

# ENVIRONMENT, WELL-BEING, AND BEHAVIOR

## Comparison of bones of 4 strains of laying hens kept in conventional cages and floor pens<sup>1,2</sup>

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**ABSTRACT** The maintenance of bone strength has been an important issue in the debate over cage use for laying hens. Bone strength depends on adequate mechanical load and cages restrict movement. Four laying crosses (Lohmann White, Lohmann Brown, H&N White, and Rhode Island Red × Barred Plymouth Rock cross hens) were housed in conventional cages or in floor pens equipped with perches and nest boxes to measure the effect of the housing system on bone strength. Approximately 15 hens of each genotype from each housing system were killed at 50 wk of age and the radius and tibia of each were removed for analysis. There were no differences between the Lohmann White and H&N White (White Leghorn) hens, likely because of their similar genetic background. The Lohmann Brown and the cross hens (brown-egg layers) were

larger and they had heavier bones, but the bone density was not different from that of the other lines. The radius was heavier for hens kept in floor pens than for those kept in cages, but the tibia was not. When hens were kept in floor pens, both bones had greater cortical bone density and cross-sectional area, but the difference between housing systems in cortical bone cross-sectional area was much greater for the radius than it was for the tibia. Although the movement of hens in cages is limited, they spend a great deal of time standing, which puts a mechanical load on the tibia. Hens in floor pens are able to stretch their wings or fly, in contrast to hens kept in cages, which likely explains why the difference between housing systems in cortical bone was greater for the radius than for the tibia.

**Key words:** layer strain, environment, bone, radius, tibia

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## INTRODUCTION

Beginning in the 1950s, industrial laying hens were taken off of the range or out of floor pens and put into cages to simplify management, keep the eggs clean, and reduce disease. Society has debated the effects of this change on hen welfare since that time. Over the past several decades, the debate has intensified (Savory, 2004), leading to the current or planned banning of standard laying cages in the European Union, several individual European countries, California, Manitoba, and likely other jurisdictions, with various phase-in periods for their elimination. Most of the debate has been because of the small size of standard laying cages restricting certain behaviors that are expressed in wild

chickens or those kept in extensive systems. However, a significant factor in the move to ban cages is the effect that cage housing has on bone strength (Leyendecker et al., 2005; Fleming et al., 2006).

Bone strength is a particular problem for modern layers (Gregory and Wilkins, 1989; Budgell and Silversides, 2004) because of the intense selection that the strains have been subjected to and the demands that the extremely high egg production places on calcium and calcium metabolism. Although bone strength was not likely part of the industrial selection programs during the greater part of the last 60 yr, the genetic determination of bone strength is high, as determined by selection experiments (Bishop et al., 2000; Fleming et al., 2006) and the observation of differences between lines (Hocking et al., 2003; Silversides et al., 2006).

The environmental restriction placed on hens in standard laying cages that is most important for bone strength is the space limitation that limits wing movement and walking, although R. Singh (University of British Columbia; unpublished data) found that hens in cages spend 3 times as much time standing as those

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in floor pens. Reduced activity reduces bone strength (Knowles and Broom, 1990), although the effect is not general, it affects the bones of the specific limb that is not exercised.

Singh et al. (2009) described a trial in which they compared egg production parameters of 2 commercial white-egg layers, a commercial brown-egg layer, and an experimental cross when hens were kept in cages or floor pens. They found no difference in overall egg production between housing systems, but BW of commercial strains were greater in floor pens. The current study aimed to investigate the effects of strain and housing system on characteristics of the radius and tibia of these hens.

## MATERIALS AND METHODS

### *Experimental Design*

Female 1-d-old Lohmann White (**LW**), Lohmann Brown (**LB**), and H&N White (**HN**) chicks were obtained from Pacific Pride Chicks (Abbotsford, British Columbia, Canada). Chicks from a cross between Rhode Island Red males and Barred Plymouth Rock females (**Cross**; Silversides, 2010) were hatched at the Agassiz Research Centre (Canada) where males and females were separated based on the barring phenotype of the males and the nonbarring phenotype of the females. As described by Singh et al. (2009), chicks were either reared and housed at 18 wk of age in conventional cages with 688 cm<sup>2</sup> per bird or housed in floor pens with 6,115 to 6,990 cm<sup>2</sup> per bird. The conventional cages were equipped only with feeders and nipple waterers and had sloped floors to facilitate egg removal, whereas the floor pens were equipped with a perch assembly and nest boxes from the second wk of age. The procedures used were approved by the Animal Care Committee of the Agassiz Research Centre (Canada) and followed the guidelines of the Canadian Council of Animal Care (CCAC, 2009).

