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Research Article

Influence of Nutrition and Genetics on Bone Parameters of 1940 Leghorn and 2016 Commercial White Leghorns

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Abstract

Bones play an essential role being responsible for the support of body mass, protection of internal organs, and providing musculature attachment sites while also serving as a reservoir for eggshell mineralization during the production phase. Targeted genetic selection has contributed to body morphometry and performance potential and could be inadvertently associated with undesirable effects on bone stability. In this study, bone parameters were compared between a contemporary and heritage line for the effect of strain and dietary regimen. A total of 320 White Leghorn laying hens (69 weeks of age) of two different strains were distributed into a 2 × 2 factorial arrangement creating 4 experimental treatment groups: 1). 2016 hen on 1940 diet, 2). 2016 hen on 2016 diet, 3). 1940 hen on 1940 diet, and 4). 1940 hen on 2016 diet with 8 replicates per treatment. Keel bones were assessed for deviations and or fractures. Significant differences ($P \geq 0.05$) were observed for both deviations and/or fractures with the 2016 strain having more when compared to the 1940 strain. Humerus and tibia bones were analyzed for bone mineral density, breaking strength, and bone ash. Humerus and tibia weights which included both pre (with meat attached) and post weights (without meat attached) had significant differences ($P \leq 0.05$) in the pre-weight in the 2016 hens, however no significant differences in the post weights. Results suggest that genetics played a role in the differences observed with the bone parameters measured and nutrition had few adverse effects.

Description of Problem

Poultry nutrition programs are often designed with production parameters in mind. Nutrient levels are set to maximize the production of eggs and meat, and those requirements are set and reviewed by the National Research Council guidelines [1]. Nutrition has been identified as a critical factor impacting skeletal growth and bone strength in poultry [2]. Nutritional criteria for hens have usually been based on optimizing responses in egg production and shell quality, with less focus on bone quality. Within the layer industry, bone weakness and associated fractures represent considerable welfare and economic problems [3]. Unlike mammals, laying hens have a unique bone turnover synchronized with a day-laying cycle, indicating that rapid remodeling occurs in laying hen bones [4]. The most important nutritional factor influencing bone quality is the supply of Ca in adequate amounts and through the diet. There is substantial genetic variation in bone traits in domestic-layer chickens. Bones are dynamic tissues, and their quality is influenced by nutritional, hormonal, and physiological factors including mechanical stress and the extent of physical activity. Bone structure can adapt its mass, shape, and internal architecture according to the mechanical loading experienced within an environment [5]. Bone metabolism in female birds is special in that they produce a medullary bone, which serves as a reservoir for calcium used in the production of the eggshell. Maintaining optimum bone strength is a particular problem for modern layers because of the intense selection that the strains have been subjected to and the demands that an extremely high egg production rate places on calcium and calcium metabolism [6]. The structural skeleton of the laying hen becomes fully developed during the rearing period [7]. A dramatic adjustment occurs within the bone biology of the hen during the onset of sexual maturity, with the function of osteoblasts changing from lamellar cortical bone to the production of medullary bone [7]. Bone-breaking strength and Bone Mineral Density (BMD) are two important parameters to assess bone quality in layers. Factors influencing these traits include nutrition, sex, age, exercise, genetics, and certain diseases [8].

Keel Bones

The keel bone is prone to damage in terms of fractures and deviations due to the anatomical position, especially in modern layers with small breast muscles [9]. Keel bone fractures are characterized by sharp bends, shearing, and/or fragmented sections of the keel bone. Fractures can extend from the ventral to the dorsal surface in the sagittal plane however, can also be cranial to caudal or a combination of the two [10]. One of the major risk factors for keel bone fractures in layers is a result of a collision with housing structures combined with weakened bone strength [9]. A less often mentioned type of keel bone damage is deviation. A normal keel bone follows a straight line, but deformation may occur, leading to deviations from this line. These can be vertical or horizontal, showing an s-shaped appearance, bumps, or notches. Deviations are known to be disruptions located in the periosteal surface of the keel making them not the direct result of a fracture or potential impact injury [9]. Contrary to fractures, which typically occur amid an isolated event, the development of deviations likely takes place over a period of time due to bone remodeling in response to regular loading pressures [9]. Results from studies conducted have reported keel bone deviations in 6-59% of laying hens aged 60-62 weeks depending on the type of production and the housing system [9].

