Research Note: Effect of light intensity of calcium homeostasis in pullets

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ABSTRACT The impact of varying light intensities on layer pullets is not yet well understood. Behaviorally, brighter illumination may increase pullet activity levels by allowing better navigation in the complexity of non-cage systems. In addition, light intensity was previously demonstrated to affect the levels of calcium and phosphate regulating hormones in mice. The objective of this study was to examine how exposure of pullets to different light intensity affects their calcium and phosphorus homeostasis. Lohmann LSL-Lite and Lohmann Brown-Lite pullets were randomized into 4 individually controlled rooms with 6 pens per room, which were assigned to 10 or 50 lux light intensity supplied via white LED lighting during the photophase. After 8 and 16 wk of exposure, plasma calcium, phosphorus, and magnesium were measured by inductively coupled plasma optical emission spectrometry; and parathyroid hormone, 1,25-dihydroxyvitamin D, fibroblast growth factor 23, and markers of bone formation and resorption were measured by ELISA. Intestine and kidney samples were collected at 16 wk and gene expression of receptors for calcium and phosphate regulating hormones was assessed. The data were analyzed by one-way ANOVA. Lohmann Brown-Lite pullets exposed to 50 lux for 8 wk exhibited lower ionized Ca levels and a trend for increased bone formation markers compared to pullets reared in 10 lux. Thus, higher light intensity during rearing may beneficially affect calcium homeostasis and bone formation in young Lohmann Brown-Lite chicken.

Key words: pullets, egg-laying, light intensity, calcium, phosphorus

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INTRODUCTION

Bone in egg-laying chickens provides structural support and serves as a reservoir of calcium for eggshell formation. Poor bone health puts laying hens at risk of developing bone fractures and skeletal deformities and may negatively impact egg production. Factors important for bone health in egg-laying chickens include exercise, dietary calcium, phosphorus, and vitamin D (Bar, 2009; Casey-Trott and Widowski, 2016). However, bone fractures persist in pullets and hens in modern aviaries, free run, and free-range housing systems, which allow for wider range of exercises (Lay et al., 2011). Light intensity during growth has been suggested as a potential factor that may improve the impact of modern housing on bone health in pullets (Hester et al., 2011). The current light intensity of 10 lux is used according to the Canadian Code of Practice for Pullets and Laying Hens (NFACC, 2017). Higher light intensity may improve the birds' ability and success in jumping, flying, and other forms of exercise, positively impacting bone development (Casey-Trott and Widowski, 2016). In addition, it may also affect other factors important for bone health. In mammals, light intensity was previously demonstrated to affect the levels of calcium regulating hormones (Hiratsuka et al., 2014). Since calcium and phosphorus homeostasis are important for laying birds (Bar, 2009), it is of interest to investigate how light intensity affects these parameters in pullets.

Blood calcium and phosphate homeostasis in terrestrial animals is regulated by the number of hormones. Parathyroid hormone (**PTH**) is a primary regulator of calcium homeostasis, secreted in response to a drop in serum calcium levels (Khundmiri et al., 2016). Vitamin D derived from the diet or skin synthesis is an important regulator of calcium and phosphate gut absorption and

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bioavailability (Khundmiri et al., 2016). Vitamin D undergoes 2 enzymatic hydroxylation reactions to become an active hormone. First, 25-hydroxyvitamin D (**25OHD**) is formed in the liver, second, 25OHD is converted by kidney α-hydroxylase to the biologically active form 1,25-dihydroxyvitamin D (1,25(OH)2D, referred to as VitD in this paper). Fibroblast growth factor 23 (**FGF23**) is a phosphate-regulating hormone produced by the most abundant bone cells, osteocytes (Balani and Perwad, 2019). PTH, VitD, and FGF23 regulation is strongly interconnected, with each of the hormones affecting and being affected by the levels or activities of the others. Understanding the regulation of calcium and phosphate homeostasis in pullets exposed to different light intensities during growth may provide mechanistic insights into the effects of different environment on bone health.