Within 2 wk after the completion of the laying trial (50 wk of age), approximately 15 hens of each genotype in each housing system (total 121) were euthanized by cervical dislocation and the radii and tibiae were removed. Sampling was distributed throughout cages and pens and the birds were treated as the experimental unit. We have no reason to believe that there is common variation due to the pen or cage and have treated this statistically as randomly sampled within each environment. The bones were cleaned of skin and flesh and stored at -20°C. Bones from the right side of the birds were used for ash determination and those from the left side were used for quantitative computed tomography (**QCT**). The bones kept for ash determination were subsequently thawed, weighed, dried at 100°C for 8 h, weighed again, ashed in a muffle furnace at 600°C for 6 h, and weighed again. The bones used for QCT were thawed and immersed in 10% (wt/vol) formalin for 1 wk, then rinsed in distilled water and shipped wet to

the University of Alberta (Canada). At the University of Alberta, the bone mineral density and cross-sectional area of the total bone, trabecular bone, and cortical bone were measured as described by Korver et al. (2004) and Jendral et al. (2008) using a Stratec XCT scanner (Norland Medical Systems Inc., Fort Atkinson, WI). The total bone measurement represents the weighted average of the cortical and trabecular bone densities and the area within the external perimeter of the bone at the 1-mm X-ray beam width. Cortical bone represents the outer shell of structural bone, and trabecular bone is the bone in the trabecular space within the cortical shell and it is assumed to include medullary bone (Saunders-Blades et al., 2009). Measurements were taken at a point representing 25% of the total length of the bone relative to the proximal end. A measure of total mineral content was obtained by multiplying the mineral density (mg/mm<sup>3</sup>) by the cross-sectional area (mm<sup>2</sup>) and was extrapolated to a 1-cm length of bone. This calculation gives the amount of bone mineral contained within a 1-cm-long cross-sectional slice of bone, and it would be analogous to determining the ash content of a small portion of the bone.

The use of perches by hens in floor pens was observed at 27 to 28 wk of age for 4 consecutive days. Ten minutes before the lights going off, the hens on perches, on the floor, or elsewhere in the pen were counted.

### *Statistical Analysis*

Data were analyzed by ANOVA using the GLM procedure of SAS (version 9.1, SAS Institute Inc., Cary, NC; Littell et al., 1991) with the main effects of strain, housing system, and the interaction between the 2. Interactions were investigated with a 2-way ANOVA that included the main effect of the subgroups. Data on perch use in floor pens (average of 4 d) were analyzed with strain as a fixed effect. When an effect was significant at  $P < 0.05$ , the means were separated using Duncan's multiple range test. Although BW has an influence on bone mineral density (Williams et al., 2000; Hocking et al., 2003), it was not used as a covariate in this study to more clearly understand the effect of strain.

## RESULTS

As shown in Tables 1 and 2, the White Leghorn crosses (HN and LW) were not different from each other in BW, any of the measures of bone weight (wet, dry, and ash), or percentages of dry weight or ash. The heavier lines (LB and Cross) had heavier bones than the White Leghorns (wet, dry, and ash), but the percentages of dry weight and ash of the radius were not different from those of the White Leghorns. For the tibia, the wet, dry, and ash weights of the Cross hens were greater than those for the LB hens, as was the percentage of dry weight but not the percentage of ash. The White Leghorn crosses were heavier in floor pens

Table 1. Body weights and weights of the radius and tibia of 4 strains of laying hens kept in 2 housing systems<sup>1</sup>