Humerus and Tibia Bones

The humerus is a pneumatic bone with a hollow inner cavity contained with air instead of marrow [7]. When the bird is static, the humerus lies adjacent to the thoracic cavity and connects with the glenoid cavity of the pectoral girdle. The top end of the humerus is rounded and fits into a cup-shaped depression in the scapula, or shoulder bone, forming a ball-and-socket joint. Ball-and-socket joints allow for circular movement. The humerus is part of the upper or thoracic portion of the body (non-weight bearing). The humerus has been mainly studied in laying hens due to its different mineralization properties when compared with the tibia [11]. The tibia bone is longer in length when compared to that of the fibula bone while being much thicker at the proximal end than it is at the distal end. Historically, tibia ash has been the main method by which bone

mineralization has been determined. The amount of available calcium and phosphorus in conjunction with other ingredients within the diet causes sensitivity to tibia ash [12]. Direct interaction has been exhibited between increased bone ash, a greater supply of available calcium and phytate phosphorus in the diet, as well as increased BW gain [12]. Fractures of the tibia, humerus, and keel tend to be the most common potentially due to the thinning of cortical bone and loss of trabecular integrity resulting in bones becoming weaker, making them more susceptible to fracture as the result of trauma [7]. The aim of this study was to evaluate the relationship of bone integrity regarding the comparison of nutrition and genetic effects of laying hens fed representative diets.

Materials and Methods

Bird Management and Diet

A total of 320 16-week-old laying hens (WL40 and WL36) were transported and housed in a laying facility at the North Carolina Chicken Education Unit in Raleigh, NC. The rearing of these birds was identical and carried out in accordance with the NCSU IACUC. All birds were randomly divided into 2 hens per cage consisting of 10 replicates. The 4 experimental treatment groups: 1). 2016 hen on 1940 diet, 2). 2016 hen on 2016 diet, 3). 1940 hen on 1940 diet, and 4). 1940 hen on 2016 diet. Feed and water were provided throughout the experimental period of 69 weeks (Table 1). Feed intake and body weight gain were measured on a 28d period resulting in 12 cycles. Hens were given a 2-week acclimation period to adjust to the new environment and diets starting at 17 wks of age. All animal management and sampling procedures were in accordance with the NCSU IACUC.

Table 1: Feed Ingredients and Mash Diet1 Compositions.

IngredientS	2016 Layer Diet2	1940 Layer Diet[2]
Corn	940.5	1146.38
Soybean Meal	718.0	232.57
Alfalfa Meal		305.97
Limestone, gr.	145.5	124.2
Coarse limestone	50.0	
Fat	110.0	
Phosphate Mono/D	17.6	
Salt	6.8	5.0
D.L. Methionine	2.9	
T-Premix	1.0	
Sodium Bi-carb	2.0	
Prop Acid 505	1.0	
Choline CL 60%	1.3	4.0
Hy-D 62.5 mg/lb		
Trace Min PMX[3]	1.0	
L-Vitamin PMX[4]	1.0	
.06% Selenium[5]	1.0	
Ronozyme HI P (GT)	0.4	
Total 2000.0 2000.0		
Calculated Analysis		
Protein %	20.8	20.0
ME kcal/kg	2926	1330
Calcium %	4.1	0.90
A. Phos %	0.45	0.42
Lysine %	1.2	0.82
TSAA %	0.81	

[1]Diets were acquired from the North Carolina State University Feed Mill in mash form
[2]Lay diet fed starting no later than 17 weeks
[3]Vitamin premix supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B12, 0.08 mg; and ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL-α-tocopheryl acetate.
[4]Mineral premix supplied the following per kilogram of feed: 120 mg of Zn as $ZnSO_4H_2O$, 120 mg of Mn as $MnSO_4H_2O$, 80 mg of Fe as $FeSO_4H_2O$, 10 mg of Cu as $CuSO_4$, 2.5 mg of I as $Ca(IO_3)2$, and 1.0 mg of Co as $CoSO_4$.
[5]Selenium premix provided 0.3 ppm Se from sodium selenite.

