Bone and eggshell quality throughout an extended laying cycle in three strains of layers spanning 50 years of selection

Charlene Hanlon,[1] Kayo Takeshima, Elijah G. Kiarie, and Grégoy Y. Bédécarrats

Department of Animal Biosciences, University of Guelph, Guelph, Ontario N1G2W1, Canada

ABSTRACT Decades of intensive genetic selection in commercial layers has resulted in earlier maturation, while sustaining high production rates to 100 wks of age (**woa**). To support eggshell formation while maintaining a healthy skeletal frame, substantial adaptations of calcium metabolism in the hen are necessary. Thus, skeletal growth, bone density, and egg quality were compared in 3 strains of layers, with the Lohmann LSL-lite as the current commercial strain, the heritage Shaver white leghorn as the mid-2000s strain, and the white-leghorn derived Smoky Joes as the non-selected 1960s strain. Tibia and Femur (n = 4/strain) were collected at 12, 17, 20, 25, 45, 60, 75, and 100 woa. Bones were measured and weighed, with bone mineral density assessed within medullary (**mBMD**) and cortical (**cBMD**) regions of the tibia using micro-Computed Tomography. Egg analyses including weight, eggshell thickness (**EST**) and eggshell breaking strength (**EBS**), were conducted throughout lay. Blood samples were collected to measure plasma calcium immediately prior to lay (18 woa) and periodically throughout the laying cycle. Femur and tibia weight, or size, did not increase beyond 12 woa, indicating that all hens reached maximum skeletal size by this time. An interaction ($P = 0.005$) was observed between strain and tibia mBMD, as all three strains demonstrated an accumulation of medullary bone from 12 to 100 woa. Regarding egg weight, while Lohmann hen eggs displayed the highest quality at 26 woa, an elevation in egg weight in Lohmann and Shaver hens ($P < 0.001$) resulted in a decline in EST and EBS over time ($P < 0.01$). Yet, at 100 woa, no strain differed in EST or EBS, despite larger variations in cumulative egg numbers ($P < 0.001$). Plasma calcium levels were significantly elevated between the immature state and peak of lay but remained unchanged throughout lay in all strains. In conclusion, our results show that although genetic selection of layer hens resulted in tremendous improvement in productivity, no detrimental effects on cBMD or mBMD were observed throughout an extended laying period up to 100 woa.

Key words: calcium, metabolism, laying hen reproduction, eggshell quality, bone quality

2022 Poultry Science 101:101672
https://doi.org/10.1016/j.psj.2021.101672

INTRODUCTION

In most animal species, the purpose of bone is primarily for structural support, as well as forming a complex reservoir for minerals, such as calcium and phosphorous (for review: Datta et al. 2008). However, in the case of avian species, the additional requirement for eggshell formation necessitates greater mobilization of calcium reserves beyond levels provided by the diet. In fact, early studies showed that while approximately 60 to 75% of the calcium destined for the shell comes directly from intestinal absorption (Driggers and Comar, 1949), up to 36% could be traced to bones (Mueller et al., 1964). This bone source is critical since shell calcification occurs during the scotophase, also known as the dark period, when the hen is not feeding. Since the calcium storage capacity will influence the reproductive efficiency of the adult, proper skeletal development is integral during pullet growth. This should be achieved prior to sexual maturation, with long bones reaching their maximum length and cortical bone reaching optimal thickness (Whitehead and Fleming, 2000). Upon activation of the reproductive axis and in preparation for the laying cycle, increasing estradiol 17-beta ($\mathbf{E_2}$) concentrations shift the activity of osteoblasts toward the formation of medullary bone (Benoit and Clavert, 1945; Common et al., 1948; Miller, 1992), with cortical bone accumulation terminated (Hudson et al., 1993). This medullary bone is deposited on the interior, endosteal surface of the cortex in the marrow cavities of long bones (Bloom et al., 1958; Mccoy and Reilly, 1996), with the largest reserve in the femur, followed by the tibia (Clunies et al., 1992). Breeding programs have focused on improving bone strength,

Received August 24, 2021.
Accepted December 15, 2021.

[1]Corresponding author: chanlon@uoguelph.ca

as well as bone mineral density (**BMD**), to improve the health and welfare of hens in the context of increasing peak egg production and extending the laying period. Although the heritability ($\mathbf{h^2}$) of BMD in hens is moderately high ($h^2 = 0.40$) (Bishop et al., 2000), suggesting bone weakness could be alleviated through breeding, the incidence rate of bone fractures remains relatively high (Clark et al., 2008) and further improvements are required. This is particularly critical as the selection pressure increases for the extended laying cycle up to 100 wk of age (**woa**) (van Sambeek, 2010; Bain et al., 2016), and careful consideration must be given to calcium requirements in the diet, housing environment, and phenotypes for genetic selection.

While bone quality remains a welfare concern in layers, there are additional economic costs associated with egg quality traits (Fathi et al., 2007; Iqbal et al., 2017; Lopez et al., 2018). The eggshell is vital for the integrity and protection of the internal components of table eggs (Bain et al., 2016), as well as embryo development in breeder flocks (Qi et al., 2016; Torres and Korver, 2018), thus directly impacting market value. During an extended laying phase, the quality of eggs decreases as the hen ages (Zita et al., 2009), with the incidence of cracked eggs significantly increasing from 6 to 8% during a normal 52-wk cycle (Hamilton et al., 1979; Dunn et al., 2008) and up to 20% during this extended period (Nys et al., 1999). However, these numbers are largely dependent upon the housing conditions. The average hen requires 2.2 g of calcium for each eggshell (Bouvarel et al., 2011), yet as the hen ages, the egg weight will increase due to a longer interval between ovulations, resulting in a greater accumulation of yolk (Zakaria et al., 1983). This resulting increase in egg weight corresponds to a decline in eggshell strength (Chang-Ho et al., 2014), as calcium deposition in the shell does not proportionately increase (Roland, 1979). Although eggshell thickness (**EST**) has been considered a critical indirect measure of eggshell breaking strength (**EBS**), there is little evidence supporting such a relationship (Kemps et al., 2006; Chang-Ho et al., 2014; Sirri et al., 2018). While egg characteristics have been shown to vary between strains (Kocevski et al., 2011), few investigations monitoring improvements in eggshell quality that have occurred since the initiation of layer breeding programs within these strains exist. Conversely, multiple nutritional programs have been developed to optimize calcium levels for the purpose of bone and egg quality (Leeson and Summers, 2005; Duran et al., 2018). Many of these studies report no beneficial effects of additional calcium beyond 52 woa (Keshavarz and Nakajima, 1993) or later in the day, corresponding to the time of shell formation (Sauveur, 1991; Keshavarz, 1998a,b). However, the inclusion of calcium sources such as coarse eggshell particles and oyster shell have been shown to improve BMD of the tibia (Lee et al., 2021), indicating that the source of dietary intervention can be beneficial.

As bone quality remains critical to the successful formation of the eggshell and the overall health of the hen, recent efforts to improve reproductive capacity must continue to prioritize these traits. Therefore, this study aimed to investigate the impact of higher productivity on the skeletal integrity and eggshell quality of layers, utilizing 3 strains of hens representing a span of over 50 yr of intensive genetic selection. Thus, by subjecting all strains to the current standardized nutrition and management programs, this study will identify physiological alterations due to the genetic potential of the birds, particularly in regard to changes in calcium storage and utilization in the hen. This will not only characterize the bone and egg quality through to 100 woa, but also determine the ability of these hens to sustain bone and eggshell quality despite elevated production demands, particularly during sexual maturation and the extended laying cycle.

MATERIALS AND METHODS

This study was approved by the University of Guelph Animal Care Committee with all management conducted and samples collected in accordance with the guidelines from the Canadian Council for Animal Care (CCAC, 2009). All birds were housed at the Arkell Poultry Research Station at the University of Guelph (Guelph, ON, Canada).

Animals and Housing

Three strains of laying hens were used in this study to examine the impact of genetic selection for improved sexual maturation, production rate, and laying persistency on bone and eggshell characteristics, as described in Hanlon et al. (2021). These strains included: the modern commercial strain, Lohmann LSL lite; the 2000s-equivalent commercial strain, heritage Shaver White Leghorns, which were donated to and maintained by the University of Guelph since 2003 (Smith, 2010); and the 1960s-equivalent commercial strain, Smoky Joe White Leghorn derivative (Len et al., 1964; Baxter et al., 2014). As the Smoky Joe line has a recessive genetic mutation for retinal degeneration (Salter et al., 1997), only the sighted hens were used.

Experimental design and management protocols were previously described in Hanlon et al. (2021). Briefly, fertile Lohmann LSL-lite eggs were acquired from Archer's Hatchery (Brighton, Ontario, Canada), while Shaver and Smoky Joe eggs were collected from the breeding colony maintained at the University of Guelph. A total of 300 eggs from each strain were placed in a single incubator to ensure identical conditions. At hatch, due to differences in hatchability and unexpectedly high ratios of males: females, a total of 120 Lohmann chicks, 101 Shaver chicks, and 94 Smoky Joe chicks were included in the study. From placement (1 d of age; **doa**) until 6 woa, birds were housed in brooding cages (n = 12 cages), with Lohmann chicks placed in 5 cages (n = 24 chicks per cage), Shaver chicks placed in 4 cages (n = 25 chicks per cage), and Smoky Joe chicks placed in 3 cages

(n = 22 to 23 chicks per cage). Chick numbers were adjusted after starve out. From 6 to 12 woa, chicks were further divided into 24 cages (n = 10 Lohmann; 8 Shaver; 6 Smoky Joe cages). All pullets were transferred to 2 identical rooms at 12 woa. Each room was equipped with 80 standard unfurnished cages (18" × 10" × 18"; Ford Dickison Inc., Mitchell, ON, Canada) and 2 birds of the same strain were allocated randomly to each cage throughout the rooms. Pullets were reared under white light-emitting diode (**LED**) lights and photostimulated at 18 woa with 12 hours (**h**) of light, increasing through a step-up program by 1 h per week, up to 16 h of light (22 woa). Diets were formulated to meet or exceed NRC requirements (NRC, 1994). All birds were provided the same diet within each growth phase, including a starter crumble from 0 to 6 woa (21% crude protein, 1.06% calcium, 0.77% phosphorus), a grower crumble from 6 to 18 woa (18% crude protein, 1.00% calcium, 0.78% phosphorous), and a layer diet ration from 18 woa through to the end of the study (18% crude protein, 4.24% calcium, 0.68% phosphorous). Feed and water were provided *ad libitum* throughout the experiment.

Bone Collection and Analysis

Specific time points were selected around sexual maturation and during the extended egg laying period to analyze bone quality. These time points included an immature baseline (12 woa), pre-lay (17 woa), initiation of lay (20 woa), peak of lay (25 woa), and throughout the remainder of the laying period (45, 60, 75, and 100 woa). Two cages per strain were randomly selected, with both birds in the cage chosen for sampling at each time point (n = 4/strain). Birds were weighed, sacrificed via cervical dislocation, and the right leg was removed to collect the femur and tibia. Bones were autoclaved at 121°C for 19 min in order to loosen the muscle and remove any debris, as previously validated by Cloft et al. (2018). Bones were then left to dry overnight before recording the absolute weight. Relative weight was then calculated based on the body weight of the individual. The length and width of tibia and femur were also measured using a caliper at the mid-point of the shaft.

