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[1]

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ABSTRACT Effects of rearing cage type and dietary limestone particle size (**LPS**) on growth, apparent retention (**AR**) of nutrients, and bone quality were investigated. The treatments were arranged in a 2 × 3 factorial with cage (conventional, **CON** and furnished, **FUR**) and LPS (fine, < 0.595 mm, **F**; medium, 0.595 to < 1.68 mm, **M**; and 1:1 mixture of F and M wt/wt; **FM**). A total of 900-day-old Lohmann LSL-Lite chicks were placed in CON (20 chicks/cage) and FUR (30 chicks/cage) based on BW. The diets were formulated according to breeder's nutrient specifications for starter, grower, and developer phases. At the end of 4, 12, and 16 wk of age (**woa**), 2 pullets/cage were euthanized for samples. At 12 and 16 woa, 1 pullet/cage was transferred to metabolism cages for AR measurements. There was no interaction ($P > 0.05$) between cage type and LPS on response variables. At 4 woa, body ($P = 0.002$) and bone ($P < 0.05$) weight was higher for CON than FUR pullets, but this was reversed ($P < 0.01$) at 16 woa. Pullets fed M LPS had higher ($P < 0.05$) AR of Ca, whole body mineral density (**BoMD**), and whole body mineral content (**BoMC**) than pullets fed F LPS. However, pullets fed F LPS had higher ($P < 0.05$) femur bone mineral density (**BMD**) and tended ($P = 0.059$) to have higher tibia bone breaking strength (**BBS**) than pullets fed M LPS at 16 woa. Pullets reared in CON cages had higher ($P < 0.05$) AR of Ca than FUR pullets. At 4 woa, CON pullets had lower ($P < 0.05$) femur and tibia BMD but higher tibia (93 vs. 83 N $P = 0.012$) BBS than FUR pullets. However, at 16 woa, FUR pullets had higher ($P < 0.05$) BoMD, BoMC, and tibia BBS than CON pullets. In conclusions, cage type and dietary LPS had independent effects on Ca utilization and skeletal development. Despite poor Ca retention, FUR caged pullets showed improved bone quality at 16 woa. Finer LPS improved femur mineral density suggesting coarser LPS had limited effects on pullet bone quality.

Key words: housing, limestone particle size, long bone attribute, pullet, rearing

INTRODUCTION

Osteoporosis is one of the main causes for bone loss and subsequent fractures in laying hens (Whitehead and Fleming, 2000). The restricted movement of birds in cages is considered a major contributor to osteoporosis (Bishop et al., 2000). Recent studies suggested that rearing housing system influenced bone quality in terms of mineralization and breaking strength at onset through to the end of lay (Regmi et al., 2015; Casey-Trott et al., 2017; Rodriguez-Navarro et al., 2018; Neijat

et al., 2019). Housing plays a pivotal role in the expression of normal behavior by providing space and opportunity for exercise (e.g., running, moving, and jumping) and load bearing (e.g., perching) (Appleby, 1998). Certain cage types with lower average space per pullet such as conventional cages limit the frequency and extent of physical activity compared with furnished cages (Rodenburg et al., 2005). Rearing environment that offers more space for movement and provision of perches can have a remarkable effect on musculoskeletal development (Widowski and Torrey, 2018). Furnished cages also known as enriched cages or colonies are more spacious than conventional cages and offer more space and in-cage facilities (e.g., perches) for locomotion, exercise, and expression of natural behaviors (National Farm Animal Care Council, 2017). In addition to space, enrichment also affects movement and activity. For example, perches in furnished cages motivate pullets to jump up to the perch and down to the floor frequently

and thus increase the load bearing on tibia, femur, or humerus, which may also influence the tibial or femoral attributes (Hester et al., 2014). Consequently, all these activities affect the skeletal integrity and compactness.

Limestone is the most common source of calcium (**Ca**) in poultry diet throughout the world. The predominant mineral in bones is Ca, and as such Ca nutrition affects bone quality in poultry (Saunders-Blades et al., 2009). One of the determinants of limestone passage rate in the gastrointestinal tract is the limestone particle size (**LPS**) because it influences its solubility (Cheng and Coon, 1990a). Larger LPS releases Ca relatively slowly from gizzard to duodenum and has been shown to influence Ca retention in hens (Lichovnikova, 2007) and broilers (Anwar et al., 2017). For example, Ca retention and tibia ash concentration increased with LPS in broilers (Anwar et al., 2017). Also, Bradbury et al. (2018) showed a significant effect of LPS (< 0.5 mm vs. > 0.5 mm) on apparent ileal Ca digestibility in broilers. Calcium digestibility was higher for diet with fine limestone particles (< 0.15 mm) than medium (0.6–0.8 mm) and coarse (> 1.18 mm) (Guinotte et al., 1995). On the contrary, Anwar et al. (2017) reported significantly higher Ca digestibility in broilers fed a diet with coarse particle size (0.71 mm) than fine (0.43 mm). Studies indicated that feeding a medium to course LPS during pullet rearing was beneficial to bone health of pullets and hens compared with fine LPS (Eusebio-Balcazar et al., 2018).

Structural bone growth continues until the surge in estradiol at sexual maturity (Whitehead, 2004; Baxter and Bédécarrats, 2019). The surge of estradiol shifts the bone mineralization process of osteoblast from structural to medullary (Kalkwarf et al., 2003). It is plausible that the opportunities for exercise and load bearing (cage type) and bioavailability of dietary Ca (LPS) could have a synergistic positive influence on structural bone development during rearing. However, there is a dearth of information on the interactive effect of cage type and dietary LPS on aspects of Ca utilization and of bone quality attributes during rearing. Eusebio-Balcazar et al. (2018) compared bone quality of brown and white pullets reared in conventional cages and aviary and fed fine (0.43 mm) and blend of fine and course (0.87 mm) from 7 to 17 wk of age (**woa**). Independent of strain and housing, pullets fed blend LPS showed improved bone mineralization at the onset of sexual maturity with subsequent positive effects on keel bone quality during laying phase (Eusebio-Balcazar et al., 2018). However, the study did not determine whether the improved bone mineralization was linked to increased Ca utilization. Therefore, the aim of the present study was to investigate interactive effect of cage type and dietary LPS on growth performance, Ca utilization, and long bone quality of Lohmann Selected Leghorn (**LSL**)-Lite pullets.

MATERIALS AND METHODS

The experimental protocol (#3,634) was reviewed and approved by the University of Guelph Animal Care Committee. This experiment took place at the University of Guelph's Arkell Poultry Research Station in Guelph, ON, and birds were cared for in accordance with the Canadian Council on Animal Care guidelines (CCAC, 2009).