Sampling

At the end of the trial, 12 hens were randomly selected, and euthanized by cervical dislocation. The right humerus and tibia were excised and cleaned of connecting tissue and then placed in storage at -20 C until analyzed. Both humerus and tibia were thawed out overnight at room temperature before further analyses were conducted. Both humerus and tibia bones were measured for length and weight using a digital caliper and precision scales (0.01 g), respectively.

Keel Analysis

The keel bones were dissected, with adherent muscle tissue being removed, and stored frozen at -20 ◦C for further analysis. The dissected keel bones were scored by palpation and visual analysis for Keel Bone Deviation (KBD) and Keel Bone Fractures (KBF). Palpation was performed by running two fingers down the edge of the keel bone to detect alterations like S-shaped deviations, bumps, depressions, or proliferations. The following scoring system was used: 4 = intact keel bone, 3 = slight deviation, 2 = moderate deviation counted as fracture, and 1 = severe deviation counted as fracture. For fractured assessment, scores 3 and 4 were combined as "no fractures" vs. scores 1 and 2 as "fractured." If fractures occurred, the type of fracture (transversal or longitudinal), as well as the number of fractures (1 to 3 or ≥4), and their location in the cranial, intermediate, or caudal third of the carina sterna, were recorded [13].

Bone Mineral Density

Bone Mineral Density (BMD) is the mass of material per volume of bone, which includes both organic, representing collagen, and inorganic, representing mineral hydroxyapatite, components. The BMD was determined in excised bones of both the humerus and tibia and the correlation was determined using the Schick AccuDEXA BMD Portable Bone Densitometer - tabletop DEXA scanner.

Bone Breaking Strength

Bone breaking strength measures resistance to fracture from a force applied perpendicular to the bone at mid-shaft [14]. Bones were cradled on 2 support points measuring 3 cm apart. Using a 50-kg load cell and a crosshead speed of 100 mm/min, the force of an attached shear plate measuring 8 cm in length and 1 mm wide was applied to the midpoint of the same anteroposterior plane of each bone using the TA. HD Plus texture analyzer machine (Stable Micro Systems, Hamilton, MA). Breaking strength was recorded.

Bone Ash

Ashing of the bone yields, by burning off all organic material, the total mineral content of a given known dry weight of bone. Ash weight is a fundamental measure of bone mineral content, expressed most often as a percentage of fat-free dry weight. Both humerus and tibia bones were cleaned of surrounding muscles and soft tissues. The tibia was separated from the fibula, and both the humerus and tibia were cut into pieces to fit into a Soxhlet for ether extraction for 48h. Ether extracted bone pieces were dried and weighed and placed in crucibles, and were ashed in a Thermolyne furnace (30400; Barnstead International) at 600°C for 10 h and weighed to determine ash percentage. Bone ash concentration was calculated as bone ash weight per unit of volume. Percentage bone ash was calculated by dividing bone ash by bone weight and multiplying by 100.

Statistical Analysis

All statistical analysis was performed in SAS, version 9.4 (SAS Institute, Inc., Cary, NC). Differences were considered significant when P≤0.05. Main effects and interaction

effects were evaluated for hen strain and diet. The experiment was a completely randomized design, and all data were analyzed with a one-way analysis of variance (ANOVA). Tukey's test was applied to compare the significance of differences between the means. The results were reported as means ± SE.

Results and Discussion

Keel bone characteristics for length, deviations, and fractures are shown in Table 2. There were significant differences (P≤0.05) observed in keel bone lengths among treatment groups. Hens of the 2016 strain had longer keel bones by approximately 0.57 mm when compared to the hens of the 1940 strain. At the strain effect for deviations, hens of the 2016 strain had significantly (P≤0.05) more deviations when compared to hens of the 1940 strain. Similar results were observed for fractures at the strain effect where hens of the 2016 strain had significantly (P≤0.05) more fractures when compared to hens of the 1940 strain. At the diet effect for deviations, no significance was observed, however, fractures at the diet effect showed a significantly (P≤0.05) higher incidence of fractures when compared to hens of the 1940 strain. Significance at the interaction level between strain x diet did occur for keel bone length, deviations, and fractures. Visual representations of the keel bone deviations are observed in Figure 1.