All right tibia samples underwent micro-computed tomography (***μ*CT**) imaging, with a region of the diaphysis, or central shaft of the bone, analyzed for each hen. Scans were completed using the GE Medical Systems Locus Explore (General Electric, Milwaukee, WI) and image analyses were performed using the GE Medical Systems MicroView program (Parallax Innovations v.2.5.0, Ilderton, ON, Canada). To standardize the region of interest (**ROI**) for the diaphysis, the advanced ROI polygon option was used and 1 slice every 50 slices was outlined with 18 outlines created for each section. Once the ROIs were developed, the Bone Analysis tool was used to determine the bone mineral content (**BMC**; mg) and bone mineral density (**BMD**; mg/cc) for both the medullary (**mBMC; mBMD**) and cortical regions (**cBMC; cBMD**). Cortical bone thickness along the diaphysis of the tibia was measured at three points along the shaft (225 slices apart) at the posterior, medial, anterior, and lateral regions and averaged.

Blood Samples

Approximately 2 mL of blood was collected from the brachial vein of each of the tissue sampled hens described above and placed in 4 mL sodium heparin tubes, immediately prior to euthanasia. These samples were used to assess the relationship between bone parameters and estradiol. To assess the calcium profile over time, additional repeated blood samples were collected using the method described above from 10 focal individuals per strain at 18, 28, 42, 56, 64, 82, and 94 woa. All samples were collected between 2 and 4 h after the lights were turned on for each time point to remove circadian fluctuations, with the same sampling order maintained throughout the study. All blood samples were centrifuged (Centrifuge J6-MI, Beckman Coulter Inc., Brea, CA) for 15 min at 900 × *g* at 4°C to recover plasma. The plasma was stored at −20°C until analyses were completed.

Calcium Analysis Samples to be used for calcium analysis were then sent to the Animal Health Laboratory at the University of Guelph and plasma calcium concentration was measured using the photometric test method (Immunochemistry Analyzer, Cobas c311, Roche, Hi-780 tachi, Indianapolis, IN).

Estradiol Analysis For estradiol analysis, only the blood samples collected at the time of bone collection were used. To remove fat, the cold ethanol extraction procedure was used as outlined by Baxter et al. (2014) and extracted samples were stored at −20°C until assayed. The DetectX commercial estradiol ELISA kit was used to measure estradiol concentrations (DetectX 17β-estradiol enzyme immunoassay #K030-H5, Arbor Assays, Ann Arbor, MI). Manufacturer's protocols were followed, and samples were measured in duplicates. Optical densities were measured using a microplate spectrophotometer at 450 nm (Model 550, Bio Rad, Hercules, CA) and data was analyzed using a 4-parameter logistic curve in the MyAssays software (www.myassays.com/arbor-assays-estradiol-eia-kit.assay). The intra-assay and interassay coefficient of variance were <15%.

Reproductive Performance

Daily egg production from each cage was recorded. Cumulative egg production was calculated on a hen-housed basis, defining the cage as the unit. Laying status was calculated based on the daily egg production of all hens for a 7-d period (3 d of egg analysis ± 2 d). This was used to indicate the percentage of hens per strain which were represented during each egg analysis period.

Egg Analysis

Egg analysis was conducted at 26, 40, 60, 70, 80, 90, and 100 woa to determine the egg quality at different time

points throughout the laying cycle. Eggs were collected from all individual cages for 3 consecutive days at each time point to ensure the best possible representation of the two individuals per cage and analyzed within 12 h of their daily collection. Eggs were individually weighed, and width and length were measured at the widest and longest point of each egg using a caliper. Eggshell thickness was determined with the precision ultrasound device (Orka Egg Shell Thickness Gauge-1, Orka Food Technology Ltd., West Bountiful, UT), and EBS, defined as the breaking force in kg (**kgF**), was conducted with the Egg Force Reader (Orka Food Technology Ltd.).

Statistical Analysis

All statistical analyses were completed using a two-way analysis of variance (**ANOVA**) in SAS (v 9.4). Proc MIXED models were used with means separated (LSMEANS) using the test of least significant differences (PDIFF statement) and Tukey's multiple comparison. Data from the μCT was $\log_2$ transformed for normality. Significance was reported at the $P < 0.05$ level. Strain and age and the interaction between them were included as the fixed effects, while room, cage, tier, and location in the room were included as random effects. Pearson correlations (Proc CORR) were determined within each individual strain regarding the femur (weight, width, length, and age) and tibia (weight, width, length, age, mBMC, mBMD, cBMC, cBMD, and E_2), as well as the egg quality traits (weight, length, width, EST, and EBS).

RESULTS

Bone Measurements

Tibia and Femur Weight Absolute and relative weights of the femur and tibia demonstrated an age ($P < 0.001$), strain ($P < 0.001$), and an interaction effect ($P < 0.05$), as seen in Table 1. While there was no difference in absolute tibia weight in Lohmann hens, relative tibia weight declined between 12 and 20 woa ($P = 0.006$). In Shaver hens, absolute weight of the tibia increased between 12 and 45 woa ($P < 0.05$), while relative weight declined during this same period ($P < 0.05$). In Smoky Joe birds, weight increased between 17 and 100 woa ($P = 0.033$), despite no differences in relative weight for this strain. In terms of the femur, Lohmann birds did not demonstrate any differences in absolute weight, yet the relative weight declined from 12 to 25 woa ($P = 0.009$). There were no differences in either absolute or relative femur weight observed in Shaver birds. In Smoky Joe birds, femurs were found to be heaviest in hens at 60 and 100 woa compared to pullets from 12 to 20 woa ($P < 0.05$), yet no differences in relative weight were observed. This resulted in the Smoky Joe femur being heavier than the Lohmann hens at 60 and 100 woa ($P < 0.01$), and heavier than that of the Shaver hens at 100 woa ($P = 0.042$).

Table 1. Interaction effect of strain and age on the absolute (g) and relative (% of live body weight) weight of the tibia and femur.

			Tibia weight				Femur weight			
			Absolute (g)		Relative (%)		Absolute (g)		Relative (%)	
Effect	Strain	Age[1]	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Strain × Age	Lohmann	12	5.41	0.28	0.52^{a}	0.02	3.90	0.15	0.37^{a}	0.01
		17	5.61	0.18	0.47^{ab}	0.03	3.95	0.09	0.33^{ab}	0.02
		20	4.86	0.31	0.35^{bc}	0.01	3.72	0.27	0.26^{ab}	0.01
		25	5.23	0.17	0.33^{bc}	0.01	3.70	0.08	0.23^{b}	0.01
		45	6.19	0.37	0.33^{c}	0.02	4.81	0.53	0.25^{b}	0.03
		60	5.69	0.47	0.29^{c}	0.01	4.37	0.31	0.23^{b}	0.01
		75	5.84	0.27	0.29^{c}	0.01	4.48	0.15	0.22^{b}	0.01
		100	5.69	0.20	0.34^{bc}	0.03	4.41	0.31	0.26^{ab}	0.03
	Shaver	12	5.64^{b}	0.08	0.54^{a}	0.02	4.02	0.05	0.38	0.01
		17	6.52^{ab}	0.12	0.50^{ab}	0.02	5.01	0.17	0.39	0.02
		20	7.02^{ab}	0.35	0.42^{abc}	0.01	5.62	0.45	0.34	0.01
		25	7.04^{ab}	0.34	0.42^{abc}	0.02	4.96	0.55	0.30	0.03
		45	7.05^{a}	0.85	0.34^{bc}	0.04	5.53	0.47	0.27	0.02
		60	6.91^{ab}	0.54	0.35^{c}	0.02	5.63	0.30	0.29	0.01
		75	6.66^{ab}	0.22	0.33^{c}	0.01	5.29	0.24	0.27	0.02
		100	6.50^{ab}	0.49	0.36^{c}	0.03	5.14	0.43	0.28	0.02
	Smoky Joe	12	5.96^{b}	0.23	0.57	0.01	4.27^{c}	0.20	0.41	0.01
		17	6.20^{b}	0.21	0.51	0.01	4.65^{bc}	0.13	0.38	0.01
		20	6.64^{ab}	0.05	0.47	0.01	4.95^{bc}	0.08	0.35	0.01
		25	6.76^{ab}	0.18	0.46	0.01	5.45^{abc}	0.13	0.37	0.01
		45	7.37^{ab}	0.37	0.45	0.01	6.34^{ab}	0.45	0.39	0.02
		60	7.77^{ab}	0.19	0.47	0.01	6.89^{a}	0.17	0.41	0.02
		75	7.51^{ab}	0.83	0.56	0.10	6.35^{ab}	0.91	0.47	0.07
		100	8.11^{a}	0.33	0.43	0.03	7.07^{a}	0.32	0.38	0.02
Source of variation							*P*-value			
Age			< 0.001		< 0.001		< 0.001		< 0.001	
Strain			< 0.001		< 0.001		< 0.001		< 0.001	
Age × Strain			0.031		0.009		0.016		< 0.001	

[1]Age was recorded in weeks.

[a-c]Simple effect LSMeans are presented within each strain of the strain × age interaction. Time points within each strain lacking a common superscript are different ($P < 0.05$).

Table 2. Effect of strain and age on the length (mm) and width (mm) of the tibia and femur.

			Tibia size				Femur size			
			Length (mm)		Width (mm)		Length (mm)		Width (mm)	
Effect	Strain	Age[1]	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Strain	Lohmann		116.2[c]	0.55	6.7[a]	0.11	78.9[c]	0.43	7.2[b]	0.07
	Shaver		118.1[b]	0.57	7.2[b]	0.11	81.1[b]	0.44	7.8[a]	0.08
	Smoky Joe		120.3[a]	0.56	7.3[b]	0.11	84.0[a]	0.44	7.1[b]	0.08
Age		12	115.7[b]	0.92	6.8[ab]	0.18	77.8[c]	0.72	7.0[c]	0.12
		17	118.2[ab]	0.92	6.9[ab]	0.18	81.6[ab]	0.72	7.4[abc]	0.12
		20	116.0[ab]	0.92	6.5[b]	0.18	79.0[bc]	0.72	7.1[c]	0.12
		25	118.8[ab]	0.89	7.0[ab]	0.17	82.4[a]	0.69	7.4[abc]	0.12
		45	119.2[ab]	0.94	7.3[ab]	0.18	82.4[a]	0.69	7.4[abc]	0.12
		60	119.8[a]	0.92	7.5[a]	0.18	82.7[a]	0.72	7.8[a]	0.12
		75	118.3[ab]	0.92	7.5[a]	0.18	82.3[a]	0.72	7.7[ab]	0.12
		100	119.5[ab]	0.92	7.2[ab]	0.18	82.4[a]	0.72	7.2[b]	0.12
Source of variation						*P*-value				
Age			0.010		0.001		< 0.001		< 0.001	
Strain			< 0.001		< 0.001		< 0.001		< 0.001	
Age × Strain			0.257		0.622		0.615		0.782	

[1]Age was recorded in weeks.
[a–c]Simple effect LSMeans are presented within each strain of the strain × age interaction. Time points within each strain lacking a common superscript are different ($P < 0.05$).

Tibia and Femur Size Tibia and femur size were considered to determine changes in calcium storage capacity. As seen in Table 2, there was an effect of both strain and age for both the length and width of the tibia ($P < 0.05$). The tibiae of Smoky Joe birds were longer and wider than that of the Lohmann birds ($P < 0.01$). The age effect translated into pullets at 12 woa demonstrating the shortest tibia compared to hens at 60 woa ($P = 0.038$), and widest at 60 and 75 woa compared to 20 woa ($P < 0.01$), with no other differences present. There was also an effect of strain and age for the length and width of the femur ($P < 0.001$). Smoky Joe hens had the longest femur ($P < 0.01$), while Lohmann hens had the shortest ($P < 0.001$), with the femur width of Shaver hens being the largest ($P < 0.001$). Overall, the length of the femur increased between 20 and 25 woa, although femurs at 17 woa were similar in length to all time points beyond 20 woa. Additionally, the width was found to increase between 12 and 60 woa ($P < 0.001$). There was no interaction present between strain and age regarding tibia or femur size.