The objective of this study was to examine how exposure of Lohmann LSL-Lite and Lohmann Brown-Lite pullets from 0 to 16 wk of age to light of different intensity affects their plasma levels of electrolytes as well as calcium and phosphorus regulating hormones.

METHODS

Animal Housing and Husbandry

All experimental procedures were approved by the University of Saskatchewan Animal Care Committee (19940248). All birds were cared for as specified in the Guide to the Care and the Use of Experimental Animals by the Canadian Council of Animal Care. The data collected for this paper was part of a larger project (Chew et al., 2021a,b).

Two 16-wk blocked trials were performed using 4 individually controlled, light-tight rooms. Each room containing 6 floor pens was randomly assigned either 10 lux or 50 lux. Newly hatched Lohmann Brown-Lite (**LB**; n = 600) and Lohmann LSL-Lite (**LW**; n = 600) pullets (obtained from Clark's Poultry, Brandon, MB) were randomly assigned to strain-specific pens (n = 300 birds per treatment). The floor pens were bedded with 7 to 10 cm depth of wheat straw, and the pullets were housed at an estimated stocking density of 6.5 birds/m^2 (50 pullets per pen), which is in accordance with the recommendations in the Lohmann Management Guide (Lohmann Tierzucht, 2018). All birds had ad libitum access to water and commercial starter (2,738 kcal/kg), grower (2,750 kcal/kg), and developer (2,725 kcal/kg) appropriate for their stage of development (Lohmann Tierzucht, 2018). During the first week, pullets had access to supplemental feeders and waterers in addition to the regular tube feeders and nipple drinkers.

Light

Each room was illuminated with eight 11-watt white light-emitting diode (**LED**) lamps with similar wavelength (2821 Kelvin, Greengage Lighting Ltd., Edinburgh, UK). For the first week, the pullets were given 23 h of light and 1 h of dark. The hours of light were decreased every week until the seventh week where lights remained at 8 h of light and 16 h of dark until the end of the trial. Dawn and dusk periods were simulated over a 15-min period. For the first week, light was set at 50 lux. After that, it was adjusted according to the room-appropriate intensity treatment (10 lux or 50 lux).

Sample Collection

Whole blood (2−4 mL) was collected from brachial vein into heparin-treated collection tubes at 8 and 16 wk of age. The samples were centrifuged for 10 min at 1300× *g*, then plasma was collected, centrifuged at 2000× for 15 min, aliquoted and frozen. At the 16 wK end point, kidney, duodenum, jejunum, and ileum were dissected, snap frozen in liquid nitrogen and kept in −80° C until analysis.

Electrolytes Evaluations

Calcium, phosphorus, magnesium, sodium, and potassium were measured by inductively coupled plasma optical emission spectrometry (**ICP-OES**) as follows. Plasma (300 μL) were combined with 1.5 mL of concentrated HCl (trace metal grade) and incubated at 95°C for 1 h. Then, 2 mL of concentrated nitric acid (trace metal grade) were added, followed by incubation at 95° C for 1 h. Samples were cooled, 1 mL of H_2O_2 was carefully added, and the samples were left overnight at room temperature. Next day, H_2O was added up to 15 mL in volume, the samples were mixed, filtered through 0.45-μm filters, and processed in the Thermo Icap ICP-OES. For the calibration curve, 2.5 mL of 1,000 ppm certified standards (SCP SCIENCE) were serially diluted with 5% nitric acid. To calculate the levels of ionized calcium, albumin was determined by Bromocresol Green albumin assay kit (Sigma, Oakville, Ontario, Canada, cat N MAK 124), and the total calcium was corrected based on plasma albumin level as described (Jafri et al., 2014).

ELISA

Plasma was diluted 2-fold, and the levels of chicken parathyroid hormone, 1.25 dihydroxy vitamin D3, FGF23, TRAP 5A and protocollagen 1-N terminal peptide (**P1NP**) were evaluated by ELISA (Cat. # ESKP0579, ESKD0009, ESKF0016, ESKT0051, ESKP0585, ABclonal) according to manufacturer's recommendations.

