Effects of the housing environment and laying hen strain on tibia and femur bone properties of different laying phases of Hy-Line hens

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ABSTRACT This study aimed to determine the effect of the housing environment and laying hen strain on tibia and femur properties. A 3 × 2 factorial arrangement of 3 housing environments (conventional cages [**CC**], enriched colony cages [**EC**], and free range [**FR**]) and 2 laying hen strains (Hy-Line W-36 [**W-36**] and Hy-Line Brown [**HB**]) in a completely randomized design was conducted from 32 to 85 wk of age. Six left tibias were collected at 8 different time points (38, 45, 52, 59, 65, 72, 79, and 85 wk of age), whereas 6 left femurs were collected at 3 time points (38, 65, and 85 wk of age). Tibias were evaluated for tibia breaking strength (**TBS**) and ash percentage, whereas femurs were evaluated for bone mineral density (**BMD**), bone mineral content, bone volume as a fraction tissue volume, and porosity percentage from total, cortical, medullary, and trabecular bones. The higher TBS ($P = 0.0005$) and ash percentage ($P = 0.045$) was observed in hens raised in FR systems compared with those raised in the CC. Overall, TBS of W-36 hens was significantly greater than that of HB hens ($P < 0.0001$); however, there was no difference in the ash percentage between the strains ($P > 0.05$). An interaction between the housing environment and hen strain was observed for BMD ($P = 0.04$), wherein W-36 hens raised in the FR system had higher BMD than HB hens. Similarly, hens raised in FR systems had higher trabecular bone volume than those raised in CC ($P = 0.022$). Hen strain influenced total and cortical bone properties: BMD, bone volume as a fraction tissue volume, and porosity percentage, wherein W-36 hens had better properties than HB hens ($P < 0.05$). Trabecular BMD was higher in W-36 hens than in HB hens ($P = 0.04$), whereas bone volume was higher in HB hens ($P < 0.0001$). The results suggest that raising laying hens in alternative housing systems that have provision for exercise such as FR reduces structural bone loss, stimulate structural bone formation, and improve breaking strength of bones; however, it varies with the strain.

Key words: bone property, housing environment, laying hen strain, X-ray microtomography

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INTRODUCTION

Conventional cages (**CC**) are the predominant housing systems in the United States because of higher revenue and productivity in a small area with high stocking density (UEP, 2019). Lately, CC have been widely criticized for compromising laying hen welfare and not providing a favorable environment to perform natural behavior, which has forced the egg industry to explore alternative housing systems (Appleby, 2003). Commercial flocks of laying hens raised in the CC are likely to develop osteoporosis owing to progressive loss of structural bone, which is linked with restricted movement and high demand for calcium for eggshell formation (Fleming et al., 1994; Whitehead and Fleming, 2000). Nearly 20–40% of calcium required for eggshell formation is supplied from bones, thus affecting bone integrity (Mueller et al., 1964). Laying hens selected for egg production are more susceptible to osteoporosis owing to a negative calcium balance, which is due to the high demand for calcium during eggshell formation (Fleming et al., 1994; Rennie et al., 1997; Whitehead and Fleming, 2000; Kim et al., 2012). Furthermore, bone quality is closely associated with egg production and egg quality, wherein a negative correlation has been observed among egg production, eggshell thickness, and bone breaking strength (Bishop et al., 2000;

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Leyendecker et al., 2001; Kim et al., 2005). The selection of laying hens for egg production and quality has negative impacts on bone health (Bishop et al., 2000; Webster, 2004). Previous studies have demonstrated that rearing hens in alternative housing environments that have provision for exercises such as perching and load-bearing activities improved bone properties (Leyendecker et al., 2005; Sandilands et al., 2009). It has been speculated that providing enough space for exercise may improve skeletal integrity by stimulating the formation of structural bone (Whitehead and Fleming, 2000; Webster, 2004; Leyendecker et al., 2005). In addition, Silversides et al. (2012) observed the difference among the laying hen strains (cross of Rhode Island Red males and Barred Plymouth Rock females, Lohmann Brown, H&N, and Lohmann White) raised in the CC or floor pen for cortical and trabecular bone mineral density (**BMD**) and area of tibias. Furthermore, Regmi et al. (2016a) reported that cortical bone thickness and density of tibias and femurs were higher in the Barred Plymouth Rock than in Hy-Line Brown (**HB**) and Hy-Line Silver Brown, which might be due to difference in egg production.

X-ray microtomography (**microCT**) is a relatively new technique to assess the architectural structure of the bone. Microtomography provides a detailed structure of the internal structure of the bone without destroying the bone. Microtomography can produce 3D images of an internal structure of bone through reconstruction (Landis and Keane, 2010). Microtomography can also provide an architectural structure of the cortical, trabecular, and medullary bone, which was not previously possible with the use of other techniques such as dual-energy X-ray absorptiometry and quantitative computed tomography (Jendral et al., 2008; Saunders-Blades et al., 2009; Landis and Keane, 2010; Chen et al., 2017; White et al., 2019). Similarly, microCT quantifies the bone material and structural properties (bone volume [**BV**], BMD, bone mineral content [BMC], and number and volume of pores in the bones). Dual-energy X-ray absorptiometry and quantitative computed tomography have been widely used to study the bone properties of laying hens in alternative housing environments (Jendral et al., 2008; Saunders-Blades et al., 2009; Tactacan et al., 2009; Regmi et al., 2016a,b). However, by using the dual-energy X-ray absorptiometry method, a detailed analysis of the trabecular bone architecture is often excluded. Quantitative computed tomography can create a 3D structure of bone, allowing separation of the cortical bone and bones in trabecular space, which includes both trabecular and medullary bones. However, it is unable to distinguish between trabecular and medullary bone within the trabecular space (Jendral et al., 2008; Saunders-Blades et al., 2009). The primary function of the trabecular bone is to distribute the mechanical load, bone remodeling, and maintaining calcium homeostasis throughout the body (Nakano et al., 2005). Previous studies reported that the housing environment and laying hen strain influenced the mechanical and structural properties of the bones; however, limited information is available regarding specific types of bones (cortical, medullary, and trabecular) (Shipov et al., 2010; Silversides et al., 2012; Regmi et al., 2016b). Therefore, it is essential to know how these bones react to a change in housing environments for different laying hen strains at different stages of lay.

With increasing consumer concern for the welfare of the laying hens, United States egg industry is exploring welfare-friendly housing environments for laying hens. However, limited information is available regarding bone properties at different stages of production in different housing environments such as CC, enriched colony cages (**EC**), or free range (**FR**). Previously, bone properties of laying hens were studied in alternative housing environments such as cage-free aviaries, EC, floors, or FR systems, but using only one laying hen strain and at a single time point (Leyendecker et al., 2005; Jendral et al., 2008; Shipov et al., 2010; Regmi et al., 2017). Most of these studies evaluated only the mechanical properties such as breaking strength, ash percentage, and cortical bone properties of the bone. The complete architectural structure of the bone, including the trabecular architecture, in different housing environments and with 2 common commercial strains has not been studied. To the author's knowledge, this is the first study to evaluate the bone properties of different laying hen strains in 3 different housing environments at different points of lay. Therefore, the objective of our study was to conduct a detailed study on the tibia and femur bone properties of laying hen strains housed in either CC, EC, or FR systems.

MATERIALS AND METHODS

The experimental procedure was approved by the Institutional Animal Care and Use Committee of Mississippi State University (IACUC# 17-554).

Housing Environment, Hen Husbandry, and Experimental Design

Day-old Hy-Line W-36 (**W-36**) and HB chicks were obtained from Hy-Line International (Hy-Line, Mansfield, GA) and reared on the floor until the pullets reached 18 wk of age. The pullets were then moved to the CC until they reached 30 wk of age and then allocated to the respective treatment groups. The CC, EC, and FR systems were located in the Poultry Research Unit at Mississippi State University. The CC and EC systems were installed within the same open-sided house, whereas the FR system was approximately 250 m away from the CC and EC system. The CC system was a three-tier A-frame system, whereas the EC system was a two-tier furnished system. Both CC and EC systems had a wire mesh floor with a floor space of 772 cm^2 per bird and 1,505 cm^2 per bird, respectively. The feeder space of 15 cm per bird and 22.5 cm per bird was allocated to the CC and EC systems, respectively. In addition, the EC system was installed with a dark nesting

area covered by nontransparent plastic curtains, perches running parallel to the cage, and a scratch pad. The FR system had an indoor and outdoor area (range) that was equally divided into 12 pens (5.57 m^2 per pen) and ranges (11.6 m^2 per range), with each connected via a window. The indoor area was equipped with wooden perches and nest boxes. The floor space provided in the indoor area was 1,742 cm^2 per bird, whereas an area of 3,484 cm^2 per bird was provided for the range with a feeder space of 3.5 cm per bird. The birds were given access to the range at least 7 h a day throughout the experimental period (32 to 85 wk of age).

Ad libitum feed and water were provided throughout the experimental period. Mash feed was fed to meet the nutrient requirement of laying hens (W-36 and HB) based on phase feeding, as per the Hy-Line management guide (Hy-Line International, 2016). The diet formulation and nutritional composition were the same as previously reported by Sharma, 2020. The lighting regime 16L:8D was as per the breeder management guidelines and was consistent for all housing environments throughout the experimental period (Hy-Line International, 2016).