Micro-CT Analysis of Tibia

Cortical Bone Analysis The cBMC and cBMD within the diaphysis region of the tibia were determined via μCT scans and displayed in Table 3. There was an age effect ($P < 0.001$) observed on cBMC and cBMD. Fluctuations in cBMC were observed, with the highest content from 12 to 20 woa and at 60 and 100 woa, while the lowest content was observed at 25 and 75 woa ($P < 0.05$). Interestingly, there was no difference between 20 and 100 woa. There was also a strain effect ($P < 0.001$) for cBMC with Smoky Joe hens demonstrating the highest content overall. Unlike cBMC, the cBMD was more consistent, with a decline between 17 and 45 woa ($P = 0.011$). No interaction was present within the cortical region.

Medullary Bone Analysis The mBMC and mBMD within the diaphysis region of the tibia were analyzed via μCT scans and shown in Table 4. These parameters were used to assess the calcium availability for eggshell synthesis. Strain ($P < 0.001$) and age ($P < 0.01$) effects on mBMC and mBMD were observed. An interaction effect between strain and age was determined ($P < 0.05$), with all strains demonstrating a progressive accumulation of medullary bone in the tibia reaching significance between 12 and 100 woa ($P < 0.05$). Overall, mBMC and mBMD was higher in Smoky Joe hens than Lohmann hens only at 60 woa ($P < 0.05$).

Cortical Bone Thickness Cortical bone thickness was measured across four regions of the diaphysis of the tibia bone: the anterior, posterior, lateral, and medial sections and presented Table 5. A strain and an age effect were

Table 3. Effect of strain and age on the cortical region bone mineral content (cBMC; mg) and bone mineral density (cBMD; mg/cc) of the tibia bone.

			Cortical Region			
			cBMC (mg)		cBMD (mg/cc)	
Effect	Strain	Age[1]	Mean	SEM	Mean	SEM
Strain	Lohmann		564.6[b]	20.09	345.6	13.97
	Shaver		612.6[b]	20.35	312.6	14.14
	Smoky Joe		722.6[a]	20.35	347.4	14.14
Age		12	667.4[ab]	33.23	477.9[a]	23.10
		17	749.5[a]	33.23	456.8[a]	23.10
		20	684.8[ab]	33.23	381.1[ab]	23.10
		25	490.3[c]	32.10	272.3[ab]	22.31
		45	577.2[bc]	33.23	287.8[bc]	23.10
		60	679.4[ab]	33.23	283.5[bc]	23.10
		75	533.0[c]	33.23	257.3[c]	23.10
		100	684.4[ab]	33.23	264.9[c]	23.10
Source of variation				*P*-value		
Age			< 0.001		< 0.001	
Strain			< 0.001		0.193	
Age × Strain			0.227		0.378	

[1]Age was recorded in weeks.
[a–d]Simple effect LSMeans are presented within each strain of the strain × age interaction. Time points within each strain lacking a common superscript are different ($P < 0.05$).

Table 4. Interaction effect of strain and age on the medullary region bone mineral content (mBMC; mg) and bone mineral density (mBMD; mg/cc) of the tibia bone.

			Medullary region			
			mBMC (mg)		mBMD (mg/cc)	
Effect	Strain	Age[1]	Mean	SEM	Mean	SEM
Strain × Age	Lohmann	12	16.8[b]	10.57	25.7[b]	15.57
		17	81.7[ab]	7.48	123.5[ab]	12.24
		20	71.7[ab]	24.27	92.2[ab]	24.06
		25	88.2[ab]	45.80	96.0[ab]	46.98
		45	95.5[ab]	17.35	124.5[ab]	22.93
		60	106.5[ab]	19.70	122.5[ab]	25.07
		75	196.3[a]	42.25	253.6[ab]	57.76
		100	190.9[a]	25.85	222.5[a]	30.53
	Shaver	12	37.1[d]	4.31	51.9[c]	9.63
		17	68.5[bcd]	5.08	85.3[abc]	8.79
		20	138.3[abcd]	33.47	143.2[abc]	34.93
		25	62.4[cd]	45.73	50.7[bc]	34.90
		45	207.8[abc]	51.50	192.2[abc]	38.47
		60	274.1[ab]	24.15	245.3[ab]	24.80
		75	217.9[abc]	59.29	262.1[ab]	76.43
		100	374.1[a]	119.26	352.1[a]	76.31
	Smoky Joe	12	29.4[d]	6.23	48.0[d]	13.36
		17	50.4[cd]	14.28	73.5[cd]	21.20
		20	63.4[bcd]	13.02	74.9[bcd]	9.84
		25	144.8[abc]	14.58	178.7[abcd]	29.89
		45	186.2[abc]	33.37	276.9[ab]	37.91
		60	549.3[a]	89.28	624.7[a]	87.24
		75	269.9[abc]	153.05	344.8[abc]	22.24
		100	501.3[a]	60.84	597.6[a]	88.38
Source of variation				*P*-value		
Age			0.008		0.009	
Strain			< 0.001		< 0.001	
Age × Strain			0.020		0.005	

[1]Age was recorded in weeks.
[a-d]Simple effect LSMeans are presented within each strain of the strain × age interaction. Time points within each strain lacking a common superscript are different ($P < 0.05$).

Table 5. Interaction effect of strain and age on the cortical bone thickness (mm) of the tibia across 4 regions, including the anterior, lateral, posterior, and medial sections.

			Anterior (mm)		Lateral (mm)		Posterior (mm)		Medial (mm)	
Effect	Strain	Age[1]	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Strain x Age	Lohmann	12	0.85[a]	0.058	0.65[a]	0.016	0.65[a]	0.024	0.66[a]	0.043
		17	0.77[a]	0.057	0.70[a]	0.060	0.70[a]	0.012	0.65[a]	0.056
		20	0.98[a]	0.088	0.74[a]	0.034	0.71[a]	0.045	0.68[a]	0.030
		25	0.88[a]	0.081	0.62[a]	0.037	0.74[a]	0.030	0.69[a]	0.052
		45	1.00[a]	0.153	0.70[a]	0.065	0.70[a]	0.022	0.69[a]	0.028
		60	0.78[a]	0.089	0.59[a]	0.054	0.59[a]	0.054	0.61[a]	0.042
		75	0.68[a]	0.064	0.56[a]	0.074	0.68[a]	0.057	0.58[a]	0.061
		100	1.04[a]	0.116	0.77[a]	0.076	0.62[a]	0.050	0.70[a]	0.095
	Shaver	12	0.86[a]	0.042	0.58[b]	0.014	0.63[a]	0.015	0.69[a]	0.047
		17	0.82[a]	0.083	0.68[ab]	0.015	0.65[a]	0.025	0.63[a]	0.044
		20	0.98[a]	0.079	0.70[ab]	0.031	0.69[a]	0.029	0.72[a]	0.033
		25	0.75[a]	0.050	0.69[ab]	0.076	0.63[a]	0.030	0.60[a]	0.009
		45	1.00[a]	0.117	0.81[a]	0.023	0.69[a]	0.011	0.68[a]	0.014
		60	0.90[a]	0.092	0.68[ab]	0.054	0.63[a]	0.022	0.62[a]	0.027
		75	0.75[a]	0.120	0.70[ab]	0.103	0.72[a]	0.081	0.65[a]	0.032
		100	0.73[a]	0.051	0.53[b]	0.028	0.60[a]	0.033	0.58[a]	0.018
	Smoky Joe	12	0.74[b]	0.042	0.67[bc]	0.014	0.70[b]	0.042	0.66[b]	0.087
		17	0.99[ab]	0.021	0.73[bc]	0.027	0.75[b]	0.006	0.67[b]	0.069
		20	1.14[ab]	0.031	0.86[b]	0.034	0.74[b]	0.042	0.78[ab]	0.006
		25	1.05[ab]	0.059	0.78[bc]	0.075	0.78[b]	0.029	0.73[ab]	0.050
		45	1.08[ab]	0.066	0.86[b]	0.019	0.90[ab]	0.046	0.77[ab]	0.054
		60	1.02[ab]	0.102	0.70[bc]	0.019	0.74[b]	0.029	0.74[ab]	0.036
		75	0.76[b]	0.048	0.61[c]	0.041	0.69[b]	0.022	0.62[b]	0.021
		100	1.32[a]	0.224	1.10[a]	0.269	1.03[a]	0.267	0.92[a]	0.189
Source of variation						*P*-value				
Age			< 0.001		< 0.001		0.027		< 0.001	
Strain			< 0.001		< 0.001		< 0.001		< 0.001	
Age × Strain			0.018		< 0.001		< 0.001		< 0.001	

[1]Age was recorded in weeks.
[a-c]Simple effect LSMeans are presented within each strain of the strain × age interaction. Time points within each strain lacking a common superscript are different ($P < 0.05$).

Table 6. Effects of strain and age on the plasma calcium concentration (mmol/L).

			Calcium (mmol/L)	
Effect	Strain	Age[1]	Mean	SEM
Strain	Lohmann		6.75	0.371
	Shaver		5.61	0.373
	Smoky Joe		6.01	0.343
Age		18	1.96[c]	0.371
		28	5.35[b]	0.471
		42	6.77[ab]	0.371
		56	7.42[a]	0.371
		64	6.87[ab]	0.396
		82	7.41[a]	0.371
		94	7.07[a]	0.371
Strain × Age	Lohmann	18	2.09	0.154
		28	5.13	0.745
		42	7.27	0.313
		56	8.24	0.393
		64	7.72	0.601
		82	9.59	1.132
		94	7.40	0.715
	Shaver	18	2.08	0.262
		28	5.16	0.339
		42	7.35	0.255
		56	6.57	0.677
		64	5.67	0.785
		82	5.70	0.871
		94	6.39	0.589
	Smoky Joe	18	1.71	0.098
		28	5.46	0.842
		42	5.70	0.882
		56	7.44	0.475
		64	7.26	0.718
		82	6.94	1.007
		94	7.43	0.959
Source of variation			*P*-value	
Age			< 0.001	
Strain			0.112	
Age × Strain			0.031	

[1]Age was recorded in weeks.
[a-c]Simple effect LSMeans are presented within each strain of the strain x age interaction. Time points within each strain lacking a common superscript are different ($P < 0.05$).

observed for all regions ($P < 0.01$). An interaction between strain and age was also detected for all regions ($P < 0.05$). While the Lohmann hens did not display any differences, Shaver hens had a thicker lateral region at 45 woa, compared to 12 and 100 woa ($P < 0.05$). In Smoky Joe hens, all regions were found to be thickest at 100 woa, with no differences observed from 12 to 75 woa ($P < 0.05$). This resulted in Smoky Joe hens demonstrating the thickest cortical bone in all regions at 100 woa compared to the other strains ($P < 0.05$).

Hormone and Calcium Analysis

Plasma Calcium Plasma calcium was measured to determine the available circulating levels for both medullary bone formation and eggshell synthesis in these hens. As displayed in Table 6, while there was no strain effect, there was an age effect on calcium plasma concentration. Birds at 18 woa demonstrated the lowest concentration ($P < 0.001$), with an elevation at 28 ($P < 0.05$), and the highest concentrations present at 56, 82, and 94 woa ($P < 0.05$). Due to the lack of strain effect, the interaction between strain and age ($P = 0.031$) was disregarded.

Table 7. Interaction effect of strain and age on the plasma estradiol-17β concentration (pg/mL).

