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Research Article

Impact of Early Qualitative Feed Restriction and the Provision of Barrier Perch on Morphometric and Biomechanical Measurements of Bones in Broiler Chickens

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Abstract

An experiment investigated the effects of early qualitative feed restriction and barrier perch provision on the morphometric and biomechanical measurements of the bones in broiler chickens. A total of 504 one-day-old Ross 308 male broiler chickens were randomly assigned to a completely randomized design with a 2×2 factorial arrangement (three replicate pens/group;42 chickens/pen) of qualitative feed restriction (presence-QFR+/absence-QFR-) and the provision of barrier perch (presence-BP+/absence-BP-). On days 21 and 42, morphometric and biomechanical parameters were measured. As a result of this study, barrier perch presence showed no significant impact on morphometric and biomechanical measurements. In the QFR- group, the weight and length of femur bones in 42-day-old broiler chickens were found to be higher (P<0.0001, P=0.034, respectively), alongside increased weight (P=0.001), inner (mediolateral P=0.002 and craniocaudal P=0.040) and outer (mediolateral P=0.047) diameter of the tibiotarsus bones. Furthermore, weight-length index values of the femur and tibiotarsus bones were higher (P=0.001, P<0.0001, respectively) in the QFR- group, while robusticity index values were lower (P=0.029, P=0.006, respectively) on the 42nd day. Regarding biomechanical parameters, the ultimate force level of tibiotarsus bones was statistically higher (P=0.030) at 42 days in the QFR- group. In summary, early protein and energy restriction caused slight decreases in some measurements of the femur and tibiotarsus bones. Strong correlations were observed between specific morphometric and biomechanical parameters, demonstrating their potential to predict biomechanical measurements.

Keywords: Animal welfare, Barrier perch, Bone, Broiler chicken, Diluted diet, Three-point bending

Introduction

Poultry meat is an economical, readily available [1], and globally consumed source of protein [2]. In the past five decades, chicken meat production has increased fivefold, parallel to the doubling of the global population. FAOSTAT forecasts indicate that this upward trajectory in poultry meat consumption will persist, driven by factors like affordability, consistent quality, adaptability, and high protein/low-fat content [3]. In order to meet this rising demand within the modern poultry industry, both the production amount has been increased, and hybrids have been developed that attain slaughter weight faster, and more breast muscle yield [4]. Rapid body growth and exacerbated development of the pectoralis major muscle have shifted broiler chickens' center of gravity and altered the broiler chickens' posture and load on the skeleton, leading to biomechanical imbalances [5-7]. This situation, resulted in increased load on the leg bones, particularly the femur and tibiotarsus [8]. This imbalance in muscle and skeletal development has led to issues like compromised leg health and lameness in modern broiler strains. These skeletal problems along with poor leg health, can lead to culling, mortality, reduced feed efficiency, and growth redaction, and are recognized as the leading cause of economic losses in broiler chicken production [9]. Therefore, in recent years, studies have focused on nutritional and environmental factors to minimize the economic losses and animal welfare concerns caused by leg health problems in broiler chickens [6].

In modern broiler strains, a high growth rate leads to rapid periosteal bone deposition, compromised mineralization, altered biomechanical properties, and increased cortical bone porosity [10,11]. Low levels of mineralization have been linked to higher fracture risk, as the degree of bone mineralization directly impacts bone strength [12]. Modulating the growth rate through feed restriction has been demonstrated to enhance bone mineralization and skeletal development, thereby positively impacting bone quality [10]. Thus, various feed restriction techniques are utilized during the early stage of life to mitigate issues related to the excessive growth rates of modern strains [13-16]. These techniques include quantitative feed restriction methods, such as daily feed restriction, skip-a-day feeding, and time-restricted feeding, as well as qualitative feed restriction methods, such as diet dilution, low-protein diets, and low-energy diets [14]. Quantitative feed restriction involves limiting the amount of feed provided to animals daily [17]. Qualitative feed restriction is an effective approach that involves nutrient dilution in the diet [17-19]. Because this strategy mitigates the adverse impacts of either starvation or chronic starvation on broiler welfare [11].

Another negative result of a high growth rate is the increase in the time spent sitting in broilers together with the increase in growth rate and decrease in locomotor activities [20]. Therefore, there has been a focus on increasing mobility levels to improve animal welfare in recent years. It has been reported that increased mobility may both have positive impacts on leg health and reduce the risk of ammonia burns in broiler chickens by improving litter conditions [21]. To this end, a multitude of studies have been carried out to explore the impacts of environmental enrichments like perches [22,23], barriers [24,25], straw bales [26,27], or platforms [28,29]. Nevertheless, the literature review revealed a scarcity of studies that specifically delved into the impacts of environmental enrichments on bone measurements.

To evaluate bone quality, researchers use invasive techniques, including bone-breaking force, bone mineral density, bone mineral content, and bone ash content [30,31], and non-invasive techniques, including dual-energy X-ray absorptiometry and various imaging approaches [32]. In addition, calculations based on morphometric measurements such as cortical index, robusticity index, and weight-length index (Seedor index) are also used to measure bone mineralization [33].