Quantitative Real Time PCR

Total RNA was isolated using the RNeasy mini kit (Qiagen, Toronto, Ontario, Canada, Cat. No. 74104), and 1 μg of total RNA was reverse transcribed using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Burlington, Ontario, Canada, 4368813). Real-time PCR was performed in 7500

Applied Biosystems instrument using TaqMan probes with the universal PCR Master Mix (Life Technologies, Burlington, Ontario, Canada, Cat. No. 4304437) in a total volume of 20 μL. The following TaqMan probes were used: Vitamin D receptor (VDR, Gg03348519_m1); FGF23 receptor 1 (FGFR1 Gg03340354_g1); FGF23 receptor 2 (Gg03349074_m1); Klotho1, (Kl, Gg033290983_m1); GAPDH (GAPDH, Gg03346982_ml). Real time PCR with SYBR Select Master Mix (Applied Biosystems, Burlington, Ontario, Canada, Cat.No 44722908) in a total volume of 20 μL using primers from Invitrogen was performed for the following genes: (PTH1-R_Rv GGCCAGCAGACAATAC CA; PTH1R_Fw ATGGGATCATATCTGGTTT AT); (PTH3-R_Fw ATGGGGTCTGTGGGCAGG: PTH3-R_Rv GTTGAAGTCGTAGATGTAGTC); (1Alpha-hydroxylase_ Fw TCGTGGCAGGAATA-CAGAGA; 1Alpha −hydroxylase_RV ACTGCCA-CACATCTTTGGGTTT) and (GAPDH _Fw AGC ACCCGCATCAAAGG; GAPDH_Rv CATCATCC-CAGCGTCCA) (Invitrogen cat numbers for primers: PTH1-R- A15611, PTH3-R - A15611, GAPDH −A15611, 1alpha-hydroxylase - 10336022).

Statistical Analysis

Data are presented as means ± standard error of the mean (**SEM**) with sample size (n) indicating the number of independent experiments, or as means ± standard deviation (**SD**) with sample size (n) indicating the number of samples. Differences were assessed using GraphPad Prism7 for Windows (Version 7.04 November 28, 2017) on log-transformed samples by Student's t test or ANOVA when appropriate and accepted as statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Lohmann LSL-Lite (LW) and Lohmann Brown-Lite (LB) pullets were randomized from hatch into rooms illuminated at 10 or 50 lux light intensity supplied via white LED lighting during the photo phase. First, we examined the changes in plasma levels of calcium and magnesium. Total calcium was not affected by light intensity in pullets of both strains and ages (Table 1). In contrast, ionized calcium levels, which were calculated based on the plasma albumin levels, were reduced ($P = 0.063$) in 8-wk-old LB pullets exposed to 50 lux light intensity compared to those reared at 10 lux (Figure 1A). Magnesium levels were not affected by light intensity (Table 1). Total plasma calcium and magnesium were significantly higher in 16-wk-old compared to 8-wk-old pullets of both strains (Table 1). Total and ionized calcium were similar in LW and LB pullets of the same age, while total magnesium was higher in 8-wk-old LW pullets compared to LB pullets (Figure 1A, Table 1). Thus, higher light intensity was associated with a decrease in ionized calcium in 8-wk-old, LB pullets only.

Next, we examined the changes in plasma levels of calcium regulating hormones, VitD and PTH in the same plasma samples. Although in several samples of 8-wk-old LB pullets reared at 50 lux VitD and PTH levels were noticeably high, average plasma VitD and PTH levels were not significantly affected by light intensity (Figures 1B and 1C). With age, plasma VitD significantly increased in both strains, while PTH did not change, except for LB pullets exposed to 50 lux light intensity, in which PTH significantly decreased with age (Figure 1C). VitD levels were similar in both strains while PTH levels were significantly higher in LW pullets compared to LB pullets at 16-wk of age. Thus, higher light intensity was not associated with significant changes in plasma VitD and PTH levels, even though both hormones were increased in a proportion of 8-wk-old LB pullets reared at 50 lux.

