitting blue (480 nm), green (560 nm), and white light (400-770 nm) at an intensity of 20 lx were positioned above the cages. Throughout the experiment, the photoperiod was set at 24 h of light and 0 hours of darkness (24L:0D).

The housing density of the quails was arranged in two separate groups. A floor area of 200 cm²/animal was provided in the normal-density housing groups. In the high-density housing groups, a floor area of 100 cm²/animal was provided. In the normal housing groups, each cage compartment contained 20 quails, while in the high-density groups, each compartment contained 40 quails.

Table 1. The experimental groups for research study

Test Groups	Color	Density of Placement	Number of Males	Number of Females	Number of Animals
Group I	White Light	100 cm ² /animal	12	8	20
Group II	Blue Light	100 cm ² /animal	12	8	20
Group III	Green Light	100 cm ² /animal	12	8	20
Group IV	White Light	200 cm ² /animal	12	8	20
Group V	Blue Light	200 cm ² /animal	12	8	20
Group VI	Green Light	200 cm ² /animal	12	8	20
Total			72	48	120

Prior to the study, 20 animals were randomly selected from this group.

On the 42nd day of the study, decapitation was performed. Subsequently, the right and left legs of each quail were collected and stored in zipped plastic bags at +4°C to prevent tissue damage (for 24 h). The dissection of the legs was performed 24 h later to extract the tibiotarsus bones. The same person carefully performed the cleaning process of the bones without damaging the periosteum and bone tissue. To preserve their properties until the measurements and mechanical test stage, the bones were wrapped in gauze moistened with physiological saline and stored in Ziplock bags at -25°C [13].

Preparation of Samples for Biomechanical Test

Before the mechanical test, the bones were slowly thawed firstly at +4°C and then soaked in sterile physiological saline at +20°C. Subsequently, the lengths of the bones

were measured with a calliper, and their midpoint was marked to determine the loading points. The bones' mediolateral and craniocaudal periosteal diameters (outer diameters) were measured at the pre-determined loading point.

Mechanical Test: Three-Point Bending Test

The Zwick/Roell Z0.5 mechanical testing machine located at Aydın Adnan Menderes University, TARBIYOMER, was used for the mechanical test (Fig. 1). The distance between the support points for the three-point bending test was determined based on the length and diameter values of the obtained bones. Since the length values between the groups are very close, the support points were selected as fixed. The midpoints of the bone lengths were designated as the loading points. Two support points were selected so that the loading point was precisely in the middle, and the support distance was selected as 20 mm. During the test,

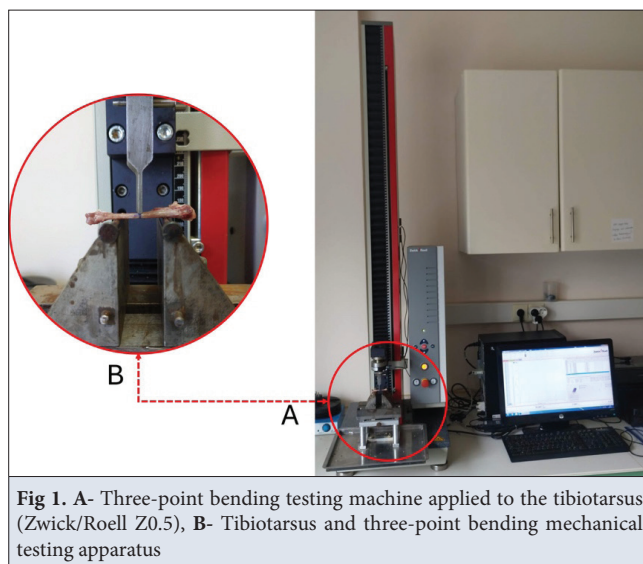


Fig 1. A- Three-point bending testing machine applied to the tibiotarsus (Zwick/Roell Z0.5), B- Tibiotarsus and three-point bending mechanical testing apparatus

Table 2. Effect of Gender, colored light, stocking density of bone morphometric measurements in the Quails^{1,2}