Birds and Cage Type

A total of 900-day-old LSL Lite chicks were procured from a commercial hatchery (Archer's Poultry Farm, Brighton, ON, Canada). The chicks were placed based on BW to 18 conventional (**CON**, 20 pullets/cage; Ford Dickison Inc., Mitchell, ON, Canada) and 18 furnished cages (**FUR**, 30 pullets/cage; Farmer Automatic, Clark Ag Systems Ltd., Caledonia, ON, Canada). The details of the CON and FUR cages used in the present study were described in our previous publications (Casey-Trott et al., 2017; Habinski et al., 2017). Briefly, the dimension (L × W × H) of CON and FUR cages were 76 cm × 71 cm × 46 cm and 239 cm × 80 cm × 75 cm, respectively. The FUR cages were outfitted with platforms and terraces to increase opportunities for load bearing exercises (e.g., jumping, perching, and flying) (Casey-Trott et al., 2017; Habinski et al., 2017). In each FUR cage, a feeder trough ran the length of the cage floor (11.4 cm W and 8.9 cm H; 23 cm from the front wall of the cage and 52 cm from the back wall) and allowed access on both sides. The water line was located behind the feeder, ran parallel to the feeder, and was equipped with 14 nipple drinkers and an additional 4 nipple drinkers over the platforms. In each CON cage, the feeder was located at the cage front (63 cm L, 8 cm W and 5.75 cm H) and allowed access on one side. The water line was located in the middle of the cage and was equipped with 2 nipple drinkers. In both cage types, the water lines and feeders were suspended from the ceiling and could be raised vertically in accordance with the pullet growth.

Limestone Particle Size and Diets

The limestone (Limestone Ground B2) sample was obtained from Pestell Minerals and ingredients (New Hamburg, ON, Canada) and separated into fine (**F**) and medium (**M**) particle size categories using Ro-Tap Sieve Shaker (W.S. TylerRX-30 E model, Hoskin Scientific Ltd, Burlington, ON, Canada). The distribution of LPS in this experiment ranged from 0.530 to 1.680 mm; ≤ 0.595 mm LPS was designated F LPS category and the rest M LPS category. The F and M categories were mixed 1:1 wt/wt to create fine-medium (**FM**) category. Diets were formulated for a three-phase program: starter (week 1–3), grower (week 4–8), and developer (week 9–16) and met or exceeded specifications for LSL (Tables 1 and 2) (Lohmann, 2019). In each phase, the 3 LPS categories were used as sources of Ca and corresponded to experimental diets F, FM, and M LPS. Particle size, if not suitable for beak size, could result in reduced feed intake and therefore weight

Table 1. Ingredients and chemical composition of diets, as fed basis.

Item	Starter (1–3 wk)	Grower (4–8 wk)	Developer (9–16 wk)
Ingredients, %			
Corn	55.00	56.50	58.54
Soybean meal	29.08	26.69	18.53
Wheat	10.00	10.00	15.00
Wheat middlings	0.00	2.08	3.00
Limestone[1]	1.83	1.87	1.76
Salt	0.45	0.45	0.45
Monocalcium phosphate	1.36	1.00	0.80
L-Lysine HCl	0.12	0.05	0.05
DL-Methionine	0.15	0.07	0.05
Vitamin and trace mineral premix[2]	1.00	1.00	1.00
Soy oil	1.00	0.20	0.52
Calculated provisions			
Dry matter, %	88.31	87.73	86.87
Crude protein, %	20.00	18.55	15.27
Ca, %	1.05	1.00	0.90
Available P, %	0.48	0.41	0.37
Na, %	0.18	0.18	0.18
AME, kcal/kg	2,860	2,800	2,850

[1]F, fine; FM, mixture of fine and medium (1:1 wt/wt) and M, medium limestone particle size (LPS). The LPS distribution was F ≤ 0.595 mm; M 0.595 < X ≤ 1.190 mm for starter phase and 0.595 < X ≤ 1.680 mm for grower and developer phase.

[2]Provided per kilogram of premix: Vitamin A = 1,200,000 IU, Vitamin D3 = 500,000 IU, Vitamin E = 8,000 IU, Vitamin B12 = 1,700 mcg, Biotin = 22,000 mcg, Menadione = 330 mg, Thiamine = 400 mg, Riboflavin = 860 mg, Pantothenic acid = 2,000 mg, Pyridoxine = 430 mg, Niacin = 6,500 mg, Folic acid = 220 mg, Choline = 60,000 mg, Iron = 6,000 mg, Copper = 1,000 mg.

gain during the rearing phase (Frikha et al., 2011). For this reason, M LPS category for starter (0.595 < X ≤ 1.190 mm) was different from grower and developer phases (0.595 < X ≤ 1.680 mm). The distribution of LPS of the limestone used for starter, grower, and developer diets is shown in Figures 2 and 3. The diets were prepared in crumbled form.

Animal Experimentations and Sampling

Diets were allocated within cage type based on BW in a completely randomized design to give 6 replicates per diet. The feed and water were provided *ad-libitum*. The cages were housed in rooms in different buildings within the Arkell Poultry Research station grounds. The rooms had similar lighting, temperature, humidity, and ventilation regimens. The room temperature was maintained at 35°C for the first 3 D, then gradually decreased to 20°C by 5 woa and held to the end of the experiment. The relative humidity was 50 to 60% throughout the experiment. Light (30 Lux) was provided continuously for the first 2 D, then the photoperiod was gradually decreased according to the lighting program for Lohmann LSL-Lite to 10L:14D at the intensity of

5 Lux. The light schedule was provided with a 20 min step up (dawn period) with full intensity at 05:30 h and a 20 min step down (dusk period) with light fully off at 19:30 h. The feed intake was determined by subtracting weight of feed remaining in the feeder plus spills from the weight of feed added in the feeder (any mortality was taken into consideration to calculate the corrected feed intake). Body weight was determined by dividing total cage weight by the number of the pullets in the cage.

Two pullets per cage were randomly selected, weighed, and sacrificed via cervical dislocation at the end of fourth, 12th and 16th woa for various samples. Gizzard was removed, weighed full and empty. Duodenum was located, and a 2 cm piece immediately proximal to the duodenal loop was excised, rinsed with saline, and stored in 10% buffered formalin for histomorphology. Right and left whole legs were excised at femur head and stored at −20°C until further analyses. One pullet per cage (18 from CON and 18 from FUR; total = 36 pullets) was transferred to metabolism cages at the end of 11th and 15th woa to determine apparent retention (**AR**) of components. The pullets were individually housed in accordance with treatment identification. The cage

Table 2. Analyzed chemical composition of experimental diets[1], as fed basis.