Item	Radius						Tibia																																																																																																																																																				
	BW50 <sup>2</sup>			Dry			Ash			Wet			Dry			Ash																																																																																																																																											
	(g)	(g)	(% of wet)	(g)	(g)	(% of dry)	(g)	(g)	(% of dry)	(g)	(g)	(g)	(g)	(g)	(% of wet)	(g)	(g)	(% of dry)																																																																																																																																									
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Cross	2,202	1,286 <sup>a</sup>	0.958 <sup>a</sup>	0.747	0.472 <sup>a</sup>	0.485	15.443 <sup>a</sup>	10.645 <sup>a</sup>	0.690 <sup>a</sup>	4.436 <sup>a</sup>	0.416	LB	1,981	1,259 <sup>a</sup>	0.941 <sup>a</sup>	0.750	0.462 <sup>a</sup>	0.491	14.459 <sup>b</sup>	9.390 <sup>b</sup>	0.650 <sup>b</sup>	3.898 <sup>b</sup>	0.414	HN	1,554	0.959 <sup>b</sup>	0.702 <sup>b</sup>	0.732	0.341 <sup>b</sup>	0.486	10.405 <sup>c</sup>	6.604 <sup>c</sup>	0.636 <sup>b</sup>	2.800 <sup>c</sup>	0.427	LW	1,562	0.982 <sup>b</sup>	0.704 <sup>b</sup>	0.720	0.341 <sup>b</sup>	0.492	10.971 <sup>c</sup>	7.015 <sup>c</sup>	0.640 <sup>b</sup>	2.950 <sup>c</sup>	0.422	SEM	38.5	0.0309	0.0223	0.0113	0.0101	0.0086	0.3430	0.2336	0.0055	0.1259	0.0093	Housing												Cage	1,763	1.063 <sup>b</sup>	0.755 <sup>b</sup>	0.714 <sup>b</sup>	0.353 <sup>b</sup>	0.469 <sup>b</sup>	12.882	8.284	0.641 <sup>b</sup>	3.270 <sup>b</sup>	0.398 <sup>b</sup>	Floor	1,881	1.178 <sup>a</sup>	0.896 <sup>a</sup>	0.761 <sup>a</sup>	0.454 <sup>a</sup>	0.507 <sup>a</sup>	12.701	8.507	0.666 <sup>a</sup>	3.762 <sup>a</sup>	0.443 <sup>a</sup>	SEM	27.2	0.0219	0.0158	0.0080	0.0072	0.0061	0.2426	0.1652	0.0039	0.0891	0.0066	P-value												Strain	0.010	<0.001	<0.001	0.279	<0.001	0.866	<0.001	<0.001	<0.001	<0.001	0.751	Housing	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.584	0.350	<0.001	<0.001	<0.001	Strain × housing	<0.001	0.122	0.389	0.694	0.353	0.757	0.109	0.117	0.501	0.809	0.836
LB	1,981	1,259 <sup>a</sup>	0.941 <sup>a</sup>	0.750	0.462 <sup>a</sup>	0.491	14.459 <sup>b</sup>	9.390 <sup>b</sup>	0.650 <sup>b</sup>	3.898 <sup>b</sup>	0.414	HN	1,554	0.959 <sup>b</sup>	0.702 <sup>b</sup>	0.732	0.341 <sup>b</sup>	0.486	10.405 <sup>c</sup>	6.604 <sup>c</sup>	0.636 <sup>b</sup>	2.800 <sup>c</sup>	0.427	LW	1,562	0.982 <sup>b</sup>	0.704 <sup>b</sup>	0.720	0.341 <sup>b</sup>	0.492	10.971 <sup>c</sup>	7.015 <sup>c</sup>	0.640 <sup>b</sup>	2.950 <sup>c</sup>	0.422	SEM	38.5	0.0309	0.0223	0.0113	0.0101	0.0086	0.3430	0.2336	0.0055	0.1259	0.0093	Housing												Cage	1,763	1.063 <sup>b</sup>	0.755 <sup>b</sup>	0.714 <sup>b</sup>	0.353 <sup>b</sup>	0.469 <sup>b</sup>	12.882	8.284	0.641 <sup>b</sup>	3.270 <sup>b</sup>	0.398 <sup>b</sup>	Floor	1,881	1.178 <sup>a</sup>	0.896 <sup>a</sup>	0.761 <sup>a</sup>	0.454 <sup>a</sup>	0.507 <sup>a</sup>	12.701	8.507	0.666 <sup>a</sup>	3.762 <sup>a</sup>	0.443 <sup>a</sup>	SEM	27.2	0.0219	0.0158	0.0080	0.0072	0.0061	0.2426	0.1652	0.0039	0.0891	0.0066	P-value												Strain	0.010	<0.001	<0.001	0.279	<0.001	0.866	<0.001	<0.001	<0.001	<0.001	0.751	Housing	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.584	0.350	<0.001	<0.001	<0.001	Strain × housing	<0.001	0.122	0.389	0.694	0.353	0.757	0.109	0.117	0.501	0.809	0.836												
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<sup>a-c</sup>Means within main effect without a common superscript are different at  $P < 0.05$ .

