

The effect of light intensity on the body weight, keel bone quality, tibia bone strength, and mortality of brown and white feathered egg-strain pullets reared in perchery systems

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ABSTRACT The development of the musculoskeletal system is influenced by bird activity, which can be impacted by light intensity (**L**). The objective of this study was to determine the effect of L on the growth and bone health of Lohmann Brown-Lite (**LB**) and Lohmann LSL-Lite (**LW**) pullets. Three L treatments (10, 30 or 50 lux, provided by white LED lights) were used in a Randomized Complete Block Design in 2 repeated trials. LB and LW (n = 1,800 per strain [**S**]) were randomly assigned to floor pens (50 pullets per pen; 12 pen replicates per L × S) within 6 light-tight rooms from 0 to 16 wk. Each pen contained 4 parallel perches and a ramp. Data collected include cumulative mortality, BW at 0, 8, and 16 wk, and uniformity, keel bone damage (**KBD**; deviations, fractures), breast muscle weight, and tibiae bone strength at 16 wk. Tibiae bone resistance to mechanical stress was assessed using a three-point-bending test. The effect of L, S, and their interactions were

analyzed using Proc Mixed (SAS 9.4) and differences were considered significant when $P < 0.05$. L did not affect BW, KBD, or mortality. An interaction between L and S was observed for bone stress (bone strength relative to bone size), however, in general, LW pullets had greater resistance to bone stress (peak noted at 30 lux) than LB (peak at 50 lux). LB pullets were heavier than LW at 8 and 16 wk. There were no S differences on KBD from palpated or dissected keel bones. LB pullets had higher breast muscle weight and heavier tibiae than LW, however relative to BW, LW had a higher percentage of breast muscle and a longer and thicker tibiae than LB. LW had higher mortality during the first wk but there was no relationship to L. Conclusively, the results suggest that L, within a range of 10 to 50 lux, does not affect pullet BW or KBD, however S may affect both parameters, as well as bone strength.

Key words: Lohmann Brown-Lite, Lohmann LSL-Lite, bone breakage test, keel bone palpation

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INTRODUCTION

Rearing pullets in complex environments has been shown to improve navigation skills, reduce fear, and enhance the musculoskeletal system (Janczak and Riber, 2015). In turn, this improves the performance and welfare of these birds during lay. Vision can play an important role in helping pullets utilize a more spacious and complex environment, and light intensity (**L**) can help with vision and navigation (McFadden, 1993; Lewis and Morris, 2006). However, there is currently no scientific evidence to suggest appropriate lighting levels for pullets, therefore a research gap exists.

The use of alternative housing systems for both pullets and adult layers has been increasing in replacement of conventional cages. Alternative housing systems include furnished (enriched) cages and noncage systems, both of which contain more space for bird movement (vertical and horizontal) and expression of natural behaviors. Alternative housing systems also provide resources such as nest boxes (for adult layers), perches, and foraging and dust bathing substrates to satisfy the bird's behavioral needs (NFACC, 2017). For growing pullets, the increased expression of natural behaviors along with increased space for exercise improve flock welfare with respect to behavioral expression, bone quality, and better adaptability to complex layer housing (Janczak and Riber, 2015; Campbell et al., 2019). For alternative housing systems to be utilized to their greatest potential, the birds must be able to see well so they can navigate their environment successfully. Light intensity plays a direct role in navigational ability, whereby L can

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improve visual acuity and help pullets navigate their environment more successfully and safely. However, high-light intensity may amplify birds' perception of colors and attraction toward plumage, potentially resulting in increased feather pecking which may lead to harmful counter effects (Kjaer and Vestergaard, 1999; Nicol et al., 2013).

Few studies have examined the impact of light intensity on layers housed in large spaces. Taylor and Scott (2002) and Taylor et al. (2003) reported that very low L (less than 1 lux) compromises visual acuity of layers, affects their willingness to jump between perches, and can lead to environmental collisions. Currently, the Canadian National Farm Animal Care Council Codes of Practice for Pullets and Laying Hens requires a minimum of 10 lux for hens housed in alternative housing systems (NFACC, 2017). However, it is unknown whether light intensity higher than 10 lux such as 30 or 50 lux can help birds navigate their surroundings even better. In addition to affecting navigational abilities, light intensity can affect hens' eating behavior (Prescott and Wathes, 2002), therefore it is also important to assess bird health, particularly bone health such as bone structure and strength, and other production parameters such as body weight and mortality.

Keel bone damage (KBD) is a major issue in commercial laying hens and may be caused by abrupt forces on the keel, such as crashing into the environment, or strong muscular contractions, such as flying or perching for long periods of time, or other factors (Sandilands et al., 2009; Harlander-Matauschek et al., 2015; Thøfner et al., 2020). Keel bone damage may be in the form of deviations which are abnormally shaped keel bones or fractures which are fragmented sections most observed at the tip of the keel bone (Fleming et al., 2004; Casey-Trott et al., 2015). Assessment of KBD in pullets is uncommon as their keel bones are not yet fully developed, however it may still be important to understand whether L can play a preliminary role in pullet keel bone morphology development.