	Starter			Grower			Developer		
Item, % LPS[1]:	F	FM	M	F	FM	M	F	FM	M
DM	87.1	87.4	87.9	87.5	87.5	87.8	88.1	87.9	87.8
Ash	5.41	5.78	5.99	5.62	5.12	5.60	4.69	4.66	4.65
Ca	0.91	0.97	1.09	1.03	0.90	0.98	0.88	0.83	0.83
Total P	0.83	0.85	0.81	0.86	0.83	0.82	0.49	0.50	0.50

[1]Limestone particle size; F, fine, < 0.595 mm, F; medium, 0.595 to < 1.68 mm, M; and FM, 1:1 mixture of F and M wt/wt.
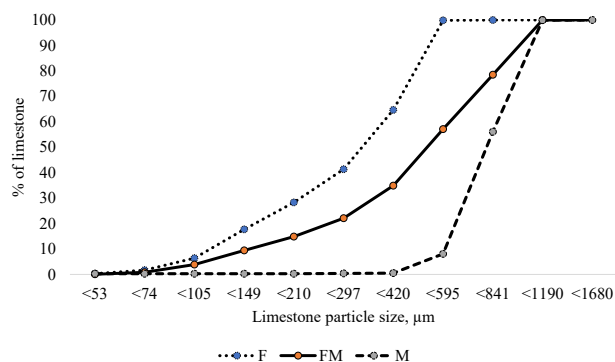
Figure 1. Limestone particle size distribution in starter diets. Limestone particle size; F, fine, < 0.595 mm, F; medium, 0.595 to <1.68 mm, M; and FM, 1:1 mixture of F and M wt/wt.

dimension and housing condition regimens were as described in our previous study (Khanal et al., 2019). The birds were acclimatized to the cages and diets for 4 D followed by 3 consecutive days of quantitative excreta collection. The pullets were given the same diets as they received in the main experiment. The diets were provided on *ad libitum* basis, topped up at 09:00 and 14:00 h. Excreta samples were collected at 11:00 and 16:00 h in rectangular aluminum foil containers daily and stored at −20°C between collections. The pullets were subsequently sacrificed and stored at −20°C for whole body densitometry.

Sample Processing and Laboratory Analyses

Limestone Solubility In vitro limestone solubility was determined as described by Zhang and Coon (1997a). Briefly, 200 mL of 0.2 N HCl in a 400 mL beaker was warmed to 42°C in a water bath (Model SW 22, Julabo GmbH, Seelbach, Germany) (oscillating at 25 rpm/minute) for 15 min. Two grams of limestone sample was placed into the solution and allowed to react with HCl for 10 min after which 80% of the supernatant was gently removed, and 200 mL of double deionized water added to stop reaction. The undissolved sample was filtered through preweighed Whatman filter (No 41; pore
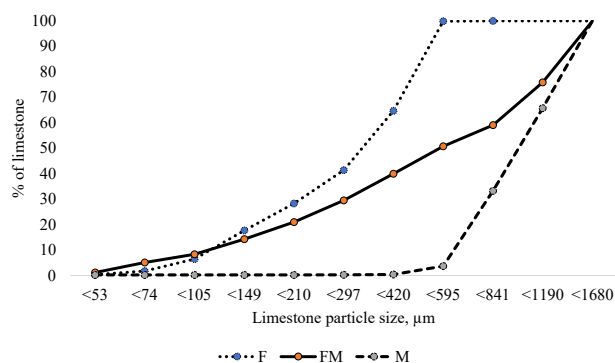

Figure 2. Limestone particle size distribution in grower and developer diets. Limestone particle size; F, fine, < 0.595 mm, F; medium, 0.595 to < 1.68 mm, M; and FM, 1:1 mixture of F and M wt/wt.

size = 20 μm) to get the undissolved limestone, which was later dried at 135°C for 2 h and weighed.

Duodenum Histomorphology The formalin fixed duodenal tissues were processed and stained (H&E stain) at Animal Health Laboratory, University of Guelph, for microscopic examination. The villi height (**VH**) and crypt depth (**CD**) were measured in 2 adjacent villi from each quadrant of transverse section (total 8 VH and CD a section). The histological slides were interpreted at 5 × magnification in Leica microscope (Leica Microsystems GmbH, Wetzlar, Germany). The images were analyzed using microscope imaging software Improvision Openlab, version 5.5.2 (Improvision Ltd, New York, NY). The software was calibrated (for regular upright scope and magnification × 5,100 pixels = 138.89 μm) to measure the VH and CD accurately. The villi height to crypt depth ratio (**VCD**) was calculated by dividing VH with CD.

Bone Attributes The legs were thawed at 5°C for 24 h and subsequently dissected to remove femur and tibia. The femur and tibia were defleshed and submitted for bone mineral density (**BMD**) and bone mineral content (**BMC**) (Baird et al., 2008; Khanal et al., 2019), bone breaking strength (**BBS**) (Park et al., 2003; Kim et al., 2012), and bone ash (Baird et al., 2008; Shim et al., 2012; Khanal et al., 2019). Femur length was taken from the edge of major trochanter to the edge of medial tibial. For the tibia, the length was taken from the edge of the tibial plateau of medial condyle to the medial malleolus. The medio-lateral diameter of both bones was taken in diaphysis exactly at the mid of the bone using the digital Vernier caliper (Mastercraft tools, Vonore, TN) with accuracy of 0.01 mm.

The BMD and BMC were determined in the right femur and tibia using a dual energy X-ray absorptiometry scanner (GE Healthcare, Madison, WI) for small animals at the Ontario Veterinary College, University of Guelph, automated with the-enCORE software, version 14.0. A three-point bending test with an Instron material tester (Model: Instron crop, Canton, MA) automated with the material test system (software BlueHill 3.0, version 3.7.7) was used to measure left femur and tibia BBS. Bones were brought to room temperature for 1 h before BBS measurement. The timing of breaking strength tests was set, so all femur and tibia samples were thawed at room temperature for 1 h. In brief, the maximum load of the compressor was set at 500 N with a cross head speed of 5 mm/second. The distance between upper and lower anvil was set to be 27 mm for all bones. For both femur and tibia at 4 woa, the spans were fixed at 3 mm from center. The spans were fixed at 4 mm and 6 mm from center for femur and tibia of both 12 and 16 woa of pullets. Femurs were kept medial side up, and tibia were kept anterior side up. The BBS was determined in Newton as provided by the apical point in the breaking strength curve. Following BBS determination, femur and tibia samples were used for ash determination. Briefly, both femur and tibia were oven dried to constant weight at 100°C for 24 h and
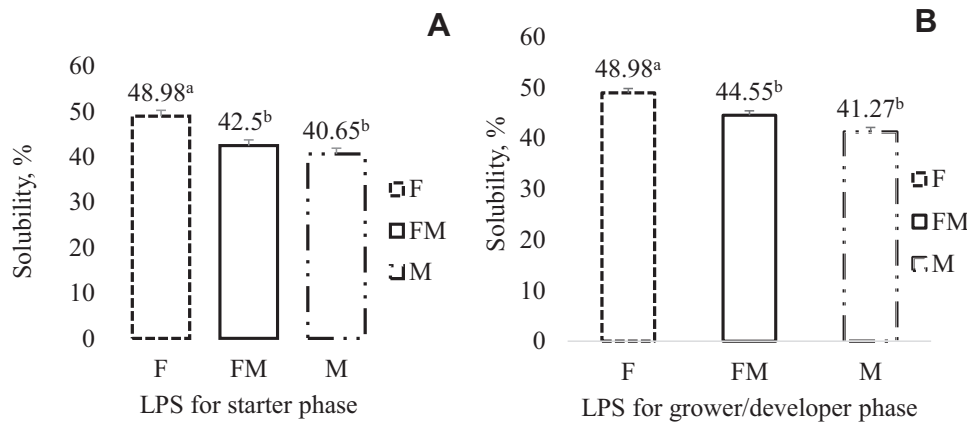
Figure 3. In vitro solubility of fine (F), fine-medium (FM), and medium (M) type limestone used in starter (A; SEM = 1.27) and grower and developer diets (B; SEM = 0.87). LSmeans assigned different letter superscripts differs, $P < 0.05$, n = 4. Abbreviation: LPS, limestone particle size.