Parameters		N	L (mm)	DExt _{ML} (mm)	DInt _{ML} (mm)	DExt _{CrCd} (mm)	DInt _{CrCd} (mm)
Gender	Male	72	52.088±0.209	3.013±0.032	1.778±0.025	2.724±0.019	1.451±0.026
	Female	48	53.613±0.256	3.221±0.040	1.774±0.031	2.839±0.023	1.463±0.032
Colored light	White	40	52.666±0.286	3.042±0.44	1.721±0.034	2.783±0.026	1.433±0.036
	Blue	40	52.512±0.286	3.120±0.44	1.759±0.034	2.750±0.026	1.468±0.036
	Green	40	53.373±0.286	3.188±0.44	1.802±0.034	2.812±0.026	1.470±0.036
Stocking density	100 cm ² / Quails	60	52.848±0.234	3.128±0.036	1.741±0.28	2.799±0.021	1.474±0.029
	200 cm ² / Quails	60	52.853±0.234	3.105±0.036	1.781±0.028	2.764±0.021	1.439±0.029
P	Gender		0.000	0.000	0.388	0.000	0.784
	Colored light		0.081	0.072	0.248	0.281	0.704
	Stocking density		0.988	0.679	0.318	0.294	0.395

¹ Data presented as Mean ± SD

² The interaction between groups was not significant for investigated traits (P>0.05)

Table 3. Effect of Gender, colored light, stocking density of bone biomechanical properties in the Quails^{1,2}

Parameters		N	Moment of Inertia (mm ⁴)	Force (N)	Deformation (mm)	Stiffness (N/mm)	Strength (MPa)	Elastic Modulus (MPa)
Gender	Male	72	2.743±0.937	45.910±0.923	0.727±0.021	83.276±1.738	119.137±3.058	5317.593±145.076
	Female	48	3.387±1.147	55.452±1.130	0.710±0.026	95.092±2.129	121.56±3.745	4921.957±177.681
Colored light	White	40	2.992±1.282	49.699±1.264	0.717±0.029	87.954±2.380	121.245±4.187	5165.824±198.653
	Blue	40	2.947±1.282	50.425±1.264	0.733±0.029	85.716±2.380	122.613±4.187	5143.890±198.653
	Green	40	3.256±1.282	51.918±1.264	0.705±0.029	93.883±2.380	117.186±4.187	5049.610±198.653
Stocking density	100cm ² / Quails	60	3.155±1.047	50.716±1.032	0.732±0.024	89.009±1.943	118.666±3.418	5022.712±162.200
	200cm ² / Quails	60	2.975±1.047	50.646±1.032	0.705±0.024	89.359±1.943	122.030±3.418	5216.838±162.200
P	Gender		0.000	0.000	0.337	0.000	0.617	0.087
	Stocking density		0.188	0.425	0.535	0.047	0.636	0.908
	Colored light		0.227	0.900	0.343	0.899	0.488	0.399

¹ Data presented as Mean ± SD² The interaction between groups was not significant for investigated traits (P>0.05)

a preload of 1N was applied, and the bones were loaded at a rate of 10mm/min until they fractured [14,15]. After the test, a Force (N) - Deformation (mm) graph was obtained for each bone. The fractured bones' mediolateral and craniocaudal endosteal diameters (inner diameters) were also measured at the loading point, for later calculations.

From the graphs obtained after the three-point bending test, the stiffness value of each bone was calculated, and the inner and outer diameter values of the bones were used to calculate the moment of inertia (inertia moments) and cortical indexes of the bones. Using this calculated moment of inertia and stiffness values, each bone's ultimate strength and elastic modulus were determined [14,15].

Statistical Analysis

Statistical data was evaluated using the SPSS statistical package program (version 22.0, SPSS Inc., Chicago, IL, US) and R Studio software (version 4.4.2, Inc, Boston, MA, USA). Normal distribution of the data was checked by Shapiro-wilk test. DExt_{ML}, I, F values that did not show normal distribution were logarithmically and DExt_{CrCd} deformation values were reverse transformed. Levene's homogeneity test was used to check whether the values were homogeneous. General linear model (Univariate-GLM) method was used for comparison between groups. Bonferroni test was used to check the significance of differences between groups. The significance of differences between groups was set at P≤0.05, shown using asterisk. All results were reported as means ± SD.