			Estradiol (pg/mL)	
Effect	Strain	Age[1]	Mean	SEM
Strain × Age	Lohmann	12	354.2[b]	44.50
		17	652.2[ab]	99.63
		20	656.2[ab]	59.29
		25	867.4[ab]	75.93
		45	944.6[a]	145.63
		60	890.7[ab]	99.85
		75	867.0[ab]	123.58
		100	887.0[ab]	245.64
	Shaver	12	266.3[c]	18.34
		17	512.8[bc]	112.34
		20	736.7[abc]	36.69
		25	850.5[ab]	151.67
		45	889.5[ab]	113.61
		60	900.6[ab]	115.55
		75	1,178.7[a]	118.59
		100	1,102.9[ab]	73.69
	Smoky Joe	12	268.8[b]	58.92
		17	465.0[ab]	44.86
		20	976.6[a]	98.57
		25	604.3[ab]	23.88
		45	693.0[ab]	100.05
		60	740.0[ab]	61.57
		75	455.4[ab]	28.50
		100	758.2[ab]	108.49
Source of variation			*P*-value	
Age			0.001	
Strain			< 0.001	
Age × Strain			0.007	

[1]Age was recorded in weeks.
[a-c]Simple effect LSMeans are presented within each strain of the strain × age interaction. Time points within each strain lacking a common superscript are different ($P < 0.05$).

Estradiol Plasma concentration of E_2 was dependent on strain ($P = 0.001$) and age ($P < 0.001$), as seen in Table 7. There was a significant interaction between age and strain ($P = 0.007$). Lohmann hens displayed an elevation between 12 and 45 woa ($P = 0.010$), while this increase occurred between 12 and 25 woa in Shaver hens ($P < 0.05$) and between 12 and 20 woa in Smoky Joe hens ($P = 0.002$).

Correlations Across Bone Parameters

The femur (Table 8) and tibia (Tables 9 and 10) of all strains demonstrated moderate correlations between weight, length, and width ($P < 0.05$). Very strong associations between mBMD and mBMC ($P < 0.001$) were observed for the tibia in all 3 strains, as well as moderate correlations between cBMD and cBMC ($P < 0.001$) in Lohmann and Shaver hens. Conversely, cBMD was found to have a moderately negatively correlation with mBMC and mBMD in all strains ($P < 0.001$), as well as a moderate positive correlation with cBMC in Lohmann and Shaver hens ($P < 0.05$). While tibia weight, length, and width were not correlated with any BMC or BMD traits in Lohmann or Shaver hens, there was a positive, moderate correlation between tibia weight and cBMC ($r^2 = 0.382$; $P < 0.05$) in Smoky Joe hens (not shown).

Additionally, Lohmann and Shaver hens demonstrated moderate negative correlations between E_2 and

Table 8. Correlations between femur weight, length, and width in the Lohmann, Shaver, and Smoky Joe hens.

	Lohmann				Shaver				Smoky Joe			
Trait	Weight	Length	Width	Age	Weight	Length	Width	Age	Weight	Length	Width	Age
Weight	1	0.391*	0.369*	0.393*	1	0.490**	0.538***	0.229	1	0.586***	0.591***	0.746***
Length		1	0.572***	0.421*		1	0.619***	0.279		1	0.641***	0.385
Width			1	0.310			1	0.120			1	0.239
Age				1				1				1

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 9. Correlations between tibia weight, length, width, and age in Lohmann, Shaver, and Smoky Joe hens.

	Lohmann				Shaver				Smoky Joe			
Trait	Weight	Length	Width	Age	Weight	Length	Width	Age	Weight	Length	Width	Age
Weight	1	0.420*	0.468**	0.289	1	0.778***	0.506**	0.077	1	0.638***	0.479**	0.674***
Length		1	0.365*	0.398*		1	0.369*	0.044		1	0.755***	0.298
Width			1	0.372*			1	0.435*			1	0.295
Age				1				1				1

*$P < 0.05$, ** $P < 0.01$, ***$P < 0.001$.

cBMD ($P < 0.05$), and moderate positive correlations between E_2 and mBMD ($P < 0.05$). While only Shaver hens were found to have a moderate negative correlation between E_2 and cBMC ($P = 0.046$), E_2 and mBMC were positively correlated in all three strains ($P < 0.05$).

Production Performance

Cumulative Egg Number The average number of cumulative eggs per hen at the time of egg quality analysis was recorded per strain and shown in Table 11. There was an interaction between strain and age ($P < 0.001$), with Lohmann hens laying the greatest number of cumulative eggs at each time point and Smoky Joe hens laying the least ($P < 0.001$). This resulted in a final cumulative egg total at the end of the study (100 woa) of 523, 408, and 240 eggs for the Lohmann, Shaver, and Smoky Joe, respectively ($P < 0.001$).

Laying Status The percentage of hens per strain laying during the 3 consecutive days of collection for egg analysis ± 2 d (7 d total) was determined and additionally reported in Table 11. There was an effect of age, strain, and an interaction ($P < 0.001$). All Lohmann hens remained in lay throughout the study. Shaver hens demonstrated a decline in the number of hens laying at 80 woa, with some returning to lay by 90 woa and further declining by 100 woa ($P < 0.05$). This fluctuation was similar to Smoky Joe hens, in which hens dropped out of lay between 80 and 90 woa ($P < 0.05$), with some returning to lay by 100 woa. Apart from 60 woa, Lohmann and Shaver hens had a higher percentage of laying birds than the Smoky Joe ($P < 0.05$). In the case of Smoky Joe hens, this was associated with spontaneous moulting, as reported in Hanlon et al., (2021).

Egg Analysis

Egg Size Egg weight, length and width were recorded at 26, 40, 60, 70, 80, 90, and 100 woa and are summarized in Table 12. There was an effect of strain and age on weight, length, and width ($P < 0.001$). For egg length, Smoky Joe eggs remained shorter than that of the other strains throughout ($P < 0.001$), while length was only observed to increase between 26 and 40 woa across strains ($P < 0.001$). Egg weight and width were also found to have a significant interaction of age and strain ($P < 0.05$), while this was not significant with length. The weight of eggs from Lohmann hens increased from 26 to 40 woa, 40 to 60 woa, as well as 80 to 90 woa ($P < 0.01$). Similarly, the width increased from 26 to 40 and 80 to 90 woa ($P < 0.05$). Weight of eggs from Shaver hens increased from 26 to 40 woa and from 40 to 60 woa ($P < 0.001$), with an elevation in width occurring between 26 to 40 woa and 40 to 90 woa ($P < 0.01$). Eggs from Smoky Joe hens only displayed an elevation in weight and width between 26 and 40 woa ($P < 0.001$),

Table 10. Correlations between tibia cortical bone mineral content (cBMC), cortical bone mineral density (cBMD), medullary bone mineral content (mBMC), medullary bone mineral density (mBMD), and estradiol (E_2) in Lohmann, Shaver, and Smoky Joe hens.

	Lohmann					Shaver					Smoky Joe				
Trait	cBMC	cBMD	mBMC	mBMD	E2	cBMC	cBMD	mBMC	mBMD	E2	cBMC	cBMD	mBMC	mBMD	E2
cBMC	1	0.605***	0.220	0.303	−0.190	1	0.624***	−0.143	−0.117	−0.355*	1	0.291	0.202	0.246	0.198
cBMD		1	−0.527**	−0.400*	−0.391*		1	−0.739***	−0.668***	−0.658***		1	−0.684***	−0.652***	−0.101
mBMC			1	0.976***	0.406*			1	0.979***	0.570***			1	0.988***	0.375*
mBMD				1	0.376*				1	0.584***				1	0.331
E2					1					1					1

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 11. Cumulative number of eggs and percentage of hens in lay at 26, 40, 60, 70, 80, 90, and 100 wk of age (woa) in Lohmann, Shaver, and Smoky Joe hens.

			Cumulative number of eggs		Percentage of hens in lay (%)	
Effect	Strain	Age[1]	Mean	SEM	Mean	SEM
Strain × Age	Lohmann	26	43.4[g]	0.42	100.0[a]	2.39
		40	138.4[f]	0.16	100.0[a]	2.39
		60	274.2[e]	0.26	100.0[a]	2.43
		70	339.9[d]	0.36	100.0[a]	2.48
		80	402.8[c]	0.42	100.0[a]	2.54
		90	463.5[b]	0.43	100.0[a]	2.60
		100	523.4[a]	0.45	96.5[a]	2.60
	Shaver	26	39.2[g]	0.49	98.8[a]	2.63
		40	124.5[f]	0.31	97.6[a]	2.63
		60	236.0[e]	0.45	96.2[a]	2.73
		70	287.0[d]	0.40	95.9[a]	2.80
		80	331.7[c]	0.56	77.1[b]	2.90
		90	369.4[b]	0.66	85.3[ab]	2.92
		100	408.4[a]	0.64	80.3[b]	2.96
	Smoky Joe	26	19.9[f]	0.56	82.7[a]	3.34
		40	71.5[e]	0.59	82.0[a]	3.41
		60	149.0[d]	0.51	95.5[a]	3.63
		70	182.1[c]	0.50	80.0[ab]	3.81
		80	206.3[bc]	0.66	50.0[c]	4.01
		90	222.0[ab]	0.46	52.8[c]	4.01
		100	240.2[a]	0.78	61.1[bc]	4.01
Source of variation				*P*-value		
Age				< 0.001		< 0.001
Strain				< 0.001		< 0.001
Age × Strain				< 0.001		< 0.001

[1]Age was recorded in weeks.
[a-g]Simple effect LSMeans are presented within each strain of the strain × age interaction. Time points within each strain lacking a common superscript are different ($P < 0.05$).

remaining unchanged thereafter. While eggs from Lohmann and Shaver hens were significantly heavier and wider than Smoky Joe eggs at all time-points throughout the study ($P < 0.001$), eggs from Lohmann hens were also heavier than from Shaver hens at 100 woa ($P < 0.05$).

Eggshell Quality Eggshell quality was assessed through measurements of EST and EBS (Table 12). A

Table 12. Interaction effects of strain and age on egg weight (g), length (mm), width (mm), eggshell thickness (EST), and eggshell breaking strength throughout the laying cycle.