The experimental design was a completely randomized design with a 3 × 2 factorial arrangement for 3 housing environments (CC, EC, and FR) and 2 laying hen strains (W-36 and HB) with a split-plot in time resulting in 6 treatments groups. The six treatment groups were W-36 White Leghorns in CC, HB in CC, W-36 White Leghorns in EC, HB in EC, W-36 White Leghorns in FR, and HB in FR. A total of 1,152 laying hens of both strains were weighed and equally allocated into each of the treatment groups (192 hens per group). Each treatment group consisted of 6 replicates. For the CC system, 8 adjacent cages, each cage containing 4 birds, were considered a replicate, whereas for the EC and FR system, each pen containing 32 birds was considered one replicate unit.

Tibia Breaking Strength and Tibia Ash

One bird per replicate was randomly selected and humanely euthanized every 7 wk (38, 45, 52, 59, 65, 72, 79, and 85 wk of age) with the use of CO_2 gas, and the weight of the bird was recorded. The left leg was separated from the hen and preserved at −20°C until further processing. All the surrounding muscle tissue and fibula bones were separated later, and left tibias thus obtained were wrapped in the wet cheesecloth and aluminum foil. The bones were preserved at −20°C until the bone breaking strength and tibia ash procedures were conducted. Before preserving the bone samples, weight was taken using a digital scale (Ohaus, Parsippany, NJ), whereas the length and diameter of the bone were measured with the use of a vernier caliper. The diameter of the tibia was measured at 3 different points, 1 cm below the proximal and distal heads, and at the middle of the shaft, and the average measurement was taken. To measure the tibia breaking strength (**TBS**), the bones were thawed overnight at room temperature. Bone breaking strength was measured using an Instron Universal Testing Machine (Instron Inc., Norwood, MA). The three-point bending procedure was followed with a crosshead speed of 10 mm/min using a 50-kg load cell (Asabe, 2005). Each bone sample after bone breaking strength data were acquired was further analyzed for tibia ash content. The bones were dried at 105°C for 24 h and then defatted using petroleum ether in a Soxhlet apparatus for 12 h. After the extraction of fat, the bones were air-dried, weighed along with the crucibles, and placed in the muffle furnace (600°C) for 24 h. After 24 h, tibia ash along with the crucible weight was recorded. Tibia ash percentage was calculated using the following formula:

$$\text{Tibia ash \%} = (\text{weight of crucible} + \text{ash} - \text{weight of crucible}) / (\text{weight of crucible} + \text{dry bone} - \text{weight of crucible}) \times 100\%$$

Analysis of Femur Bone

The femur bones, collected at 38, 65, and 85 wk of age, were removed and cleaned of all soft tissues. The femur bones were then wrapped with a wet cheesecloth and aluminum foil and kept at −20°C until further processing. The femur bones were thawed at 4°C overnight, and bone properties were analyzed by Skyscan X-ray microtomography (Bruker MicroCT, Billerica, MA) at the Department of Poultry Science at the University of Georgia. The bones were wrapped in the cotton cloth and fixed in a plastic cone (50-mL conical cone) to prevent desiccation. The X-ray source was set at 80 kV and 125 μA. The pixel size was fixed at 25 μm, the rotation angle of 0.4° was applied at each step, and 4 images per rotation were captured. A series of 2D images were captured, which were later used to reconstruct a 3D image using N-Recon (Bruker MicroCT, Billerica, MA). The 3D image was then straightened using Data Viewer software (Bruker MicroCT, Billerica, MA), and the volume of interest was selected using CTAn software (Bruker MicroCT, Billerica, MA). The volume of interest is defined as the section of the bone from which morphometry and density measurement were analyzed, and it was selected from a distal supracondylar region from which a total of 300 slides (7.5 mm) were analyzed. Two phantoms (8-mm diameter) of known density (0.25 and 0.75 g/cm^3) for calcium hydroxyapatite ($Ca_5(PO_4)_3(OH)$) were scanned to allow for calibration of BMD.

Microtomography was performed on the distal epiphyses of the femur, and a part of the distal supracondylar region was selected as a volume of interest wherein all 3 bone sections (cortical bone, medullary bone, and trabecular bone) were present. The description of the

Table 1. Definition and description of the measured parameters for femur microarchitecture.

Abbreviation	Variable	Description of variables	Standard unit
TV	Tissue volume	Volume of the entire region of interest	mm^3
BV	Bone volume	Volume of the bone segment	mm^3
BV/TV	Bone volume fraction	Bone volume segment volume as a fraction of tissue volume from the region of interest	%
BMD	Bone mineral density	Measure the bone mineral content per unit of volume	g/cm^3
BMC	Bone mineral content	Measure the bone mineral content of the tissue	G
VCP	Volume of closed pores	Volume of closed pores	mm^3
VOP	Volume of open pores	Volume of open pores	mm^3
TVP	Total volume of pores	Total pore volume (closed pores and open pores)	mm^3
PP	Porosity percentage	Volume of pores (TVP, mm^3)/total volume of bone (BV, mm^3)	%
Tb.N	Trabecular number	Average number of trabeculae per unit of length	1/mm
Tb.Th	Trabecular thickness	Mean thickness of trabeculae measured using 3D methods	Mm
Tb.Sp	Trabecular separation	Mean distance between trabeculae measured using 3D methods	Mm
Tb.Th.SD	SD of trabecular thickness	Measure of the homogeneity of trabecular thickness, assessed using direct 3D methods	Mm
Tb.Pf	Trabecular pattern factor	Indicates the degree of trabecular branching	1/mm
Conn.	Connectivity	Redundancy of trabecular connection	
Conn.Dn	Connectivity density	A measure of the degree of connectivity of trabeculae normalized by TV	$1/mm^3$
SMI	Structure model index	An indicator of the structure of trabeculae; the SMI will be 0 for parallel plates and 3 for cylindrical rods.	
DA	Degree of anisotropy	1 = isotropic, >1 = anisotropic	

measured parameters is shown in Table 1, adapted from the study by Bouxsein et al. (2010). Tissue volume (**TV**), BV, BV as a fraction of TV (**BV/TV**), BMD, and BMC were measured from the whole volume of interest, cortical bone, medullary bone, and trabecular bone sections. The volume of interest and cortical bone section was also measured for the volume of pores (volume of the closed pore and open pores) and porosity percentage (**PP**). From the trabecular bone, trabecular thickness (**Tb.Th**), trabecular number (**Tb.N**), structure model index, degree of anisotropy (**DA**), connectivity (**Conn.**), trabecular separation, and SD of Tb.Th were also measured. The BMC of the bone was calculated using the following formula:

$$\text{BMC} = \text{BMD}\left(\text{g/cm}^3\right) \times \text{TV}\left(\text{cm}^3\right)$$

Eggshell Quality

To correlate bone properties with eggshell quality parameters, such as eggshell thickness, eggshell percentage, and eggshell breaking strength, 3 different time points were selected (38, 65, and 85 wk of age). A total of 36 eggs (6 from each replicate) from each treatment were collected randomly to measure the eggshell parameters over the selected time points. Eggshell thickness was measured using an Ames micrometer (B. C. Ames Incorporated, MA) at the top, equator, and bottom of the eggshell, and the average measurement was obtained. Eggshell breaking strength was measured using an Instron Universal Testing Machine model 3,345 (Instron Inc., Norwood, MA), at a constant crosshead speed of 20 mm/min using a 100-N static load cell and a 35-mm circular steel probe as a compression device (Clerici et al., 2006; Sharma et al., 2020). To calculate the eggshell percentage, eggs were broken, and the eggshell was rinsed with tap water to clear the albumin and then allowed to dry for 2 d. Eggshell percentage was calculated by dividing the eggshell weight by egg weight and multiplying by 100 (Sharma et al., 2020).

Statistical Analysis

Data were analyzed using the PROC GLM procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC) as a completely randomized design with a split-plot in time. Means were separated using Fisher's LSD test, with the significance level set as $P \leq 0.05$. Correlations among the bone parameters and eggshell qualities were evaluated using PROC CORR (Pearson correlation) of SAS version 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Overall, hens housed in CC (2.05 kg) and EC (2.03 kg) were heavier than those house in the FR system (1.97 kg; $P = 0.0009$). Among the laying hen strains, HB (2.25 kg) hens were heavier than W-36 hens (1.79 kg; $P < 0.0001$; data not shown). The initial and final BW for HB hens were 2.03 kg and 2.17 kg, respectively; for W-36 hens, the respective BW were 1.65 kg and 1.78 kg.

Tibia Properties

The effect of housing environments and laying hen strains on tibia properties is shown in Table 2. An interaction among the housing environments and laying hen strains was observed for tibia weight as a percentage of BW ($P = 0.004$). The highest tibia weight as a percentage of BW was observed in HB hens raised in the FR system (0.57%) and lowest in W-36 hens raised in CC

Table 2. Effect of housing environments and laying hen strains on various tibia bone properties.