The study has been designed considering the issues observed in the poultry industry' described above. Given that normal bone development in healthy broiler chickens reaches its peak within the initial three weeks of life [34], it was hypothesized that slowing the growth rate during this period could promote better bone development. To this purpose, qualitative feed restriction was applied during the first three weeks of life. Barrier perch was preferred to increase the mobility level of broiler chickens, and therefore, it was placed between the feeder and the drinker. In addition, its widespread usability and cost in the chicken industry were considered. In line with the information, this study aimed to investigate the impacts of implementing early-life qualitative feed restriction to limit weight gain and the use of a barrier perch to enhance mobility on morphometric and biomechanical measurements.

Material and Methods

Ethical Statement

All experimental procedures conducted in this study received approval from the Local Ethics Committee for Animal Experiments at Aydın Adnan Menderes University (approval no: 64583101/2023/57).

Experimental Design and Groups

An experiment was conducted based on a completely randomized design involving a 2x2 factorial arrangement of groups. A total of 504 one-day-old Ross 308 male broiler chickens (initial body weights: 46.73±0.17) were randomly allotted four groups and three replicates (3 replicates/group; 42 birds/pen). The experimental design is explained in detail in *Table 1*. The groups were categorized based on the application of early qualitative feed restriction (qualitative feed restriction-QFR+/without qualitative feed restriction-QFR-) and the provision of barrier perches (the presence of barrier perch-BP+/the absence of barrier perch-BP-).

All diets used in the experiment were formulated based on corn and soybean meal. The diets were prepared in three phases: starter, grower, and finisher, following the recommendations provided in the Ross 308 commercial

***Table 1.** Experimental design*[1]

QFR	BP	Replicates	Birds Per Pen	Total Per Group
-	-	3	42	126
-	+	3	42	126
+	-	3	42	126
+	+	3	42	126
Total				504

[1]*QFR: qualitative feed restriction, BP: barrier perch.*

hybrid catalog [35]. In the QFR- group, the broiler chickens were fed a starter diet containing 3000 kcal/kg metabolizable energy (ME) and 23% crude protein (CP) from day 0 to 10. From day 11 to 24, they received a grower diet with 3100 kcal/kg ME and 21.5% CP. Finally, from day 25 to 42, they were provided with the finisher diet containing 3200 kcal/kg ME and 19.5% CP. For the QFR+ group, the feeding regimen was modified during the first 21 days. The quantity of soybean meal and vegetable oil in the diet was reduced, while the inclusion of wheat bran was increased to 15%. As a result, the CP content of the diet was reduced by 20% (18.6% and 17.2% CP), and the ME level was reduced by 10% (2700 and 2790 kcal/kg ME). From day 21 to 42, the broiler chickens in the QFR+ group were fed the same diet as the QFR- group. In the groups with BP+, a wooden perch barrier measuring 1.8 cm in width and 5 cm in height was installed between the feeder and the drinker from the 3rd day to the 14th day of the experiment. From the 14th day until the end of the experiment, a barrier perch measuring 1.8 cm in width and 15 cm in height was used in the same location.

Housing and Management

Broiler chickens were raised in floor pens (measuring 2 m in width, 2 m in depth, and 0.75 m in height) and located at the Poultry Research Unit, Aydın, Türkiye, for 42 days. Each pen was furnished with two drinker lines and two round feeders, ensuring ad libitum access to feed and water. Bedding material consisting of wood shavings (6 cm in depth) was utilized. The stocking density (33 kg/m^2) and lighting regimen (24 h of light for the initial seven days and 18 h of light with 6 h of darkness for the subsequent days) were established following the European Union Directive (2007/43/EC) [36]. The broiler chickens were maintained under optimal management conditions, which included temperature and humidity control [37].

Data Collection and Measurement Procedures

On day 21, a total of 60 broiler chickens (15 chickens per group), and on day 42, a total of 80 broiler chickens (20 chickens per group) were randomly selected and slaughtered. A total of 140 right femur and tibiotarsus bones were collected, and the surrounding tissues were removed. These bones were then stored in a deep freezer at -20°C for further measurements.

Morphometric measurements: Geometric properties (length, weight, mediolateral external diameter-DE_{ML}; mediolateral internal diameter-DI_{ML}; craniocaudal external diameter-DE_{CrCau}; craniocaudal internal diameter-DI_{CrCau}) and index measurements (cortical index-CI; weight-length index-WLI; robusticity index-RI) were determined on the tibiotarsus bones. The femur bones were measured for weight, length, DE_{ML}, RI, and WLI. The weights of the bones were measured using a digital weight scale (Scaltec SBP52, Heiligenstadt, Germany), while the length and diameter were measured using a digital caliper (Mitutoyo, Model No: CD-15CP, Code No: 500-181 U, Absolute Digital Caliper, Tokyo, Japan). Index measurements of the bones were calculated using the following formulas [38].

$$Cortical\ index\ (CI) = \frac{ML\ external\ diameter - ML\ internal\ diameter}{ML\ external\ diameter}\ x\ 100$$

$$Weight - length\ index\ (WLI) = \frac{bone\ weight\ (mg)}{bone\ length\ (mm)}$$

$$Robusticity\ index\ (RI) = \frac{bone\ length}{\sqrt[3]{bone\ weight}}$$

Biomechanical measurements: The right tibiotarsus bones (n=140) underwent a three-point bending test to evaluate their biomechanical properties. These tests were performed using a three-point bending test apparatus (ANSI/ASAE S459) on the Zwick/Roell Z 0.5 (Zwick Roell, Ulm, Baden-Württemberg, Germany) testing device at Aydın Adnan Menderes University Agricultural Research Center (TARBİYOMER). The span length was set at 50 mm. The device operated at the speed of 10 mm/min, with a preload force of 2 N applied. The testXpert II software was used to record the measured data. Data on bone deformation under pressure were obtained graphically, and the following parameters were determined from graph [39].