Plasma levels of total P and FGF23 were examined in pullets reared at different light intensities. Plasma P was not affected by light intensity, age or strain of pullets (Table 1). Average plasma FGF23 was not affected by light intensity in pullets of both strains and ages (Figure 1D). Plasma FGF23 significantly increased with age in LW pullets, but not in LB pullets. Plasma FGF23 was also higher in LW pullets compared to LB pullets at 16-wk of age (Figure 1D).

Calcium and phosphorus absorption in intestine are critical for regulating calcium homeostasis (Bar, 2009; Khundmiri et al., 2016). Normally, when plasma Ca^{2+} levels are decreased, VitD, activated by alpha-hydroxylase, will stimulate Ca^{2+} absorption in the small intestine (Bar, 2009; Khundmiri et al., 2016). For most birds, calcium is absorbed prior to reaching the lower ileum due to the proximal intestine's high efficiency to absorb Ca^{2+} (Wasserman, 2004). PTH is considered to affect intestinal absorption through its action on VitD (Bar, 2009; Khundmiri et al., 2016). The duodenum, jejunum and ileum intestine regions of LB and LW 16-wk-old pullets reared at 10 lux and 50 lux light intensities were isolated and the expression of VDR, PTH1R, FGFR1, and KL were examined. Light intensity resulted in higher VDR expression in duodenum of LW pullets ($P = 0.073$), lower PTH1R expression in jejunum of LB pullets ($P = 0.046$) and higher KL expression in ileum of LW pullets ($P = 0.0003$) reared at 50 lux compared to 10 lux (Table 1). VDR expression was similar between the strains. PTH1R expression was higher in jejunum and ileum of LW compared to LB pullets when reared at 50 lux. FGFR1 expression was significantly higher in jejunum of LW compared to LB pullets at both light intensities but was lower in ileum of LW pullets reared at 10 lux, compared to corresponding LB pullets. KL expression levels were significantly higher in LW pullets compared to LB pullets at 10 lux in the duodenum, whereas, in the jejunum, it was significantly lower in LW pullets compared to LB pullets at 50 lux. Thus, exposure to higher light intensity only significantly affected 2 parameters: LB pullets displayed lower PTH1R levels in the jejunum, and LW pullets exhibited higher level of FGF23 coreceptor Klotho in

Table 1. Effect of light intensity during rearing on plasma levels of electrolytes and gene expression in intestine and kidney. Electrolytes were measured in plasma of 8- and 16-wk-old pullets using ICP-OES.