Treatments	BW (%)	BL (cm)	BD (cm)	DB (g)	DBP (%)	TBS (KgF)	AP (%)
Environment							
CC	0.48	11.78	0.71	5.58^{b}	57.03	23.05^{b}	55.64
EC	0.50	11.78	0.72	5.75^{b}	57.72	24.97^{a}	55.67
FR	0.53	11.80	0.72	6.02^{a}	58.87	26.47^{a}	56.47
SEM	0.006	0.31	0.005	0.075	0.45	0.554	0.25
Strain							
HB	0.53	12.12^{a}	0.77	6.52^{a}	56.01^{b}	22.81^{b}	55.77
W-36	0.48	11.48^{b}	0.67	5.06^{b}	59.74^{a}	26.86^{a}	56.09
SEM	0.005	0.03	0.003	0.061	0.37	0.452	0.20
Age of the birds							
38 wk	0.51	11.73^{b,c}	0.74^{a}	5.43^{c}	54.97	22.25^{d}	56.04
45 wk	0.51	11.84^{a,b}	0.70^{c}	5.36^{c}	55.16	22.92^{c,d}	57.76
52 wk	0.49	11.81^{a,b}	0.71^{c}	5.50^{c}	56.31	24.81^{a,b,c,d}	57.51
59 wk	0.49	11.76^{b,c}	0.71^{c}	5.54^{c}	56.68	24.85^{a,b,c}	57.44
65 wk	0.51	11.66^{b}	0.73^{a,b}	5.94^{b}	58.06	25.70^{a,b}	56.52
72 wk	0.50	11.94^{a}	0.72^{b,c}	6.19^{b}	59.09	27.14^{a}	57.03
79 wk	0.50	11.81^{b}	0.71^{b,c}	5.93^{a,b}	59.79	24.26^{c,d,e}	53.91
85 wk	0.52	4.76^{c}	0.72^{b,c}	6.35^{a}	62.54	26.24^{a,b}	51.43
SEM	0.009	0.06	0.005	0.11	0.67	0.91	0.44
Environment × strain							
CC × HB	0.50^{b}	12.13	0.76	6.30	55.27	21.13	55.40
CC × W-36	0.46^{c}	11.44	0.66	4.89	58.76	24.84	55.87
EC × HB	0.51^{b}	12.10	0.78	6.52	56.20	22.94	55.76
EC × W-36	0.49^{b}	11.46	0.67	5.00	59.21	26.95	55.60
FR × HB	0.57^{a}	12.11	0.77	6.73	56.54	24.23	56.14
FR × W-36	0.50^{b}	11.51	0.67	5.29	61.25	28.93	56.81
SEM	0.008	0.02	0.005	0.11	0.64	0.78	0.35
Environment × age of birds							
CC × 38 wk	0.46	11.67	0.73	5.15	54.61^{d,e}	18.62	55.70^{e,f}
CC × 45 wk	0.48	11.70	0.69	5.03	53.93^{e}	20.23	56.43^{c,d,e,f}
CC × 52 wk	0.46	11.83	0.71	5.07	53.82^{e}	21.10	57.14^{a,b,c,d,e,f}
CC × 59 wk	0.47	11.71	0.70	5.43	56.66^{b,c,d,e}	24.42	57.09^{a,b,c,d,e,f}
CC × 65 wk	0.51	11.81	0.72	5.67	56.49^{b,c,d,e}	22.63	56.60^{c,d,e,f}
CC × 72 wk	0.48	12.02	0.72	6.20	58.59^{b,c,d,e}	26.45	55.50^{f,g}
CC × 79 wk	0.47	11.89	0.71	5.65	56.83^{b,c,de}	23.52	56.04^{c,d,e,f}
CC × 85 wk	0.50	11.63	0.70	6.35	64.65^{a}	26.02	50.72^{i,j}
EC × 38 wk	0.53	11.68	0.74	5.46	54.56^{d,e}	24.03	56.47^{c,d,e,f}
EC × 45 wk	0.51	11.95	0.72	5.27	54.82^{d,e}	23.01	58.90^{a}
EC × 52 wk	0.48	11.72	0.71	5.69	57.86^{b,c,d,e}	26.25	58.47^{a,b}
EC × 59 wk	0.47	11.75	0.71	5.51	56.24^{b,c,d,e}	24.91	57.72^{a,b,c,d,e}
EC × 65 wk	0.51	11.60	0.74	5.74	57.49^{b,c,d,e}	26.39	56.21^{c,d,e,f}
EC × 72 wk	0.50	11.91	0.73	5.86	57.74^{b,c,d,e}	25.32	57.54^{a,b,c,d,e,f}
EC × 79 wk	0.49	11.76	0.71	6.07	61.34^{a,b,c}	24.16	53.11^{h}
EC × 85 wk	0.51	11.87	0.72	6.34	61.20^{a,b,c}	25.47	53.46^{g,h}
FR × 38 wk	0.56	11.88	0.74	5.62	55.66^{c,d,e}	23.65	55.87^{d,e,f}
FR × 45 wk	0.54	11.89	0.71	5.76	56.70^{b,c,d,e}	25.52	57.97^{a,b,c,d}
FR × 52 wk	0.52	11.87	0.71	5.71	57.19^{b,c,d,e}	27.08	56.82^{a,b,c,d,e,f}
FR × 59 wk	0.52	11.83	0.71	5.68	57.18^{b,c,d,e}	25.25	57.53^{a,b,c,d,e,f}
FR × 65 wk	0.52	11.54	0.72	6.41	60.19^{a,b,c,d}	28.09	56.76^{b,c,d,e,f}
FR × 72 wk	0.53	11.89	0.71	6.51	60.94^{a,b,c}	29.64	58.06^{a,b,c}
FR × 79 wk	0.55	11.78	0.71	6.05	61.20^{a,b,c}	25.08	52.57^{h,i}
FR × 85 wk	0.54	11.77	0.73	6.37	61.77^{a,b}	27.23	50.12^{j}
SEM	0.016	0.09	0.010	0.19	1.16	1.58	0.77
Strain × age of birds							
HB × 38 wk	0.56	12.03	0.80^{a}	6.10	53.27	19.85	55.60^{c,d}
HB × 45 wk	0.53	12.20	0.75^{c,d}	6.08	53.33	22.36	58.92^{a}
HB × 52 wk	0.50	12.14	0.76^{b,c,d}	6.26	54.31	24.21	57.78^{a,b}
HB × 59 wk	0.50	12.09	0.76^{b,c,d}	6.15	54.58	23.74	57.31^{a,b,c}
HB × 65 wk	0.52	11.85	0.78^{a,b}	6.69	56.48	23.17	56.29b^{c,d}
HB × 72 wk	0.52	12.39	0.77^{b,c}	6.98	56.50	24.92	57.75^{a,b}
HB × 79 wk	0.53	12.15	0.74^{d}	6.64	57.87	21.16	52.72^{e}
HB × 85 wk	0.54	12.07	0.78^{a,b}	7.17	61.19	23.07	50.07^{f}
W-36 × 38 wk	0.47	11.47	0.68^{e}	4.80	56.58	24.50	56.45^{b,c,d}
W-36 × 45 wk	0.49	11.51	0.66^{e,f}	4.67	56.89	23.57	56.66^{b,c,d}
W-36 × 52 wk	0.48	11.48	0.66^{f}	4.73	58.32	25.33	57.23^{a,b,c}
W-36 × 59 wk	0.47	11.42	0.65^{f}	4.88	58.91	26.04	57.58^{a,b}
W-36 × 65 wk	0.50	11.45	0.67^{e,f}	5.19	59.63	28.24	56.75^{b,c,d}
W-36 × 72 wk	0.48	11.51	0.67^{e,f}	5.40	61.68	29.35	56.32^{b,c,d}
W-36 × 79 wk	0.47	11.47	0.68^{e}	5.21	61.71	27.35	55.10^{d}
W-36 × 85 wk	0.50	11.45	0.66^{e,f}	5.53	63.90	29.41	52.80^{e}
SEM	0.013	0.08	0.003	0.16	0.94	1.29	0.62

(continued on next page)

Table 2. (*continued*)

Treatments	BW (%)	BL (cm)	BD (cm)	DB (g)	DBP (%)	TBS (KgF)	AP (%)
P-value							
Housing environment	<0.0001	0.6683	0.0941	0.0014	0.0168	0.0005	0.0449
Strain	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.2843
Age of the birds	0.0917	0.0164	0.0006	<0.0001	<0.0001	0.0026	<0.0001
Environment × strain	0.0044	0.6655	0.9859	0.8830	0.5256	0.7903	0.4234
Environment × age of birds	0.5255	0.1433	0.7541	0.4342	0.0332	0.5546	0.0048
Strain × age of birds	0.3633	0.1632	0.0351	0.9473	0.9310	0.3051	0.0005
Environment × strain × age of birds	0.3477	0.9413	0.0003	0.7350	0.5433	0.5348	0.1931

[a–j]Values within columns not sharing the superscripts are significantly different at $P \leq 0.05$.

Abbreviations: AP, tibia ash percentage; BW, bone weight; BD, bone diameter; BL, bone length; CC, conventional cage; DB, dried bone weight; DBP, dried bone percentage; EC, enriched colony cage; FR, free range; HB, Hy-Line Brown; TBS, tibia breaking strength; W-36, Hy-Line W-36.