Light intensity may also play an indirect role in breast muscle (*Pectoralis major* and *Pectoralis minor*) weight and bone strength. The keel bone anchors breast muscles used for wing motion which influences bird flight (Fleming et al., 2004; Casey-Trott et al., 2015). The breast muscles are also used for static loading when a bird rests on a perch (Hughes and Appleby, 1989; Newman and Leeson, 1998). With increased locomotory behavior, perching, and wing use, breast muscles may increase in size, contributing toward musculoskeletal system development (Newman and Leeson, 1998; Casey-Trott et al., 2017). To date, no studies on the effect of L on laying hen or pullet breast weight have been reported. The effect of L on bone strength has also not been well studied in laying hens. Bone strength can be influenced by many variables, such as nutrition, genetics, and bird activity. Load-bearing exercises such as mounting and dismounting perches can increase bone structure, enhance mineral composition, and improve overall bone composition (Hester et al., 2013; Regmi et al., 2015;

Casey-Trott et al., 2017). Therefore, because higher L can increase locomotory activity of pullets, bone strength and breast muscle mass may increase simultaneously, and are important factors to evaluate and understand.

In addition, bird strain (S) may affect the aforementioned measured parameters. Brown- and white-feathered birds differ genetically, anatomically, and behaviorally (Riczu et al., 2004; Kozak et al., 2016a; Fawcett et al., 2020). Brown-feathered layers are heavier than white-feathered layers, which several studies suggested may affect locomotory activity (Wall and Tauson, 2007; Mohammed and Said, 2016). In fact, white-feathered birds performed more aerial locomotion (Kozak et al., 2016a), used more wing-assisted locomotion and were able to ascend ramps more easily (LeBlanc et al., 2018) when compared to browns. Bird musculoskeletal composition and bird activity may simultaneously influence each other, and may also be associated with genetic predispositions (Kozak et al., 2016a; Fawcett et al., 2020; Pufall et al., 2021). However, research in this area has been limited.

Although L during rearing may affect the birds' locomotory activity, behavior, and musculoskeletal development, there is no scientific evidence that demonstrates what L level is appropriate for pullets reared in complex environments. This study aimed to understand whether L at 10, 30 or 50 lux can affect pullet bone health, integrity, and strength without affecting regular performance indicators such as BW and mortality. The objectives of this study were to determine whether L influences BW, keel bone damage, breast muscle mass, bone strength, and mortality of 2 common egg-laying strains, the Lohmann Brown-Lite (LB), and Lohmann LSL-Lite (LW) pullets reared in a complex environment. The data presented here are part of a larger project, with a second publication to report successfulness of landings and behavioral expression of these same pullets (Chew, 2020).

MATERIALS AND METHODS

The experimental protocols were approved by the University of Saskatchewan Animal Care Committee (AUP #19940248). All birds were cared for as specified in the Guidelines on the Care and Use of Farm Animals in Research, Teaching and Testing by the Canadian Council on Animal Care (2009). This experiment examined the effects of 3 L (10, 30, 50 lux) during pullet rearing on BW, keel bone health, tibia bone strength, and mortality of 2 pullet strains (Lohmann Brown-Lite and Lohmann LSL-Lite). Two 16 wk experiments blocked by trial were conducted from May to August 2018 and 2019.

Housing and Management

LB (n = 900 per trial) and LW (n = 900 per trial) female pullets (total N = 3,600) were randomly assigned

to floor pens (50 pullets per pen; 4.0 m × 2.3 m) within 6 environmentally controlled rooms (6 pens per room, 6 rooms per trial) from 0 to 16 wk. Stocking density was 0.15 m²/bird, in accordance with the Lohmann Management Guide (Lohmann Tierzucht, 2018). Each pen was bedded with wheat straw to a depth of 7 to 10 cm and equipped with one perching system (height 0.56 m × width 1.16 m × length 2.18 m), one ramp (length 81.3 cm × width 48.3 cm, at an angle of 38°), 2 pan feeders (0.36 m diameter and 1.13 m circumference before 6 wk, and 0.44 m diameter and 1.38 m circumference after 6 wk), and one drinker line with 6 nipples (Lubing Systems LP, Cleveland, TN). The perching system consisted of 4 horizontal wooden perches spaced 30 cm apart. Each of the 4 perches was a rectangle (width 3.8 cm × height 3.5 cm) with the top corners angled for easy grasping. The ramps were made of 14-gauge wire (2.54 cm × 2.54 cm dimensions) and were added to the perch at 14 d of age to prevent pullets' toes and legs from getting caught in the wire. During the first week, supplemental feeders and drinkers were used. All birds had ad libitum access to water and commercial feed appropriate for their stage of development. At 8 wk, all pullets were wing-banded for identification purposes. Birds were vaccinated against Marek's Rispens, HVT-IBD, Newcastle Bronchitis, *Salmonella typhimurium*, and *Salmonella enteritidis*.