Measured compound	Location	Breed	Age (wk)	10 lux	50 lux
Electrolytes concentrations (mg/L)					
Calcium	Plasma	LB	8	122.12 (1.43) ####	124.12 (2.17) ####
			16	144.40 (7.38) ####	149.45 (2.89) ####
	Plasma	LW	8	123.04 (5.45) ####	117.18 (4.35) ####
			16	150.15 (0.82) ####	145.47 (5.31) ####
Potassium	Plasma	LB	8	182.60 (3.70) &	163.00 (9.60) &
			16	170.79 (10.00)	162.03 (3.31)
	Plasma	LW	8	149.28 (9.66) &	133.24 (10.70) &
			16	154.23 (3.27)	152.27 (4.82)
Magnesium	Plasma	LB	8	14.88 (0.39) ####, &&	14.79 (0.78) ####, &&
			16	18.83 (1.31) ####	19.63 (0.60) ####
	Plasma	LW	8	16.27 (0.24) ####, &&	16.21 (0.19) ####, &&
			16	20.05 (0.21) ####	18.84 (0.71) ####
Sodium	Plasma	LB	8	3,091.90 (43.48)	3,048.52 (47.83)
			16	2,817.82 (106.90)	3,031.34 (38.93)
	Plasma	LW	8	2,794.65 (142.57)	2,673.41 (160.31)
			16	2,970.28 (41.00)	3,011.72 (64.16)
Phosphorus	Plasma	LB	8	72.85 (1.46)	72.65 (3.47)
			16	71.79 (2.35)	73.55 (1.79)
	Plasma	LW	8	80.07 (3.03)	77.49 (2.68)
			16	73.29 (3.18)	73.24 (2.55)
Gene expressions					
FGFR1	Duodenum	LB	16	0.87 (0.29)	1.17 (0.37)
		LW	16	0.78 (0.11)	0.72 (0.18)
	Jejunum	LB	16	0.90 (0.30) &	0.77 (0.19) &
		LW	16	5.44 (1.94) &	5.00 (1.92) &
	Ileum	LB	16	0.85 (0.07) &&	0.91 (0.11)
		LW	16	0.31 (0.18) &&	1.15 (0.26)
	Kidney	LB	16	0.85 (0.12)	0.98 (0.10)
		LW	16	0.97 (0.29)	1.31 (0.76)
PTH1R	Duodenum	LB	16	4.47 (2.18)	9.53 (5.31)
		LW	16	2.07 (1.28)	4.68 (2.17)
	Jejunum	LB	16	0.29 (0.19) *	0.2 (0.1) *, &&&
		LW	16	0.46 (0.14)	0.43 (0.10) &&&
	Ileum	LB	16	1.41 (0.45)	1.26 (0.06)
		LW	16	3.98 (3.07)	1.70 (1.20)
	Kidney	LB	16	31.92 (12.07) &	52.75 (9.64)
		LW	16	1.94 (1.10) **, &	37.24 (9.35) **
KL	Duodenum	LB	16	0.42 (0.16) &	0.65 (0.25)
		LW	16	1.78 (0.36) &	1.86 (0.56)
	Jejunum	LB	16	0.72 (0.28)	0.57 (0.17) &
		LW	16	0.27 (0.18)	0.14 (0.04) &
	Ileum	LB	16	0.66 (0.10)	0.71 (0.16)
		LW	16	0.34 (0.17) ***	1.95 (0.33) ***
	Kidney	LB	16	0.75 (0.11)	0.66 (0.10) &
		LW	16	1.23 (0.15)	2.39 (1.26) &
VDR	Duodenum	LB	16	0.91 (0.15)	1.41 (0.21)
		LW	16	0.66 (0.14)	1.15 (0.13)
	Jejunum	LB	16	1.39 (0.42)	2.53 (0.55)
		LW	16	2.84 (0.48)	3.51 (0.66)
	Ileum	LB	16	0.62 (0.13)	0.27 (0.07) &
		LW	16	0.86 (0.38)	1.10 (0.30) &
	Kidney	LB	16	0.97 (0.05)	0.93 (0.07) &&
		LW	16	0.66 (0.09)	0.54 (0.10) &&
CYP17A1	Kidney	LB	16	1.13 (0.14)	1.05 (0.15)
		LW	16	0.73 (0.19)	0.43 (0.14)

Sixteen-wk-old pullets were euthanized, duodenum, jejunum, and ileum intestinal regions, and kidneys were dissected and the expression of VDR, PTH1R FGFR2, FGFR1, KL, and CYP17A1 (in kidney only) were examined by RT-PCR.

Indicated are average values with SEM in the parentheses, n = n = 4−6 pullets/group.

Statistical significance was assessed using ANOVA and is indicated as follows: * the effect of the light intensity, # the effect of age, and & the effect of breed. The number of symbols corresponds to the significance level.

ileum. The relevance of this change needs to be examined in the future studies, because both PTH and FGF23 are assumed to exert their action on intestinal absorption of calcium and phosphorus respectively through VitD.