<sup>1</sup>The number of samples is 13 to 17 for each combination of strain and housing system.

<sup>2</sup>BW50 = BW at 50 wk of age.

<sup>3</sup>Cross = Rhode Island Red × Barred Plymouth Rock; LB = Lohmann Brown; HN = H&N; LW = Lohmann White.

than in cages (Table 2) and the Cross hens weighed less in the floor pens than cages. The BW of the LB hens was not different between the 2 housing systems. The housing system affected all bone weights and the ash of the radius, with the floor pens resulting in higher values. The housing system did not affect wet or dry weight of the tibia, but the percentage of dry weight, ash weight, and the percentage of ash were all greater for the tibia of birds kept on the floor. The differences in the radius and tibia among lines and housing systems were investigated further by measuring the total, trabecular, and cortical bone cross-sectional areas, densities, and mineral contents.

Total bone measurements of the radius (Table 3) for the 2 White Leghorn crosses were not different from each other, but the heavier strains differed for several measurements. The cross-sectional area of the radius was greater for the 2 heavier strains than for the White Leghorns, and the Cross hens had a greater cross-sectional area than the LB hens in the cages but not in the floor pens (Table 2). Hens in floor pens had greater radius bone density than those in cages (Table 3). The White Leghorn strains were not different for trabecular or cortical bone densities or cross-sectional areas. The LB hens had greater trabecular bone density than the other breeds, and the cross-sectional area of trabecular bone was greatest for the heavier breeds, with no difference between the Cross and HN hens. The area of trabecular bone in the radius was greater for hens kept in the floor pens than in the cages. The LB and Cross hens in cages had greater cortical bone density compared with that of the White Leghorn hens, but the strains were equal in the floor pens (Table 3). The area of cortical bone was greatest for the Cross hens, intermediate for the LB hens, and lowest for the White Leghorn hens.

The total density of the tibia was lowest for the LB hens (Table 4), but the cross-sectional area of the tibia of both heavy breeds was greater than that of the White Leghorns. The total bone density was greatest in the floor pens, but the cross-sectional area of the tibia was not affected by the housing system. The heavier breeds had lower trabecular bone density than the White Leghorns, with that of the Cross hens being lowest and with no difference between the LB and LW hens. The cross-sectional area of trabecular bone in the tibia was highest for the LB hens and lowest for the White Leghorn hens, with that of the Cross hens being intermediate. In the tibia, the housing system did not affect trabecular bone density, but the trabecular area was higher for birds in the cages compared with that of those in the floor pens.

The cortical density of the tibia was the same for all strains in the floor pens, and the values for the Cross and LB hens were not different between the floor pens and the cages (Table 2). However, the White Leghorn hens had greater cortical density when kept in floor pens than when kept in cages. The White Leghorns were not different for the cortical area of the tibia and

**Table 2.** Interactions between strain of layer and housing system (cage vs. floor) for bone measures shown in Tables 1, 3, and 4<sup>1</sup>