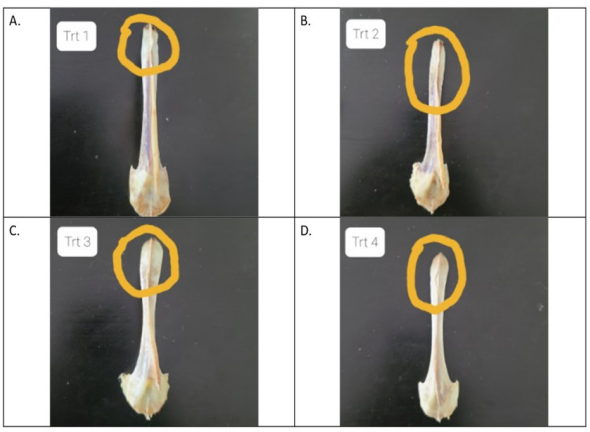

Figure 1: Visual Representation of Keel Bone Deviations of all Treatment Groups.

Legend: Treatment groups were as follows: 1). 2016 hen on 1940 diet; 2). 2016 hen on 2016 diet; 3). 1940 hen on 1940 diet; and 4). 1940 hen on 2016 diet

Table 2: Keel Bone Characteristics.

	Length (cm)	Deviations	Fractures
Main Effects			
Strain (S)			
2016	10.34[a]	1.2[a]	0.95[a]
1940	9.76[b]	0.7[b]	0.42[b]
SEM	0.115	0.429	0.395
p-value	0.003	0.051	0.022
Diet (D)			
2016	10.11[a]	0.95[a]	0.67[b]
1940	9.98[b]	0.95[a]	0.70[a]
SEM	0.116	0.214	0.403
p-value	0.004	0.457	0.038
Interaction			
S x D	0.016	0.014	0.012

[1]Values are presented as Means
Note. Mean values down the column having the same alphabets are not significantly different at P≤0.05 according to Tukey's tests.

Humerus bone weight, mineral density, breaking strength and length and diameter measurements are displayed in Table 3. There were no significant differences (P ≤0.05) in humerus bone weights for either the strain or diet main effects. Similar results were seen for the bone mineral density as well displaying no significance for the strain or diet effect, however, hens of the 2016 strain and hens fed on the 2016 diet had a higher bone mineral density when compared to hens of the 1940 strain and hens fed on the 1940 diet. Breaking strength, which is separated by bending moment expressed as kg/mm, peak force expressed as kg, and diameter expressed as mm was identical for both strains and for hens fed on both diets resulting in no significance. Humerus length showed significance (P≤0.05) at the strain effect with hens of the 2016 strain having longer humerus lengths when compared to hens of the 1940 strain. However, different results were observed on the diet effect with hens fed on the 1940 diet having longer humerus lengths when compared to hens fed on the 2016 diet. The diameter showed no significance (P≤0.05) between hens of either strain and for hens fed on either diet. The only interaction between strain x diet was observed for the length of the humerus bone. Tibial bone weight, mineral density, breaking strength and length and diameter measurements are displayed in Table 4.

Table 3: Humerus Bone Mineral Density, breaking strength and measurements.

	Weight (g)	BMD Test Results (g/cm²)	Breaking strength (g/mm²)	Length (mm)	Diameter (mm)
Main Effects					
Strain (S)					
2016	3.20[a]	0.29[a]	0.05[a]	71.13[a]	40.92[a]
1940	3.17[a]	0.27[a]	0.05[a]	69.09[b]	40.96[a]
SEM	0.663	0.21	0.012	0.179	0.59
P value	0.323	0.649	0.291	0.322	0.303
Diet (D)					
2016	3.24[a]	0.29[a]	0.05[a]	81.08[b]	40.98[a]
1940	3.20[a]	0.28[a]	0.05[a]	85.85[a]	40.89[a]
SEM	0.473	0.2	0.011	0.017	0.582
P value	0.308	0.648	0.297	0.0314	0.319
Interaction					
S x D	0.341	0.233	0.591	0.043	0.323

[1]Values are presented as Means
Note. Mean values down the column having the same alphabets are not significantly different at P≤0.05 according to Tukey's tests.
*Bone mineral density test results were provided by the accuDEXA Bone Densitometry Report

Table 4: Tibial Bone Mineral Density, breaking strength and measurements.