			Weight (g)		Length (mm)		Width (mm)		EST (mm)		EBS (kgF)	
Effect	Strain	Age[1]	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Strain × Age	Lohmann	26	57.57[d]	0.34	56.2	0.15	42.7[d]	0.09	0.440[a]	0.002	4.894[a]	0.060
		40	62.93[c]	0.37	58.2	0.35	43.7[c]	0.14	0.428[ab]	0.003	4.623[b]	0.079
		60	64.62[b]	0.45	60.1	0.23	43.8[c]	0.11	0.411[bc]	0.003	3.854[c]	0.081
		70	65.02[b]	0.63	60.5	0.23	44.0[bc]	0.13	0.401[cd]	0.003	3.706[c]	0.102
		80	65.59[b]	0.45	60.3	0.19	44.0[bc]	0.22	0.400[cd]	0.002	3.498[d]	0.060
		90	67.55[a]	0.38	61.5	0.16	44.6[a]	0.09	0.394[cd]	0.002	3.264[e]	0.056
		100	68.76[a]	0.48	62.1	0.19	44.6[ab]	0.25	0.382[d]	0.003	2.970[f]	0.062
	Shaver	26	56.04[c]	0.48	56.3	0.54	42.3[c]	0.12	0.410[a]	0.004	3.769[a]	0.051
		40	61.38[b]	0.60	60.4	0.29	43.6[b]	0.18	0.393[ab]	0.003	3.395[b]	0.070
		60	63.58[a]	0.71	59.0	0.30	44.0[ab]	0.19	0.414[a]	0.003	2.945[c]	0.073
		70	64.24[a]	0.67	59.1	0.60	44.2[ab]	0.18	0.388[ab]	0.005	2.967[c]	0.098
		80	62.95[ab]	0.68	58.8	0.30	43.9[ab]	0.16	0.382[ab]	0.004	2.836[cd]	0.082
		90	65.18[a]	0.75	60.3	0.24	44.6[a]	0.15	0.373[b]	0.005	2.744[cd]	0.068
		100	66.09[a]	1.04	60.2	0.74	44.7[a]	0.18	0.373[b]	0.004	2.583[d]	0.067
	Smoky Joe	26	42.35[b]	0.99	51.6	0.51	38.6[b]	0.23	0.354	0.006	3.547[ab]	0.071
		40	49.40[a]	0.50	54.2	0.41	40.2[a]	0.49	0.374	0.004	3.843[a]	0.122
		60	50.78[a]	0.69	55.2	0.50	40.3[a]	0.25	0.374	0.006	3.508[ab]	0.095
		70	50.53[a]	1.00	54.3	1.67	40.8	0.32	0.370	0.009	3.463[ab]	0.241
		80	51.39[a]	1.08	55.2	0.61	41.1[a]	0.30	0.363	0.010	3.029[bc]	0.189
		90	49.98[a]	1.25	58.5	0.71	40.0[ab]	0.38	0.349	0.008	2.752[c]	0.180
		100	51.97[a]	0.47	56.3	0.57	41.1[a]	0.21	0.377	0.004	3.562[ab]	0.124
Source of variation							*P*-value					
Age			< 0.001		<0.001		< 0.001		< 0.001		< 0.001	
Strain			< 0.001		<0.001		< 0.001		< 0.001		< 0.001	
Age × Strain			< 0.001		0.475		0.029		< 0.001		< 0.001	

[1]Age was recorded in weeks.
[a-f]Simple effect LSMeans are presented within each strain of the strain × age interaction. Time points within each strain lacking a common superscript are different ($P < 0.05$).

strain and an age effect were determined on both parameters ($P < 0.001$). Additionally, there was an interaction between strain and age for both parameters ($P < 0.001$), as EST and EBS progressively declined over the course of the study in Lohmann hens ($P < 0.01$). In fact, EST declined between 26 and 60 woa, and 60 and 100 woa, while EBS declined consistently, with only 60 and 70 woa displaying similar EBS. Eggs from Shaver hens also demonstrated a steady decline in EST and EBS over time ($P < 0.05$), although to a lesser extent, with a decline in EST between 60 and 90 woa, and a decline in EBS between 26 and 40, 40 and 60, and 60 and 100 woa. While there was no decline in EST in eggs from Smoky Joe hens over time, there was a significant decline in EBS between 40 and 80 woa ($P < 0.01$), with a significant elevation at the end of the study between 90 and 100 woa ($P < 0.05$). This resulted in a higher EST and EBS for eggs from Lohmann hens at 26 and 40 woa compared to the other strains ($P < 0.01$). At 60 woa, EST and EBS of eggs from Smoky Joe hens were significantly lower than that of eggs from Lohmann and Shaver birds ($P < 0.05$) and this pattern persisted for EBS at 70 woa. From 80 to 90 woa, EBS of eggs from Lohmann hens was higher than from Shaver hens, while eggs from the Smoky Joe were intermediate ($P < 0.05$). Interestingly, by the end of the study, at 100 woa, EBS for eggs from Smoky Joe hens was higher than that of the remaining strains ($P < 0.05$).

Relationship Between Egg Quality Parameters The correlations between egg quality traits are listed in Table 13. For all 3 strains, egg weight was found to be positively correlated with the length and width ($P < 0.001$), and egg length displayed low to moderate correlations with egg width ($P < 0.01$). Interestingly, while EST was found to have a weak negative correlation with weight, length, and width in eggs from Lohmann hens ($P < 0.001$), EST had a weak positive correlation with weight and length in Smoky Joe hens ($P < 0.001$), and no correlation was present in eggs from Shaver hens. EST was also found to have a weak to moderate positive correlation with EBS among all three strains ($P < 0.001$). Additionally, EBS was also negatively correlated with width and weight in eggs from Shaver and Lohmann hens ($P < 0.001$) and only eggs from Lohmann hens demonstrated a negative correlation between EBS and length ($P < 0.001$). Furthermore, the age of all strains was positively correlated with weight and width ($P < 0.05$; not shown). This resulted in a negative correlation between age and EBS in all strains ($P < 0.001$; not shown). Intriguingly, EST was negatively correlated with age for Lohmann and Shaver hens, but no correlation was observed for Smoky Joe hens (not shown).

DISCUSSION

As the integrity of the skeletal system is critical not only for the health of the laying hen but also for eggshell quality, it is essential to ensure hens can maintain proper calcium homeostasis from the pullet stage to the end of an extended production cycle. In birds, due to the presence of the medullary bone, calcium storage capacity will be influenced by the size of the bones. Therefore, this study was conducted to assess and compare these parameters across different strains of White Leghorns laying hens, which represent different levels of genetic selection as the breeding objectives within the industry primarily focused on increasing the rate of production and the overall cumulative egg numbers (van Sambeek, 2010; Bain et al., 2016).

Interestingly, regardless of strain, the femur and tibia were not found to grow in length or width beyond 12 woa within each strain. As it has been previously proposed that tibial length is the best indicator of structural body height and size (Rising and Somers, 1989), our results suggest that these long bones, and thus the skeletal structure height, have completed their growth prior to sexual maturation. Previous studies have indicated that the initiation of lay and the initial rise in E_2 are responsible for switching the deposition of calcium from structural bone formation to medullary bone in preparation for lay and thus terminating growth (Whitehead and Wilson, 1992; Hudson et al., 1993; Fleming et al., 1998). However, our data indicate growth is terminated prior to the elevation in E_2 concentration in all strains. Interestingly, the strain differences in bone length suggest differences in structural size, with Lohmann hens having the shortest femur and tibia, and the Smoky Joe hens having the longest. This may suggest that while selection focused on production efficiency, this resulted in a smaller overall skeletal structure, as per the size of long bones. This is consistent with a study conducted by Hocking et al. (2003), who observed that the tibial length in traditional strains was greater than in commercial layers. This smaller structure was also

Table 13. Correlation between egg weight, length, width, eggshell thickness (EST), and eggshell breaking strength (EBS) in Lohmann, Shaver, and Smoky Joe hens.

	Lohmann					Shaver					Smoky Joe				
Trait	Weight	Length	Width	EST	EBS	Weight	Length	Width	EST	EBS	Weight	Length	Width	EST	EBS
Weight	1	0.720***	0.507***	−0.222***	−0.324***	1	0.146***	0.787***	0.045	−0.263***	1	0.622***	0.401***	0.246***	0.012
Length		1	0.370***	−0.305***	−0.397***		1	0.093**	0.001	−0.007		1	0.168**	0.196***	−0.080
Width			1	−0.136***	-0.165***			1	0.016	−0.227***			1	0.076	0.081
EST				1	0.551***				1	0.140***				1	0.400***
EBS					1					1					1

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

reflected in the overall weight of both the femur and tibia of Lohmann hens, with values similar to the dry weight previously reported in numerous studies (Kim et al., 2004; Akbari Moghaddam Kakhki et al., 2019, 2020a; Khanal et al., 2019, 2020a, 2021). Furthermore, the relative weight of the femur and tibia to the bodyweight of Lohmann birds decreased over time as body weight is known to increase during maturation. The relative tibia weight of the Smoky Joe did not change throughout, while the relative tibia weight of Lohmann and Shaver hens declined up to 20 and 45 woa, respectively. This is likely a reflection of a combination of the slower growth rate of the Smoky Joe established in Hanlon et al. (2021) and lower cumulative production. As Smoky Joe hens demonstrate delayed entry into lay and significantly lower cumulative egg numbers throughout, the positive correlation between tibia weight and cBMC suggests that cortical bone deposition is responsible for this elevation in bone weight at the end of the study. This is further supported by the increased thickness in all regions of the cortical bone observed in this strain. The findings in tibia and femur dry weight are consistent with several studies which reported no weight differences throughout maturation and an extended laying period, despite various nutritional interventions to stimulate growth of the long bones, such as increasing calcium, phosphorous and other nutrient inclusion rates in the diet (Frost and Roland, 1991; Kim et al., 2004, 2005; Safaa et al., 2008; Pastore et al., 2012).

While many of the previous studies assessing BMC and BMD in laying hens were conducted using quantitative CT (**QCT**; Korver et al., 2004) or dual-energy x-ray absorptiometry (**DEXA**; Schreiweis et al., 2003, 2005), the current study was completed using μCT analysis. This recently validated method was established as a highly accurate indicator of bone-breaking strength and mineral status in chickens (Donkó et al., 2018). We selected the tibia, as this bone is the most commonly studied in laying hens due to its unique rapid growth and utilization during lay. Since the tibia is highly susceptible to calcium imbalance (Cloft et al., 2018), it is able to provide an adequate overview of the skeletal health of the individual. However, it is important to note that the tibia, while recognized as the second largest reserve of medullary bone, is only one of many sources (Clunies et al., 1992), and our results represent only a subset of the medullary content available to the hen during egg formation. During sexual maturation, no significant changes in tibia bone thickness, cBMC, or cBMD were observed regardless of strain. This signifies that in addition to the lack of cortical bone accumulation during the later growth period, this bone source was not being reabsorbed at this time (van de Velde et al., 1984; Dacke et al., 1993; Kerschnitzki et al., 2014). Furthermore, despite a numerical increase in all 3 strains, no significant differences were observed in mBMC or mBMD during entry into lay (17−20 woa). This indicates that, contrary to previous reports (Whitehead and Fleming, 2000), while the medullary bone in the diaphysis of the tibia does begin to accumulate, this was not a rapid process shown to be synchronized with the initial rise in E_2 and the corresponding onset of lay in any strain. Accordingly, these results reveal that the sexual maturation process did not impact the overall skeletal structure in our study. It is also important to note that both the blood and bone samples were collected following oviposition, approximately 3 to 6 h after the lights turned on, meaning that while eggshell formation was not concurrently occurring, the hens had not yet consumed all of the dietary calcium necessary to replenish the medullary content (van de Velde et al., 1984; Dacke et al., 1993). While variation due to the influence of time was controlled in this study, further studies should consider the 24 h variation in mBMC and mBMD within the tibia and other medullary bone sources in the laying hen. As expected, since Smoky Joe hens displayed the lowest production rate, the mBMD of these hens was greatest overall by 100 woa. However, cBMD was not found to differ between strains. This indicates that while displaying a smaller bone at maturity and maintaining the highest production rate throughout, the cortical density of the tibia in Lohmann hens was not negatively impacted.

While the influence of estrogen on the period surrounding medullary bone formation has been well studied (Ali, 1992; Whitehead and Fleming, 2000; Whitehead, 2004), the impact of intensive selection on this relationship is less clear. Previous studies have considered the immediate effects of increasing E_2 levels throughout maturation compared to treatment with the aromatase inhibitor, letrozole (**LZ**; reviewed by: Haynes et al., 2003; Dowsett et al., 2005), with LZ shown to reduce circulating E_2 levels by 33 to 50% (Deng et al., 2010). Overall, LZ treatment in pullets increased cortical bone area and cBMD at 6 and 9 woa compared to untreated animals (Li et al., 2019). Meanwhile, plasma calcium concentration was lower in LZ treated hens (Deng et al., 2010), with a reduction in calcium-binding protein in the duodenum (Li et al., 2018) and a decline in estrogen receptors (Deng et al., 2010; Li et al., 2018). Taken together, these studies demonstrate that higher levels of E_2 are associated with medullary bone formation, while lower levels support the formation of cortical bone. This is in line with the results of the current study, with E_2 found to be positively correlated with mBMC in all strains, as well as positively associated with mBMD in Lohmann and Shaver hens, while negative correlations were observed between E_2 and cBMC in Shaver hens and between E_2 and cBMD in the Lohmann and Shaver strains. This was accompanied by an anticipated negative correlation between cortical and medullary parameters in all strains.

