Spectrum Lighting During Pullet Rearing and Its Impact on Subsequent Production Performance in Layers

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Primary Audience: Poultry Producers, Flock Supervisors, Researchers

SUMMARY

Artificial lighting is used to control growth and reproduction. Lighting protocols are defined by the quantity (photoperiod, intensity) and the quality (wavelength) of light. Recently, with the introduction of light emitting diode (LED) bulbs, interest has grown in investigating the effect of spectrum lighting. Thus, the aim of this study was to examine the effect of red and green light on growth during pullet rearing, and on possible carry-over effects during the subsequent adult phase. Lohmann Brown-Lite chicks were raised in a 2-story free run barn divided into 4 sections and exposed to either 60% red LED (RL) or 60% green LED (GL) light treatments. At 19 wk, all birds were moved to an adult free-run barn with RL and GL pullets placed on separate halves of the barn. In the adult barn, all birds were exposed to RL. Body weight, egg production, ovarian morphology, estradiol and calcium levels, as well as bone structure were recorded until 70 wk. Although no consistent significant difference was observed in body weight or general reproductive parameters, RL pullets tended to sexually mature earlier. As well, no carry-over effect was detected. Regardless of pullet treatment, egg production remained high throughout, especially towards the end of lay. In conclusion, spectrum lighting during the rearing of layer pullets did not impact growth or subsequent production performance; however, exposing adult hens to RL may be beneficial to maintain high egg production.

Key words: laying hen, light, wavelength, growth, egg production

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DESCRIPTION OF PROBLEM

Growth rate and feed efficiency during the pullet phase as well as reproductive status during the laying phase are 2 of the main parameters underlying the performance of commercial laying hens; each of these can be influenced by artificial lighting. Birds are capable of perceiving light through photoreceptors in the retina, pineal gland, and hypothalamus [1, 2]. As seasonal breeders, commercial layers rely on photoperiodic cues to initiate sexual maturation [3] via the activation of the hypothalamo–pituitary–gonadal (**HPG**) axis as reviewed by Bédécarrats and by Bain et al. [4, 5]. Briefly, the reproductive axis and hence egg production are regulated by

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the hypothalamic neuropeptides, gonadotropin inhibitory hormone (**GnIH**), and gonadotropin releasing hormone (**GnRH-I**) [4]. During the rearing phase, exposure to short photoperiod results in higher melatonin production which in turn allows GnIH to maintain the reproductive axis in an inactive state [6, 7]. At this stage, structural bone (cortical and trabecular bone tissues) develop and grow to provide support and serve as frame for the musculoskeletal system [8]. Once pullets are ready for sexual maturation, increased photoperiod stimulates the release of GnRH-I, while reducing the expression of GnIH, and the HPG axis is activated [3, 9–11]. The production of GnRH-I results in increased gonadotropin release (luteinizing hormone [**LH**] and follicle stimulating hormone [**FSH**]) from the anterior pituitary gland, which then stimulates the ovary to undergo follicular maturation and steroidogenesis with the synthesis of estradiol (E_2) and progesterone required for preparing the hen for egg production and to trigger ovulation, respectively [4, 5, 12, 13]. More specifically, E_2 is essential for stimulating the development of the reproductive tract and secondary sex characteristics, the liver to produce yolk lipids, and the switch from structural bone to medullary bone deposition of calcium in bones, such as the femur and tibia [4, 5, 8]. As egg formation is initiated, shell deposition mostly occurs during the dark period, at a time when a limited amount of feeding takes place and limited amount of calcium is available from the gut [5, 8]. The consequent low plasma calcium concentration stimulates the production of parathyroid hormone, and together with E_2, these hormones convert vitamin D into the active form ($1,25(OH)_2D_3$) to release calcium from the bones by osteoclasts [8, 14, 15]. Osteoclasts can act on both medullary and structural bones, potentially putting high-producing hens at risk, especially as growth of structural bone ceases when hens reach sexual maturity [8, 15, 16]. Maintaining a balance between osteoblastic (bone-forming) and osteoclastic (bone resorbing) activity is important for bone remodeling and integrity. However, bone resorption over the laying period can lead to progressive, widespread loss of structural bone, resulting in fragile bones highly susceptible to fractures, also known as osteoporosis. The major cause of osteoporosis in laying hens is due to the cessation of structural bone formation, switching to medullary bone formation at onset of sexual maturity, with a progressive loss of structural bone over the laying cycle [16, 17]. Although the occurrence of fractures cannot be completely prevented, it is important to provide sufficient calcium and vitamin D to help minimize the incidence of osteoporosis [16].