Strain <sup>2</sup>	BW50 <sup>3</sup> (g)		Radius total cross-sectional area (mm <sup>2</sup> )		Radius cortical density (mg/cm <sup>3</sup> )		Tibia cortical density (mg/cm <sup>3</sup> )		Tibia cortical cross-sectional area (mm <sup>2</sup> )	
	Cage	Floor	Cage	Floor	Cage	Floor	Cage	Floor	Cage	Floor
Cross	2,286 <sup>a</sup>	2,130 <sup>b</sup>	8.01 <sup>b</sup>	9.31 <sup>a</sup>	1,018 <sup>c</sup>	1,054 <sup>ab</sup>	953 <sup>b</sup>	979 <sup>b</sup>	29.47 <sup>b</sup>	35.90 <sup>a</sup>
LB	1,949 <sup>c</sup>	2,019 <sup>bc</sup>	7.23 <sup>c</sup>	9.31 <sup>a</sup>	1,039 <sup>b</sup>	1,063 <sup>a</sup>	947 <sup>b</sup>	973 <sup>b</sup>	22.18 <sup>c</sup>	26.43 <sup>b</sup>
HN	1,406 <sup>e</sup>	1,692 <sup>d</sup>	5.39 <sup>d</sup>	7.55 <sup>bc</sup>	990 <sup>d</sup>	1,057 <sup>ab</sup>	946 <sup>b</sup>	1,049 <sup>a</sup>	20.71 <sup>c</sup>	19.76 <sup>c</sup>
LW	1,464 <sup>e</sup>	1,682 <sup>d</sup>	5.32 <sup>d</sup>	7.35 <sup>c</sup>	1,005 <sup>cd</sup>	1,053 <sup>ab</sup>	968 <sup>b</sup>	1,046 <sup>a</sup>	20.70 <sup>c</sup>	20.72 <sup>c</sup>
SEM	54.1		0.168		7.0		13.7		1.415	

<sup>a-c</sup>Means of measurements without a common letter are different at  $P < 0.05$ .

<sup>1</sup>The number of samples is 13 to 17 for each combination of strain and housing system.

<sup>2</sup>Cross = Rhode Island Red × Barred Plymouth Rock; LB = Lohmann Brown; HN = H&N; LW = Lohmann White.

<sup>3</sup>BW50 = BW at 50 wk of age.

there was no difference between these strains when kept in pens or floor pens. However, values for the Cross and LB hens were both greater when hens were kept in floor pens than when kept in cages.

The Cross hens had the greatest and the White Leghorn hens the least total and cortical bone mineral content in both the radius and the tibia (Table 5). Radius trabecular bone mineral content was greatest in the LB and lowest in the LW hens, and tibia trabecular bone mineral content was lowest in Cross hens. Hens housed in floor pens had greater mineral content in all fractions of bone except for the trabecular bone in the tibia. Tibia trabecular bone mineral content of LB hens was greater than that of White Leghorn hens in cages, but this difference was not significant when the hens were housed in floor pens (Table 6). In the radius, the cage by strain interaction for the trabecular bone mineral content was not significant ( $P = 0.06$ ) but a similar

effect was seen, with much lower values for the White Leghorns in cages.

Use of perches (Table 7) just before lights out by white-egg strains (LW and HN) was much higher than that by brown-egg strains (LB and Cross). Nearly all of the brown-egg hens were found on the floor when observations were made.

## DISCUSSION

The tibia is often used by researchers (Knowles and Broom, 1990; Zhang and Coon, 1997; Tactacan et al., 2009) to represent the leg of a chicken, but both the humerus (Knowles and Broom, 1990; Tactacan et al., 2009) and the radius (Clark et al., 2008) have been studied to represent the wing. In this study, the tibia was used to represent the leg and the radius was used for the wing because the humerus can have variable

**Table 3.** Total bone mineral density and cross-sectional area of the radius of 4 strains of laying hens kept in 2 housing systems to 50 wk of age<sup>1</sup>