(0.46%). Tibia weight was higher in hens raised in the EC and FR systems than in those raised in CC, whereas for the HB hens, hens raised in the FR system had higher tibia weight than those raised in CC and EC. The three housing environments (CC, EC, and FR) were not different with regard to tibia length ($P = 0.09$) and diameter ($P = 0.668$). Among the 2 laying hen strains, HB hens had higher tibia length (12.12 cm vs. 11.48 cm; $P < 0.0001$) and diameter (0.767 cm vs. 0.665 cm; $P < 0.0001$) than W-36 hens. The longest tibia length was observed at 72 wk of age and smallest at 65 wk of age ($P = 0.016$). Similarly, the largest tibia diameter was observed at 38 wk of age and lowest at 45, 52, and 59 wk of age ($P = 0.0006$). The effect of the housing environment was observed for dry weight ($P = 0.001$) and dry weight percentage of the tibia ($P = 0.016$). Hens raised in the FR system had more dried tibia weight (6.02 g) than those raised in CC (5.58 g). Likewise, FR hens had higher dried tibia weight percentage (58.87%) than the hens raised in CC (57.03%). In addition, laying hen strains differed in terms of dried weight and dried bone percentage (both; $P < 0.0001$). Dried tibia weight was observed to be higher in the HB hens (6.52 g) than in the W-36 hens (5.06 g), whereas dried bone percentage was higher in W-36 (59.74%) hens than in HB hens (56.01%). The highest dried tibia weight and percentage were observed at 85 wk of age (both; $P < 0.0001$). Both dried tibia weight and percentage gradually increased as the hens aged.

An interaction among the housing environment and age of the birds ($P = 0.004$) and hen strain and age of the birds ($P = 0.0005$) was observed for tibia ash percentage. Overall, ash percentage was highest in hens raised in the FR system (56.47%) than in those raised in CC (55.64%) and EC (55.67%; $P = 0.044$). Tibia ash percentage was similar among the housing environments until 72 wk of age. At 79 wk of age, hens raised in CC had a higher ash percentage than hens raised in the EC and FR systems, and at 85 wk of age, hens raised in the FR system had higher ash percentage than hens raised in CC and EC. At 45 wk of age, HB hens had higher ash percentage than W-36 hens; however, toward the end of the trial, at 79 and 85 wk of age, W-36 hens had higher ash percentage than HB hens. The highest TBS was observed in the FR (26.47 kgF) and EC (24.97 kgF) hens compared with hens raised in CC (23.05 kgF; $P = 0.0005$). The lowest TBS was observed for the HB hens (22.81 kgF) compared with W-36 hens (26.86 kgF; $P < 0.0001$). Tibia breaking strength gradually increased as the hens aged and was the highest at 72 wk of age (27.14 kgF), and then, it decreased at 79 wk of age (24.26 kgF; $P < 0.0001$).

Femur Properties

Total Volume of Interest. The effects of housing environments and laying hen strains for total volume of interest properties are shown in Table 3. An interaction between the housing environment and hen strain for BMD ($P = 0.040$) and between hen strain and age of the birds for BMC ($P = 0.0372$) was observed. The main effect of housing environments was not observed for the measured variables (BMD, BMC, TV, BV, BV/TV, VCP, VOP, TVP, and PP; $P > 0.05$). The highest BMD was observed in W-36 hens raised in the FR system (0.458 g/cm^3) compared with W-36 hens raised in EC and CC. However, there was no difference in BMD in HB hens raised in all 3 housing environments. Likewise, BMD showed an increasing trend with the age of the birds, with the highest BMD observed in W-36 hens at 85 wk of age. However, it decreased for the HB hens from 65 (0.445 g/cm^3) to 85 (0.402 g/cm^3) wk of age. An interaction was observed for BMC between the housing environment and laying hen strain ($P = 0.030$), wherein HB hens had higher BMC than W-36 hens raised in CC and EC systems; however, there was no difference in hens raised in the FR system. Overall, BMD increased with the age of the birds from 38 (0.191 g/cm^3) to 65 wk of age (0.239 g/cm^3) and remained constant afterward ($P < 0.0001$).

The highest TV was observed in the HB hens (629.1 mm^3) compared with W-36 hens (467.0 mm^3; $P < 0.0001$). There was a trend observed in the interaction between the housing environment and hen strain for BV ($P = 0.059$). The highest BV was observed in HB hens raised in CC (216.1 mm^3) and lowest in W-36 hens raised in CC (146.3 mm^3). Bone volume increased from 163.6 to 199.8 mm^3 from 38 to 65 wk of age ($P = 0.0006$). Bone volume percentage as a fraction of TV had a similar pattern as BV. The BV/TV increased from week 38 (29.95%) to week 65 (37.04%). The BV/TV at 85 wk of age (35.80%) was not statistically different from that at week 65 ($P = 0.0003$).

Table 3. Effect of housing environments and laying hen strains on femur bone properties (total volume of interest).

Treatments	BMD (g/cm^3)	BMC (g)	TV (mm^3)	BV (mm^3)	BV/TV (%)	BS (mm^2)
Environment						
CC	0.403	0.216	535.7	181.2	33.67	1,284
EC	0.413	0.224	549.0	187.1	34.52	1,432
FR	0.416	0.226	554.3	188.7	34.66	1,304
SEM	0.015	0.008	7.65	8.1	1.47	49
Strain						
HB	0.392	0.246	629.1^{a}	206.6^{a}	33.04	1,526^{a}
W-36	0.428	0.200	467.0^{b}	165.7^{b}	35.50	1,160^{b}
SEM	0.012	0.007	6.2	6.6	1.20	40
Age of the birds						
38 wk	0.350	0.191^{b}	549.8	163.6^{b}	29.95^{b}	1,204^{b}
65 wk	0.442	0.239^{a}	542.4	199.8^{a}	37.04^{a}	1,463^{a}
85 wk	0.439	0.236^{a}	547.4	193.4^{a}	35.80^{a}	1,350^{a,b}
SEM	0.012	0.007	6.5	7.3	1.31	54
Environment × strain						
CC × HB	0.411^{a,b}	0.255^{a}	620.8	216.1	34.89	1,537
CC × W-36	0.394^{b}	0.177^{d}	450.7	146.3	32.45	1,031
EC × HB	0.393^{b}	0.244^{a,b}	625.0	203.0	32.80	1,600
EC × W-36	0.431^{a,b}	0.207^{c,d}	481.5	173.7	36.06	1,282
FR × HB	0.374^{b}	0.239^{a,b,c}	640.6	200.8	31.51	1,448
FR × W-36	0.458^{a}	0.214^{b,c}	468.1	176.6	37.81	1,159
SEM	0.021	0.011	10.8	11.5	2.08	69
Strain × age of birds						
HB × 38 wk	0.332^{d}	0.208	628.0	179.6	28.64	1,333
HB × 65 wk	0.445^{a,b}	0.275	621.1	227.0	36.86	1,744
HB × 85 wk	0.402^{b,c}	0.255	637.3	214.0	33.80	1,513
W-36 × 38 wk	0.367^{c,d}	0.173	471.7	147.6	31.25	1,074
W-36 × 65 wk	0.439^{a,b}	0.207	472.5	175.6	37.21	1,213
W-36 × 85 wk	0.475^{a}	0.217	457.2	172.9	37.79	1,188
SEM	0.018	0.010	9.2	10.4	1.85	77
P-value						
Housing environment	0.8777	0.7455	0.2026	0.8973	0.9524	0.0632
Strain	0.1044	<0.0001	<0.0001	<0.0001	0.2667	<0.0001
Age of the birds	<0.0001	<0.0001	0.8979	0.0006	0.0003	0.0033
Environment × strain	0.0401	0.0306	0.2795	0.0594	0.0677	0.2411
Environment × age of birds	0.3459	0.4538	0.8146	0.2726	0.2779	0.2962
Strain × age of birds	0.0372	0.1080	0.1571	0.4281	0.3658	0.1858
Environment × strain × age of birds	0.3325	0.5815	0.1081	0.3471	0.1962	0.5500