	Weight (g)	BMD Test Results (g/cm²)	Breaking strength (g/mm²)	Length	Diameter
Main Effects					
Strain (S)					
2016	6.21[a]	0.71[a]	0.20[a]	117.34[a]	32.02[a]
1940	5.79[b]	0.71[a]	0.11[b]	112.76[b]	30.91[b]
SEM	0.185	0.021	0.218	0.422	0.733
p-value	0.053	0.7	0.041	0.045	0.049
Diet (D)					
2016	6.01[a]	0.72[a]	0.12[b]	114.19[a]	31.29[a]
1940	5.95[a]	0.69[a]	0.19[a]	113.61[a]	31.63[a]
SEM	0.185	0.01	0.161	0.422	0.734
p-value	0.348	0.631	0.053	0.351	0.218
Interaction					
S x D	0.243	0.378	0.045	0.056	0.121

[1]Values are presented as Means

Note. Mean values down the column having the same alphabets are not significantly different at P≤0.05 according to Tukey's tests.

*Bone mineral density test results were provided by the accuDEXA Bone Densitometry Report

Tibia weights expressed in grams showed significance (P≤0.05) with the strain effect with hens of the 2016 strain having heavier weights when compared to hens of the 1940 strain. However, there were no significant differences (P≤0.05) with the diet effect for tibia weights, but hens fed on the 2016 diet had slightly heavier tibia bones when compared to hens fed on the 1940 diet. No significant differences (P≤0.05) were exhibited among the main effects of strain or diet for tibia bone mineral density. However, hens fed on the 2016 diet had a higher bone mineral density when compared to hens fed on the 1940 diet. Tibia breaking strength which is separated by bending moment expressed as kg/mm, peak force expressed as kg, and diameter expressed as mm showed that hens of the 2016 strain had a stronger breaking strength when compared to hens of the 1940 strain, however, with the diet effect, hens fed on the 1940 diet had stronger breaking strength when compared to hens fed on the 2016 diet.The length of the tibia at the strain effect was longer in hens of the 2016 strain resulting in significance (P≤0.05) but, there was no significance observed between hens fed on the 2016 diet or 1940 diet at the diet effect. Similar results were observed for the diameter measurements at the strain effect of the tibia in which hens of the 2016 strain had a significantly (P≤0.05) bigger diameter when compared to hens of the 1940 strain, however, no significance was observed between hens fed on the 2016 diet and those hens fed on the 1940 diet. Interactions between strain x diet were observed for both breaking strength and tibia bone length.

Percent bone ash of the humerus and tibia are displayed in Table 5. The aim of this study was to evaluate the genetic variation of bone properties of the keel, humerus, and tibia bones of hens representing different eras and diet regimens. Concerns acquired from egg producers, veterinarians, nutritionists, and geneticists have been placed on bone health along with the bone metabolism of laying hens. Bone health crucially impacts skeletal support along with eggshell quality deeming it vital in meeting these industry targets. The most important factors influencing these features are locally acting stresses and strains created by intrinsic muscle forces as well as external loads. Body mass or physical activity which can lead to an increase in bone load can stimulate bone formation as well as increase bone mass. Decreased bone load or hypoactivity can induce bone loss or reduced mass in chickens because of modeling and remodeling that is mediated by the activity of osteoblasts and osteoclasts. Bone strength was not deemed a top productive trait in past selection programs making it possible that over several generations of genetic selection for production traits pertaining to high egg production has resulted in a negative impact with hens having genetically weaker bones [3]. The occurrence of bone breakage and bone quality is understood to vary among different laying hen strains.

Table 5: Percent Bone Ash of Humerus and Tibia.