Item	Radius					
	Total <sup>2</sup>		Trabecular <sup>3</sup>		Cortical <sup>4</sup>	
	Density (mg/cm <sup>3</sup> )	Area (mm <sup>2</sup> )	Density (mg/cm <sup>3</sup> )	Area (mm <sup>2</sup> )	Density (mg/cm <sup>3</sup> )	Area (mm <sup>2</sup> )
Strain <sup>5</sup>						
Cross	788	8.66	197 <sup>b</sup>	2.48 <sup>ab</sup>	1,036	6.30 <sup>a</sup>
LB	783	8.27	241 <sup>a</sup>	2.67 <sup>a</sup>	1,051	5.63 <sup>b</sup>
HN	751	6.53	196 <sup>b</sup>	2.15 <sup>bc</sup>	1,027	4.51 <sup>c</sup>
LW	785	6.26	191 <sup>b</sup>	1.81 <sup>c</sup>	1,025	4.55 <sup>c</sup>
SEM	14.5	0.119	11.8	0.150	4.9	0.116
Housing						
Cage	762 <sup>b</sup>	6.48	195	1.97 <sup>b</sup>	1,013	4.58 <sup>b</sup>
Floor	791 <sup>a</sup>	8.39	218	2.60 <sup>a</sup>	1,057	5.92 <sup>a</sup>
SEM	10.2	0.084	8.3	0.106	3.5	0.082
<i>P</i> -value						
Strain	0.184	<0.001	0.012	<0.001	<0.001	<0.001
Housing	0.040	<0.001	0.050	<0.001	<0.001	<0.010
Strain × housing	0.301	0.043	0.645	0.055	0.016	0.498

<sup>a-c</sup>Means within main effect without a common letter are different at  $P < 0.05$ .

<sup>1</sup>The number of samples is 13 to 17 for each combination of strain and housing system.

<sup>2</sup>Weighted average of trabecular and cortical bone mineral density within the cross-sectional area of the bone at the point of measurement.

<sup>3</sup>Density and cross-sectional area of bone mineral contained within the interior of the cortical bone shell.

<sup>4</sup>Density and cross-sectional area of bone mineral contained in the cortical (outer) shell of the bone.

<sup>5</sup>Cross = Rhode Island Red × Barred Plymouth Rock; LB = Lohmann Brown; HN = H&N; LW = Lohmann White.

**Table 4.** Bone mineral density and cross-sectional area of the tibia of 4 lines of chickens kept in 2 housing systems to 50 wk of age<sup>1</sup>

Item	Tibia					
	Total <sup>2</sup>		Trabecular <sup>3</sup>		Cortical <sup>4</sup>	
	Density (mg/cm <sup>3</sup> )	Area (mm <sup>2</sup> )	Density (mg/cm <sup>3</sup> )	Area (mm <sup>2</sup> )	Density (mg/cm <sup>3</sup> )	Area (mm <sup>2</sup> )
Strain <sup>5</sup>						
Cross	591 <sup>a</sup>	59.94 <sup>a</sup>	106 <sup>c</sup>	24.3 <sup>b</sup>	966	32.7
LB	515 <sup>b</sup>	58.57 <sup>a</sup>	167 <sup>b</sup>	29.9 <sup>a</sup>	960	24.3
HN	598 <sup>a</sup>	40.36 <sup>b</sup>	200 <sup>a</sup>	19.2 <sup>c</sup>	1,000	20.2
LW	608 <sup>a</sup>	41.20 <sup>b</sup>	186 <sup>ab</sup>	18.4 <sup>c</sup>	1,005	20.7
SEM	14.6	0.835	10.5	1.10	9.7	1.00
Housing						
Cage	542 <sup>b</sup>	50.10	161	24.2 <sup>a</sup>	953	23.3
Floor	613 <sup>a</sup>	49.90	168	21.8 <sup>b</sup>	1,012	25.7
SEM	10.3	0.590	7.4	0.720	6.9	0.71
<i>P</i> -value						
Strain	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Housing	<0.001	0.803	0.535	0.025	<0.001	0.017
Strain × housing	0.743	0.210	0.118	0.152	0.009	0.032

<sup>a-c</sup>Means within main effect without a common letter are different at  $P < 0.05$ .

<sup>1</sup>The number of samples is 13 to 17 for each combination of strain and housing system.

<sup>2</sup>Weighted average of trabecular and cortical bone mineral density within the cross-sectional area of the bone at the point of measurement.

<sup>3</sup>Density and cross-sectional area of bone mineral contained within the interior of the cortical bone shell.