Each room (6 per trial) was randomly assigned to one of 3 L treatments (10, 30, 50 lux). Each room was illuminated by eight 11-watt white light-emitting diode (LED) lamps (2,821 Kelvin, Greengage Agritech Limited, Roslin Innovation Centre, Midlothian, UK) which were positioned so that L was similar in all pens. For the first week only, L was set to 50 lux in all rooms to allow all chicks to easily locate feed and water (NFACC, 2017). Every 2 wk, L was measured at bird level in the middle of each pen with a lux meter (ExTech LT300; ExTech Instruments, Montreal, Quebec, Canada) and adjusted if necessary. The pullets were reared under a photoperiod of 23 Light:1 Dark (L:D) for the first 7 d, and then gradually decreased weekly until they reached 8L:16D at 7 wk, which was maintained for the remainder of the trial (NFACC, 2017). Dawn and dusk periods (15 min each) were simulated daily. On the first d, temperature was set at 33°C and gradually decreased each day until 7 wk, where room temperatures then remained constant at 20°C (Lohmann Tierzucht, 2018). Heat was provided via hot water pipes along the interior walls, and all rooms were ventilated through a negative pressure inlet-fan system.

Data Collection

Body weight. Body weight was recorded on a pen basis at 0 and 8 wk. Uniformity (individual weights on all birds) was assessed at 16 wk.

Keel bone assessment. At 16 wk, 10 pullets per pen (12 pen replicates per L × S over both trials, n = 720) were palpated for keel bone fractures and deviations

using the Simplified Keel Assessment Protocol (Casey-Trott et al., 2015). Two trained and blinded individuals assessed each bird and mutually agreed on the keel bone status. An additional 9 pullets per pen (12 pen replicates per L × S over both trials, n = 648) were euthanized via injection of T-61 (0.4 mL mebezonium iodide/tetracaine per kg; Intervet Canada Corp, Kirkland, QC, Canada) into the brachial vein. The right *Pectoralis major* and *Pectoralis minor* (*supracorcoideus*) muscles were removed and weighed. Breast muscle weights were reported as absolute values and calculated relative to body weight. The keel bones were scored for fractures and deviations. Similar to the palpation data, a mutual agreement was reached between the same 2 assessors.

Bone strength assessment. After keel bone removal, right tibiae were removed, cleaned of tendons and muscles and frozen at -20°C until further assessment. Bones were thawed at 4°C for 24 h before the bone strength assessment. The length and width measurements perpendicular and parallel to the direction of the applied force, were recorded using a 150 mm electronic caliper with digital display (Mastercraft 58-6800-4; Mastercraft Tools, Toronto, Canada).

To determine bone breaking strength, an Instron Universal Testing machine (Instron 3366; Instron Corp., Norwood, MA) was used to perform a 3-point bending test. The Instron machine was fitted with a 50 kg load cell and set to a loading rate of 30 mm/min. Each bone was placed dorsal side up on supports placed 5 cm apart. For each bone, the maximum flexure load (reported in Newtons [N]) was recorded as the ultimate breaking force required to break the tibia. Postbreakage, the internal widths, perpendicular (wide side of tibia) and parallel (narrow side of tibia) to the direction of the applied force, at the inflection point of the tibia were measured with digital calipers. These measurements were used to calculate the distance between the neutral axis of the bone and the extreme outer fiber, which are points along the plane of the bone (C, measured in cm), and the moment of inertia (Crenshaw et al., 1981). For each measurement, the absolute value was used for calculation. In addition, relative values were calculated to adjust for body weight of the birds. To calculate for bone strength in resistance to mechanical stress relative to bone size (stress, kg/cm², Crenshaw et al., 1981), the flexure load was converted to kilograms (1 N = 0.010971621 kg) and the following equations from Crenshaw et al. (1981) were used:

$$\text{Stress} \left(\frac{\text{kg}^2}{\text{cm}} \right) = \frac{\text{force (kg)} \times \text{length (cm)} \times C \text{ (cm)}}{4 \times \text{moment of inertia (cm}^2\text{)}}$$

$$C = \frac{D}{2}$$

$$\text{Moment of inertia} = 0.0491 (BD^3 - bd^3)$$

where C is the distance between the neutral axis of the bone and the extreme outer fiber, which are the points

along the plane of the bone; D is the external diameter of the bone at the point of loading and parallel (narrow side of bone) to the direction of the applied force; B is the external diameter of the bone at the point of loading and perpendicular (wide side of bone) to the direction of the applied force; b is the internal diameter of the bone at the point of loading and perpendicular (wide side of bone) to the direction of the applied force; and d is the internal diameter of the bone at the point of loading and parallel (narrow side of bone) to the direction of the applied force.