Surprisingly, no changes in cBMC or cBMD were observed in the tibia of Lohmann hens during the extended laying period, indicating an absence of cortical bone breakdown despite an enhanced cumulative egg number. This finding, combined with the lack of changes in cortical bone thickness during the persistent laying cycle, suggests a lack of osteoporosis in the diaphysis of

the tibia, as defined by Whitehead and Fleming (2000). While this contrasts with a previous report showing a loss of structural bone, especially the overall cBMD, from the initiation of lay to the end of the cycle regardless of strain (Wilson et al., 1992), a study by Kim et al. (2007), using a more contemporary strain reported no changes in cBMD regardless of laying status. This may be explained by the moderate heritability of BMD reported in hens ($h^2 = 0.35$ to 0.40; Bishop et al., 2000; Guo et al., 2017) and its possible association with body size and egg production (Rennie et al., 1997; Fleming et al., 2004), as both of these traits are part of the current breeding programs. In fact, genetics have been estimated to be responsible for up to 40% of the variation in bone quality (Bishop et al., 2000). Nonetheless, in addition to genetics, improvements in diet and housing have also contributed to successfully alleviating osteoporosis in laying hens (Whitehead, 2000; Bouvarel et al., 2011; Sokołowicz et al., 2018).

Beyond sexual maturation and the lay of the first egg, our study identified significant differences between strains throughout the laying cycle in the medullary bone. Specifically, mBMC significantly increased by 45 woa in Shaver and 25 woa in Smoky Joe hens while it did not increase until 75 woa in Lohmann hens. At this age, Shaver and Smoky Joe hens were producing at a lower rate than the Lohmann, resulting in a lower requirement for calcium to be transported to the oviduct, hence reducing bone resorption (Whitehead, 2004; Mazzuco and Hester, 2005). Intriguingly, all 3 strains displayed the highest mBMC and mBMD at 100 woa, suggesting that as reported by Whitehead (2004), these hens continued to deposit calcium as medullary bone throughout the laying cycle. Interestingly, in Smoky Joe hens, this corresponded to a period (75−100 woa) when spontaneous moult was observed with only one sampled hen remaining in lay at 75 woa and 2 having initiated a second laying cycle at 100 woa. Spontaneous moult triggering bone remodeling could have resulted in a secondary deposition of cortical bone observed at 100 woa. This is supported by Mazzuco and Hester (2005), who showed that total BMD declines during moult, recovering only once the second laying cycle has begun. Surprisingly, while a previous study by Pongmanee et al. (2020) reported a declining thickness in the cortical region, which was then replaced with medullary bone, this was not the case in our study.

The ability to maintain bone quality throughout the extended laying cycle is critical as layers require a substantial amount of calcium for each eggshell. In fact, the ability of the bone to store this calcium for deposition when the dietary source is insufficient will determine egg quality. As classified by the Canadian Food Inspection Agency (CFIA, 2019), Smoky Joe hens laid pee-wee (less than 42 g) to small (42−49 g) eggs at 26 woa, corresponding to the age at which all hens had entered lay. While smaller eggs during the initial weeks of the cycle were anticipated, this continuous production of pee-wee eggs by Smoky Joe hens beyond the peak of lay is indicative of the breeding objectives at that time, with no emphasis placed on early egg production rate or egg weight. In contrast, eggs from Lohmann and Shaver hens were classified as large (56−63 g) by 26 woa, which, in combination with an earlier entry in lay, is indicative of the success of breeding programs and consistent with previous studies (Akbari Moghaddam Kakhki et al., 2020b; Khanal et al., 2020b). This is no surprise, as an increase in egg weight from initiation to 37 woa was found to be moderately heritable ($h^2 = 0.36$), although the heritability of early egg weight and egg weight at 37 woa were independently determined ($h^2 = 0.39$ and 0.45, respectively) (Poggenpoel and Duckitt, 1988). Additionally, genetic correlations have been observed between egg weight increase and 2 traits of interest, age of first egg and production rate (Poggenpoel and Duckitt, 1988). However, while the correlation was positive for production rate up to 273 d (0.44), the genetic correlation between egg weight increase and age of first egg was negative (−0.50) (Poggenpoel and Duckitt, 1988). Furthermore, deviation from pullet target body weight has been shown to influence the mean egg weight during the early laying phase, with a correlation of 0.85. This translated to egg weight increasing by 0.7 g per 100-g increase in pullet live body weight at the onset of lay (Bouvarel et al., 2011). This may explain the smaller size of the eggs at the beginning of the cycle and partly explain the continuation of small eggs produced by Smoky Joe hens as they remained lighter than the other strains during peak production (Hanlon et al., 2021). With Smoky Joe hens and eggs remaining lighter throughout the entire laying cycle, the present study suggests that body weight may play a larger role in controlling egg weight throughout the production cycle than initially suspected, as demonstrated by Leeson and Summers (2005). Although, rather than body weight, body composition appears more important to directly influence egg weight (Bouvarel et al., 2011). Beyond weight, our results also show that eggs from Lohmann hens displayed the highest shell quality during the early laying phase with a moderate, positive correlation between EST and EBS. Interestingly, De Ketelaere et al. (2002) found an EST of 0.370 mm at 36 woa in Lohmann LSL hens, lower than any of the measurements presented in this study for this strain. This suggests that, due to the heritability of this trait ($h^2 = 0.446 \pm 0.126$) (Sreenivas et al., 2013), early eggshell quality has improved over the last 8 yr for this strain. These improvements are further highlighted when considering a study by Tyler and Geake (1958), which reported white leghorn eggs with an EST of 0.340 mm, prior to the inclusion of shell quality in the breeding programs and improvements in dietary supplementation.

Shell thickness was found to decrease in both Lohmann and Shaver hens, consistent with previous literature suggesting that a constant quantity of calcium and minerals is supplied to the shell gland at the time of deposition, despite the increasing egg weight or yolk size (Roland, 1979; Buss, 1988). While the decrease in shell quality of eggs produced beyond 72 woa has been a

concern in commercial strains (De Ketelaere et al., 2002), based on our results and more recent literature (Chang-Ho et al., 2014; Molnár et al., 2016), this may become less of an issue. Nonetheless, Molnár et al. (2016) reported a significant weekly decline in EST of 0.23 μm between 60 and 80 woa. Notably, the EST reported during the early laying cycle in the study mentioned above was similar to the current findings, demonstrating values well above those reported in older studies (De Ketelaere et al., 2002; Kemps et al., 2006). Similar to EST, EBS declined over time in all strains, as previously reported (Chang-Ho et al., 2014; Sirri et al., 2018; Fathi et al., 2019). However, while Chang-Ho et al. (2014) reported no correlation between EST and EBS, Sirri et al. (2018) found a strong positive correlation (0.456) between these two traits, in agreement with the current study. This relationship has allowed breeding companies to use EST as a noninvasive method to study the shell strength of their flocks (Bain et al., 2016). Though EBS was found to decline steadily over time in Lohmann and Shaver hens, this decline was less consistent in the Smoky Joe. This could potentially be due to the spontaneous moult observed for this strain and the very low productivity, allowing more time for the formation of a stronger shell. Overall, this study hypothesizes that the lack of differences observed between strains at 100 woa was due to the relatively recent incorporation of the extended laying period into commercial breeding programs with further room for improvement. This is critical as the strength of the eggshell continues to be an economically important trait (Fathi et al., 2007; Iqbal et al., 2017; Lopez et al., 2018). Nonetheless, it is imperative to highlight that Lohmann hens were the only strain that did not undergo a spontaneous moult while maintaining similar eggshell quality at 100 woa.

While insufficient dietary calcium would have an adverse effect on eggshell quality (Classen and Scott, 1982; Hartel, 1990) and bone strength (Whitehead, 2004), the lack of changes in plasma calcium levels during the entire laying cycle for all three strains indicates that the hens in this study met their calcium requirements. As anticipated, plasma calcium levels were lowest at 18 woa, prior to photostimulation and the initiation of lay. This corresponded to the last sampling period during which hens were provided with a grower diet with a 1.0% calcium inclusion. At 19 woa, hens were switched to a breeder diet containing 4.24% calcium in preparation for lay. This change in the diet most likely contributed to the elevated levels in plasma calcium observed from 28 to 94 woa in all strains. This is similar to the findings of Bar et al. (1996), who showed plasma calcium levels were approximately 2.5 mmol/L during the immature state, rising to 5 to 6 mmol/L for the remainder of the laying period. These findings were attributed to a combination of dietary changes and the rise in E_2 associated with the formation of medullary bone, leading to an increase in mobilized calcium. In fact, treatment with E_2 was shown to elevate plasma calcium levels, while the opposite result occurred when the hens were treated with the anti-estrogen, Tamoxifen (Bar et al., 1996). Therefore, the elevation in plasma calcium observed between 18 and 28 woa, partly due to the increased dietary calcium content, was also associated with the elevated E_2 concentrations observed during maturation. Without correcting the dietary calcium levels for the production rate, the lack of detected differences in plasma calcium levels aligns with previous findings (Bacon et al., 1980; Qin and Klandorf, 1995). Furthermore, the lack of change in plasma calcium levels within the Lohmann and Shaver throughout the remainder of the laying cycle corresponded to an increase in mBMD, while eggshell thickness decreased and egg size increased. As previously reported by Roland (1979) and Buss (1988), our results indicate that the quantity of eggshell deposited remains constant regardless of egg size, leading to thinning of the shell with an increase in surface area. This occurs despite the availability of the medullary source, as additional calcium content is not mobilized within the plasma. This is further supported by the lack of change in calcium concentration observed in Smoky Joe hens, as EST and egg weight remained constant throughout lay, despite an increase in mBMD. Interestingly, it has been shown that calcium absorption declines in the ageing hen, contributing to the deterioration of shell and bone quality (Abe et al., 1982; Joyner et al., 1987) . In our study, plasma calcium levels did not decline over time, while medullary bone content increased in all strains at the end of the study. This suggests that even if absorption was reduced, the constant plasma calcium levels were maintained through bone reabsorption. In fact, despite displaying increased productivity, current commercial hens demonstrate the potential to maintain calcium absorption better than previous studies have suggested.

In conclusion, results from this study highlight that modern commercial laying hens sustained high production rates, reaching over 500 eggs in 100-wk, with no apparent detrimental impact to medullary and cortical BMC or BMD in the long bones assessed. In fact, despite producing half of the cumulative number of eggs in the same laying cycle, the Smoky Joe hens demonstrated a larger overall skeletal structure, yet no significant improvements in bone quality over the modern commercial strain. In addition, egg quality was found to be significantly improved during the early laying cycle in Lohmann hens, and while a decline in shell quality due to increasing egg weight was observed over time, the overall quality remained higher in Lohmann hens than both Shaver and Smoky Joe hens in the latter part of the cycle. Altogether, this study demonstrates that despite the elevated production rate of the current commercial hens throughout a 100-wk study, improvements in eggshell quality, along with the maintenance of the skeletal structure, have been achieved, allowing for a successful persistent laying period.