	Humerus (%)	Tibia (%)
Main Effects		
Strain (S)		
2016	8.82[a]	46.03[a]
1940	8.59[a]	46.92[a]
SEM	0.685	0.7
p-value	0.28	0.289
Diet (D)		
2016	9.05[a]	46.67[a]
1940	8.36[b]	46.29[a]
SEM	0.685	0.705
p-value	0.053	0.244
Interactions		
S x D	0.395	0.283

[1]Values are presented as Means ± SEM

Note . Mean values down the column having the same alphabets are not significantly different at P≤0.05 according to Tukey's tests.

Overall Effects

Several factors have been identified that can affect the strength and integrity of bones in laying hens. The focus in this study was related to genetics and nutrition on the bone parameters that were measured. The main effects in this study were strain and diet followed by interaction of strain x diet. Genetics plays a role in the functionality of the skeletal system of laying hens. A study conducted by Bishop et al., (2000) reported that approximately 40% of bone strength variation can be accounted for due to genetic differences among laying hens. Another study conducted by Stratmann et al., (2016) reported that genetic lines that were selected for high bone strength had fewer keel bone fractures and higher bone mineral density. In the past selection programs, bone quality was not accounted for, unlike other traits such as egg production. Therefore, it has been assumed that modern laying hens have been negatively affected by genetic selection resulting in weaker bones. Surprisingly, the results in this current contradicted the studies previously mentioned related to bone mineral density of the humerus and tibia showing no significant difference in both genetic lines suggesting that both lines are more alike than different genetically and diet was not a major contributing factor. Similar results were presented in the breaking strength of both the humerus and tibia of both lines as well as the keel bone analysis of deviations and fractures. Dietary effects weren't apparent in comparison to genetic effects. With the results from this study, it can be determined that genetic selection can provide a more permanent solution for improving bone quality of laying hens being of both a welfare and economic concern in comparison to nutritional interventions. However, the issue can also be addressed with proper nutrition. The interactions of the bones analyzed in this study are mainly additive and can contribute to overall bone quality either independently or jointly. It should be noted that sampling took place at one-time point, at the end of the trial, so the lack of variation could be attributed to the absence of measurements throughout the hens' lifetime during the single lay cycle.

Keel Bone Analysis

Several production parameters are potentially connected to Keel Bone Fractures (KBF); particularly being an early onset of lay [14]. In a study conducted by Schütz et al. [15], it was reported that the White Leghorn hybrid came into production relatively earlier (19.9 weeks) when compared to the Red Jungle fowl (24.9), thus theorizing that the ossification of the caudal part of the keel was not completed until around 30-40 weeks of age further suggesting that the modern hens started laying before the keel was mature [15]. Results from this study were in agreement with this theory due to the hens of the 2016 strain coming into production earlier than the hens of the 1940 strain. Due to the

higher impact force when experiencing a collision incident, heavier hens are more likely to suffer from Keel Bone Damage (KBD) [16]. The prevalence of keel bone fractures depends not only on the housing condition but also on the rearing system that was used during the pullet development, alongside age and strain. Keel bone deviations and fractures are assumed to have a different etiology, with deviations believed to be a result of extended perching and long-term pressure exhibited on the keel bone [14]. In this study, the prevalence of KBD and KBF were observed in both strains and in both representative diets. It was demonstrated that KBD occurred more in the lighter strain hens (1940 strain) when compared to the heavier strain hens (1940 strain) suggesting that the weight of those hens had an impact on their ability to move more freely when compared to the heavier hens thus resulting in keel damage. Due to the lack of significance with diet, it can be suggested that the nutritional composition had little effect on preventing or reducing the damage of the keel bone. KBF was also observed in both strains, but hens of the 2016 strain had a higher occurrence suggesting that the positive correlation was due to body weight due to the 2016 strain being heavier than the 1940 strain. It can also be concluded that diet did impact the severity of KBF with hens fed on the 1940 diet being presented with more fractures than hens fed on the 2016 diet due to being unable to meet the high calcium demands for egg production. According to Bain et al. [17], this is a classical explanation for justifications in KBFs as this phenomenon potentially induces resorption or breakdown of the bone matrix, thus releasing contained material and leaving the bones weak and brittle.