ashed in a muffle furnace at 600° for 12 h (Khanal et al., 2019).

Whole Body Densitometry Whole body mineral density (**BoMD**) and whole body mineral content (**BoMC**) at 4, 12 and 16 woa were assessed through dual energy X-ray absorptiometry as described above.

Diets and Excreta Analyses Excreta samples were oven dried at 60°C for 48 h and along with air dried diets ground using a coffee grinder (KitchenAid BCG 111OB, Whirlpool Corp, Benton Harbor, MI). Dry matter was determined with method 930.15 (AOAC, 2005) and ash in a muffle furnace at 600°C for 12 h. For Ca and P content in diet and excreta, samples were ashed and digested with a mixture of 5 mL concentrated HCl and 50 μL concentrated HNO_3 in a pyrex tube for 20 h at 120°C. The tube content was poured into a 100 mL volumetric flask, filled with double deionized water (dd H_2O) up to meniscus, covered with parafilm, inverted 2 to 3 times to mix well and settled down overnight. An aliquot of 15 mL was taken for determination of Ca and P using inductively coupled atom emission spectrometry (ICP-OES, Varian Inc, Palo Alto, CA).

Calculations and Statistical Analyses

The geometric mean diameter and geometric standard deviation of LPS in experimental diets was calculated according to Wilcox et al., (1970). Limestone solubility was calculated as follows:

$Solubility, \% = \frac{\text{Dissolved limestone, g}}{\text{Total limestone, g}} \times 100$

Apparent retention of DM, organic matter (**OM**), ash, Ca, and P was calculated as follows:

distribution of data before statistical analyses. The model was $Y_{ijk} = \mu + a_i + b_j + ab_{ij} + \varepsilon_{ijk}$, where, Y_{ijk} was the response variable, a_i was cage type, b_j was the LPS, ab_{ij} was the interaction between cage type and LPS, and E_{ijk} is the error term. One-way ANOVA was used for the in vitro solubility of LPS data. The LSmeans were separated using Tukey test, and significance was declared at $P < 0.05$, and a tendency to a pattern was declared at $0.05 \leq P \leq 0.1$.

RESULTS

For the starter diet, the geometric mean (±SD) diameter of LPS for F, FM, and M was 0.286 ± 0.0013, 0.468 ± 0.0020, and 0.790 ± 0.0015 mm, respectively. The corresponding values of LPS used in the grower and developer diets were 0.287 ± 0.0013, 0.477 ± 0.0031, and 0.984 ± 0.0011 mm, respectively. The analyzed concentration of DM, ash, Ca, and P in the experimental diets are presented in Table 2.

In Vitro Limestone Solubility

The solubility decreased with increasing LPS (Figures 3A and 3B). The solubility of F particles in starter and grower/developer diets was significantly higher ($P < 0.01$) than that of FM and M categories. The solubility of M LPS used in grower/developer tended ($P = 0.06$) to be lower than that of FM.

$$AR, \% = \frac{\text{Total component intake, g} - \text{total component in excreta, g}}{\text{Total component intake, g}} \times 100$$

where component can be DM, OM, ash, Ca, or P.

The cage was the experimental unit, and data were analyzed using Proc GLM procedures of SAS software 9.4. Shapiro–Wilk test was employed to test the normal

Feed Intake, Body Weight, and Gizzard

There was no ($P > 0.05$) interactions between cage type and dietary LPS on feed intake and BW

Table 3. Effects of cage type and dietary limestone particle size (LPS) on feed intake, body weight, and gizzard weight in LSL-Lite pullets.

Item	Feed intake, g			Body weight, g				Gizzard, g/kg BW			Gizzard content, g		
Age, week:	1–4	5–12	13–16	0	4	12	16	4	12	16	4	12	16
Cage[1]													
CON	689.3[a]	3,832.3	1,612.6[b]	35.0	262.5[a]	993.6[b]	1,160.5[b]	28.1	16.6	144.2[a]	3.1	3.6	2.2[b]
FUR	599.3[b]	3,813.1	1,810.0[a]	34.9	254.3[b]	1,025.2[a]	1,235.0[a]	28.0	16.2	13.5[b]	3.0	3.8	2.7[a]
SEM	6.4	53.1	13.4	0.1	1.7	4.3	9.1	0.4	0.3	0.2	0.1	0.2	0.2
LPS[2]													
F	646.3	3,849.3	1,724.0	34.9	253.1[a]	1,015.3	1,209.1	29.4[a]	16.6	13.5	3.2	3.7	2.2
FM	643.2	3,711.0	1,693.4	35.0	260.0[a,b]	1,007.0	1,185.4	27.9[b]	16.5	14.3	3.1	3.8	2.5
M	643.5	3,847.8	1,716.5	34.9	262.1[b,c]	1,005.9	1,198.1	27.1[b]	16.1	13.8	3.0	3.5	2.5
SEM	7.8	65.2	16.5	0.1	2.1	5.4	11.1	0.6	0.3	0.3	0.1	0.2	0.2
P-values													
Cage	<0.01	0.801	<0.01	0.181	0.002	<0.01	<0.01	0.877	0.327	0.030	0.785	0.467	0.041
LPS	0.953	0.631	0.404	0.336	0.015	0.416	0.337	0.023	0.497	0.143	0.408	0.671	0.567
Cage × LPS	0.728	0.679	0.657	0.374	0.486	0.372	0.553	0.253	0.368	0.073	0.785	0.566	0.060

[a-c]Within a column by response criteria, LSmeans assigned different letter superscripts differs, $P < 0.05$, n = 6.
[1]CON, conventional cage 76 cm × 71 cm × 46 cm; FUR, furnished cage 239 cm × 80 cm × 75 cm outfitted with platforms and terraces to increases opportunities for load bearing exercises (e.g., jumping, perching, flying) (Casey-Trott et al., 2017; Habinski et al., 2017).
[2]F, fine; FM, mixture of fine and medium (1:1 wt/wt) and M, medium limestone particle size. Gizzard weight and content are average of 2 pullets per cage.