Mortality. Birds were monitored daily. Pullets found dead or culled due to sickness or injury were sent to an independent diagnostic laboratory (Prairie Diagnostic Services, Saskatoon, SK, Canada) for necropsy and determination of cause of death. As L was constant at 50 lux in all rooms for the first 7 d, mortality was split into 2 periods for analyses: Wk 0 to 1 and Wk 1 to 16.

Statistical Analyses

The experiment was designed as a 3 (L) × 2 (S) factorial arrangement, with room nested within L, in a Randomized Complete Block Design. Percentage data were checked for normality using the Univariate procedure of SAS 9.4 and normalized using log transformation (data log +1). Data were analyzed using the mixed procedure with room as the replicate unit for L (2 room repetitions per L treatment per trial) and nested within L, pen as the replicate unit for S (3 pen replicates per S per room per trial), and trial as block. A Tukey's range test was used to separate means when significant differences were detected. Significance was declared when $P < 0.05$ and trends were noted at $0.05 \leq P < 0.10$.

RESULTS

Body Weight

L did not affect pullet body weight (Table 1). The 2 S used in this experiment differed in body weight at 8 and 16 wk; LB pullets were heavier than LW pullets at 8 (785.50 g vs. 708.72 g; $P < 0.001$) and 16 wk (1.46 kg vs. 1.17 kg, $P < 0.001$). At 16 wk, LW pullets were more uniform in body weight within 5, 10, and 15% of the pen average, and had a lower coefficient of variation

($P = 0.016$, $P < 0.001$, $P = 0.004$, Table 1). No interactions were noted between L and S.

Keel Bone Damage

Overall, no impact of L, S, nor their interactions were noted for deviations or fractures of palpated or dissected keel bones (Table 2).

Breast Muscle Weight

LB pullets had heavier *Pectoralis major* (65.28 g vs. 58.43 g; $P < 0.001$) and *Pectoralis minor* (20.99 g vs. 18.55 g; $P < 0.001$) muscles than LW pullets. Relative to body weight, LW pullets had a larger percentage of *P. major* (5.00% vs. 4.52%; $P < 0.001$) and *P. minor* (1.59% vs. 1.45%; $P < 0.001$) than LB pullets (Table 3).

Bone Strength Assessment

There was an interaction between L and S with respect to bone stress (Table 4). Bone stress refers to bone strength relative to bone size. LW pullets' tibiae were more resistant to mechanical stress than LB pullets under all tested light intensities, but peak resistance to mechanical stress was noted under 30 lux for the LW strain, and under 50 lux for LB (Table 5). L did not affect other tibia bone characteristics. The main effects of S on tibia bone characteristics are shown in Table 4. For absolute measurements, the tibiae of LB pullets were heavier (12.87 g vs. 9.81 g; $P < 0.001$), longer (11.66 cm vs. 11.39 cm; $P < 0.001$), and tended to be slightly thicker (wide side, 0.18 cm vs. 0.17 cm; $P = 0.064$) than LW pullets. Relative to body weight, LB pullets still had heavier tibiae (0.93% vs. 0.81%; $P < 0.001$) than LW pullets. However, LW pullets had longer (9.47% vs. 8.39%; $P < 0.001$) and thicker (wide side, 0.14% vs. 0.13%; $P < 0.001$ and narrow side, 0.12% vs. 0.10%; $P < 0.001$) tibiae than LB pullets.

Mortality

Table 6 shows the mortality data. Mortality was divided into 2 periods: the first week of age (Wk 0–1) during which all L settings were at 50 lux, and Wk 1 to 16 when L settings differed. During the first period (Wk

Table 1. Body weight at 0, 8, and 16 wk of age and uniformity at 16 wk of Lohmann Brown-Lite (LB) or Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30 or 50 lux.

Weeks of age	Light intensity (L)			P-value	Strain (S)		P-value	L × S P-value	SEM
	10	30	50		LB	LW			
BW 0 (g)	34.04	34.17	33.92	0.405	33.97	34.11	0.360	0.528	0.176
BW 8 (g)	753.92	743.08	744.33	0.419	785.50	708.72	<0.001	0.381	5.151
BW 16 (kg)	1.32	1.31	1.31	0.739	1.46	1.17	<0.001	0.436	0.018
Uniformity at 16 wk (% within <i>x</i> of the mean)									
5	56.31	59.52	56.82	0.240	55.47	59.64	0.016	0.977	0.848
10	87.49	89.37	89.76	0.293	86.25	91.50	<0.001	0.614	0.709
15	97.94	97.69	97.92	0.967	97.06	98.64	0.004	0.426	0.278
CV	6.61	6.27	6.29	0.263	6.86	5.92	<0.001	0.932	0.107

Table 2. Frequency of keel bone deviations and fractures (%) determined by palpation and dissection of Lohmann Brown-Lite (LB) and Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30 or 50 lux at 16 wk of age.