The kidneys also play a critical role in calcium and phosphorus homeostasis. In the kidneys, PTH targets 1-alpha-hydroxylase which activates vitamin D by converting 25-hydroxy VD_3 (calcidiol) to 1,25-dihydroxyvitamin D_3 (calcitriol). As a result, VitD can then stimulate various mechanisms to restore plasma Ca^{2+} levels, including increasing reabsorption of Ca^{2+} in the proximal convoluted tubules (Bar, 2009; Khundmiri et al., 2016). PTH independently affects the distal convoluted tubules to reabsorb Ca^{2+} (Khundmiri et al., 2016). We examined potential contribution of kidney by assessing

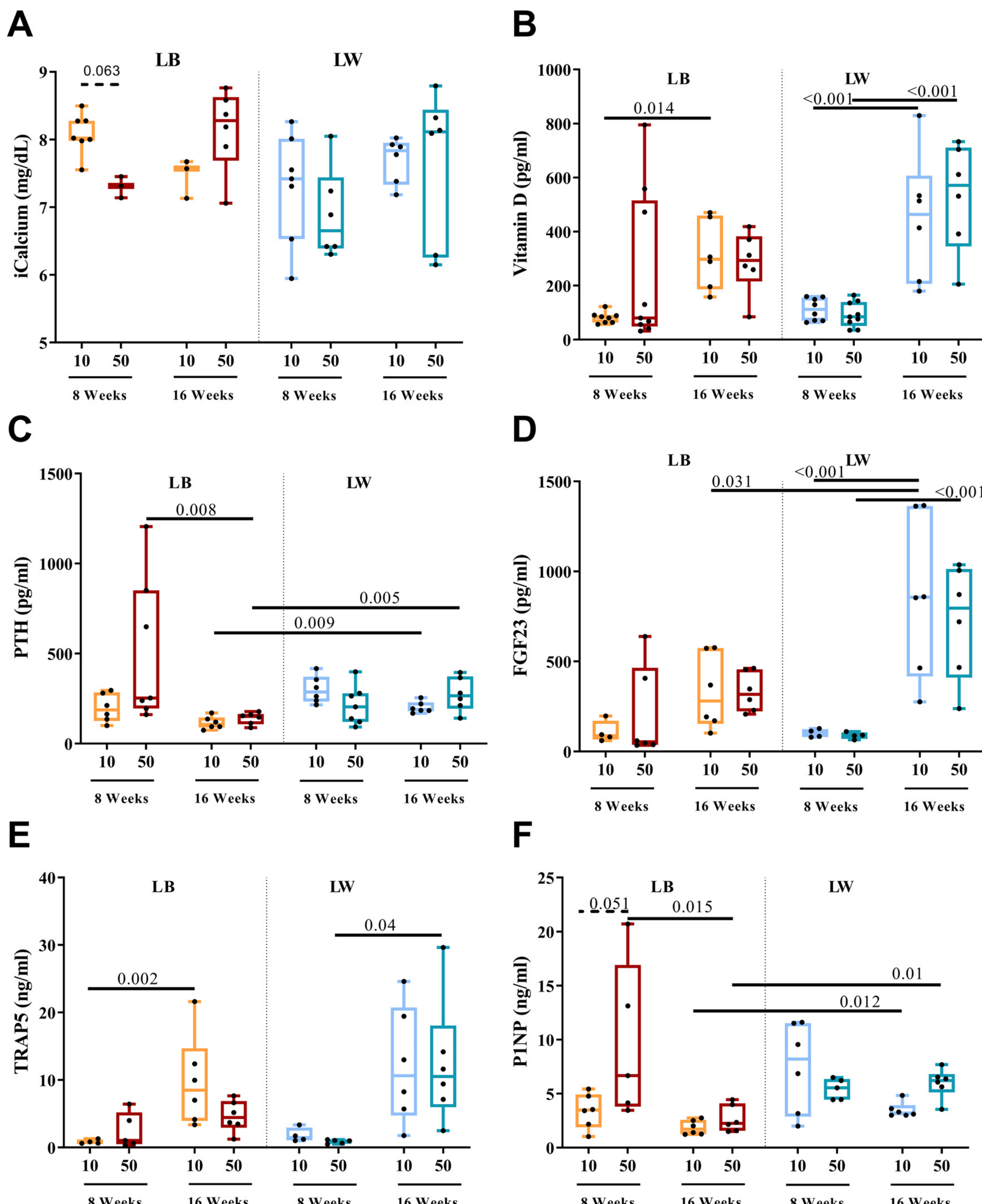

Figure 1. Changes in parameters of calcium and phosphate homeostasis in pullets reared at different light intensities. Pullets of Lohmann Brown-Lite (LB, yellow and red bars) and Lohmann Selected Leghorn Lite (LW, blue and green bars) strains were reared in floor pens in light intensity of 10 (yellow and blue bars) or 50 (red and green bars) lux, and blood samples were taken at 8 (left) and 16 (right) weeks of age. Plasma Ca^{2+} was measured using ICP-OES, and was normalized to albumin levels to estimate ionized Ca^{2+}. Hormonal levels and bone turnover markers were measured using ELISA. Shown are average levels of A) Ionized Ca^{2+}; B) VitD; C) PTH; D) FGF23; E) osteoclast marker TRAP5; F) osteoblast marker P1NP. Data are means ± SEM, n = 3−6 pullets/group, statistical significance was assessed using ANOVA.

the plasma electrolyte levels and kidney expression of VDR, CYP17A1 (gene name for α-hydroxylase), as well as PTH1R, FGFR1, and KL. Plasma levels of total Na^+ and K^+ were examined in 8- and 16-wk-old LW and LB pullets reared at 10 lux and 50 lux light intensities. Plasma Na^+ and K^+ levels were not affected by light intensity (Table 1). Plasma Na^+ and K^+ levels were not affected by age and strain, except for higher K^+ in LB compared to LW in 8-wk-old pullets. Light intensity did not affect the expression of VDR, α- hydroxylase, FGFR1, or KL, while PTH1R expression was higher in LW pullets reared at 50 lux compared to 10 lux (Table 1). LB pullets displayed higher levels of VDR and α-hydroxylase compared to LW pullets at 50 lux as well as higher PTH1R expression at 10 lux, while LW pullets had higher KL expression compared to LB pullets at 50 lux. Thus, higher light intensity was associated with higher plasma Na^+ in LB 16-wk-old pullets and higher kidney PTH1R expression levels in LW 16-wk-old pullets.