RESULTS

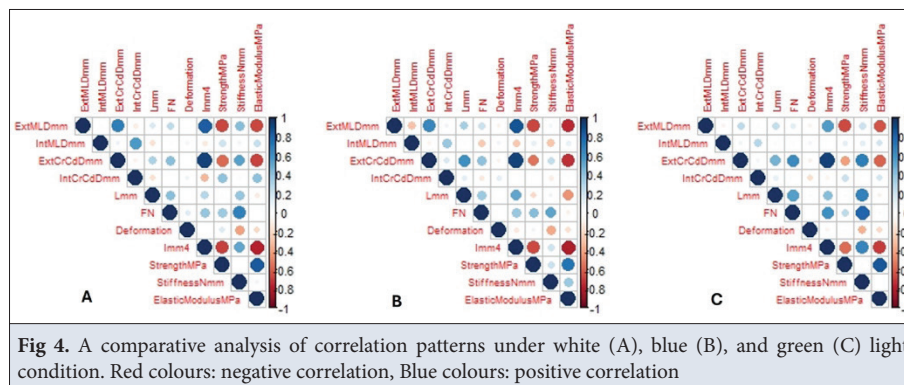
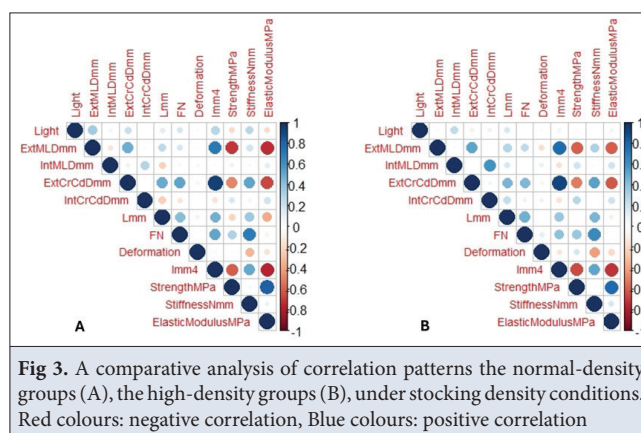
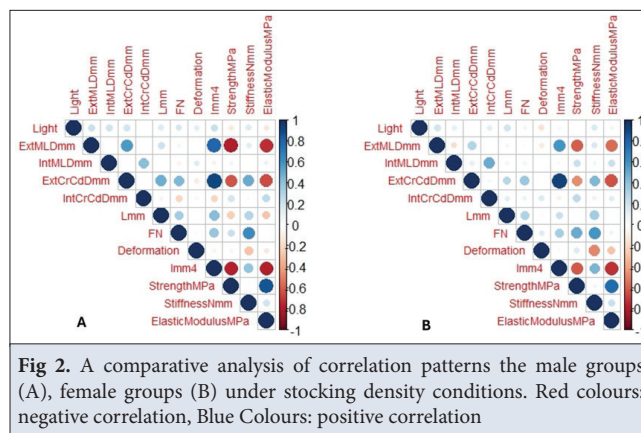
In this study, the morphometric measurements (L, DExt)

and mechanical properties (moment of inertia, bone breaking force, and stiffness values) of quail bones, along with their variations according to gender, light color, and stocking density, are detailed in [Table 2](#) and [Table 3](#).

According to the [Table 2](#) and [Table 3](#), the morphometric (L, DExt) and mechanical properties of quail bones (moment of inertia, bone breaking force and stiffness values) differed between genders (P<0.000). In addition, although there was a significant difference in stiffness of bones among the light groups (P=0.047), but the used post hoc test could not measure the differences among the groups. No significant difference was seen in the results of all the parameters among differently stocked groups. In addition, the statistical analysis also showed that there were no interactions between the two provided stocking density rates on biomechanical parameters of quail bones.

Correlation analyses were performed separately for sex, stocking density, and light colour groups ([Fig. 2](#), [Fig. 4](#))

The correlation matrices revealed distinct gender-specific patterns in the relationships between structural and mechanical properties. In males, external measurements demonstrated robust correlations with mechanical properties, particularly the DExt_{ML} showing strong negative correlations with Elastic Modulus (r ≈ -0.8) and Strength (r ≈ -0.6). Similarly, in males, DExt_{CrCd} exhibited substantial negative correlations with Elastic Modulus (r ≈ -0.7) and Strength (r ≈ -0.5). Conversely, females displayed generally weaker correlations, with DExt_{ML} showing moderate negative correlations with Elastic Modulus (r ≈ -0.6) and Strength (r ≈ -0.4). The Imm⁴ parameter demonstrated notable positive correlations with mechanical properties in both genders, though the relationship was more pronounced in males (r ≈ 0.7 with



Strength) compared to females ($r \approx 0.6$ with Strength). Internal measurements ($DInt_{ML}$ and $DInt_{CrCd}$) exhibited weaker correlations compared to external measurements across both genders (Fig. 2).