Ultimate force (F_{max}): The maximum force applied to the bone at the time of fracture. It's extrinsic strength properties of bone (Newton-N).

Stiffness (S): The resistance required to flex the bone is calculated using the slope of the elastic portion of the force-deformation curve. It's intrinsic properties of bone (N/mm).

Ultimate strength (σ): The maximum strength that a bone can withstand. It's intrinsic properties of bone (MegaPascal-MPa).

Elastic modulus (E): The resistance required to flex the bone is calculated using the slope of the elastic portion of the stress-strain curve. It's intrinsic properties of bone (MegaPascal-MPa).

Moment of inertia (l_x): Calculated based on the cross-section of the bone (inner and outer bone diameter at the bending point) to determine the bending strength and elastic modulus of the bone (mm^4).

Statistical Analysis

The data obtained from the study were analyzed using the SPSS software package (version 22.0, SPSS Inc., Chicago, IL, USA). To assess the normal distribution of the variables, the Shapiro-Wilk test/Kolmogorov-Smirnov test was employed. Non-normally distributed variables were subjected to logarithmic, square, and reverse transformation. The assumption of homogeneity of variances was evaluated using Levene's test. Bone measurements were subjected to analyses using a general linear model procedure, and means were compared using the least square difference (LSD) method. The experimental model for the design was defined as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + E_{ijk}$$

Where Y_{ijk}=the observed value, μ=the overall mean, α_i=the effect of qualitative feed restriction (QFR- and QFR+), β_j= the effect of barrier perch (BP- and BP+), $(\alpha\beta)_{ij}$= the interaction between qualitative feed restriction and barrier perch, and E_{ijk}= the test error per observation. Since the interaction between groups was determined to be insignificant for the investigated traits, it was not included in the tables.

The correlation analyses and scatterplots were carried out between biomechanical measurements and geometric properties/index measurements of the tibiotarsus of broiler chickens aged 42 days old using the ggpairs function from the GGally package (version 2.1.2) of RStudio software (version 4.3.1, Inc, Boston, MA, USA). P value less than 0.05 was considered statistically significant.

Results

Morphometric Measurements

To determine the effect of feed restriction applied during the first 21 days, lower values were obtained in the group of QFR+ in all geometric properties made on the femur and tibiotarsus bones of the 21-day-old broiler chickens (*Tables 2, Table 3*). The differences detected in all measurements except the length and internal diameter measurements (length, DI_{ML} and DI_{CrCau}) of the tibiotarsus were found to be statistically significant (femur: weight P<0.0001; length P=0.012; DE_{ML} P<0.0001; tibiotarsus: weight P<0.0001; DE_{ML} P<0.0001; DE_{CrCau} P<0.0001). Similarly, in the

Table 2. *Effects of QFR and BP on geometric properties of femur in broiler chickens[1,2,3]*

Day	Measurements[4]	Qualitative Feed Restriction			Barrier Perch			SEM
		QFR-	QFR+	P	BP-	BP+	P	
21st day	Weight (g)	4.92	4.09	<0.0001	4.57	4.44	0.450	0.12
	Length (mm)	50.31	48.70	0.012	49.70	49.39	0.658	0.46
	DE_{ML} (mm)	6.50	5.79	<0.0001	6.16	6.12	0.724	0.08
42nd day	Weight (g)	18.92	17.40	<0.0001	18.09	18.23	0.721	0.29
	Length (mm)	76.37	75.30	0.034	75.82	75.85	0.943	0.35
	DE_{ML} (mm)	10.51	10.21	0.059	10.33	10.39	0.737	0.11

[1] Data presented as the least square means; [2] The sample size is 60 on the 21st day and 80 on the 42nd day; [3] The interaction between groups was not significant for investigated traits (P>0.05); [4] DE_{ML}: medio-lateral external diameter

Table 3. *Effects of QFR and BP on geometric properties of tibiotarsus in broiler chickens[1,2,3]*