	Light intensity (L)			<i>P</i> -value	Strain (S)		<i>P</i> -value	L × S <i>P</i> -value	SEM
	10	30	50		LB	LW			
Palpation									
Deviations	3.33	4.58	5.42	0.481	5.56	3.33	0.370	0.640	0.860
Fractures	1.25	2.50	0.00	0.361	0.83	1.67	0.787	0.437	0.557
Dissection									
Deviations	6.48	5.56	9.26	0.728	8.02	6.17	0.630	0.394	1.061
Fractures	0.00	0.00	0.00	-	0.00	0.00	-	-	0.000

n = 12 pen replicates per light intensity × strain.

0–1), LW pullets had higher mortality (2.78% vs. 0.78%; $P < 0.001$) than LB pullets, with yolk sac infection (2.28% vs. 0.28%; $P < 0.001$) being the primary cause.

In the second period (Wk 1–16), there was no effect of L or interaction between L × S on mortality and morbidity (average of 0.75% mortality across all L treatments). LW pullets had a higher mortality (1.11% vs. 0.39%; $P = 0.015$) than LB pullets. The primary causes of mortality were yolk sac infection (0.39% vs. 0.06%; $P = 0.026$) and polyserositis (0.28% vs. 0.00%; $P = 0.038$).

DISCUSSION

The objectives of this study were to determine whether L influences the body weight, keel bone damage, breast muscle mass, bone strength, and mortality of Lohmann Brown-Lite and Lohmann LSL-Lite pullets reared in a complex environment.

Light Intensity

Based on the results of the present study, L did not affect body weight. This is in agreement with Dorminey et al. (1970) who studied L between one and 32 lux in White Leghorn pullets reared in floor pens.

Light intensity between 10 and 50 lux did not affect KBD in pullets and there are several possible explanations for this. L at 10 lux may have been bright enough for pullets to navigate their surroundings safely. KBD can be caused by crashes, unequal wing-loading, and perch use (Tauson and Abrahamsson, 1994; Sandilands et al., 2009; Stratmann et al., 2015a, b).

Previous studies demonstrated that L of less than 1 lux may compromise visual acuity and prevent successful jumps in hens (Taylor and Scott, 2002; Taylor et al., 2003). Therefore, the 10 lux treatment in the present study may not have been low enough to inhibit safe jumping in pullets (Chew, 2020) resulting in no KBD differences across treatments. In addition, pullet age may have played a factor in the absence of KBD. At 16 wk, pullet's keel bones have not yet fully ossified and are still cartilaginous (Buckner et al., 1949; Casey-Trott, 2016). Any damage to the keel bone during this stage of development would not be as severe as when fully ossified (Nicol et al., 2006; Rufener and Makagon, 2020). Additionally, L treatments of 10, 30, or 50 lux did not differ from each other enough to influence locomotory activity (Chew, 2020) and subsequently musculoskeletal system development of these pullets. Nonetheless, results of this study demonstrate that L between 10 and 50 lux did not negatively affect pullet KBD.

Despite different L treatments not affecting KBD, results of the study revealed numerically more recorded fractures in palpated than dissected pullets, and numerically more recorded deviations in pullets that were dissected than palpated (not statistically analyzed). These differences in results may have been due to random error as different pullets were used for both evaluation methods, however there is also the possibility for misdiagnosis of false positives in fractures and false negatives in deviations from the palpation technique. Even though the palpation technique may lead to misdiagnoses (Casey-Trott et al., 2015), several steps can be taken to increase accuracy in the data. These steps include adequate training, practice, and inter-observer reliability of more than 80% prior to assessments, all of which were

Table 3. Pectoralis major and minor weights of Lohmann Brown-Lite (LB) or Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30 or 50 lux at 16 wk of age.

Weight	Light intensity (L)			<i>P</i> -value	Strain (S)		<i>P</i> -value	L × S <i>P</i> -value	SEM
	10	30	50		LB	LW			
BW (kg)	1.32	1.30	1.31	0.403	1.45	1.17	<0.001	0.461	0.018
<i>Pectoralis major</i> (g)	62.89	61.59	61.09	0.539	65.28	58.43	<0.001	0.659	0.617
% BW	4.79	4.78	4.70	0.754	4.52	5.00	<0.001	0.314	0.052
<i>Pectoralis minor</i> (g)	19.61	20.01	19.70	0.841	20.99	18.55	<0.001	0.717	0.231
% BW	1.49	1.55	1.52	0.469	1.45	1.59	<0.001	0.548	0.018

n = 12 pen replicates per light intensity × strain.