ACKNOWLEDGMENTS

The authors would like to acknowledge and thank our funding agencies, Livestock Research Innovation Corporation (through Egg Farmers of Ontario and the Poultry Industry Council; Project #331), Natural Sciences and Engineering Research Council (NSERC) through the discovery program, the Ontario Ministry of Agriculture and Rural Affairs (OMAFRA; Project #27311) for access to the Arkell Research Station through their Tier II funding and Canada First Research Excellence Fund (CFREF) for their contributions via the Food from Thought program (Agricultural Systems for a Healthy Planet) at the University of Guelph. We would also like to thank the summer students involved in this project (Emily Deignan, Quinn Rausch, Virginia Madsen, and Bryn Van Winters), as well as the Staff of the Arkell Poultry Research Station for their time and dedication to making this trial a success.

DISCLOSURES

The authors affirm that there is no conflict of interest.

REFERENCES

Abe, E., H. Horikawa, T. Masumaura, M. Sugahara, and T. Suda. 1982. Disorders of cholecalciferol metabolism in old egg-laying hens. Journal of Nutrition 112:436–446.

Akbari Moghaddam Kakhki, R., T. Heuthorst, A. Wornath-Vanhumbeck, M. Neijat, and E. Kiarie. 2019. Medullary bone attributes in aged lohmann lsl-lite layers fed different levels of calcium and top-dressed 25-hydroxy vitamin D 3. Can. J. Anim. Sci. 99:138–149.

Akbari Moghaddam Kakhki, R., K. R. Price, J. Moats, G. Bédécarrats, N. A. Karrow, and E. G. Kiarie. 2020a. Impact of feeding microalgae (Aurantiochytrium limacinum) and co-extruded mixture of full-fat flaxseed as sources of n-3 fatty acids to ISA brown and Shaver white breeders and progeny on pullet skeletal attributes at hatch through to 18 weeks of age. Poult. Sci. 99:2087–2099.

Akbari Moghaddam Kakhki, R., V. L. Shouldice, K. R. Price, J. Moats, and E. G. Kiarie. 2020b. n-3 fatty acids fed to ISA brown and Shaver white breeders and their female progeny during rearing: Impact on egg production, eggshell, and select bone attributes from 18 to 42 weeks of age. Poult. Sci. 99:3959–3970.

Ali, S. 1992. Matrix formation and mineralisation in bone. Pages 19−38 in Bone Biology and Skeletal Disorders in Poultry. C. C. Whitehead, ed. Carfax Publishing Co, Oxfordshire, UK.

Bacon, W. L., K. I. Brown, and M. A. Musser. 1980. Changes in plasma calcium, phosphorus, lipids, and estrogens in turkey hens with reproductive state. Poult. Sci. 59:444–452.

Bain, M. M., Y. Nys, and I. C. Dunn. 2016. Increasing persistency in lay and stabilising egg quality in longer laying cycles. What are the challenges? Br. Poult. Sci. 57:330–338.

Bar, A., E. Vax, W. Hunziker, O. Halevy, and S. Striem. 1996. The role of gonadal hormones in gene expression of calbindin (Mr 28,000) in the laying hen. Gen. Comp. Endocrinol. 103:115–122.

Baxter, M., N. Joseph, V. R. Osborne, and G. Y. Bédécarrats. 2014. Red light is necessary to activate the reproductive axis in chickens independently of the retina of the eye. Poult. Sci. 93:1289–1297.

Benoit, J., and J. Clavert. 1945. Role indispensable des parathyroids dans la calcification de l'osfolliculinque chez le canard domestique. Cr. Seanc. Soc. Biol. 139:743.

Bishop, S. C., R. H. Fleming, H. A. Mccormagk, D. K. Flock, and C. C. Whitehead. 2000. Inheritance of bone characteristics affecting osteoporosis in laying hens. Br. Poult. Sci. 41:33–40.

Bloom, M. A., L. V Domm, A. Nalbandov, and W. Bloom. 1958. Medullary bone of laying chickens. Am. J. Anat. 102:411–453.

Bouvarel, I., Y. Nys, and P. Lescoat. 2011. Hen nutrition for sustained egg quality. Pages 261−290 in Improving the Safety and Quality of Eggs and Egg Products, Vol 1: Egg Chemistry, Production and Consumption. Y. Nys, M. Bain and F. Van Immerseel, eds. Woodhead Publishing Ltd, Cambridge, UK.

Buss, E. G. 1988. Correlation of egg shell weight with egg weight, body weight, and percent shell. Pages 348−349 in Proceedings of the XVIII World's Poultry Congress.

CCAC, 2009. CCAC: Guidelines on the Care and Use of Farm Animals in Research, Teaching and Testing. Canadian Council on Animal Care, Ottawa, ON, Canada (2009), pp. 1−168.

CFIA. 2019. Operational procedure: shell egg product inspection. Can. Food Insp. Agency, Gov. Canada, Canada. Accessed December 2021. https://www.inspection.gc.ca/food-safety-for-industry/compliance-continuum/guidance-for-inspectors/sip/shell-egg-product-inspection/eng/1541106195931/1541106196158#a72.

Chang-Ho, K., J. Song, J. Lee, and K. Lee. 2014. Age-Related changes in egg qulaity of Hy-Line brown hens. Int. J. Poult. Sci. 13:510–514.

Clark, W. D., W. R. Cox, and F. G. Silversides. 2008. Bone fracture incidence in end-of-lay high-producing, noncommercial laying hens identified using radiographs. Poult. Sci. 87:1964–1970.

Classen, H. L., and T. A. Scott. 1982. Self-selection of calcium during the rearing and early laying periods of White Leghorn pullets. Poult. Sci. 61:2065–2074.

Cloft, S. E., C. I. Robison, and D. M. Karcher. 2018. Calcium and phosphorus loss from laying hen bones autoclaved for tissue removal. Poult. Sci. 97:3295–3297.

Clunies, M., D. Park, and S. Leeson. 1992. Calcium and phosphorous metabolism and eggshell formation of hens fed different amounts of calcium. Poult. Sci. 71:482–489.

Common, R. H., N. A. Rutledge, and R. W. Hale. 1948. Observation on mineral metabolism of pullets. VIII. The influence of gonadal hormones on the nutrition of calcium and phosphorous. J. Agric. Sci. 38:64–78.

Dacke, C. G., S. Arkle, D. J. Cook, I. M. Wormstone, S. Jones, M. Zaidi, and Z. a. Bascal. 1993. Medullary bone and avian calcium regulation. J. Exp. Biol. 88:63–88.

Datta, H. K., W. F. Ng, J. A. Walker, S. P. Tuck, and S. S. Varanasi. 2008. The cell biology of bone metabolism. J. Clin. Pathol. 61:577–587.

Deng, Y. F., X. X. Chen, Z. L. Zhou, and J. F. Hou. 2010. Letrozole inhibits the osteogenesis of medullary bone in prelay pullets. Poult. Sci. 89:917–923.

Donkó, T., A. Tischler, Csóka, G. Kovács, M. Emri, Petneházy, A. Szabó, V. Halas, J. Tossenberger, R. Garamvölgyi, and G. Bajzik. 2018. Estimation of bone mineral density and breaking strength of laying hens based on scans of computed tomography for body composition analysis. Br. Poult. Sci. 59:365−370.

Dowsett, M., L. A. Martin, I. Smith, and S. Johnston. 2005. Mechanisms of resistance to aromatase inhibitors. J. Steroid Biochem. Mol. Biol. 95:167–172.

Driggers, J. C., and C. L. Comar. 1949. The secretion of radioactive calcium (Ca45) in the hen's egg. Poult. Sci. 28:420–424.

Dunn, I. C., N. T. Joseph, M. Bain, A. Edmond, P. W. Wilson, P. Milona, Y. Nys, J. Gautron, M. Schmutz, R. Preisinger, and D. Waddington. 2008. Polymorphisms in eggshell organic matrix genes are associated with eggshell quality measurements in pedigree Rhode Island Red hens. Anim. Genet. 40:110–114.

Duran, M. R., C. Chen, and W. K. Kim. 2018. Effects of vitamin d and calcium for the prevention of osteoporosis at various stages of life of laying hens-review. Int. J. Poult. Sci. 17:405–409.

Fathi, M., A. El-Dein, S. El-Safty, and L. Radwan. 2007. Using scanning electron microscopy to detect the ultrastrucutral variations in eggshell quality of Fayoumi and Dandarawi chicken breeds. Int. J. Poult. Sci. 6:236–241.

Fathi, M. M., A. Galal, U. M. Ali, and O. K. Abou-Emera. 2019. Physical and mechanical properties of eggshell as affected by chicken breed and flock age. Br. Poult. Sci. 60:506–512.

Fleming, R., H. McCormack, L. Mcteir, and C. C. Whitehead. 1998. Medullary bone and humeral breaking strength in laying hens. Res. Vet. Sci. 64:63–67.