Day	Measurements[4]	Qualitative Feed Restriction			Barrier Perch			SEM
		QFR-	QFR+	P	BP-	BP+	P	
21st day	Weight (g)	7.00	6.00	<0.0001	6.48	6.51	0.914	0.17
	Length (mm)	69.88	68.45	0.130	69.51	68.83	0.468	0.66
	DE_{ML} (mm)	6.03	5.36	<0.0001	5.72	5.68	0.735	0.08
	DI_{ML} (mm)	2.75	2.66	0.254	2.73	2.69	0.668	0.06
	DE_{CrCau} (mm)	5.23	4.76	<0.0001	5.05	4.94	0.337	0.08
	DI_{CrCau} (mm)	2.60	2.55	0.568	2.58	2.57	0.843	0.06
42nd day	Weight (g)	26.09	24.29	0.001	25.25	25.13	0.811	0.36
	Length (mm)	107.62	106.85	0.248	106.91	107.56	0.326	0.46
	DE_{ML} (mm)	10.10	9.75	0.047	9.90	9.95	0.753	0.12
	DI_{ML} (mm)	5.76	5.37	0.002	5.51	5.62	0.368	0.08
	DE_{CrCau} (mm)	8.39	8.12	0.064	8.31	8.20	0.456	0.10
	DI_{CrCau} (mm)	5.14	4.90	0.040	5.04	5.00	0.728	0.08

[1] Data presented as the least square means; [2] The sample size is 60 on the 21st day and 80 on the 42nd day; [3] The interaction between groups was not significant for investigated traits (P>0.05); [4] DE_{ML}: medio-lateral external diameter, DI_{ML}: medio-lateral internal diameter, DE_{CrCau}: cranio-caudal external diameter, DI_{CrCau}: cranio-caudal internal diameter

Table 4. *Effects of QFR and BP on index measurements of bones in broiler chickens*[1,2,3]

Day	Measurements[4]	Qualitative Feed Restriction			Barrier Perch			SEM
		QFR-	**QFR+**	**P**	**BP-**	**BP+**	**P**	
21^{st} day	**Tibiotarsus**							
	CI	54.29	50.18	0.001	52.00	52.47	0.699	0.86
	WLI	99.69	87.34	<0.0001	92.82	94.21	0.602	1.87
	RI	3.66	3.78	<0.0001	3.74	3.70	0.118	0.02
	Femur							
	WLI	97.39	83.57	<0.0001	91.44	89.52	0.452	1.79
	RI	2.97	3.06	<0.0001	3.01	3.02	0.778	0.02
42^{n} day	**Tibiotarsus**							
	CI	42.86	44.81	0.054	44.25	43.42	0.405	0.70
	WLI	242.28	227.18	<0.0001	236.06	233.40	0.521	2.91
	RI	3.63	3.69	0.006	3.65	3.68	0.199	0.02
	Femur							
	WLI	247.59	230.85	0.001	238.35	240.08	0.711	3.29
	RI	2.87	2.91	0.029	2.89	2.89	0.676	0.01

[1] Data presented as the least square means; [2] The sample size is 60 on the 21^{st} day and 80 on the 42^{nd} day; [3] The interaction between groups was not significant for investigated traits (P>0.05); [4] CI: cortical index, WLI: weight-length index, RI: robusticity index

geometric properties of the femur and tibiotarsus bones of the 42-day-old broiler chickens, higher values were observed in the group of QFR-. Statistically significant differences were detected in all geometric properties (femur: weight P<0.0001; length P=0.034; tibiotarsus: weight P=0.001; DE_{ML} P=0.047; DI_{ML} P=0.002; DI_{CrCau} P=0.040) except for the DE_{ML} of the femur and the length and DE_{CrCau} of the tibiotarsus.

In the tibiotarsus of 21-day-old broiler chickens, the CI was significantly higher (P=0.001) in the QFR- group, whereas in the tibiotarsus of 42-day-old broiler chickens, it was higher (P=0.054) in the QFR+ group (*Table 4*). The WLI of tibiotarsus bones of 21 and 42-day-old broiler chickens with qualitative feed restriction was found to be lower (P<0.0001) while the RI was higher (P<0.0001; P=0.006, respectively). The WLI of the femur of 21 and 42-day-old broiler chickens with qualitative feed restriction was lower (P<0.0001; P=0.001, respectively) while the RI was higher (P<0.0001; P=0.029, respectively). The presence of the barrier perch did not affect the morphometric measurements (geometric and index) of the tibiotarsus and femur bones in 21 and 42-day-old broiler chickens.

Table 5. *Effects of QFR and BP on biomechanical measurements of tibiotarsus in broiler chickens*[1,2,3]

Day	Measurements[4]	Qualitative Feed Restriction			Barrier Perch			SEM
		QFR-	**QFR+**	**P**	**BP-**	**BP+**	**P**	
21^{st} day	F_{max} (N)	166.46	123.43	<0.0001	147.53	142.37	0.512	5.53
	S (N/mm)	77.81	67.67	0.004	72.30	73.18	0.794	2.38
	σ (MPa)	81.96	84.14	0.450	82.28	83.83	0.589	2.02
	E (MPa)	1141.26	1503.32	<0.0001	1278.03	1366.55	0.346	65.87
	I_x (mm^4)	41.70	27.20	<0.0001	35.88	33.02	0.403	2.18
42^{nd} day	F_{max} (N)	315.35	287.57	0.030	305.15	297.77	0.598	9.04
	S (N/mm)	87.67	84.12	0.208	84.45	87.34	0.303	1.97
	σ (MPa)	79.46	78.60	0.799	78.35	79.71	0.688	2.37
	E (MPa)	1644.29	1753.85	0.235	1621.94	1776.20	0.207	80.64
	I_x (mm^4)	261.23	231.88	0.076	250.06	243.05	0.669	11.54