(Table 3). At the end of 4 woa, the cumulative feed intake was higher for the pullets reared in CON ($P < 0.0001$) compared with pullet reared in FUR cages. On the contrary, the feed intake was higher ($P < 0.0001$) for the pullets reared in FUR than in CON cages at the end of developer phase (16 woa). There was no cage effect ($P = 0.801$) on feed intake from 5 to 12 woa. The cage type affected ($P < 0.01$) BW at 4, 12, and 16 woa. In this context, pullets of CON were heavier ($P = 0.002$) than the pullets of FUR at the end of 4 woa; however, FUR pullets were heavier ($P < 0.0001$) than CON pullets at the end of 12 and 16 woa. Limestone particle size affected the BW at the end of 4 woa ($P < 0.015$) with birds fed M diet showing higher BW (262 vs. 253 g) than birds fed F diet, whereas BW (260 g) of FM birds was intermediate and similar ($P > 0.05$) to F and M birds. There were no LPS effects ($P > 0.05$) on BW at 12 and 16 woa (Table 3). At an early age (4 woa), the

LPS affected the relative gizzard weight ($P = 0.030$) such that gizzard was heavier for fine LPS fed pullets. At 16 woa, cage type tended to interact ($P = 0.073$) with diets on relative gizzard weight such that M LPS fed pullets had heavier (14.73 g/kg BW) gizzard in FUR but lighter (12.88 g/kg BW) in CON. The LPS did not affect ($P > 0.05$) the gizzard weight and content. However, the gizzard content was 25% higher ($P = 0.041$) for FUR pullets compared with CON pullets at the end of developer phase.

Duodenal Histomorphology and Apparent Retention of Calcium and Phosphorous

There was no interaction ($P > 0.05$) between cage type and LPS or main effect of LPS on duodenal VH, CD, and VCD (Table 4). The cage type affected VH or

Table 4. Effect of cage type and dietary limestone particle size (LPS) on duodenal villi height (VH) and crypt depth (CD) in LSL-Lite pullets.

Item	Cage[1]		LPS[2]			SEM		P-values		
	CON	FUR	F	FM	M	Cage	LPS	Cage	LPS	Cage × LPS
At 4 wk of age										
VH, μm	1,663.0	1,724.9	1,644.2	1,730.3	1,707.4	41.7	51.1	0.302	0.476	0.580
CD, μm	240.5[b]	277.5[a]	258.7	260.4	257.8	7.0	8.6	0.001	0.976	0.449
VCD	6.9[a]	6.2[b]	6.4	6.7	6.7	0.2	0.2	0.016	0.597	0.994
At 12 wk of age										
VH, μm	2,244.8	2,085.7	2,202.2	2,143.1	2,150.5	40.4	49.5	0.009	0.659	0.945
CD, μm	227.8	240.5	246.4	222.4	233.7	7.1	8.7	0.216	0.167	0.773
VCD	9.9[a]	8.8[b]	9.1	9.7	9.3	0.2	0.3	0.009	0.461	0.792
At 16 wk of age										
VH, μm	1,781.4	1,733.1	1,762.1	1,760.9	1,748.9	22.2	27.2	0.136	0.931	0.616
CD, μm	232.14[a]	214.1[b]	222.5	221.1	225.6	4.0	4.9	0.003	0.811	0.407
VCD	7.70	8.13	7.55	8.02	7.79	0.1	0.2	0.079	0.724	0.815

[a,b]Within a row by factor of analysis (cage or LPS), LSmeans assigned different letter superscripts differs, $P < 0.05$ (n = 6).
[1]CON, conventional cage 76 cm × 71 cm × 46 cm; FUR, furnished cage 239 cm × 80 cm × 75 cm outfitted with platforms and terraces to increases opportunities for load bearing exercises (e.g., jumping, perching, flying) (Casey-Trott et al., 2017; Habinski et al., 2017).
[2]F, fine; FM, mixture of fine and medium (1:1 wt/wt); and M, medium limestone particle size. VCD, villi height to crypt depth ratio.

Table 5. Effects of cage type and dietary limestone particle size (LPS) on apparent retention of components in LSL pullets.

	At the end of 12 wk of age, %					At the end of 16 wk of age, %				
Item	DM	OM	Ash	Ca	P	DM	OM	Ash	Ca	P
Cage[1]										
CON	75.8	78.6	25.2[a]	37.1[a]	26.3[a]	81.3[a]	83.7[a]	37.7[a]	41.1[a]	28.8
FUR	75.6	78.8	20.9[b]	21.6[b]	17.3[b]	79.5[b]	82.4[b]	28.3[b]	34.7[b]	25.7
SEM	0.48	0.47	1.03	1.46	1.20	0.47	0.39	2.02	2.12	2.13
LPS[2]										
F	75.2	78.3	21.5	27.7[b]	22.7	80.0	82.8	29.9	30.9[b]	24.8
FM	75.3	78.3	22.6	27.2[b]	21.2	80.8	83.3	35.1	41.8[a]	28.3
M	76.5	79.4	25.1	33.2[a]	21.5	80.5	83.1	34.0	40.9[a]	28.6
SEM	0.59	0.57	1.31	1.79	1.48	0.58	0.48	2.4	2.6	2.60
P-values										
Cage	0.768	0.820	0.006	<0.01	<0.01	0.013	0.02	0.002	0.041	0.306
LPS	0.265	0.336	0.132	0.044	0.733	0.608	0.71	0.301	0.010	0.522
Cage × LPS	0.528	0.489	0.938	0.876	0.881	0.072	0.08	0.057	0.098	0.167

[a,b]Within a column by response criteria, LSmeans assigned different letter superscripts differs, $P < 0.05$, n = 6.
[1]CON, conventional cage 76 cm × 71 cm × 46 cm; FUR, furnished cage 239 cm × 80 cm × 75 cm outfitted with platforms and terraces to increases opportunities for load bearing exercises (e.g., jumping, perching, flying) (Casey-Trott et al., 2017; Habinski et al., 2017).
[2]F, fine; FM, mixture of fine and medium (1:1 wt/wt) and M, medium limestone particle size.