Bone Mineral Density

Dual-Energy X-Ray Absorptiometry (DEXA) consists of a moving X-ray generator that produces photons at 2 energy levels. A collimated scintillation detector moves simultaneously on the opposite side of the bone measuring flux. As the beam passes through the limb or bone, photon output is filtered to produce 2 distinct peaks that distinguish soft tissue from bone, generating bone density values. It should also be noted that the positioning of the bone being measured is critical in determining BMD when using the DEXA due to its 2-dimensional display according to Markel et al., (1994). In chickens, there has been a positive correlation between bone-breaking force ($r = 0.58$ to 0.68; $P < 0.001$) and bone ash weight ($r = 0.73$ to 0.99; $P < 0.001$) with bone mineralization as determined through utilization of the DEXA [18]. The humerus representing the wing, has one of the greatest fracture rates of all bones [19] and had a lower total bone mineral density measurement when compared to the humerus in this current study. According to Jendral MJ, et al. [20], the lower total BMD likely reflects excessive bone mineral loss by birds whose movements were highly restricted due to heavier size, however, in this current study, the results contradicted those claims due to insignificance of humerus BMD of both strains in identical cage systems. According to research conducted by Schreiweis MA et al. [21] adult White Leghorn hens consuming diets that have decreased calcium levels approximately around 5.4, 3.6, and 1.8% Ca, have shown a linear decrease in both tibia and humeral Bone Mineral Density (BMD) together with bone mineral content. The tibia had higher BMD when compared to the humerus. The tibia possesses a greater medullary component when compared to the humerus which most likely contributed to the results of higher BMD. The similarities of the interaction between strain and diet could have been a result of bone mineralization not being affected over the course of the single lay cycle. The BMD of both humerus and tibia was not influenced significantly by the main effects of hen strain or diet.

Breaking Strength

According to Leyendecker et al., (2001), genetics can influence bone stability and bone-breaking strength in addition to exercise and nutrition, however, Bishop et al., (2000) showed that bone stability could potentially be enhanced via selection occurring within a few generations [22]. Bone-breaking strength using material testing equipment is a common means of determining the functional characteristics of bone as a material. The humerus, known to normally be a pneumatized bone, devoid of mineral within its trabecular space with various degrees of humeral pneumatization, have been reported by Flemming et al. [23] to have the presence of medullary bone which in turn appears to increase humeral density and bone strength [24]. The results from this study demonstrated that humeral density was potentially increased to promote greater bone strength without negative implications from either strain or diet due to the lack of significance. The tibia is often used by researchers to represent the leg of a chicken. The tibiae of heavy hens were found to have greater breaking strength, diameter, length, bone weight, and total bone volume compared to the tibiae of lighter hens in research conducted by Kolakshyapati et al. [16] which agreed with results from this current study. Comparable differences can be attributed to the overall body size with the knowledge that bone geometry responds to the changes in body weight as well as additional body mass thus increasing the loading strain that is applied to the skeletal system, and therefore its composition and strength [16]. The results in this study demonstrated that the tibia breaking strength differed significantly among the genetic groups and diets but did not for humerus breaking strength suggesting that layer lines do differ with respect to body size. The results could also be triggered by both environment and nutrition in conjunction with underlying genetic factors.

Bone Ash

Utilizing the ash content of a bone typically provides estimates of the total mineral content [24]. Bone ash occurs when bones calcinate due to being heated at high temperatures. Ash is known as the inorganic residue resulting after water along with other materials are vaporized and organic substances are burned in the presence of oxygen [24]. Although the percentage of tibia ash is commonly and routinely used as a measure of bone mineralization, the actual weight of the tibia ash may be a more sensitive indicator of bone mineralization [12]. In conventional cages, all of these activities are constrained by both the small surface area of the cages and the absence of a suitable amenity, and movement is likely insufficient to prevent the loss of mineralized bone [20,25-35]. The results from this study suggest diet had a stronger influence on humerus ash compared to strain, but pressure of strain or diet did not impact bone ash of the tibia [36-44].

Conclusion and Applications

Quantity and quality are both important for overall bone health. Although bone strength was not likely part of the genetic selection programs during the greater part of the last 70+ years, the genetic determination of bone strength is high. Since skeletal problems in laying hens are important economic, welfare, and health issues for the poultry industry, a better understanding of bone metabolism in laying hens is important to enhance productivity and improve animal welfare.

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