Finally, we assessed how exposure to different light intensities affects plasma bone turnover markers in pullets. Osteoclast marker, tartrate resistant phosphatase type 5 (**TRAP5**), was not affected by light intensity in pullets of both strains and ages (Figure 1E). TRAP5 was increased with age in LB and LW pullets and was similar between the strains. Osteoblast marker, procollagen type 1 N-terminal propeptide (**P1NP**) exhibited a trend for higher levels in 8-wk-old LB pullets exposed to 50 lux compared to 10 lux (Figure 1F). P1NP levels were decreased with age in LB pullets reared at 50 lux but were not affected by age in LW pullets or LB pullets reared at 10 lux. P1NP levels were similar in young, 8-wk-old LB and LW pullets, however, P1NP levels were higher in 16-wk old LW compared to LB pullets at both 10 and 50 lux (Figure 1F). Thus, higher light intensity was potentially associated with higher levels of osteoblast marker P1NP only in 8-wk-old LB pullets.

Overall, this study demonstrates that exposure of LB chicken to 50 lux altered calcium homeostasis in young, 8-wk-old pullets, while light intensity did not affect parameters of calcium and phosphorus homeostasis in LW pullets. Though light intensity had some effects on gene expression of factors related to calcium and phosphorus homeostasis in intestine and kidney, only bone formation markers demonstrated a trend for consistent increase in 8-wk-old LB pullets. Taken together, these data suggest that increasing light intensity during rearing of egg-laying pullets has a potential to improve their wellbeing through beneficial changes in calcium homeostasis and bone development. These changes may be mediated by direct effects of light on hormonal regulation of calcium and phosphate homeostasis or can be due to higher physical activity because of improved vision-driven navigation. Further studies may help in better understanding the mechanisms of these effects and in finding the optimal values light intensity that provides the most practical benefits.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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