CD; at 4 woa, crypts were deeper ($P = 0.001$) in FUR pullets; however, at 12 woa, the villi were ($P = 0.009$) longer in CON Pullets. At 16 woa, the VH was similar ($P > 0.05$) for both FUR and CON pullets, but the CD were deeper ($P = 0.003$) in CON pullets. The VCD was notably higher for pullets reared in CON cages at 4 wk ($P = 0.016$) and 12 wk ($P = 0.009$) of age, but the opposite was observed at 16 woa, where VCD tended ($P = 0.08$) to be higher for pullets reared in FUR cages ($P = 0.079$).

There was no interaction ($P > 0.05$) between cage type and LPS on AR of DM, OM, ash, Ca, and P (Table 5). However, the cage type tended to interact with diet on AR of DM ($P = 0.072$), OM ($P = 0.082$), ash ($P = 0.057$), and Ca ($P = 0.098$) at 16 woa (Table 5). In this context, the AR of Ca for M LPS (48.9%) in CON cages was higher than for F LPS (31.6%) in CON and F (30.3%) and M (32.9%) in FUR

cages. The AR of ash was higher ($P < 0.05$) for CON than for FUR pullets at 12 and 16 woa. There was an effect ($P < 0.05$) of both the cage type and diet on AR of Ca at 12 and 16 woa. The AR of Ca was higher for CON pullets than FUR pullets at 12 woa (37.2 vs. 21.6%, $P < 0.0001$) and at 16 woa (41.1 vs. 34.7%, $P = 0.04$). The main effect ($P \leq 0.44$) of diet on AR of Ca was such that pullets fed M had higher AR of Ca than pullets fed either F or FM at 12 woa, whereas AR of Ca was higher for FM and M than for F at 16 woa. The AR of P was only affected by the cage type at 12 woa with CON pullets showing higher ($P < 0.0001$) AR of P than FUR pullets.

Long Bones and Whole Body Attributes

Cage type and LPS did not interact ($P > 0.05$) on femur and tibia diaphyseal diameter and length (data

Table 6. Effects of cage type and dietary limestone particle size (LPS) on femur and tibia weight in LSL-Lite pullets.

Item	Absolute femur wt., g			Absolute tibia wt, g			Relative femur wt, g/kg BW			Relative tibia wt, g/kg BW		
Age, week:	4	12	16	4	12	16	4	12	16	4	12	16
Cage[1]												
CON	0.726[a]	3.436	3.935[b]	1.046[a]	4.476	5.445[b]	2.566	3.445	3.543	3.698	4.778	4.900
FUR	0.649[b]	3.556	4.447[a]	0.869[b]	4.888	5.975[a]	2.477	3.497	3.654	3.323	4.812	4.910
SEM	0.013	0.067	0.081	0.016	0.085	0.105	0.043	0.053	0.052	0.069	0.075	0.065
LPS[2]												
F	0.658[b]	3.539	4.115	0.927[b]	4.884	5.639	2.432	3.521	3.526	3.426	4.863	4.836
FM	0.676[b]	3.428	4.189	0.933[b]	4.750	5.639	2.545	3.428	3.687	3.505	4.754	4.968
M	0.729[a]	3.521	4.269	1.013[a]	4.844	5.850	2.589	3.464	3.581	3.600	4.768	4.911
SEM	0.016	0.083	0.099	0.02	0.104	0.128	0.053	0.065	0.064	0.084	0.091	0.080
P-values												
Cage	0.003	0.220	<0.01	<0.01	0.311	0.001	0.158	0.493	0.147	0.001	0.756	0.914
LPS	0.011	0.602	0.552	0.008	0.655	0.418	0.116	0.603	0.217	0.358	0.663	0.516
Cage × LPS	0.515	0.436	0.818	0.909	0.370	0.699	0.446	0.232	0.715	0.943	0.258	0.382

[a,b]Within a column by response criteria, LSmeans assigned different letter superscripts differs, $P < 0.05$, n = 6.
[1]CON, conventional cage 76 cm × 71 cm × 46 cm; FUR, furnished cage 239 cm × 80 cm × 75 cm outfitted with platforms and terraces to increases opportunities for load bearing exercises (e.g., jumping, perching, flying) (Casey-Trott et al., 2017; Habinski et al., 2017).
[2]F, fine; FM, mixture of fine and medium (1:1 wt/wt) and M, medium limestone particle size.

Table 7. Effects of cage type and dietary limestone particle size (LPS) on femur and tibia mineral density and mineral content in LSL-Lite pullets.

| Item | Bone mineral density, g/cm² | | | | | | Bone mineral content, g | | | |
| | Femur | | | Tibia | | | Femur | | Tibia | |
Age, week:	4	12	16	4	12	16	12	16	12	16
Cage[1]										
CON	0.076[b]	0.116	0.120	0.077[b]	0.155	0.159	0.480	0.652	0.944	1.061
FUR	0.117[a]	0.115	0.122	0.105[a]	0.157	0.158	0.516	0.702	0.991	1.108
SEM	0.007	0.002	0.002	0.005	0.002	0.002	0.017	0.02	0.018	0.029
LPS[2]										
F	0.089	0.115	0.130[a]	0.096	0.157	0.163	0.479	0.725	0.958	1.125
FM	0.094	0.117	0.117[b]	0.088	0.154	0.157	0.516	0.662	0.979	1.062
M	0.108	0.115	0.117[b]	0.089	0.156	0.156	0.500	0.645	0.966	1.066
SEM	0.009	0.002	0.003	0.006	0.003	0.003	0.021	0.025	0.023	0.035
P-values										
Cage	0.001	0.661	0.646	0.001	0.417	0.899	0.156	0.095	0.088	0.264
LPS	0.370	0.849	0.016	0.645	0.742	0.293	0.476	0.079	0.816	0.400
Cage × LPS	0.715	0.992	0.420	0.553	0.637	0.317	0.916	0.972	0.494	0.894

[a,b]Within a column by response criteria, LSmeans assigned different letter superscripts differs, $P < 0.05$, n = 6.
[1]CON, conventional cage 76 cm × 71 cm × 46 cm; FUR, furnished cage 239 cm × 80 cm × 75 cm outfitted with platforms and terraces to increases opportunities for load bearing exercises (e.g., jumping, perching, flying) (Casey-Trott et al., 2017; Habinski et al., 2017).
[2]F, fine; FM, mixture of fine and medium (1:1 wt/wt); and M, medium limestone particle size.

not shown). Similarly, the cage type did not affect diaphyseal diameter of femur (7.22 vs. 7.35 mm; $P = 0.206$) and tibia (6.53 vs. 6.58 mm, $P = 0.130$) and length of femur (80.07 vs. 81.11 mm; $P = 0.122$) and tibia (114.59 vs. 114.47 mm; $P = 0.915$) for the pullets reared in CON and FUR cages, respectively. As well, LPS did not affect these morphometric characters of femur and tibia. Femur and tibia weights at 4, 12, and 16 woa are shown in Table 6. There was no interaction ($P > 0.05$) between cage type and LPS on absolute and relative femur and tibia weight (Table 6). At 4 woa, pullets reared in CON cages had higher ($P < 0.05$) femur (absolute) and tibia (absolute and relative) weight than pullets reared in FUR cages. However, at 12 woa, pullets reared in FUR cages had heavier ($P = 0.001$) absolute weight of tibia than pullets reared in CON cages (Table 6). The LPS affected the absolute femur and tibia weight at an earlier age (4 woa) such that pullets fed M LPS had heavier femur ($P = 0.011$) and tibia ($P = 0.008$) than pullets fed F and FM LPS.