Fleming, R. H., H. A. McCormack, L. McTeir, and C. C. Whitehead. 2004. Incidence, pathology and prevention of keel bone deformities in the laying hen. Br. Poult. Sci. 45:320–330.
Frost, T. J., and D. A. Roland. 1991. The influence of various calcium and phosphorus levels on tibia strength and eggshell quality of pullets during peak production. Poult. Sci. 70:963–969.
Guo, J., C. Sun, L. Qu, M. Shen, T. Dou, M. Ma, K. Wang, and N. Yang. 2017. Genetic architecture of bone quality variation in layer chickens revealed by a genome-wide association study. Sci. Rep. 7:1–10.
Hamilton, R. M. J., K. G. Hollands, P. W. Voisey, and A. A. Grunder. 1979. Relationship between egg shell quality and shell breakage and factors that affect shell breakage in the field—a review. Worlds. Poult. Sci. J. 35:177–190.
Hanlon, C., K. Takeshima, and G. Y. Bédécarrats. 2021. Changes in the control of the hypothalamic-pituitary gonadal axis across three differentially selected strains of laying hens (Gallus gallus domesticus). Front. Physiol. 12:1–20.
Hartel, H. 1990. Evaluation of the dietary interaction of calcium and phosphorus in the high producing laying hen. Br. Poult. Sci. 31:473–494.
Haynes, B. P., M. Dowsett, W. R. Miller, J. M. Dixon, and A. S. Bhatnagar. 2003. The pharmacology of letrozole. J. Steroid Biochem. Mol. Biol. 87:35–45.
Hocking, P. M., M. Bain, C. E. Channing, R. Fleming, and S. Wilson. 2003. Genetic variation for egg production, egg quality and bone strength in selected and traditional breeds of laying fowl. Br. Poult. Sci. 44:365–373.
Hudson, H. A., W. M. Britton, G. N. Rowland, and R. J. Buhr. 1993. Histomorphometric bone properties of sexually immature and mature white leghorn hens with evaluation of fluorochrome injection on egg production traits. Poult. Sci. 72:1537–1547.
Iqbal, J., N. Mukhtar, Z. U. Rehman, S. H. Khan, T. Ahmad, M. S. Anjum, R. H. Pasha, and S. Umar. 2017. Effects of egg weight on the egg quality, chick quality, and broiler performance at the later stages of production (week 60) in broiler breeders. J. Appl. Poult. Res. 26:183–191.
Joyner, C., M. Peddie, and T. Taylor. 1987. The Effect of Age on Egg Production in the Domestic Hen. General and Comparative Endocrinology 65:331–336.
Kemps, B. J., T. Govaerts, B. De Ketelaere, K. Mertens, F. R. Bamelis, M. M. Bain, E. M. Decuypere, and J. G. De Baerdemaeker. 2006. The influence of line and laying period on the relationship between different eggshell and membrane strength parameters. Poult. Sci. 85:1309–1317.
Kerschnitzki, M., T. Zander, P. Zaslansky, P. Fratzl, R. Shahar, and W. Wagermaier. 2014. Rapid alterations of avian medullary bone material during the daily egg-laying cycle. Bone 69:109–117.
Keshavarz, K. 1998a. Investigation on the possibility of reducing protein, phosphorus, and calcium requirements of laying hens by manipulation of time of access to these nutrients. Poult. Sci. 77:1320–1332.
Keshavarz, K. 1998b. Further investigations on the effect of dietary manipulation of protein, phosphorus, and calcium for reducing their daily requirement for laying hens. Poult. Sci. 77:1333–1346.
Keshavarz, K., and S. Nakajima. 1993. Re-evaluation of calcium and phosphorus requirements of laying hens for optimum performance and eggshell quality. Poult. Sci. 72:144–153.
De Ketelaere, B., T. Govaerts, P. Coucke, E. Dewil, J. Visscher, E. Decuypere, and J. De Baerdemaeker. 2002. Measuring the eggshell strength of 6 different genetic strains of laying hens: techniques and comparisons. Br. Poult. Sci. 43:238–244.
Khanal, T., G. Y. Bedecarrats, and E. G. Kiarie. 2021. Cage type and mineral nutrition had independent impact on skeletal development in Lohmann LSL-Lite pullets from hatch to 16 weeks of age. Anim. Nutr. 7:631–640.
Khanal, T., G. Y. Bédécarrats, T. Widowski, and E. G. Kiarie. 2020a. Rearing cage type and dietary limestone particle size: I, effects on growth, apparent retention of calcium, and long bones attributes in Lohmann selected Leghorn-Lite pullets. Poult. Sci. 99:4454–4465.
Khanal, T., G. Y. Bédécarrats, T. M. Widowski, and E. G. Kiarie. 2020b. Rearing cage type and dietary limestone particle size: II, effects on egg production, eggshell, and bone quality in Lohmann selected Leghorn-Lite hens. Poult. Sci. 99:5763–5770.
Khanal, T., T. Widowski, G. Y. Bédécarrats, and E. Kiarie. 2019. Effects of pre-lay dietary calcium (2.5 vs. 4.0%) and pullet strain (Lohmann Brown vs . Selected Leghorn LSL-Lite) on calcium utilization and femur quality at 1st through to the 50th egg. Poult. Sci. 98:4919–4928.
Kim, W. K., L. M. Donalson, S. A. Bloomfield, H. A. Hogan, L. F. Kubena, D. J. Nisbet, and S. C. Ricke. 2007. Molt performance and bone density of cortical, medullary, and cancellous bone in laying hens during feed restriction or alfalfa-based feed molt. Poult. Sci. 86:1821–1830.
Kim, W. K., L. M. Donalson, P. Herrera, L. F. Kubena, D. J. Nisbet, and S. C. Ricke. 2005. Comparisons of molting diets on skeletal quality and eggshell parameters in hens at the end of the second egg-laying cycle. Poult. Sci. 84:522–527.
Kim, W. K., L. M. Donalson, P. Herrera, C. L. Woodward, L. F. Kubena, D. J. Nisbet, and S. C. Ricke. 2004. Effects of different bone preparation methods (fresh, dry, and fat-free dry) on bone parameters and the correlations between bone breaking strength and the other bone parameters. Poult. Sci. 83:1663–1666.
Kocevski, D., N. Nikolova, and A. Kuzelov. 2011. The influence of strain and age on some egg quality parameters of commercial laying hens. Biotechnol. Anim. Husb. 27:1649–1658.
Korver, D. R., J. L. Saunders-Blades, and K. L. Nadeau. 2004. Assessing bone mineral density in vivo: quantitative computed tomography. Poult. Sci. 83:222–229.
Lee, W. Do, D. Kothari, K. M. Niu, J. M. Lim, D. H. Park, J. Ko, K. Eom, and S. K. Kim. 2021. Superiority of coarse eggshell as a calcium source over limestone, cockle shell, oyster shell, and fine eggshell in old laying hens. Sci. Rep. 11:1–10.
Leeson, S., and J. D. Summers. 2005. Commercial Poultry Nutrition. 3rd ed. University Books, Guelph, Ontario.
Len, R., H. Abplanalp, and E. A. Johnson. 1964. Second year production of force molted hens in the California random sample test. Poult. Sci. 43:638–646.
Li, Q., J. Xia, S. Wang, Z. Zhou, and Z. Li. 2019. Letrozole induced changes in bone mineral properties and mechanical functions of laying hens. Poult. Sci. 98:2562–2569.
Li, Q., X. Zhao, S. Wang, and Z. Zhou. 2018. Letrozole induced low estrogen levels affected the expressions of duodenal and renal calcium-processing gene in laying hens. Gen. Comp. Endocrinol. 255:49–55.
Lopez, J. C., L. Kitto, and R. M. Hulet. 2018. Effect of eggshell temperature on survival rate, development at hatch, and 7-day growth. J. Appl. Poult. Res. 27:249–252.
Mazzuco, H., and P. Y. Hester. 2005. The effect of an induced molt and a second cycle of lay on skeletal integrity of White Leghorns. Poult. Sci. 84:771–781.
Mccoy, M. A., and G. A. C. Reilly. 1996. Density and breaking strength of bones of mortalities among caged layers. Res. Vet. Sci. 60:185–186.
Miller, S. C. 1992. Calcium homeostasis and mineral turnover in the laying hen. Pages 103—116 in Bone Biology and Skeletal Disorders in Poultry. C. C. Whitehead, ed. Carfax Publishing Co., Oxfordshire, UK.
Molnár, A., L. Maertens, B. Ampe, J. Buyse, I. Kempen, J. Zoons, and E. Delezie. 2016. Changes in egg quality traits during the last phase of production: is there potential for an extended laying cycle? Br. Poult. Sci. 57:842–847.
Mueller, W. J., R. Schraer, and H. Scharer. 1964. Calcium metabolism and skeletal dynamics of laying pullets. J. Nutr. 84:20–26.
NRC (National Research Council). 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.
Nys, Y., M. T. Hincke, J. M. Garcia-ruiz, and S. Solomon. 1999. Avian eggshell mineralization. Poult. Avian Biol. Rev. 10:143–166.
Pastore, S. M., P. C. Gomes, H. S. Rostagno, L. F. T. Albino, A. A. Calderano, C. R. Vellasco, G. da, S. Viana, and R. L. de Almeida. 2012. Calcium levels and calcium: available phosphorus ratios in diets for white egg layers from 42 to 58 weeks of age. Rev. Bras. Zootec. 41:2424–2432.
Poggenpoel, D. G., and J. S. Duckitt. 1988. Genetic basis of the increase in egg weight with pullet age in a white leghorn flock. Br. Poult. Sci. 29:863–867.
Pongmanee, K., I. Kühn, and D. R. Korver. 2020. Effects of phytase supplementation on eggshell and bone quality, and phosphorus and calcium digestibility in laying hens from 25 to 37 wk of age. Poult. Sci. 99:2595–2607.

Qi, X., D. Tan, C. Wu, C. Tang, T. Li, X. Han, J. Wang, C. Liu, R. Li, and J. Wang. 2016. Deterioration of eggshell quality in laying hens experimentally infected with H9N2 avian influenza virus. Vet. Res. 47:1–10.

Qin, X., and H. Klandorf. 1995. Effect of estrogen on egg production, shell quality and calcium metabolism in molted hens. Comp. Biochem. Physiol. − Part C 110:55–59.

Rennie, J. S., R. H. Fleming, H. A. Mccormack, C. C. Mccorquodale, and C. Whitehead. 1997. Studies on effects of nutritional factors on bone structure and osteoporosis in laying hens. Br. Poult. Sci. 38:417–424.

Rising, J., and K. Somers. 1989. The measurement of overall body size in birds. Auk Ornithol. Adv. 106:666–674.

Roland, D. A. 1979. Factors influencing shell quality of aging hens. Poult. Sci. 58:774–777.

Safaa, H. M., M. P. Serrano, D. G. Valencia, M. Frikha, E. Jiménez-Moreno, and G. G. Mateos. 2008. Productive performance and egg quality of Brown egg-laying hens in the late phase of production as influenced by level and source of calcium in the diet. Poult. Sci. 87:2043–2051.

Salter, D. W., W. S. Payne, D. T. Ramsey, M. Blair, and J. A. Render. 1997. A new inherited ocular anomaly in pigmented White Leghorn chickens. J. Vet. Diagn. Investig. 9:407–409.

van Sambeek, F. 2010. Longer production cycles from a genetic perspective. Prog. Layer Genet. 10:1–5.

Sauveur, B. 1991. Effect of method of rearing fowls on egg characters. Prod. Anim. 4:123–130.

Schreiweis, M. A., J. I. Orban, M. C. Ledur, and P. Y. Hester. 2003. The use of densitometry to detect differences in bone mineral density and content of live white leghorns fed varying levels of dietary calcium. Poult. Sci. 82:1292–1301.

Schreiweis, M. A., J. I. Orban, M. C. Ledur, D. E. Moody, and P. Y. Hester. 2005. Validation of dual-energy X-ray absorptiometry in live White Leghorns. Poult. Sci. 84:91–99.

Sirri, F., M. Zampiga, A. Berardinelli, and A. Meluzzi. 2018. Variability and interaction of some egg physical and eggshell quality attributes during the entire laying hen cycle. Poult. Sci. 97:1818–1823.

Smith K., 2010. Accessed Aug. 2020. https://docplayer.net/24990397-The-history-of-shaver-Q10breeding-farms.html.

Sokołowicz, Z., J. Krawczyk, and M. Dykiel. 2018. The effect of the type of alternative housing system, genotype and age of laying hens on egg quality. Ann. Anim. Sci. 18:541–555.

Sreenivas, D., M. Prakash, M. Mahender, and R. Chatterjee. 2013. Genetic analysis of egg quality traits in White leghorn chicken. Veterinary World 6(5):263–266.

Torres, C. A., and D. R. Korver. 2018. Influences of trace mineral nutrition and maternal flock age on broiler embryo bone development. Poult. Sci. 97:2996–3003.

Tyler, C., and F. H. Geake. 1958. Studies on egg shells. IX.—The influence of individuality, breed and season on certain characteristics of egg shells from pullets. J. Sci. Food Agric. 9:473–483.

van de Velde, J. P., J. P. W. Vermeiden, J. J. A. Touw, and J. P. Veldhuijzen. 1984. Changes in activity of chicken medullary bone cell population in relation to the egg-laying cycle. Metab. Bone. Dis. Rel. Res. 5:191–193.

Whitehead, C. 2000. Genotype and nutrition interactions in relation to bone strength. Lohmann Inf 23:15–20.

Whitehead, C. C. 2004. Overview of bone biology in the egg-laying hen. Poult. Sci. 83:193–199.

Whitehead, C. C., and R. H. Fleming. 2000. Osteoporosis in Cage Layers. Poult. Sci. 79:1033–1041.

Whitehead, C. C., and S. Wilson. 1992. Characteristics of osteopenia in hens. Pages 265−280 in Bone Biology and Skeletal Disorders in Poultry. C. C. Whitehead, ed. Carfax Publishing Co., Oxfordshire, UK.

Wilson, S., S. R. I. Duff, and C. C. Whitehead. 1992. Effects of age, sex and housing on the trabecular bone of kaying strain domestic fowl. Res. Vet. Sci. 53:52–58.

Zakaria, A. H., T. Miyaki, and K. Imai. 1983. The effect of aging on the ovarian follicular growth in laying hens. Poult. Sci. 62:670–674.

Zita, L., E. Tůmová, and L. Štolc. 2009. Effects of genotype, age and their interaction on egg quality in brown-egg laying hens. Acta Vet. Brno 78:85–91.