Treatments	NCP	VCP (mm^3)	CPP (%)	VOP (mm^3)	OPP (%)	TVP (mm^3)	PP (%)
Environment							
CC	873	2.10	1.22	352.4	65.94	354.5	66.33
EC	964	2.02	1.13	359.9	65.11	361.9	65.48
FR	745	1.88	0.99	363.7	65.01	365.6	65.34
SEM	113	0.17	0.10	10.8	1.47	10.9	1.47
Strain							
HB	964	2.42^{a}	1.22	420.1^{a}	66.58	422.2^{a}	66.96
W-36	757	1.60^{b}	1.01	299.8^{b}	64.16	301.4^{b}	64.50
SEM	92	0.14	0.08	8.8	1.20	8.9	1.20
Age of the birds							
38 wk	664	2.28^{a}	1.39^{a}	384.0^{a}	69.64^{a}	386.2^{a}	70.05^{a}
65 wk	930	1.58^{b}	0.82^{c}	341.1^{b}	62.67^{b}	342.6^{b}	62.96^{b}
85 wk	974	2.13^{a}	1.13^{b}	351.72^{b}	63.82^{b}	353.9^{b}	64.20^{b}
SEM	112	0.15	0.09	8.9	1.31	8.9	1.31
Environment × strain							
CC × HB	1,158	2.41	1.22	402.2	64.72	404.6	65.11
CC × W-36	588	1.80	1.23	302.5	67.15	304.3	67.55
EC × HB	982	2.35	1.20	419.7	66.83	422.1	67.20
EC × W-36	947	1.73	1.07	306.7	63.59	308.5	63.94
FR × HB	764	2.48	1.24	437.3	68.10	439.8	68.49
FR × W-36	726	1.28	0.75	290.2	61.92	291.4	62.19
SEM	160	0.24	0.14	15.3	2.08	15.4	2.08
P-value							
Housing environment	0.3721	0.6340	0.2631	0.6338	0.9611	0.6441	0.9524
Strain	0.0781	0.0004	0.1309	<0.0001	0.2741	<0.0001	0.2667
Age of the birds	0.0647	0.0042	<0.0001	0.0023	0.0003	0.0020	0.0003
Environment × strain	0.1143	0.3141	0.1263	0.2028	0.0712	0.1979	0.0677
Environment × age of birds	0.6664	0.2880	0.1965	0.2415	0.2861	0.2342	0.2779
Strain × age of birds	0.9275	0.1988	0.2664	0.1016	0.3631	0.1006	0.3658
Environment × strain × age of birds	0.5003	0.7362	0.7085	0.0473	0.2021	0.0453	0.1962

[a–c]Values within columns not sharing the superscripts are significantly different at $P \leq 0.05$.

Abbreviations: BMD, bone mineral density; BMC, bone mineral content; BS, bone surface; BV, bone volume; BV/TV, bone volume fraction as a fraction of tissue volume; CC, conventional cage; CPP, closed pore percentage; EC, enriched colony cage; FR, free range; HB, Hy-Line Brown; NCP, number of closed pores; OPP, open pore percentage; PP, porosity percentage; TV, tissue volume; TVP, total volume of pores; VCP, volume of closed pores; VOP, volume of closed pores; W-36, Hy-Line W-36.

Hy-Line Brown hens had a higher VCP (2.42 mm^3) and VOP (420.10 mm^3) than W-36 hens (1.60 mm^3 and 299.76 mm^3; $P = 0.0004$ and $P < 0.0001$). The highest VCP and VOP was observed at week 38 (2.28 mm^3 and 386.24 mm^3), and the lowest volume was observed at week 65 (1.58 mm^3 and 341.06 mm^3; $P = 0.0042$ and $P = 0.002$). Similarly, total volume of pores was higher in the HB (422.5 mm^3) hens than in W-36 (301.4 mm^3) hens and was highest at 38 wk of age (386.2 mm^3; $P < 0.0001$). Laying hen strains did not differ in terms of PP ($P = 0.266$), but the main effect of age was observed ($P = 0.0003$). The highest PP was observed at 38 wk of age (70.05%).

Cortical Bone. The effects of housing environments and laying hen strains on cortical bone properties are shown in Table 4. Housing environments did not have any effect on measured cortical bone parameters; however, laying hen strains had an effect on most of the measured variables. Cortical BMD was observed higher in the W-36 (0.985 g/cm^3) hens than in HB hens (0.927 g/cm^3; $P < 0.0001$). The highest BMD was observed at 65 wk of age (0.981 g/cm^3), and the lowest BMD was observed at 38 wk of age (0.916 g/cm^3; $P < 0.0001$). Hy-Line Brown hens had higher BMC (0.129 g) than W-36 hens (0.145 g; $P = 0.033$). Bone mineral content was observed higher at 65 and 85 wk of age (0.146 g), and it was lowest at wk 38 (0.116 g: $P = 0.0003$).

The greatest TV and BV were observed in the HB hens (156.2 mm^3 and 146.4 mm^3) compared with W-36 hens (130.4 mm^3 and 123.8 mm^3; $P = 0.003$ and $P = 0.007$); however, BV/TV was greater in W-36 (94.90%) hens than in HB hens (93.57%; $P = 0.0103$). Tissue volume was higher at 85 (151.6 mm^3) and 65 wk of age (149.7 mm^3) than at wk 38 (127.4 mm^3; $P = 0.008$). Similar results were observed for BV ($P = 0.006$) and BV/TV ($P = 0.049$).

While comparing the laying hen strains for the VCP, HB hens had higher volume (1.54 mm^3) than W-36 hens (1.06 mm^3; $P = 0.001$), and the highest volume was observed at 38 (1.415 mm^3) and 85 wk of age (1.425 mm^3) compared with 65 wk of age (1.041 mm^3; $P = 0.018$). Similarly, HB hens had a higher VOP (8.29 mm^3) than W-36 hens (5.54 mm^3; $P = 0.0003$). Laying hen strains differed in terms of PP, wherein HB hens had higher pores (6.43%) than W-36 hens (5.10%; $P = 0.010$). Laying hens at 38 wk of age had a higher PP (6.40%) than the hens at 65 wk of age (4.87%; $P = 0.049$).

Medullary Bones. The effects of housing environments and laying hen strains on medullary bone are shown in Table 5. When comparisons were made across the housing environments, measured medullary bone parameters did not differ ($P > 0.05$), but laying hen strains affected these properties. Bone mineral density was not different among the hen strains ($P = 0.501$); however, it increased with the age of the hens. The highest BMD was observed at 65 and 85 wk of age (0.186 g/cm^3 and 0.172 g/cm^3, respectively), and the lowest BMD was observed at 38 wk of age (0.115 g/cm^3; $P < 0.0001$). Hy-Line Brown hens had higher BMC (0.064 g) than W-36 hens (0.044 g; $P < 0.0001$). Overall, BMC was observed to be highest at 65 wk of age (0.063 g) and lowest at 38 wk of age (0.043 g). The highest medullary TV was observed in HB (417.67 mm^3) hens compared with W-36 hens (293.8 mm^3; $P < 0.0001$). The highest BV and BV/TV were observed in HB hens (15.20 mm^3 and 4.35%) compared with W-36 hens (7.32 mm^3 and 2.66%; $P = 0.0001$ and $P = 0.023$). The highest BV and BV/TV were observed at 65 wk of age (15.61 mm^3 and 4.81%) and were the lowest at 38 wk of age (6.06 mm^3 and 1.60%; $P = 0.004$ and $P = 0.004$).

Trabecular Bone. The effects of housing environments and laying strains on trabecular bone are shown in Table 6. Trabecular BMD was only numerically higher in hens raised in the FR system (0.766 g/cm^3) than in those raised in CC (0.760 g/cm^3) and EC (0.751 g/cm^3; $P = 0.084$). Trabecular BMD was lowest for the HB hens (0.753 g/cm^3) compared with W-36 hens (0.765 g/cm^3; $P = 0.040$). Bone mineral density increased with the age of the birds and was highest at 85 wk of age (0.782 g/cm^3) and lowest at 38 wk of age (0.728 g/cm^3; $P < 0.0001$). Trabecular BMC was observed to be higher in hens raised in the FR system (0.013 g) than in hens raised in CC (0.011 g; $P = 0.025$), and between the hen strains, HB (0.014 g) hens had higher BMC than W-36 hens (0.010 g; $P < 0.0001$). The highest BMC was observed at 65 wk of age (0.013 g; $P = 0.057$).

The hens raised in the FR system had higher BV (17.27 mm^3) than hens raised in both the CC (13.97 mm^3) and EC system (16.25 mm^3; $P = 0.022$). Overall, HB hens had higher BV (18.94 mm^3) than W-36 hens (12.89 mm^3; $P < 0.0001$). A trend was observed for Tb.Th for the housing environment ($P = 0.062$). The hens raised in the FR system had higher Tb.Th (0.146 mm) than hens raised in the EC (0.138 mm) and CC (0.140 mm) systems. Trabecular thickness did not differ among the hen strains ($P < 0.696$). The hens raised in the EC system had a greater Tb.N (7.35 per millimeter) than hens raised in the FR system (6.93 per millimeter; $P = 0.091$). Greater Tb.N was observed at 38 (7.54 per millimeter) and 85 wk of age (7.27 per millimeter; $P = 0.011$). Housing environments and laying hen strains did not have any effect on trabecular pattern factor ($P > 0.05$). Trabecular pattern factor ($P = 0.0213$) and SD of Tb.Th ($P = 0.013$) were both higher at 65 wk of age.

Structure model index and Conn. did not differ in hens raised in either the CC, EC, or FR system ($P > 0.05$). Trabecular Conn. was observed to be higher in the HB hens (1106.6) than in W-36 hens (612.5; $P < 0.0001$). Degree of anisotropy was observed to be lowest for the hens raised in the FR system (1.635) compared with those raised in CC (1.763) and EC (1.736; $P = 0.040$). A higher DA was observed in the W-36 hens (1.840) than in HB hens (1.575; $P < 0.0001$).

Correlation Analysis

The correlation between BW, femur bone properties, tibia bone properties, and eggshell qualities is shown in

Table 4. Effect of housing environments and laying hen strains on femur bone properties (cortical bone).