There was no interaction ($P > 0.05$) between cage type and LPS on femur and tibia BMD and BMC (Table 7). The cage-type effect was such that femur ($P = 0.0009$) and tibia ($P = 0.001$) BMD was higher for pullets reared in FUR cages than those reared in CON cage at 4, 12, and 16 woa. Pullets reared in FUR cages tended to have higher femur ($P = 0.10$) and tibia ($P = 0.09$) BMC than pullets reared in CON cages. Diet only affected femur BMD at 16 woa with birds fed FM and M LPS having lower BMD than birds fed F diet. Femur BMC tended ($P = 0.079$) to be higher in the pullets fed F diet relative to pullets fed other diets. The cage type did not interact ($P > 0.05$) with LPS on BoMD and BoMC (Table 8). Cage or LPS had no effect on BoMD and BoMC at 4 and 16 woa. However, BoMD and BoMC was influenced ($P < 0.05$) by cage type and LPS at 16 woa. In this context, pullets reared in FUR

cages had higher BoMD ($P = 0.001$) and BoMC ($P = 0.017$) than pullets reared in CON cages. The diet effect was such that diet FM had higher ($P = 0.045$) BoMD than F diet, whereas BoMD of diet M was intermediate and similar ($P > 0.05$) to that of the other diets. The BoMC was higher ($P = 0.029$) for diet M relatively to diets F and FM.

The cage type and LPS did not interact ($P > 0.05$) on femur and tibia BBS and ash concentration (Table 9). Neither cage type nor LPS affected ($P > 0.05$) femur BBS; however, at 4 woa, pullets reared in CON cages tended ($P = 0.067$) to have higher femur BBS relative

Table 8. Effect of cage type and dietary limestone particle size (LPS) on whole body mineral density and body mineral content in LSL-Lite pullets.

| Item | Whole body mineral density, g/cm2 | | | Whole body mineral content, g | | |
Age, week:	4	12	16	4	12	16
Cage[1]						
CON	0.158	0.230	0.214[b]	3.933	18.27	23.25[b]
FUR	0.158	0.222	0.235[a]	3.883	19.09	25.40[a]
SEM	0.003	0.004	0.004	0.117	0.460	0.601
LPS[2]						
F	0.163	0.224	0.213[b]	4.000	18.37	23.19[b]
FM	0.153	0.232	0.232[a]	3.854	18.60	23.80[b]
M	0.154	0.222	0.228[a,b]	3.870	19.11	25.98[a]
SEM	0.003	0.005	0.005	0.143	0.572	0.736
P-values						
Cage	0.852	0.232	0.001	0.765	0.224	0.017
LPS	0.134	0.406	0.045	0.735	0.630	0.029
Cage × LPS	0.413	0.360	0.671	0.608	0.172	0.438

[a,b]Within a column by response criteria, LSmeans assigned different letter superscripts differs, $P < 0.05$, n = 6.
[1]CON, conventional cage 76 cm × 71 cm × 46 cm; FUR, furnished cage 239 cm × 80 cm × 75 cm outfitted with platforms and terraces to increases opportunities for load bearing exercises (e.g., jumping, perching, flying) (Casey-Trott et al., 2017; Habinski et al., 2017).
[2]F, fine; FM, mixture of fine and medium (1:1 wt/wt); and M, medium limestone particle size.

Table 9. Effect of cage type and dietary limestone particle size (LPS) on femur and tibia ash and bone breaking strength in LSL pullets.

	Breaking strength, N						Ash, %					
Item	Femur			Tibia			Femur			Tibia		
Age, wk:	4	12	16	4	12	16	4	12	16	4	12	16
Cage[1]												
CON	102.4	175.9	176.0	93.37[a]	148.7	183.4[b]	37.8	32.51	35.15[a]	36.87[b]	37.10	36.92
FUR	94.92	180.8	167.1	83.35[b]	159.7	197.9[a]	37.2	32.55	33.36[b]	38.23[a]	37.50	36.16
SEM	2.80	6.16	5.54	2.66	4.59	4.62	0.37	0.37	0.51	0.38	0.39	0.37
LPS[2]												
F	95.43	177.2	180.8	84.78	154.0	198.1	37.38	32.57	34.55	36.78	36.99	36.31
FM	97.08	176.9	165.3	88.44	156.0	194.5	37.84	32.68	34.22	38.28	37.39	36.69
M	103.5	180.9	168.8	91.87	152.7	179.3	37.29	32.36	34.02	37.59	37.52	36.62
SEM	3.43	7.54	6.779	3.26	5.62	8.72	0.45	0.45	0.62	0.47	0.48	0.46
P-values												
Cage	0.067	0.573	0.264	0.012	0.098	0.035	0.264	0.941	0.018	0.017	0.479	0.165
LPS	0.230	0.914	0.251	0.32	0.917	0.059	0.664	0.875	0.829	0.091	0.716	0.830
Cage × LPS	0.773	0.471	0.403	0.931	0.973	0.932	0.627	0.996	0.482	0.217	0.804	0.629

[a,b]Within a column by response criteria, LSmeans assigned different letter superscripts differs, $P < 0.05$, n = 6.

[1]CON, conventional cage 76 cm × 71 cm × 46 cm; FUR, furnished cage 239 cm × 80 cm × 75 cm outfitted with platforms and terraces to increases opportunities for load bearing exercises (e.g., jumping, perching, flying) (Casey-Trott et al., 2017; Habinski et al., 2017).

[2]F, fine; FM, mixture of fine and medium (1:1 wt/wt); and M, medium limestone particle size.

to pullets reared in FUR cages. Cage type effects on tibia BBS was such that CON pullets showed higher BBS (93.4 vs. 83.4 N; $P = 0.012$) than FUR pullets at 4 woa. However, at 16 woa, FUR pullets had higher tibia BBS (197.9 vs. 183.4 N; $P = 0.035$) than CON pullets. Moreover, FUR pullets tended ($P = 0.098$) to show higher tibia BBS than CON pullets at 12 woa. Although LPS had no effect ($P > 0.05$) femur and tibia BBS, pullets fed diet M tended ($P = 0.06$) to have lower tibia BBS than birds fed F. Pullets reared in CON cages showed more (35.2 vs. 33.4%; $P = 0.018$) femur ash than pullets reared in FUR cages at 16 woa. Pullets reared in FUR cages had higher (38.2 vs. 36.9%, $P = 0.017$) tibia ash than pullets reared in CON cages (Table 9). There was a tendency ($P = 0.091$) for a diet effect on tibia ash at 4 woa in which case birds fed FM diet tended to have higher ash concentration than F birds.