<sup>4</sup>Density and cross-sectional area of bone material contained in the cortical (outer) shell of the bone.

<sup>5</sup>Cross = Rhode Island Red × Barred Plymouth Rock; LB = Lohmann Brown; HN = H&N; LW = Lohmann White.

amounts of medullary bone (Clark et al., 2007), which can confound the results.

Two commercial White Leghorn crosses (LW and HN) were included in this trial and were not different for any measure of the bones studied. This similarity could be because the crosses both originated from the White Leghorn breed, because industrial white-egg layers likely all include Mount Hope breeding (Hunton,

2008), or because the 50-yr association between Lohmann and H&N (Tierzucht, 2009) may have led to similar selection histories.

There is a positive association between BW and bone size in some housing systems (Knowles and Broom, 1990). In a previous analysis (F. G. Silversides, unpublished), including BW as a covariate eliminated the effect of the strain because the strains were of different

**Table 5.** Mineral content of the radius and tibia of 4 strains of laying hens kept in 2 housing systems to 50 wk of age<sup>1,2</sup>

Item	Mineral content (mg/cm)					
	Radius			Tibia		
	Total <sup>3</sup>	Trabecular <sup>4</sup>	Cortical <sup>5</sup>	Total <sup>3</sup>	Trabecular <sup>4</sup>	Cortical <sup>5</sup>
Strain <sup>6</sup>						
Cross	6,823 <sup>a</sup>	486 <sup>b</sup>	6,535 <sup>a</sup>	35,443 <sup>a</sup>	2,764	31,561 <sup>a</sup>
LB	6,446 <sup>b</sup>	629 <sup>a</sup>	5,921 <sup>b</sup>	30,067 <sup>b</sup>	5,027	23,319 <sup>b</sup>
HN	4,909 <sup>c</sup>	402 <sup>bc</sup>	4,690 <sup>c</sup>	24,050 <sup>c</sup>	3,707	19,953 <sup>c</sup>
LW	4,905 <sup>c</sup>	336 <sup>c</sup>	4,651 <sup>c</sup>	24,909 <sup>c</sup>	3,362	20,745 <sup>c</sup>
SEM	110.5	34.8	119.3	858.0	290.4	899.6
Housing						
Cage	4,912 <sup>b</sup>	370 <sup>b</sup>	4,644 <sup>b</sup>	26,860 <sup>b</sup>	3,786	21,996 <sup>b</sup>
Floor	6,630 <sup>a</sup>	558 <sup>a</sup>	6,253 <sup>a</sup>	30,346 <sup>a</sup>	3,656	25,766 <sup>a</sup>
SEM	78.2	24.6	84.4	606.7	205.4	636.1
<i>P</i> -value						
Strain	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Housing	<0.001	<0.001	<0.001	<0.001	0.604	<0.001
Strain × housing	0.894	0.058	0.430	0.481	0.045	0.010

<sup>a-c</sup>Means within main effect without a common letter are different at  $P < 0.05$ .

<sup>1</sup>The number of samples is 13 to 17 for each combination of strain and housing system.

<sup>2</sup>Bone mineral content was obtained by multiplying the mineral density (mg/mm<sup>3</sup>) by the cross-sectional area (mm<sup>2</sup>), and extrapolated to a 1-cm length of bone.

<sup>3</sup>Weighted average of trabecular and cortical bone mineral density within the cross-sectional area of the bone at the point of measurement.

<sup>4</sup>Density and cross-sectional area of bone mineral contained within the interior of the cortical bone shell.

<sup>5</sup>Density and cross-sectional area of bone material contained in the cortical (outer) shell of the bone.

<sup>6</sup>Cross = Rhode Island Red × Barred Plymouth Rock; LB = Lohmann Brown; HN = H&N; LW = Lohmann White.

weights. In this case, the strain and BW are confounded, so either the BW can be included as a covariate, which may eliminate the effect of strain, or the effect that the BW difference between strains might have on bone strength can be discussed. We have taken the second approach that allows us to investigate the effect of the strain. As expected, the larger brown-egg layers had heavier bones, both in weight and total area, than the White Leghorn crosses, and the larger Cross hens had heavier tibias than those of the LB hens. The heavier bones resulted in greater values for dry weight and ash, but the percentage ash of dry weight and cortical bone density were not affected by strain, suggesting that bone mineralization was similar for all 4 strains.