[1] Data presented as the least square means;[2] The sample size is 60 on the 21^{st} day and 80 on the 42^{nd} day;[3] The interaction between groups was not significant for investigated traits (P>0.05); [4] F_{max}: ultimate force, S: stiffness, σ: ultimate strength, E: elastic modulus, I_x: moment of inertia

Biomechanical Measurements

In the tibiotarsus of 21-day-old broiler chickens, the ultimate force, stiffness, and moment of inertia were higher in the QFR- group (P<0.0001; P=0.004; P<0.0001, respectively), whereas ultimate strength and elastic modulus were higher in the QFR+ group (P=0.450; P<0.0001, respectively). When examining the biomechanical measurements of the tibiotarsus bones of 42-day-old broiler chickens, all parameters, except for ultimate force (P=0.030), showed statistically insignificant differences between the QFR groups. The presence of barrier perch did not impact the biomechanical measurements of the tibiotarsus in 21 and 42-day-old broiler chickens *(Table 5)*.

Correlation Analysis Between Morphometric and Biomechanical Measurements

The study adopted the correlation coefficient standards described by Hayran and Hayran [40], where coefficients ranging from 0.05 to 0.30 are considered insignificant, those between 0.30 and 0.40 are categorized as low, those between 0.40 and 0.60 are categorized as moderate, those between 0.60 and 0.70 are categorized as good, those between 0.70 and 0.75 are categorized as very good, and those between 0.75 and 1.00 are categorized as indicating a strong correlation. The correlation coefficients (r) between geometric properties and biomechanical measurements of 42-day-old tibiotarsus bones are presented in *Fig. 1*. A strong positive correlation was found between outer diameters (DE_{ML} and DE_{CrCau}) and moment of inertia (r=0.877, P<0.001; r=0.970, P<0.001, respectively). Conversely, elastic modulus and outer diameters had a strong negative correlation (r=-0.789, P<0.001; r=-0.863, P<0.001). These results suggest that as outer diameters increase, there is a corresponding increase in the moment of inertia while a decrease in elastic modulus is observed. It was found that DE_{ML} showed a good negative correlation with ultimate strength (r=-0.659, P<0.001), while DE_{CrCau} exhibited a moderate negative correlation with ultimate strength (r=-0.557, P<0.001). Conversely, there was a good positive correlation (r=0.643, P<0.001) between DE_{CrCau} and ultimate force. The analysis revealed a moderate negative correlation between DI_{ML} and ultimate strength (r=-0.501, P<0.001). Furthermore, there was a moderate positive correlation noted between DI_{ML} and moment of inertia (r=0.512, P<0.001), as well as between DI_{CrCau} and moment of inertia (r=0.522, P<0.001).

The correlation coefficients (r) between the index and biomechanical measurements of 42-day-old tibiotarsus bones are shown in *Fig. 2*. The correlations between CI and biomechanical measurements were observed to be