Currently, the effect of lighting spectra on production performance has been a topic of great interest for both growth in broilers and egg production in layers. Exposing broiler chicks to shorter wavelengths, such as green (550 nm) and blue (450 nm) light, results in the enhancement of growth, through increased proliferation of satellite cells, and subsequent increase in muscle mass compared to birds exposed to longer wavelengths [18–20]. However, broilers and layers have been genetically selected for divergent traits and little evidence exists on the effects of light spectrum on the growth of layer pullets. Furthermore, exposure of broiler breeders and layers to green light has been suggested to have an inhibitory effect on reproductive performance by stimulating retinal photoreceptors; however, specific underlying mechanisms have yet to be determined [21–23]. On the other hand, longer wavelengths, such as red (660 nm) light, have been reported to stimulate sexual maturation and advance overall reproductive performance in both layers and broiler breeders [21, 23–25]. The stimulatory effect of red light during sexual maturation has been shown to also result in an increased concentration of E_2, with an earlier elevation [21, 23–25], which as mentioned above is critical to mediate the physiological changes necessary to switch from growth to egg production.

However, most studies on the impact of light spectrum in layers focused on adult hens and egg production, while limited research has been conducted on the rearing of commercial layer pullets. Thus, this study investigated whether 60% red **LED** (light emitting diode) light or 60% green LED light (hereinafter RL and GL, respectively) during the rearing phase has the ability to impact pullet growth and sexual maturation, and whether it will result in any carry-over effect on the production performance of adult hens later in life.