Treatments	BMD (g/cm^3)	BMC (g)	TV (mm^3)	BV (mm^3)	BV/TV (%)	BS (mm^2)
Environment						
CC	0.954	0.133	139.5	131.1	93.89	770.3
EC	0.948	0.135	143.2	135.1	94.20	788.2
FR	0.967	0.141	146.3	138.2	94.64	746.0
SEM	0.006	0.007	7.6	7.5	0.38	18.6
Strain						
HB	0.927^b	0.145^a	156.2^a	146.4^a	93.57^b	851.9^a
W-36	0.985^a	0.129^b	130.4^b	123.8^b	94.90^a	686.8^b
SEM	0.005	0.006	6.2	6.1	0.31	15.2
Age of the birds						
38 wk	0.916^b	0.116^b	127.4^b	119.2^b	93.60^b	752.5
65 wk	0.981^a	0.146^a	149.7^a	142.3^a	95.13^a	759.9
85 wk	0.971^a	0.146^a	151.6^a	142.6^a	94.03^{a,b}	789.5
SEM	0.007	0.006	6.67	6.4	0.43	19.1
Environment × strain						
CC × HB	0.921	0.151	163.6	153.0	93.09	882.2
CC × W-36	0.988	0.114	115.3	109.2	94.69	658.4
EC × HB	0.934	0.143	152.7	143.5	93.98	815.4
EC × W-36	0.999	0.139	139.9	133.0	95.29	676.6
FR × HB	0.925	0.140	152.2	142.5	93.62	860.6
FR × W-36	0.968	0.131	135.3	128.5	94.72	723.9
SEM	0.009	0.010	10.8	10.7	0.54	26.3
P-value						
Housing environment	0.0983	0.8083	0.9210	0.9101	0.4323	0.2588
Strain	<0.0001	0.0335	0.0035	0.0078	0.0103	<0.0001
Age of the birds	<0.0001	0.0003	0.0086	0.0065	0.0497	0.3497
Environment × strain	0.4357	0.1215	0.1209	0.1387	0.9406	0.1483
Environment × age of birds	0.3531	0.4200	0.4880	0.4754	0.8114	0.8109
Strain × age of birds	0.2256	0.7011	0.6932	0.7504	0.0609	0.0982
Environment × strain × age of birds	0.9967	0.3361	0.4108	0.4119	0.9628	0.7226

Treatments	NCP	VCP (mm^3)	CPP (%)	VOP (mm^3)	OPP (%)	TVP (mm^3)	PP (%)
Environment							
CC	664	1.375	1.091	6.97	5.08	8.34	6.11
EC	763	1.304	1.015	6.84	4.84	8.14	5.80
FR	605	1.215	0.882	6.87	4.52	8.08	5.36
SEM	87	0.110	0.090	0.57	0.34	0.63	0.38
Strain							
HB	743	1.540^a	1.099	8.29^a	5.4^a	9.83^a	6.43^a
W-36	611	1.060^b	0.892	5.54^b	4.25^b	6.60^b	5.10^b
SEM	71	0.089	0.070	0.47	0.27	0.51	0.31
Age of the birds							
38 wk	557	1.415^a	1.182^a	6.74	5.29	8.15	6.40^a
65 wk	687	1.041^b	0.762^b	6.36	4.15	7.40	4.87^b
85 wk	778	1.425^a	1.035^a	7.54	4.99	8.96	5.97^{a,b}
SEM	86	0.103	0.079	0.66	0.40	0.71	0.43
Environment × strain							
CC × HB	860	1.567	1.114	9.06	5.88	10.63	6.92
CC × W-36	468	1.182	1.069	4.87	4.29	6.05	5.31
EC × HB	618	1.573	1.110	7.69	4.97	9.26	6.02
EC × W-36	592	0.856	0.653	6.04	4.08	6.90	4.71
FR × HB	759	1.478	1.071	8.16	5.37	9.63	6.38
FR × W-36	765	1.149	0.964	5.67	4.37	6.82	5.28
SEM	123	0.154	0.125	0.81	0.48	0.89	0.54
P-value							
Housing environment	0.4465	0.6200	0.3096	0.9885	0.5396	0.9583	0.4323
Strain	0.1321	0.0010	0.0908	0.0003	0.0107	0.0002	0.0103
Age of the birds	0.1516	0.0188	0.0012	0.4673	0.1397	0.3437	0.0497
Environment × strain	0.1453	0.3482	0.1628	0.3116	0.8543	0.4549	0.9406
Environment × age of birds	0.7767	0.6358	0.7767	0.9571	0.8401	0.9239	0.8114
Strain × age of birds	0.9705	0.3309	0.2967	0.1268	0.0608	0.1212	0.0609
Environment × strain × age of birds	0.5764	0.5727	0.2895	0.8703	0.9091	0.9459	0.9628

[a–c]Values within columns not sharing the superscripts are significantly different at $P \leq 0.05$.

Abbreviations: BMD, bone mineral density; BMC, bone mineral content; BS, bone surface; BV, bone volume; BV/TV, bone volume fraction as a fraction of tissue volume; CC, conventional cage; CPP, closed pore percentage; EC, enriched colony cage; FR, free range; HB, Hy-Line Brown; NCP, number of closed pores; OPP, open pore percentage; PP, porosity percentage; TV, tissue volume; TVP, total volume of pores; VCP, volume of closed pores; VOP, volume of closed pores; W-36, Hy-Line W-36.

Table 7. BW was highly correlated with BMC (0.525; $P < 0.0001$) and eggshell percentage (0.244; $P < 0.05$). Bone mineral density of the volume of interest was highly positively correlated with BMC (0.752; $P < 0.0001$), BV (0.898; $P < 0.0001$), dried tibia bone percentage (0.458; $P < 0.0001$), TBS (0.606; $P < 0.0001$), and tibia ash percentage (0.206; $P < 0.01$) but negatively correlated with eggshell percentage (−0.339; $P < 0.001$)

Table 5. Effect of housing environments and laying hen strains on femur bone properties (medullary bone).

Treatments	BMD (g/cm^3)	BMC (g)	TV (mm^3)	BV (mm^3)	BV/TV (%)	BS (mm^2)
Environment						
CC	0.158	0.054	349.1	12.20	3.86	740.5^{b}
EC	0.168	0.058	356.5	11.91	3.83	914.2^{a}
FR	0.148	0.051	357.8	9.53	2.81	797.0^{a,b}
SEM	0.011	0.003	10.3	1.58	0.67	53.8
Strain						
HB	0.159	0.064^{a}	417.7^{a}	15.20^{a}	4.35^{a}	983.7^{a}
W-36	0.156	0.044^{b}	293.8^{b}	7.32^{b}	2.66^{b}	656.4^{b}
SEM	0.009	0.003	8.4	1.29	0.55	43.9
Age of the birds						
38 wk	0.115^{b}	0.043^{b}	374.4^{a}	6.06^{b}	1.60^{b}	663.3^{b}
65 wk	0.186^{a}	0.063^{a}	342.3^{b}	15.61^{a}	4.81^{a}	1014.0^{a}
85 wk	0.172^{a}	0.056^{a}	347.3^{b}	11.83^{a,b}	4.03^{a,b}	775.8^{b}
SEM	0.011	0.004	8.0	2.05	0.74	65.5
Environment × strain						
CC × HB	0.176	0.067	403.0	19.01	5.88	973.8
CC × W-36	0.138	0.040	295.2	5.39	1.85	507.3
EC × HB	0.137	0.058	431.6	11.49	2.77	916.9
EC × W-36	0.159	0.044	284.0	7.57	2.85	677.2
FR × HB	0.166	0.067	417.6	15.32	4.49	1069.3
FR × W-36	0.169	0.049	302.2	8.89	3.25	776.4
SEM	0.016	0.005	14.6	2.24	0.95	76.1
P-value						
Housing environment	0.4924	0.3141	0.6774	0.3855	0.3935	0.0598
Strain	0.5010	<0.0001	<0.0001	0.0001	0.0237	<0.0001
Age of the birds	<0.0001	0.0028	0.0093	0.0048	0.0041	0.0008
Environment × strain	0.1098	0.3285	0.2667	0.0857	0.0734	0.2863
Environment × age of birds	0.7762	0.9618	0.3838	0.2859	0.1628	0.2103
Strain × age of birds	0.0857	0.1889	0.1354	0.1635	0.2314	0.0890
Environment × strain × age of birds	0.6385	0.9960	0.0557	0.4265	0.1634	0.5156

[a,b]Values within columns not sharing the superscripts are significantly different at $P \leq 0.05$.

Abbreviations: BMD, bone mineral density; BMC, bone mineral content; BV, bone volume; BV/TV, bone volume fraction as a fraction of tissue volume; BS, bone surface; CC, conventional cage, EC, enriched colony cage; FR, free range; HB, Hy-Line Brown; TV, tissue volume; W-36, Hy-Line W-36.

and eggshell thickness (−0.243; $P < 0.05$). Femur BMC was highly correlated with BV (0.703; $P < 0.0001$) and TBS (0.310; $P < 0.01$). A strong positive correlation was observed between femur BV and TBS (0.607; $P < 0.0001$). Eggshell percentage was negatively correlated with femur BV (−0.251; $P < 0.05$), dried tibia bone percentage (−0.511; $P < 0.0001$), TBS (−0.415; $P < 0.0001$), and tibia ash percentage (−0.242; $P < 0.05$). Similarly, eggshell thickness was also negatively correlated with dried tibia bone percentage (−0.525; $P < 0.0001$), TBS (−0.329; $P < 0.001$), and tibia ash percentage (−0.370; $P < 0.0001$).