Table 4. Tibia bone parameters of Lohmann Brown-Lite (LB) or Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 10, 30 or 50 lux at 16 wk of age.

	Light intensity (L)			<i>P</i> -value	Strain (S)		<i>P</i> -value	L × S <i>P</i> -value	SEM
	10	30	50		LB	LW			
Absolute									
Tibia weight (g)	11.34	11.43	11.25	0.298	12.87	9.81	<0.001	0.928	0.188
Length (cm)	11.57	11.51	11.49	0.212	11.66	11.39	<0.001	0.708	0.022
Outer width (W ¹ , cm)	0.74	0.73	0.73	0.282	0.79	0.67	<0.001	0.213	0.007
Inner width (W ¹ , cm)	0.56	0.55	0.56	0.189	0.61	0.50	<0.001	0.095	0.007
Thickness (W ¹ , cm)	0.18	0.17	0.17	0.116	0.18	0.17	0.064	0.433	0.002
Outer width (N ² , cm)	0.59	0.59	0.59	0.499	0.63	0.55	<0.001	0.561	0.005
Inner width (N ² , cm)	0.45	0.45	0.45	0.372	0.49	0.41	<0.001	0.556	0.005
Thickness (N ² , cm)	0.14	0.14	0.14	0.722	0.14	0.14	0.657	0.687	0.001
Force (kg)	19.90	19.57	19.72	0.404	19.64	19.82	0.296	0.104	0.093
Relative ³									
Weight (% BW)	0.87	0.88	0.86	0.668	0.93	0.81	<0.001	0.241	0.012
Length (cm/kg)	8.93	8.96	8.90	0.964	8.39	9.47	<0.001	0.214	0.114
Outer width (W ¹ , cm/kg)	0.57	0.56	0.56	0.998	0.57	0.56	0.571	0.141	0.006
Inner width (W ¹ , cm/kg)	0.43	0.43	0.43	0.870	0.44	0.42	0.021	0.203	0.005
Thickness (W ¹ , cm/kg)	0.14	0.13	0.13	0.309	0.13	0.14	<0.001	0.142	0.002
Outer width (N ² , cm/kg)	0.46	0.45	0.45	0.966	0.45	0.45	0.813	0.193	0.005
Inner width (N ² , cm/kg)	0.35	0.35	0.35	0.988	0.35	0.34	0.093	0.286	0.004
Thickness (N ² , cm/kg)	0.11	0.11	0.10	0.727	0.10	0.12	<0.001	0.160	0.002
Stress (kg/cm ²)	1523.35	1551.86	1553.63	0.832	1276.96	1816.23	<0.001	0.0497	35.201

¹W – wide. The diameters are perpendicular to the direction of the applied force.

²N – narrow. The diameters are parallel to the direction of the applied force.

³Relative. Tibia bone characteristics were adjusted for BW.n = 12 pen replicates per light intensity × strain.

practiced in the study. In this case, these tools were sufficient for the authors to see that L at 10, 30, or 50 lux did not affect pullet KBD. However, for future research, more training on palpation to prevent false positives and negatives may be warranted. Other methods of KBD evaluation, such as radiography, ultrasonography, and peripheral quantitative computed tomography (pQCT) may result in greater accuracy than palpation (Casey-Trott et al., 2015).

Development of the musculoskeletal system and the mechanical strain forces that cause bone modeling and remodeling come from muscles. In birds, the breast muscles anchor the keel bone and make flights possible (Duncker, 2000; Fleming et al., 2004). Therefore, it is important to measure both keel bone damage and breast muscle mass. In this study, L did not affect KBD, and breast muscle weight was also unaffected. In Red jungle fowl, the breast muscle constitutes 20% of the bird's body weight (Duncker, 2000; Casey-Trott, 2016). In the present study, the *Pectoralis major* and *Pectoralis minor* only made up 5.97% and 6.59% of body weight in LB and LW pullets, respectively. This is due to selection for productive traits in modern commercial laying hens and not muscle composition, resulting in a more exposed

keel bone and reduced control in flight, which may influence KBD (Jackson and Diamond, 1996; Fleming et al., 2004). In the case of this study, KBD was not observed and is likely due to the underdeveloped keel bones in the pullets.