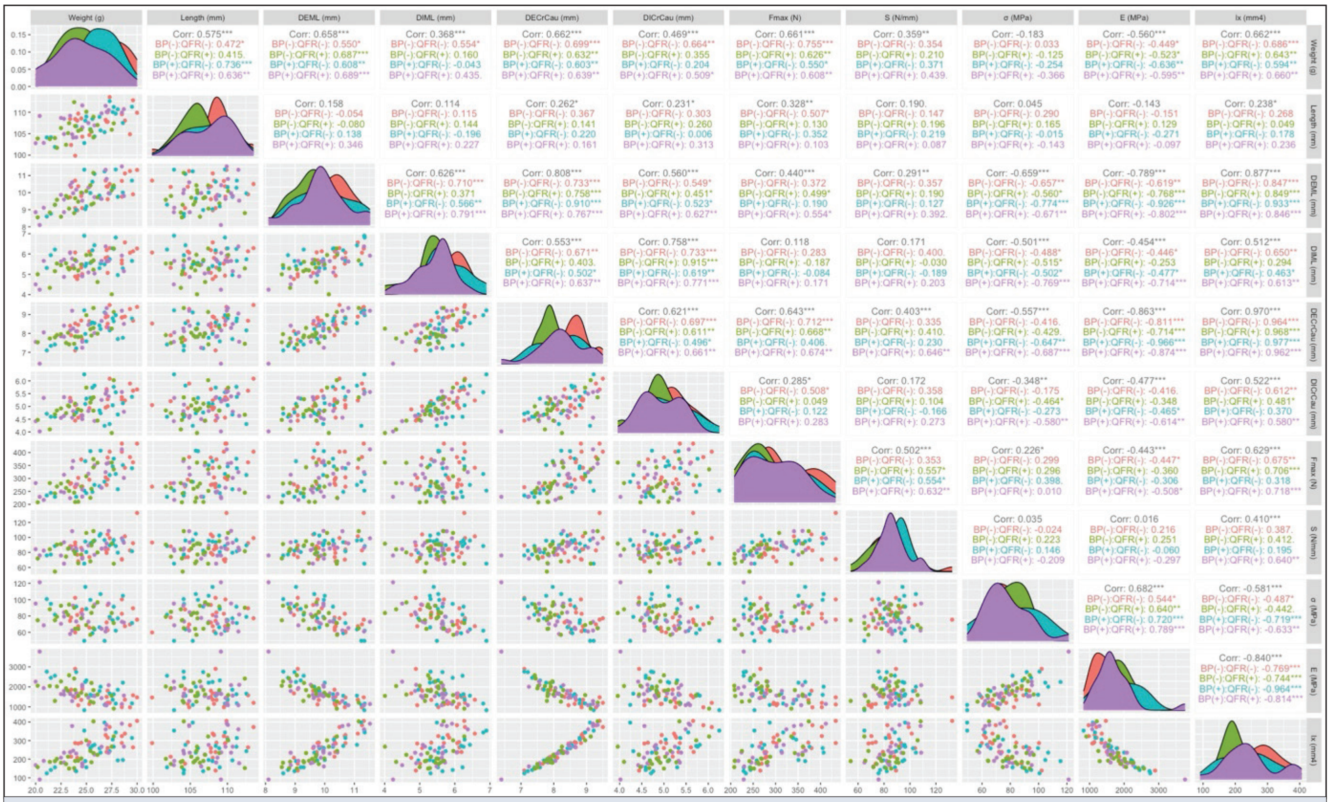

Fig 1. Scatterplots showing the correlation matrix results for biomechanical measurements and geometric properties of broiler chickens[1,2] (Created in RStudio version 4.3.1. using ggpairs function from the GGally package version 2.1.2). [1]DE_{ML}: medio-lateral external diameter, DI_{ML}: medio-lateral internal diameter, DE_{CrCau}: cranio-caudal external diameter, DI_{CrCau}: cranio-caudal internal diameter, F_{max}: ultimate force, S: stiffness, σ: ultimate strength, E: elastic modulus, I_x: moment of inertia; [2]A total of 80 tibiotarsus bones from 42-day-old broiler chickens were used in the correlation analysis