DISCUSSION

Skeletal health and its metabolism in laying hens are of prime importance for optimum performance as 20–40% of calcium for eggshell formation is mobilized from bones (Mueller et al., 1964). The occurrence of osteoporosis is more likely in laying hens raised in the CC than in those raised in the extensive housing systems such as aviaries or the FR system (Regmi et al., 2016a; Casey-Trott et al., 2017). Lately, laying hens are being raised in extensive housing environments such as barns, cage-free aviaries, or the FR system, thereby providing opportunities for physical activities and better skeletal health.

Previous studies have compared tibia bone properties of laying hens raised in different housing environments and observed poor bone quality (breaking strength, BMD, and BMC) in hens raised in the CC compared with hens raised in the aviary, floor, or FR system (Newman and Leeson, 1998; Jendral et al., 2008; Shipov et al., 2010; Silversides et al., 2012; Regmi et al., 2016b; Yilmaz Dikmen et al., 2016). The compositional characteristics of the tibias, including tibia weight as a percentage of BW, dried tibia bone percentage, and ash percentage, differed among the housing environments (Silversides et al., 2012; Regmi et al., 2016b; Yilmaz Dikmen et al., 2016) and laying hen strains (Silversides et al., 2012; Regmi et al., 2016a). Tibia weight as a percentage of BW increased with increasing floor space in both laying hen strains, which might be due to increased locomotor activities stimulating bone formation (Whitehead and Fleming, 2000). In our study, hens raised in the FR sytem had a higher dried tibia bone percentage than hens raised in CC, which was similar to the previous results (Silversides et al., 2012; Regmi et al., 2016b; Yilmaz Dikmen et al., 2016). The change in tibia ash percentage over the laying period in both housing environments and laying hen strains might be due to the age-related biochemical changes in the collagen matrix. Rath et al. (2000) explained that the post-translational modification of the collagen matrix might have affected the bone mineralization process, making changes in ash content of the bones over time.

In our study, the highest TBS was observed for the hens housed in the FR and EC system compared with

Table 6. Effect of housing environments and laying hen strains on femur bone properties (trabecular bone).

Treatments	BMD (g/cm^3)	BMC (g)	BV (mm^3)	BS (mm^2)	Tb.Th (mm)	Tb.N (1/mm)
Environment						
CC	0.760	0.011^{b}	13.97^{b}	352.6^{b}	0.140	7.28
EC	0.751	0.012^{a,b}	16.25^{a,b}	422.3^{a}	0.138	7.35
FR	0.766	0.013^{a}	17.27^{a}	410.3^{a,b}	0.146	6.93
SEM	0.005	0.001	0.85	21.8	0.002	0.14
Strain						
HB	0.753^{b}	0.014^{a}	18.94^{a}	475.3^{a}	0.142	7.18
W-36	0.765^{a}	0.010^{b}	12.89^{b}	318.4^{b}	0.141	7.19
SEM	0.004	0.001	0.69	17.8	0.002	0.12
Age of the birds						
38 wk	0.728^{c}	0.011	14.81	375.6	0.134^{b}	7.54^{a}
65 wk	0.766^{b}	0.013	17.25	417.8	0.150^{a}	6.74^{b}
85 wk	0.782^{a}	0.012	15.53	392.9	0.140^{b}	7.27^{a}
SEM	0.005	0.001	0.86	21.2	0.003	0.15
Environment × strain						
CC × HB	0.755	0.013	17.33	439.5	0.140	7.33
CC × W-36	0.766	0.008	10.60	265.8	0.140	7.24
EC × HB	0.750	0.014	19.33	500.9	0.140	7.20
EC × W-36	0.751	0.010	13.51	352.5	0.130	7.48
FR × HB	0.754	0.015	20.10	486.5	0.140	7.02
FR × W-36	0.778	0.011	14.43	334.1	0.150	6.84
SEM	0.007	0.001	1.20	30.8	0.003	0.20
P-value						
Housing environment	0.0847	0.0255	0.0222	0.0517	0.0624	0.0911
Strain	0.0404	<0.0001	<0.0001	<0.0001	0.6961	0.9835
Age of the birds	<0.0001	0.0579	0.1514	0.3023	0.0012	0.0113
Environment × strain	0.3695	0.8232	0.8942	0.9207	0.3789	0.5051
Environment × age of birds	0.1793	0.7282	0.8130	0.7886	0.2665	0.4088
Strain × age of birds	0.2606	0.7322	0.6793	0.3059	0.2455	0.2194
Environment × strain × age of birds	0.1890	0.3331	0.4448	0.8538	0.1330	0.0943

Treatments	Tb.Pf (1/mm)	SMI	DA	Conn.	ConnD (1/mm^3)	Tb.Th.SD (mm)
Environment						
CC	6.94	1.712	1.763^{a}	791.3	66.3	0.052
EC	6.73	1.674	1.736^{a}	1001.5	63.7	0.050
FR	5.59	1.607	1.635^{b}	776.2	45.4	0.050
SEM	0.58	0.060	0.030	93.3	10.1	0.000
Strain						
HB	6.31	1.608	1.575^{b}	1106.6^{a}	67.4	0.052
W-36	6.49	1.717	1.840^{a}	612.5^{b}	49.4	0.049
SEM	0.47	0.051	0.027	76.1	8.2	0.001
Age of the birds						
38 wk	5.13^{b}	1.421^{b}	1.772^{a}	706.3	48.4	0.047^{b}
65 wk	7.19^{a}	1.876^{a}	1.612^{b}	977.2	58.6	0.053^{a}
85 wk	6.85^{a}	1.693^{a}	1.744^{a}	879.4	67.13	0.052^{a}
SEM	0.60	0.084	0.040	94.3	8.8	0.001
Environment × strain						
CC × HB	7.46	1.728	1.636	1102.3	85.9	0.052
CC × W-36	6.41	1.697	1.890	480.2	46.8	0.051
EC × HB	6.37	1.591	1.576	1261.3	68.7	0.052
EC × W-36	7.04	1.748	1.877	770.5	59.3	0.048
FR × HB	5.15	1.509	1.516	972.9	48.8	0.052
FR × W-36	6.02	1.705	1.755	579.4	41.9	0.048
SEM	0.82	0.088	0.047	131.9	14.3	0.002
P-value						
Housing environment	0.1618	0.3965	0.0404	0.1583	0.2298	0.6499
Strain	0.9438	0.2015	<0.0001	<0.0001	0.0932	0.0338
Age of the birds	0.0213	0.0014	0.0124	0.0617	0.2170	0.0132
Environment × strain	0.3454	0.3227	0.7768	0.6795	0.3439	0.7570
Environment × age of birds	0.5071	0.9295	0.8491	0.3136	0.4227	0.3383
Strain × age of birds	0.6277	0.4148	0.7219	0.2082	0.5664	0.9776
Environment × strain × age of birds	0.1193	0.2598	0.5514	0.2150	0.1241	0.7810

[a–c]Values within columns not sharing the superscripts are significantly different at $P \leq 0.05$.

Abbreviations: BMD, bone mineral density; BMC, bone mineral content; BV, bone volume; BS, bone surface; CC, conventional cage; Conn., trabecular connectivity; ConnD, trabecular connectivity density; DA, degree of anisotropy; EC, enriched colony cage; FR, free range; HB, Hy-Line Brown; SMI, structure model index; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Pf, trabecular pattern factor; Tb.Th.SD, SD of trabecular thickness; W-36, Hy-Line W-36.

hens housed in CC. Similar results were observed by previous researchers, wherein hens housed in the FR system, aviaries, litter, or EC had higher TBS compared with hens housed in CC (Newman and Leeson, 1998; Leyendecker et al., 2005; Fleming et al., 2006; Shipov et al., 2010; Regmi et al., 2016b; Yilmaz Dikmen et al., 2016). Hens housed in the CC had relatively limited space for locomotor activities, whereas hens housed in

Table 7. The correlation among total volume of interest properties (BMD, BMC, and BVP), tibia bone properties (DBP, TBS, and AP), and eggshell qualities (SP and EBS).