Correlation analysis revealed distinct patterns between normal-density (A) and high-density (B) bone specimens. In normal-density specimens, external measurements ($DExt_{ML}$) showed strong negative correlations with mechanical properties, particularly with Modulus of Elasticity ($r \approx -0.8$) and Strength ($r \approx -0.6$). The $DExt_{CrCd}$ parameter also showed significant correlations with mechanical properties (with Modulus of Elasticity $r \approx -0.7$). Similarly, high-density specimens showed generally

weaker correlations between external measurements and mechanical properties; $DExt_{ML}$ showed moderate correlations with Modulus of Elasticity ($r \approx -0.6$) and Strength ($r \approx -0.4$). Interestingly, the Imm^4 parameter showed strong positive correlations with mechanical properties in both density groups. However, it was slightly more pronounced in normal density specimens (with $r \approx 0.7$ Strength) than in high density specimens ($r \approx 0.6$). Internal measurements ($DInt_{ML}$ and $DInt_{CrCd}$) showed relatively weak correlations in both density groups, suggesting that external dimensions may be more reliable predictors of mechanical properties, especially in normal density bone specimens (Fig. 3).

Correlation matrices provide insights into the structural-mechanical relationships of bone samples by revealing distinct patterns across the three lighting conditions. Under all lighting conditions (A), external measurements showed the strongest correlations with mechanical properties. Specifically, $DExt_{ML}$ showed robust negative correlations with Elastic Modulus and Strength, while $DExt_{CrCd}$ showed similarly strong correlations with mechanical parameters. Similarly, under blue light conditions (B), correlations between external measurements and mechanical properties weakened moderately. The $DExt_{ML}$ parameter maintained negative correlations with Elastic Modulus and Strength, albeit with reduced strength. Green light conditions (C) revealed a pattern in which correlation strengths generally remained weaker than those observed under white and blue light. The relationship between external measurements and mechanical properties remained negative. Internal measurements ($DInt_{ML}$ and $DInt_{CrCd}$) showed relatively consistent, but weak correlations across all three lighting conditions, while the Imm^4 parameter consistently showed strong negative correlations with mechanical properties across all lighting conditions, with slightly varying magnitudes (Fig. 4).

DISCUSSION

The findings of this study shed light on the intricate relationship between light exposure and bone health in Japanese quails. They reveal that the colour of LED light can significantly impact bone stiffness, albeit without marked effects on bone strength and elastic modulus. These results align with the hypothesis that environmental factors, such as light wavelength, play a critical role in avian development and welfare.

The gender effect on bones revealed that the moment of inertia was significantly higher in females than in male quail bones. This result was supported by the previous observation that the diaphysis cross-sectional area of bone was greater in females than in male quails [16]. Our study was also consistent with research findings on broiler males and females, which showed higher densitometric and geometric parameters in females compared to male broilers [17,18]. There was the possibility of female quail adaptation to bear a heavy weight by bone geometrical changes, which resulted in increased diameters. Although strength and stiffness were found to be significantly ($P < 0.000$) higher in female quails than in male quails, bone strength was not affected by gender. In a previous study by Rath et al. [19], bone strength in broilers was not affected by gender. Our study was also consistent with the findings of studies conducted on female and male chickens, which showed higher densitometric and geometric parameters in female chickens compared to male chickens [17], and that bones are more sensitive to physical loads during growth;

therefore, the results are generally characterized by an increase in bone mass through periosteum and endosteal apposition, with or without changes in mineral density [20].

It has been reported that bone density increases with age in females compared to males [17] and that the effects of the egg-laying mechanism and hormones increase bone density [21]. There were findings that the effects on sex-related bone traits became more pronounced in the later stages of the birds' lives [22], which may be an important factor affecting the bone strength of these quails. The different LED lights increased bone stiffness in the quails exposed to "Green" light, which is particularly intriguing. Specifically, the linear model analysis reveals a statistically significantly higher stiffness in the green group, suggesting that the wavelength or intensity of green light may promote bone quality aspects that are not immediately apparent through gross morphological measurements. The reason could be the fact that birds possess high peak sensitivity between wavelengths of 545 to 575 nm (green light). This result was in accordance with the previous findings of Rozenboim et al. [3], who found that green light was associated with increased growth of broiler birds. This finding was also in line with prior research focused primarily on blue and green wavelengths [23,24], which suggested that a broader spectrum of light colours, including those closer to the yellow wavelength, may influence avian bone physiology. That's why, in this study, green light also influenced the biomechanical properties of the bones to some extent.