The LB hens had higher trabecular bone density in the radius than other hens, and the Cross hens had lower trabecular bone density in the tibia. Trabecular bone makes a minor contribution to bone strength (Fleming et al., 1998) and it cannot be distinguished from medullary bone using QCT (Korver et al., 2004). The lower trabecular bone density for the Cross hens may thus reflect their lower rate of egg production (Singh et al., 2009) rather than a difference in bone strength.

The radius, representing the wing, was heavier for hens in floor pens than for those in cages by all measures, but the tibia, representing the leg, was not. This difference between radius and tibia is reflected by the total bone area and is similar to what was observed by Knowles and Broom (1990). The major difference was in the cortical bone area, with a strain by housing system interaction for the tibia because the White Leghorns were not affected by the housing system and the heavier lines had greater tibia cortical bone area in the floor pens. All 4 lines had higher radius cortical bone density in the floor pens than in cages, with a greater effect of housing system for the White Leghorns, but the tibia cortical density was not affected by the housing system for the heavy lines. Bone size is affected by loading (New, 2001), and these data suggest that loading of the wing, represented by the radius, was greater in the floor pens than in cages. This has been observed

**Table 6.** Interaction between strain of laying hen and housing system<sup>1</sup>

Strain <sup>2</sup>	Trabecular mineral content (mg/cm)	
	Cage	Floor
Cross	3,369 <sup>cd</sup>	2,158 <sup>d</sup>
LB	5,340 <sup>a</sup>	4,714 <sup>ab</sup>
HN	3,202 <sup>cd</sup>	4,160 <sup>abc</sup>
LW	3,232 <sup>cd</sup>	3,503 <sup>bc</sup>
SEM	410.6	

<sup>a-d</sup>Means within main effect without a common letter are different at  $P < 0.05$ .

<sup>1</sup>Bone mineral content was obtained by multiplying the mineral density (mg/mm<sup>3</sup>) by the cross-sectional area (mm<sup>2</sup>), extrapolated to a 1-cm length of bone.

<sup>2</sup>Cross = Rhode Island Red × Barred Plymouth Rock; LB = Lohmann Brown; HN = H&N; LW = Lohmann White.

**Table 7.** Location of hens just before lights off for 4 layer strains housed in floor pens<sup>1</sup>

Strain <sup>2</sup>	Location of hens (% of total)		
	Perches	Floor	Other
LW	76.3 <sup>a</sup>	12.6 <sup>b</sup>	11.1
HN	64.5 <sup>a</sup>	29.5 <sup>b</sup>	6.0
LB	6.8 <sup>b</sup>	92.4 <sup>a</sup>	0.8
Cross	8.6 <sup>b</sup>	90.6 <sup>a</sup>	0.9
SEM	7.49	7.76	5.67
<i>P</i> -value	<0.001	<0.001	0.532

<sup>a,b</sup>Means in a column without a common superscript differ at  $P < 0.05$ .

<sup>1</sup>Total number of observations is the average of 4 daily observations (3 for H&N) in 20 pens. Observations occurred on 4 consecutive days between 27 and 28 wk of age.

<sup>2</sup>Cross = Rhode Island Red × Barred Plymouth Rock; LB = Lohmann Brown; HN = H&N; LW = Lohmann White.

by others (Knowles and Broom, 1990) and is explained by the greater ability of the hens in floor pens to use their wings. Although the hens in cages walked less than those in floor pens, they spent more time standing (R. Singh, unpublished data), which would load the tibia and reduce the difference between housing systems. To this effect, the area of the radius of the White Leghorns, which used their perches in the floor pens, was increased substantially more than that of the brown-egg layers, which did not. In a similar fashion, the cortical density of the radius of the White Leghorns was affected more by the housing system than that of the brown-egg layers. The White Leghorns appeared to use their space, both horizontal and vertical, more effectively than the brown-egg layers when it was provided and this likely contributed to better bone characteristics when they were kept in floor pens than when they were kept in cages.

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