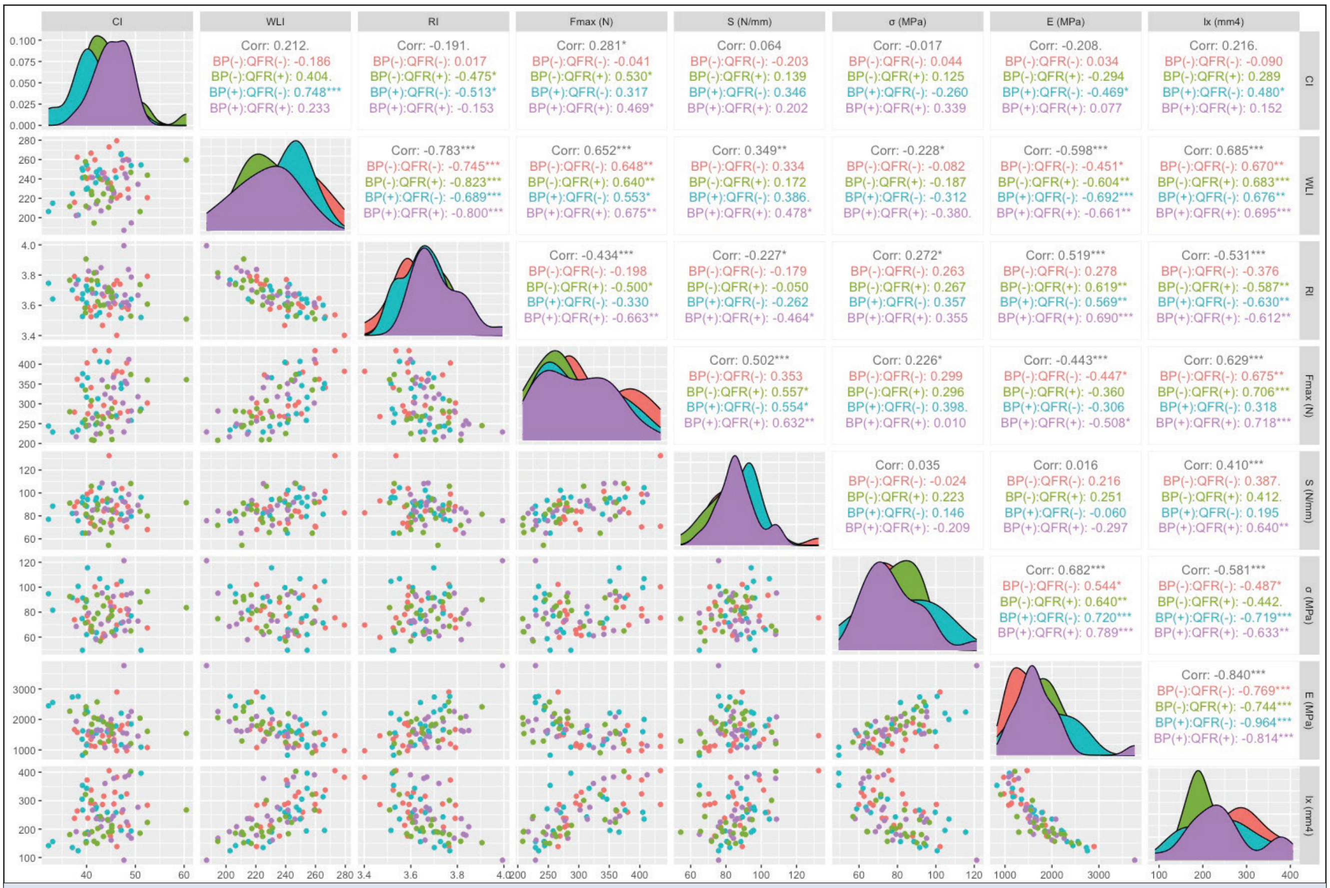

Fig 2. Scatterplots showing the correlation matrix results for biomechanical and index measurements of broiler chickens[1,2] (Created in RStudio version 4.3.1. using ggpairs function from the GGally package version 2.1.2). [1]CI: cortical index, WLI: weight-length index, RI: robusticity index, F_{max}: ultimate force, S: stiffness, σ: ultimate strength, E: elastic modulus, I_x: moment of inertia; [2]A total of 80 tibiotarsus bones from 42-day-old broiler chickens were used in the correlation analysis

low or insignificant (r<0.40). Conversely, a good positive correlation was identified between WLI and ultimate force (r=0.652, P<0.001), as well as moment of inertia (r=0.685, P<0.001). Additionally, a moderate negative correlation was found between elastic modulus and WLI (r=-0.598, P<0.001). RI and elastic modulus were observed to have a moderate positive correlation (r=0.519, P<0.001). A moderate negative correlation was identified between RI and ultimate force (r=-0.434, P<0.001), as well as moment of inertia (r=-0.531, P<0.001).

Discussion

In the research findings, qualitative feed restriction was observed to decrease the length and weight of femur bones of 42-day-old broiler chickens while leaving the diameter unaffected. Similar to the present findings, Yalçın et al.[41] reported that broiler chickens subjected to protein restriction exhibited shorter femur length. In contrast, Bruno et al.[42] reported that early qualitative feed restriction had no impact on the weight and length of femur bones but, resulted in a decrease in femur bone diameter in broiler chickens. Furthermore, Bruno et al.[43] indicated that early quantitative feed restriction did not affect the weight of femur bones, but led to reductions in both the length and diameter of femur bones in broiler chickens. In the study, it was found that qualitative feed restriction resulted in decreases in all geometric properties of the tibiotarsus bones of 42-day-old broiler chickens although the reductions in length and diameter (DE_{CrCau}) were not statistically significant. Consistent with the study findings, El-Faham et al.[44] and Pirzado et al.[45] reported that low energy levels did not affect the tibia length of 42-day-old broiler chickens. Similarly, Bruno et al.[42] found that early qualitative feed restriction did not influence tibia length. Conversely, Bruno et al.[43] stated that early quantitative feed restriction significantly reduced tibia length. Additionally, contrary to the present study findings, previous studies reported that tibia weight was not affected by low protein/energy levels [45,46] or early qualitative/quantitative feed restriction [42,43] in broiler chickens. Venalainen et al.[47] stated that tibia length, external diameter, and weight were greater in broiler chickens given high ME diets than in those given low ME diets. In the study, better results were obtained in 42-day-old broiler chickens in the group without feed restriction in terms of WLI and RI. Conversely, El-Faham et al.[44]

reported that energy restriction did not affect levels of WLI and RI. This discrepancy among study results might be related to differences in methods, duration, or levels of feed restriction. When the findings of the current trial were evaluated, it was observed that qualitative feed restriction reduced or tended to reduce all geometric properties of the femur and tibiotarsus on day 42. This situation is thought to be due to the limitation of growth caused by feed restriction. This notion is also evident from the 240.53 g body weight difference observed between the QFR groups on days 0-42 in the present study (data not shown).

The research findings align with previous studies that reported no significant effect on the presence of barrier perch on the weight, length, and diaphysis diameter of tibiotarsus bones of 42-day-old broiler chickens [22,48]. Similarly, Ventura et al.[49] observed in their study that the use of barriers did not affect the diameter and length of the tibia bones in broiler chickens. In contrast to these findings, Türkyılmaz et al.[50] stated that perch use significantly increased the broiler chickens' tibia weight, length, and diameter. Bizeray et al.[24] reported that the utilization of barriers did not have an impact on broiler chickens' tibia length but led to an increase in diameter. Consistent with the research results, Karaarslan and Nazlıgül [38] and Dereli Fidan et al.[48] noted that the provision of perch did not affect index measurements of the tibiotarsus bones (CI, WLI, RI) of 42-day-old broiler chickens. It is thought that the inconsistent findings might be due to differences in the designs and configurations of the perches used.

It was determined that implementing protein and energy level restrictions to decelerate the growth rate of broiler chickens did not result in the anticipated levels of difference in bone biomechanical measurements among the groups. The limited impact of the feed restriction is attributed to its application solely during the initial 21 days, followed by a return to a normal diet for the subsequent 21 days. The effect of early qualitative feed restriction on biomechanical measurements of tibiotarsus bones in 42-day-old broiler chickens was statistically insignificant, except for ultimate force. However, there was a visible trend towards an increase in the elastic modulus value. Unlike research outcomes, some researchers reported that protein and/or energy restriction impacts on breaking strength (ultimate force) were insignificant [45,46,51]. The inconsistency observed among study results may stem from variations in the methodologies, durations, or levels of feed restriction implemented. Further research exploring these factors in greater detail may help elucidate the underlying reasons for the discrepancies observed in the literature.