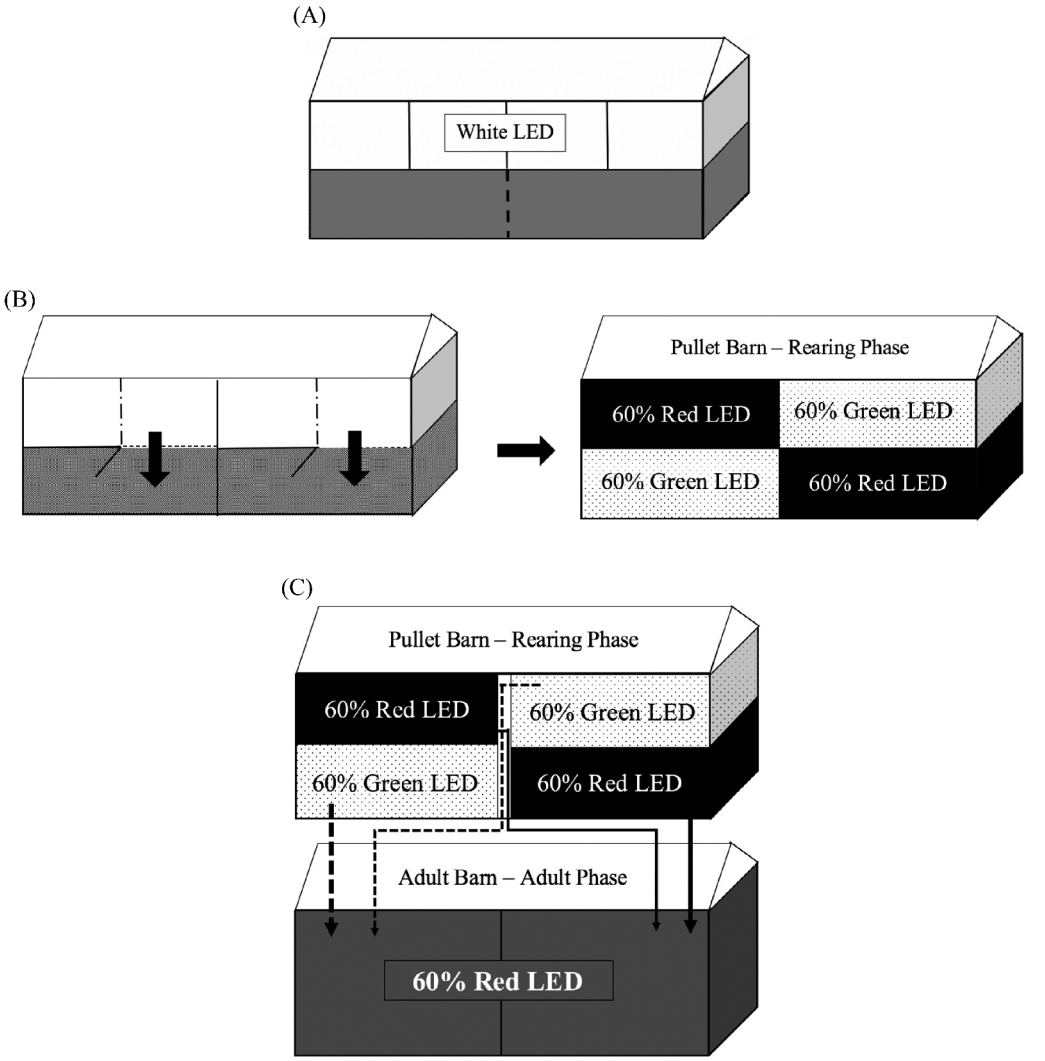

Figure 1. Diagrams of the housing condition. (A) Represents the housing condition for the first 2 wk, where chicks were maintained under white LED light in 2 sections on the top story. (B) At 14 of age, half of the pullets from the top story were moved to the bottom and 60% Red and 60% Green LED light treatments commenced. (C) Represents the transfer to the adult barn from the pullet barn at 19 wk of age. Birds reared under 60% Green LED were moved to one section of the adult barn, and birds reared under 60% Red LED were moved to the remaining section of the adult barn. All birds were kept under 60% Red LED from 19 to 70 wk of age.

MATERIALS AND METHODS

Experimental Birds, Housing Conditions, and Lighting

This study was conducted at Burnbrae Farms [26] and adhered to the guidelines outlined by the National Farm Animal Care Council [27]. A total of 14,500 Lohmann Brown-Lite day-old chicks [28] were housed in the top story of a 2-floor, free-run pullet barn separated into 2 light isolated sections. During the first 2 wk, chicks were maintained under white LED lights following an intermittent lighting program for the first week (4 h light and 2 h dark), and a 16 h photoperiod from day 7 to 14. At 2 wk of age (**woa**), half of the chicks were randomly moved to the bottom floor (also split into 2 sections), resulting in a quadrant layout (2 sections per story; Figure 1). Simultaneously, experimental lights were turned

on with one replicate on each floor illuminated with either RL (640 to 660 nm [29]) or GL (515 to 525 nm [30]) separated by light isolating partitions. RL was used to investigate whether the rate of sexual maturation could be enhanced, whereas GL was used to investigate any effect on pullet growth during the rearing phase and whether it may result in delayed sexual maturation. Photoperiod was gradually decreased each week until 9 h was reached at 6 woa, and this photoperiod was maintained until 15 woa. Once flock BW average reached the 15 woa target (1.236 kg; [31]), photoperiod was increased by 2 h per week up to 14 h (reached at 18 woa). The housing temperature was adjusted according to the Lohmann Brown-Lite management guide [31] and set to 34°C from time of chick placement to 5 d of age, and gradually decreased by 0.5°C daily until 24°C was reached. At 19 woa, pullets were transferred to a 1-story free-run laying barn, divided into 2 sections by a wire mesh and with nest boxes placed in the center of the barn. In the laying barn, pullets from RL treatment were placed in one section while pullets from GL treatment were placed in the other, with all birds exposed to the RL with a 14-h photoperiod regardless of pullet treatment. Hens were kept in the adult barn until 70 woa, with feed and water provided ad libitum according to NRC requirements [31–33].

Data and Sample Collection

Body weights were recorded weekly during the rearing phase (from 8 to 18 woa) on 50 randomly selected pullets per quadrant (n = 2 sections per treatment; n = 50 birds per section). After transfer to the adult barn, BW was recorded weekly until 30 woa and biweekly thereafter on 50 randomly selected birds per treatment. Egg production was recorded daily from 19 to 70 woa for each treatment and was expressed as weekly production rate (eggs per hen housed). Additionally, the number of cracked eggs and mortality were recorded.