Interestingly, S reacted to L differently in terms of bone stress. Demonstrated through a 3-point bending test, the bone breakage assessment is a gauge for skeletal health and is associated with growth and egg production in layers (Rath et al., 2000). A higher stress value indicates a greater ability for the bone to withstand stress (increase in strength) and therefore a healthier skeletal system. Many factors can affect bone strength, and at a young age, exercise is one of the primary contributors to the development of the musculoskeletal system (Fleming et al., 1994; Janczak and Riber, 2015). The results of the present study reported greater bone resistance to mechanical stress in LW than LB pullets. Within S, numerically, there was an increase in bone resistance to mechanical stress with increasing L for LB pullets, while bone resistance to mechanical stress peaked at 30 lux for LW pullets. Although there was an interaction between L and S, bone resistance to mechanical stress within S was not significantly different between L treatments, therefore this may have been due to a random effect. Another explanation could be that LW pullets reared at 30 lux increased in their locomotory activity, however, at 50 lux they may have become visually stimulated to do another activity, such as preening (Chew, 2020). Overall, the results from this study suggest that L of 10 lux is bright enough for pullets to navigate their environment safely, and L of 30 lux or 50 lux may result in an increase in locomotory activity and may numerically but not significantly improve the skeletal system and bone strength for LB pullets.

Table 5. Interaction between light intensity (10, 30 or 50 lux) and strain (Lohmann Brown-Lite [LB] and Lohmann Selected Leghorn Lite [LW]) on tibia bone stress (kg/cm²) of pullets at 16 wk of age (12 pen replicates per light intensity × strain).

Strain	Light intensity		
	10	30	50
LW	1802.63 ^a	1842.74 ^a	1802.17 ^a
LB	1244.07 ^b	1260.99 ^b	1325.81 ^b

^{a-b}Means with different letters indicate a significant difference ($P < 0.05$).

Table 6. Overall mortality (%) and cause of mortality (%) of Lohmann Brown-Lite (LB) and Lohmann Selected Leghorn Lite (LW) pullets reared in floor pens in light intensity of 50 lux from 0 to 1 wk of age, and in 10, 30 or 50 lux from 1 to 16 wk of age.

	Light intensity (L)			<i>P</i> -value	Strain (S)		<i>P</i> -value	L × S <i>P</i> -value	SEM
	10	30	50		LB	LW			
Wk 0–1									
Overall mortality	-	-	1.78	-	0.78	2.78	<0.001	-	0.273
Infectious	-	-	1.39	-	0.33	2.45	<0.001	-	0.249
Yolk sac infection	-	-	1.28	-	0.28	2.28	<0.001	-	0.232
Polyserositis	-	-	0.06	-	0.00	0.11	0.139	-	0.039
Osteomyelitis	-	-	0.06	-	0.06	0.06	1.000	-	0.039
Peritonitis	-	-	0.00	-	0.00	0.00	-	-	0.000
Metabolic – ascites	-	-	0.00	-	0.00	0.00	-	-	0.000
Skeletal – rotated tibia	-	-	0.00	-	0.00	0.00	-	-	0.000
Emaciation/dehydration	-	-	0.31	-	0.33	0.28	0.866	-	0.094
Other/no visible lesions	-	-	0.08	-	0.11	0.06	0.541	-	0.047
Other	-	-	0.03	-	0.06	0.00	0.315	-	0.028
No visible lesions	-	-	0.06	-	0.06	0.06	1.000	-	0.039
Wk 1–16									
Overall mortality	0.50	0.75	1.00	0.691	0.39	1.11	0.015	0.155	0.150
Infectious	0.25	0.42	0.67	0.377	0.06	0.83	0.001	0.775	0.120
Yolk sac infection	0.08	0.25	0.33	0.369	0.06	0.39	0.026	0.650	0.075
Polyserositis	0.17	0.00	0.25	0.321	0.00	0.28	0.038	0.321	0.072
Osteomyelitis	0.00	0.17	0.00	0.331	0.00	0.11	0.102	0.071	0.039
Peritonitis	0.00	0.00	0.08	0.445	0.00	0.06	0.319	0.371	0.028
Metabolic – ascites	0.00	0.08	0.00	0.428	0.00	0.06	0.315	0.365	0.028
Skeletal – rotated tibia	0.00	0.08	0.08	0.645	0.06	0.06	1.000	0.225	0.039
Emaciation/dehydration	0.00	0.00	0.00	-	0.00	0.00	-	-	0.000
Other/no visible lesions	0.25	0.17	0.25	0.872	0.28	0.17	0.461	0.175	0.075
Other	0.00	0.17	0.08	0.373	0.06	0.11	0.566	0.718	0.047
No visible lesions	0.25	0.00	0.17	0.352	0.22	0.06	0.154	0.134	0.060

One concern with increasing L is the possibility of increased flock aggression and incidence of cannibalism, thereby potentially increasing the level of mortality (Kjaer and Vestergaard, 1999). The results of the present study show that L between 10 and 50 lux did not affect mortality of pullets up to 16 wk. This is in agreement with several studies conducted on broilers (Downs et al., 2006; Kristensen et al., 2006; Lien et al., 2007) and layers (Huber-Eicher and Audigé, 1999; Kjaer and Sørensen, 2002).