It was observed that the barrier perches, utilized to enhance bone strength by promoting mobility, did not achieve the anticipated effect on the biomechanical measurements of the tibiotarsus. Despite observing higher values of stiffness, ultimate strength, and elastic modulus in the tibiotarsus bones of broiler chickens raised in pens with barrier perches, these values fell below expectations. This outcome is attributed to the low levels of perching ratio of 2% observed in broiler chickens (data not shown). The effect of providing barrier perch on all biomechanical parameters of tibiotarsus bones in 21- and 42-day-old broiler chickens was statistically insignificant. It is consistent with the research results described by Bizeray et al.[24], who reported that the effect of barrier provision on the breaking strength and stiffness of tibia bones was statistically insignificant. Aksit et al.[22] revealed similar findings' showing that perch use had no significant effect on breaking strength. Similarly, Dereli Fidan et al.[48] observed that the presence of a perch did not affect the moment of inertia value. Moreover, Türkyılmaz et al.[50] stated that the use of perch did not affect the ultimate strength and elastic modulus. However, contrary to research findings, Türkyılmaz et al.[50] reported conflicting results stating that perch use led to a significant increase in ultimate force, stiffness, and moment of inertia. The inconsistencies in findings are thought to arise from variations in the designs and configurations of the perches employed in the studies.

The center of gravity in broiler chickens shifted towards the front of the body due to increased breast meat yield and growth rate, consequently altering the biomechanics of the leg bones [5]. For this reason, recent studies have primarily focused on the parameters of the tibia and femur bones. Yalçın et al.[41] reported a significant correlation between bone-breaking strength and bone weight, as well as bone length, suggesting that the bone-breaking strength can be predicted from these variables. Similarly, in line with the research findings, a positive and good correlation was found between bone weight and breaking strength (ultimate force). Additionally, in the present study, a substantial positive and good correlation was identified between weight and moment of inertia. Furthermore, according to the study results, as the outer diameter of the tibiotarsus bone (DE_{ML}, DE_{CrCau}) increased, a decrease in the level of elastic modulus and an increase in moment of inertia were observed. In light of these results, predictions can be made regarding ultimate force, moment of inertia, and elastic modulus based on the variables' weight and outer diameter.

The strength of a bone depends on its geometric properties, cortical thickness, porosity, and trabecular framework. Understanding bone strength often involves assessing bone density through techniques such as DEXA (Dual-energy X-ray absorptiometry) or using a variety of imaging methods. Additionally, geometric indices such as the cortical index, robusticity index, and weight-length index also provide valuable information. Biomechanical tests, such as three-point bending, offer more precise insights into bone strength [23]. In this context, it was examined that the correlation between these indices and biomechanical measurements to gain a comprehensive understanding of bone strength [52]. Therefore, it should be evaluated in light of this information when examining the correlation levels between the RI and biomechanical measurements. Although there is a significant correlation between the RI and all biomechanical measurements, in terms of the degree of correlation, it was determined that there was only a moderate negative correlation with ultimate force and moment of inertia and a moderate positive correlation with elastic modulus. In line with this result, it can be said that as the RI value decreases, ultimate force and moment of inertia increase, and elastic modulus decreases. Similarly, significant correlations were identified between the WLI and all biomechanical measurements. Specifically, a significant positive correlation was observed with ultimate force and moment of inertia, alongside a moderate negative correlation with elastic modulus. It can be said that, as the WLI value increases, ultimate force and moment of inertia increase, but elastic modulus decreases. Conversely, no remarkable correlation was discerned between the CI and biomechanical measurements. In light of these results, predictions can be made regarding ultimate force, moment of inertia, and elastic modulus based on the variables of RI and WLI.

In conclusion, a strong/good correlation was found between some morphometric and biomechanical parameters (DE_{ML} and $I_x/E/\sigma$, DE_{CrCau} and $I_x/E/F_{max}$, weight and F_{max}/I_x, WLI and I_x/F_{max}). It is thought that these variables can be suggested to predict biomechanical measurements. It was determined that the presence of barrier perches did not affect bone morphometric and biomechanical measurements. It was found that protein and energy restriction applied during the early period resulted in a slight decrease in some morphometric and biomechanical measurements of the femur and tibiotarsus bones. Additionally, the inconsistent results reported in previous studies for all parameters examined in this trial suggest that bone shape in broilers may exhibit a high degree of individual variation. Further research exploring these factors may help elucidate the underlying reasons for the discrepancies observed in the literature.

Declarations

Availability of Data and Materials: The data that findings of this study are available from the corresponding author (S. Karaarslan) upon reasonable request.

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Declaration of Generative Artificial Intelligence (AI)

The authors declare that the article and/or tables and figures were not written/created by AI and AI-assisted technologies.

Author Contributions: SK was responsible for the conception and design of the study. SK, MK, and OT performed the experiments. SK, MK, and FSK were biomechanical analyses in this study. SK and AN performed statistical data interpretation. SK wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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