DISCUSSION

There were differences in both the pattern of LPS distribution and geometric mean diameter in F, FM, and M categories. These differences influenced in vitro solubility; generally, the solubility of the finer particles was higher. The in vitro solubility of limestone corresponds to its particle size in agreement with other findings (Cheng and Coon, 1990b; Zhang and Coon, 1997b). The high AR of Ca for coarser limestone particles could be explained by lower solubility. The cage type affects pullet mobility and the utilization of the amenities offered (Hester et al., 2014). The BW and feed intake were higher in CON pullets at an early age, but later reversed with higher BW in FUR. At an early age, owing to the smaller body size, spacing was arguably sufficient for CON pullets. However, it is plausible that as the pullet grew, space as well as feed and water accessibility declined in CON cages. Several studies suggested high feed intake in hens housed in furnished cages compared

with those in conventional cages (Hetland et al., 2003, 2004; Valkonen et al., 2008; Tactacan et al., 2009). Meng et al. (2017) found that the locomotor activity of hens along with other activities such as drinking, preening, and even fighting were higher in furnished than in conventional cages. A thorough research is required to understand how the cage type affects feed intake and energy partitioning in growing pullets. The higher BW for M LPS fed pullets might have resulted from an interaction between Ca and other nutrients. Bradbury et al. (2018) reported a reduced BW in broilers fed fine limestone as highly soluble Ca reduced digestibility of copper and manganese. Majeed (2019) reported a lower apparent ileal digestibility of lysine in 35-day-old broilers when fed diet with highly soluble calcium source. Our in vitro solubility showed a higher solubility of fine LPS than medium LPS.

The cells in the intestinal crypts are highly mitotic (Hoopers, 1956). The increased exercise in FUR pullets is associated with increased metabolic energy demand that could stimulate intestinal growth (Daniels et al., 2016). A phenomenon that might have increased crypt mitotic activity leading to a deeper crypt and shorter villus. Daniels et al. (2016) and Gomes et al. (2016) reported a significant increase in CD in the exercised rats fed the same diet. The rate of cell turnover is governed by genetics; however, it is also affected by feeding (Hoopers, 1956). At 16 woa, we found an effect of cage type on CD but not VH and the crypts were deeper for CON pullets. The higher stocking density in CON might be a factor for the higher crypt depth. Li et al. (2017) reported deeper crypt in the jejunum of 28-day broiler reared at a higher stocking density compared with the control. Several studies have illustrated that the LPS is a leading determinant of digestibility and bioavailability of Ca (Soares, 1995; Lichovnikova, 2007; Saunders-Blades et al., 2009).

Although we did not observe any interaction between cage type and dietary LPS on bone quality, the main

effects were observed on several femur and tibia quality parameters. The higher femur and tibia weight at 4 woa in CON pullets and at 16 woa in FUR pullets could be explained by the higher BW. The higher femur and tibia weight of pullets fed M LPS at 4 woa could also be related to BW. The furnished cage and coarser LPS diet enhanced the mineral density and mineral content of the whole body. The higher body mineral density in FUR pullets might be explained by higher bone mineral content. This could be linked to higher Ca retention observed for M relative to F LPS, so that relatively more Ca was distributed in the body for the same amount of the Ca intake. The higher digestibility of Ca might be because of slow but continuous release of Ca from coarser LPS in the gizzard even during the dark phase when pullets tend to rest rather than eat. At 4 woa, the higher femur and tibia BMD in FUR pullets than that in CON pullets might be because of more mineral concentration in the bones as illustrated by bone weight. The higher femur BMD and tendency for higher femur BMC and tibia BBS for F LPS fed pullets at 16 woa was rather difficult to interpret given pullets fed this diet showed lower Ca retention and BoMD and BoMC. Nonetheless, the observations disagreed with previous study that demonstrated feeding medium LPS (0.879 vs. 0.432 mm) to white (Bovans) and brown (Lohmann) pullets during rearing enhanced bone mineralization and stemmed keel bone damage at onset of sexual maturity (Eusebio-Balcazar et al., 2018). Perhaps suggesting different strains may respond differently to dietary LPS manipulation during rearing. The higher tibia mineral density at 4 woa in FUR pullets was related to higher ash concentration. At 16 woa, femur BMC was similar in pullets reared in FUR and CON; however, the femur weight was lower for CON pullets. As ash concentration is the ratio of ash to bone weight, the higher femur ash concentration in CON pullets was likely because of lighter femur.

The impact of cage type and LPS on femur and tibia BBS was different. At an early age (4 woa), femur BBS was influenced by the cage type with stronger bones for CON pullets. This could be explained by the heavier femur observed in these pullets. At that younger age, the femur bone mineralization is at a relatively low level so the bone mineral density could not account for the differences in BBS. Although BBS is generally strongly correlated with BMD (Schreiweis et al., 2003), it does not seem to be true for younger pullet, suggesting the organic matrix and its orientation (or compactness) might be important for BBS. At 16 woa, however, the femur BS was similar across cage type and LPS, although it was numerically higher for pullet fed fine LPS. This could be partly explained by higher BMD in pullets fed fine LPS. At 16 woa, both the cage type and the LPS in the diet affected tibia BS which was higher for pullet reared in FUR and tended to be higher for pullets fed F LPS. The enlarged space and the presence of perches increased the physical activity and perching of pullets, which potentially improved leg health (Ventura et al., 2012; Norring et al., 2016).

Increased perching likely enhances mineral density and mineral content (Hester et al., 2013) by promoting the skeletal development (Yan et al., 2014). The BBS depends on thickness, collagen fibre alignment, and microstructure of cortical bone; however, we could not measure these parameters in the present study. So, future studies or experiments should focus on measuring these parameters as well.

The cage type and the LPS did not interact to influence Ca utilization or bone quality parameters. The cage type affected mineral density and mineral content of whole body and long bones, and breaking strength. All these qualities were superior in pullets reared in FUR cages at 16 woa. The femur and tibia were heavier in pullets fed medium sized LPS, but this effect was limited to the starter phase. Interestingly, the LPS influenced BoMC being higher for medium than fine LPS. However, at 16 woa, finer LPS enhanced femur mineral density and content and tended to strengthen tibia. Overall, the present study indicated that the pullets have better leg bone quality when reared in furnished cage than in conventional cage. Although feeding medium LPS improved Ca retention, finer LPS supported superior bone quality indicating coarser LPS had limited effects on skeletal development in pullets.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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