However, the influence of light colour on the birds was seen as trivial, suggesting insignificant improvement in strength and elastic modulus of the quail bones [25]. Multiple variables, including dimensions, strength, and elastic modulus of quail bones, among the different light-exposed groups (White, Blue, Green), did not reveal statistically significant differences. These findings show that while lighting conditions may influence growth and behaviour in poultry, their direct impact on the quail's bone physical and biomechanical properties may require a more nuanced understanding of a larger study group [2,26].

Our results add to the growing body of evidence that light colour has a physiological impact on bone development. This agrees with studies in broiler chickens, where light intensity and color significantly influence skeletal health [4,5]. Previous studies highlighted the role of light in modulating skeletal muscle development and possibly bone quality through mechanisms such as enhanced activity levels or altered metabolic pathways affecting bone density and strength [2,24].

Housing birds at higher densities has been found associated with leg weakness and poor walking ability [27]. However, this study did not follow the previous findings.

Further research is needed on how environmental factors synergize to affect bone health and integrity in poultry, potentially focusing on biochemical or morphological differences [28,29].

The correlation analysis of morphological and biomechanical data revealed complex relationships between bone architecture and mechanical properties. The analysis demonstrated that external dimensions serve as reliable predictors of mechanical properties in bone architecture, with higher bone stiffness specifically related to increased moment of inertia and periosteal mediolateral diameter. This finding aligns with previous research in which wider tibial bones of Lohmann Dual exhibited greater rigidity than the narrow tibia of Ross 308 chicken [30]. However, the bone strength and elastic modulus showed negative correlations with diameter and moment of inertia, which could be attributed to the dependence of strength not only on bone geometry but also on cortical thickness, porosity, and trabecular framework [14].

These structural-mechanical relationships demonstrated distinct patterns influenced by multiple factors, including gender and density variations. Gender-specific differences indicated sexual dimorphism's role, with males exhibiting stronger correlations between dimensional and mechanical parameters compared to females, suggesting the necessity of gender-specific approaches in structural measurements. Additionally, density-dependent variations showed that normal-density samples maintained stronger correlations between dimensional and mechanical parameters compared to high-density samples.

In addition, the observed relationships between structural and mechanical properties in bone analysis revealed relatively similar correlation patterns with the choice of lighting conditions, but with minor differences. This similarity in correlation patterns under different lighting conditions suggested that lighting protocols could be evaluated similarly in bone morphometry and mechanical property assessments. The illumination conditions significantly impacted these structural-mechanical relationships, with white light providing the most pronounced correlations and potentially offering optimal conditions for structural-mechanical assessments. In contrast, blue and green light conditions demonstrated moderate correlation strengths, which might be more suitable for specific analytical purposes. This variation across different lighting conditions emphasizes the critical importance of implementing standardized illumination protocols in bone morphometry and mechanical property assessments in veterinary research settings.

However, this study had several limitations that should be considered when interpreting the results. First of all, the tibiotarsus bone, which is frequently preferred in

poultry studies, was used and its effects on other bones were not investigated. Additionally, while three different LED light colors were tested, the study did not investigate the effects of different light intensities or photoperiods that could affect bone development. Finally, the study did not include biochemical markers of bone metabolism or histological analysis, which could provide deeper insights into the mechanisms underlying the observed effects of light exposure on bone properties.

In conclusion, the impact of sex on bone properties was clear, with female quail bones showing significantly higher values for moment of inertia, fracture strength, and hardness compared to male quail bones. This finding aligns with the adaptation of female quails to support heavier body weights, indicating sex-specific mechanisms in bone development. However, stocking density did not significantly affect bone parameters, which contradicts previous research and warrants further investigation. Overall, this study highlights the intricate nature of bone development and the impact of environmental factors. While lighting conditions do not appear to affect bone morphology, the differences in hardness significantly suggest that optimal light exposure is essential. This opens up avenues for future research into these interactions.

DECLARATIONS

Availability of Data and Materials: Data this used available on request from the corresponding authors (F. Sevil Kilimci).

Acknowledgments: We would like to thank Assistant Professor Dr. Solmaz KARAARSLAN for her support during the statistics phase and Associate Professor Dr. Mehmet KAYA for her support in providing materials.

Financial Support: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest: We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Declaration of Generative Artificial Intelligence (AI): The authors used AI technologies only to improve readability and language.

Author Contributions: IGY, FSK, and FTY: Conceptualization, Data curation, Formal analysis. IGY, EKY, KK, and SR: Investigation, Methodology, Project administration. FSK and FTY: Software, Resources. FSK, and IGY: Supervision, Validation, Visualization: FSK, IGY, KK, and SR: Writing – original draft, Writing - review & editing.

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