Measured parameters	BW (kg)	BMD (g/cm^3)	BMC (g)	BV (mm^3)	DBP (%)	TBS (kgF)	AP (%)
BMD (g/cm^3)	0.021						
BMC (g)	0.525****	0.752****					
BV (mm^3)	−0.031	0.898****	0.703****				
DBP (%)	−0.026	0.458****	0.239*	0.411****			
TBS (kgF)	−0.172	0.606****	0.310**	0.607****	0.461****		
AP (%)	−0.066	0.261**	0.090	0.309**	−0.207*	0.242*	
SP (%)	0.244*	−0.339***	−0.033	−0.251*	−0.511****	−0.415****	0.242*
ST (mm)	0.168	−0.243*	−0.027	−0.144	−0.525****	−0.329***	0.370****
EBS (kgF)	0.253	−0.058	0.151	−0.038	−0.352**	−0.081	0.320**

*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.
****$P < 0.0001$.
Abbreviations: AP, tibia ash percentage; BMD, bone mineral density; BMC, bone mineral content; BV, bone volume; DBP, dried bone percentage; EBS, eggshell breaking strength; SP, eggshell percentage; ST, eggshell thickness; TBS, tibia breaking strength.

the EC and FR system had an opportunity for load-bearing activities such as perching, flying, and running. The provision of exercise in the FR system contributed to higher breaking strength (Whitehead and Fleming, 2000; Leyendecker et al., 2005). Furthermore, Fleming et al. (1994) observed excessive endosteal erosion of the cortical bone when limited exercise was provided to the hens housed in CC. They explained this as an adaptive bone remodeling, wherine external physical stimuli such as exercise or load-bearing activities promote maintenance of the bone mass, thus improving breaking strength. Similarly, Newman and Leeson (1998) observed higher TBS when laying hens were moved from the CC to the aviary system, suggesting that some mechanism might have been involved in stimulating the formation of structural bone rather than inhibiting calcium resorption. Besides, higher breaking strength in hens raised in the FR system might also be correlated with higher ash percentage, which measures the bone's total mineral contents, including calcium, which is a structural component of bone. Rowland et al. (1972) and Fleming et al. (2006) observed the difference among the laying hen strains in terms of TBS. They correlated breaking strength with egg production and ash percentage; the lower the egg production, the higher the breaking strength. In our case, overall egg production was higher in the HB hens (Sharma et al., 2020) than in W-36 hens, whereas ash percentage was similar in both strains. Sparke et al. (2002) reported that tensile strength of the bone is provided by intermolecular cross-linking of collagen fibers, which is affected by the genetics of the hen. The difference in breaking strength among the laying hen strains in our study might be due to the cross-linking of collagen fibers, which were beyond the scope of our study.

In our study, we did not observe significant differences in the measured parameters from total volume of interest and medullary bone of the femur among any of the housing environments. However, we observed a trend for cortical and trabecular BMD, which were higher in hens raised in the FR system than in hens raised in CC and EC. Silversides et al. (2012) observed higher tibial BMD in hens raised in the floor pen relative to hens raised in the CC but did not observe any difference in cortical and trabecular bone. Regmi et al. (2016a) observed different results for BMD when different sections of the bone were analyzed and explained this difference might be due to the site-specific skeletal response and anisometric nature of the bone. Insertion points of muscles along with other soft tissues and natural curvatures of the bones might create differences in structural composition within a bone known as site-specific skeletal responses (Regmi et al., 2016b). Comparatively, lower cortical and trabecular BMD in hens raised in the CC and EC might be due to the excessive endosteal bone loss due to restricted movement (Jendral et al., 2008; Shipov et al., 2010). Mechanical strength of the bone is not only solely dependent on BMD but also depends on the complex architecture of the trabecular bone whose primary purpose is to distribute the mechanical load and provide strength to the bone (Hordon et al., 2000). The architectural structure of the trabecular bone was mostly affected by the housing environments. Trabecular BV, BMC, and Tb.Th were all higher in hens raised in the FR system than in hens raised in CC, which might have given the strength to the tibias. In addition, trabecular BV and thickness was lower in the hens raised in CC relative to the hens raised in the FR system. Lower trabecular BV and thickness in the hens raised in CC might be due to the excessive resorption of calcium by osteoclast cells rather than deposition by osteoblast cells (Belanger, 1963; Taylor and Belanger, 1969; Wilson, 1994). A strong correlation between trabecular BMD and eggshell percentage ($R = -0.475$) and BMD and eggshell thickness ($R = -0.418$) was observed in our result, which also supports this hypothesis. Greater Tb.N was observed in the hens raised in the CC, which might be to regulate calcium homeostasis in the body for structural calcium loss and distribute the mechanical load as hens in the CC spend most of the time standing.

Based on the results from the microCT analysis, W-36 hens had superior microstructural architecture of the total volume of interest, cortical bone, and trabecular bone of the femur throughout the experimental period

compared with HB hens. However, higher values for medullary bone properties were observed in the HB hens compared to W-36 hens. Cortical and trabecular BMD was higher in W-36 hens than in HB hens; however, total and trabecular BMC was higher in HB hens than in W-36 hens. These differences might be due to the difference in egg production (Sharma et al., 2020), wherein overall hen day egg production eggshell percentage, and eggshell thickness were higher in the HB hens than in W-36 hens. Although total and cortical BV was higher in the HB hens, higher BV/TV was observed in the W-36 group, which further supports the fact that higher mobilization of bone for egg production might occur. In addition, total volume of pores and VOP were higher in the HB hens, which might have formed during resorption of bone for eggshell formation. Mature osteocytes help in bone resorption by attacking the tissues from the inside of calcified tissue, which might have formed pores in the cortical bones (Belanger, 1963). For medullary bone properties, BMC, BV, and BV/TV were higher in HB hens than in W-36 hens, which further supports the hypothesis that there might be some variation for mobilization of bone for eggshell formation. Formation and resorption of medullary bone is a sequential process and is related to egg production (Bloom et al., 1958). Medullary bone formation occurs at the expense of the structural bone in response to estrogen, and some part of calcium is derived from structural bones for formation of medullary bones (Hurwitz 1964). A higher level of estrogen stimulates osteoblastic function and inhibits osteoclastic function. The increase in the estrogen level during the laying period changes the function of osteoblasts toward the formation of medullary bone rather than structural bone. When egg production decreases or reproductive function ceases, formation of medullary bone decreases, whereas formation of structural bone increases in response to the level of estrogen (Whitehead, 2004). Both the estrogen level and reproductive activity of the laying hen have an impact on the volume of the medullary bone (Gilbert, 1983). The difference in the amount of the medullary bone depends on absorption, storage, and utilization of calcium for eggshell formation (Bloom et al., 1958). Wilson (1994) observed widespread medullary bone in hens, which were in the middle of the lay, and bone remodeling process during the egg laying cycle resulted in increased BV. In our study, in addition to egg production, there might be a higher rate of bone remodeling in HB hens than in W-36 hens because of the difference in eggshell properties, which might have affected BV, BV/TV, and BMC of medullary bone. Furthermore, a negative correlation was observed between total and cortical BMD and BV with respect to eggshell properties (eggshell percentage and eggshell thickness). Previously, Silversides et al. (2012) observed the variation due to strains while comparing the 4 strains of laying hens (Lohmann Brown, Lohmann White, H&W White, and a cross of Rhode Island Red and Barred Plymouth Rock hens). In their study, higher total and trabecular BMD was observed in the crossbreed, Lohmann White, and H&N White than in Lohmann Brown. However, BMC was higher in the Lohmann Brown and crossbreed than in white strains, which might be related to the difference in egg production. Similarly, Regmi et al. (2016a) observed differences in cortical bone density and thickness among the Barred Plymouth Rock, HB, and Hy-Line Silver Brown. Barred Plymouth Rock had higher cortical thickness and BMD than the others, but had lower egg production. Besides, genetic selection of the hens for more egg production resulted in reduction of bone properties (Bishop et al., 2000; Webster 2004). Previously, bone properties such as breaking strength, BMD, and cortical area were compared between 2 lines (egg production and bone properties). Higher bone properties were observed for hens selected for bone properties rather than egg production (Bishop et al., 2000; Sparke et al., 2002; Fleming et al., 2006). Although most of the measured tibia and femur properties were observed to be higher in the hens raised in the FR sytem and W-36 hens, we did not observe any signs of lameness or osteoporosis in any of the housing environments or laying hen strains.

Most of the measured femur bone properties, including BV, BMD, BMC, and BV/TV, increased with age from 38 to 65 wk of age. A previous study by Hudson et al. (1993) did not observe any structural bone remodeling (growth) based on the osteon's activity (secondary osteons) after hens get sexually matured. However, cortical and trabecular BV, BV/TV, BMD, and BMC were increased in our study as the hens aged, suggesting that the structural bone growth still occurs after the formation of the medullary bone. For the medullary bone, BV, BV/TV, BMD, and BMC were lowest at 38 wk of age, highest at 65 wk of age, and in between at 85 wk of age. The increase in the medullary bone properties at an older age might be related to a lower level of estrogen owing to decrease in egg production, thus inhibiting osteoclastic activity (Whitehead, 2004). Similarly, total and cortical volume of pores and PP were lower at 65 and 85 wk of age, whereas Tb.Th, pattern factor, Conn., and DA were higher at 65 and 85 wk of age, which might be related to higher TBS at these ages.

The results of this study provide further evidence to support that the housing environment and laying hen strain influence architectural and mechanical properties of bones. The results further suggest that raising laying hens in the extensive housing systems with provision for exercise reduces structural bone loss, stimulates structural bone formation, and improves breaking strength of the bones. In addition, there might have been some differences in calcium mobilization from bones for eggshell formation between HB and W-36 hens, and future research is needed to study this phenomenon. Further studies might be needed on cross-linking collagen molecules to understand the difference in mechanical strength among such house environments and laying hen strains.

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DISCLOSURES

The authors have no conflict of interests toward submission and publication of this manuscript.

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