Strain

One of the objectives of this study was to understand how different L treatments affect layer pullets from different S. Throughout the experiment, pullet body weight was consistent with the Lohmann performance guide (Lohmann Tierzucht, 2018). LB pullets were heavier than LW pullets, which is also in agreement with other studies (Tauson et al., 1999; Vits et al., 2005). As a result, LB pullet breast muscle weight and tibiae were heavier and larger than LW pullets. However, despite the larger difference, KBD was similar between S. In addition, although keel bone deviations were thought to be affected by perching behavior in laying hens (Tauson and Abrahamsson, 1996), LW pullets, who spent more time on perches than LB pullets (Mohammed and Said, 2016; Chew, 2020) had no more deviations than LB pullets. This suggests that both LB and LW pullets were able to navigate their surroundings safely, or as mentioned earlier, were too young for KBD to be evident (Casey-Trott, 2016). LW pullets also

expressed higher locomotory activity than LB pullets (Kozak et al., 2016b; LeBlanc et al., 2018; Chew, 2020; Pufall et al., 2021) which may develop the musculoskeletal system and result in increased breast muscle mass (Casey-Trott et al., 2017; Chew, 2020). This may explain the larger breast muscle to body-weight ratio in LW compared to LB pullets observed in this study. On the other hand, this may also be due to genetic differences in anatomical traits between brown- and white-feathered strains (Fawcett et al., 2020). Further research between the anatomical and behavioral characteristics of brown- and white-feathered egg-laying strains may be helpful to better understand and explain strain variances.

The results for pullet tibia strength may also be related to bird activity or genetic differences. In the present study, despite LB pullets having heavier and larger tibiae than LW pullets, relative to body weight, LW pullets had longer and stronger tibiae bones than LB pullets. Stronger tibiae may be associated with higher locomotory activity, which was observed more frequently in white-feathered than brown-feathered layers (Mohammed, 2012; Chew, 2020). Perching may also influence bone formation as the activity requires mechanical loading during mounting and dismounting of perches, and static loading for perch balance (Hughes and Appleby, 1989; Newman and Leeson, 1998; Casey-Trott et al., 2017). Since white-feathered pullets typically spend more time on perches than brown-feathered pullets (Tauson and Abrahamsson, 1996; Wall and Tauson, 2007; Chew, 2020), it is possible that LW pullets in the present study developed a stronger musculoskeletal system than LB pullets. However, this may also

be due to genetic differences; LW pullets could be genetically predisposed for stronger bones resulting in more perching, as opposed to having stronger bones due to high perching activity. Previous studies have reported lower bone strength in white-feathered hens than brown-feathered hens (Riczu et al., 2004; Vits et al., 2005), however, the values reported were not corrected for body weight. In contrast, other studies that calculated for bone strength relative to body weight reported stronger bones in white-feathered layers (Fawcett et al., 2020; Pufall et al., 2021). This is in agreement with the present study, where LW pullets had higher bone strength than LB pullets. However, neither S was affected by L treatments.

Mortality was higher in LW pullets than LB pullets but there was no relationship to L. The highest causes of mortality were due to infections occurring early in life, namely yolk sac infection and polyserositis, which is in agreement with Olsen et al. (2012). Yolk sac infections are acquired in-ovo and are due to translocation of bacteria from the bloodstream, air sacs or intestine (Olsen et al., 2012). Polyserositis is an inflammation of the serous membranes and is also primarily caused by bacterial infections (Srinivasan et al., 2014). Possible explanations for higher mortality between S may be due to transmission from the parent flock, spread of bacteria during hatch or transport, or parent flock age.

In conclusion, the results of this study suggest that L at 10, 30, or 50 lux did not affect body weight, keel bone health or mortality of egg-strain pullets reared in floor pens containing a perchery system to 16 wk. There was an interaction between L and S for bone stress, however results showed only an effect of S whereby LW pullets had greater bone stress than LB pullets. S also played a role in each of the measured parameters. LB pullets had a higher body weight and heavier tibiae, while relative to body weight, LW pullets had a larger breast muscle weight, thicker tibiae, higher bone strength, and higher mortality than LB pullets. S did not affect the incidence of KBD. Overall, the results indicate that an environment of 10, 30, or 50 lux does not negatively affect the body weight, KBD, and bone health of LW and LB pullets. In addition, body weight and KBD were unaffected by L, possibly due to either L ranges not being large enough or due to the young age of the pullets. Therefore, increasing L treatment ranges would be of interest for future research to determine the extent of high L on pullet health parameters. Additionally, it would be interesting to see the effect of different L past the pullet phase and into the adult production phase, including evaluations on the musculoskeletal system and eggshell quality of layers.

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DISCLOSURES

The authors declare no conflicts of interest.

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