During flock health care checks, BW records, blood samples, and carcasses of 10 individuals per treatment (n = 5 pullets per quadrant during the rearing phase) were recovered from Burnbrae Farms at 7, 11, 18, 25, 41 and 69 woa. From the carcasses, the left ovary and right femur were collected for further analysis, and relative weights of liver and ovary (without F1 to F6) were calculated. Ovary samples (without F1 to F6; hierarchal follicles) were soaked in 70% ethanol [34] overnight to visualize follicles in order to count and categorize based on size.

Hormone Extraction and Enzyme Immunoassay

Plasma was recovered from each blood sample [35], and samples were extracted with ethanol to increase E_2 retention and reduce nonspecific interference of the components within the sample following the protocol of Baxter et al. [23, 36]. E_2 concentrations in extracted plasma samples were then determined using a commercial enzyme immunoassay kit according to the manufacturer's instructions [37]. Additionally, calcium concentration in the remaining plasma samples was analyzed at the Animal Health Laboratory of the University of Guelph using immunochemistry [38].

Bone Quantitative Computed Tomography

Prior to analysis, the right femur from each bird at 18, 25, 41, and 69 woa was fixed in 10% phosphate buffered formalin [39] for 2 wk following the protocol modified from Silversides et al. [40] and Ariyamuni [41]. Bone quality was determined by quantitative computed tomography (**QCT**) according to the protocol developed at the University of Alberta and previously described by Korver et al. [42, 43]. The bone mineral density and cross-sectional area of the total, cortical, and trabecular space (containing both trabecular and medullary bone; [44]) were determined at the mid-point of each femur. Bone mineral content was calculated as the amount of bone mineral contained within a 1 mm-thick longitudinal section of the femur at the scan location [44].

Statistical Analysis

Pairwise comparisons of BW, organ weight, bone mineralization parameter, as well as plasma E_2 and calcium concentration means were analyzed using the LSMEANS statement of the MIXED procedure of SAS [45], where

significance was considered when $P \leq 0.05$. Tukey's post hoc test was used to compare treatment means. Fixed effects included age, treatment, story-level (during rearing period), and an interaction between age and treatment, while the assigned section of the barn was the random effect. Furthermore, Pearson product-moment correlation coefficients were determined for BW, ovary weight, liver weight, E_2 concentration, trabecular and cortical density, as well as area and mineral contents. Egg data collected throughout this study could not be statistically analyzed due to egg production of each treatment corresponded to the average from the entire section of the barn and are reported as means only.

RESULTS AND DISCUSSION

Effect of Spectrum Lighting on Body Weight

Body weight was used as an indicator of growth for the purposes of this trial. During the rearing phase, BW was dependent on age ($P < 0.001$) and story level ($P < 0.001$). As shown in Figure 2A, pullets from the bottom story were significantly heavier than pullets from the top story at 14 (1.34 kg $\pm$ 0.01 vs. 1.29 kg $\pm$ 0.01) and 18 woa (1.58 kg $\pm$ 0.01 vs. 1.51 kg $\pm$ 0.01). Further review showed that temperature in the top story was on average 2°C warmer than the bottom at both 14 and 18 woa. Birds typically react to higher ambient temperature by reducing their feed intake, thereby decreasing heat production which in turn results in decreased BW gain [46]. Although feed consumption was not recorded in this trial, a temperature effect could explain the differences observed. A significant interaction between age and treatment on BW ($P < 0.001$) was observed; however, individual significant difference was observed only at 18 woa between light treatments, when pullets exposed to RL (1.57 kg $\pm$ 0.01) were heavier than GL (1.52 kg $\pm$ 0.01) (Figure 2B). Interestingly, the significant difference observed at 18 woa was at the time of transfer to the adult barn, and this may be attributed to an earlier sexual maturation of pullets reared under RL as the egg production at 19 and 20 woa of birds exposed to RL was observed to be 5 and 15% greater than birds exposed to GL, respectively. During sexual maturation, the development of the reproductive tract and ovary are associated with an increase in BW [47]. This can be supported by the positive correlation of both liver and ovary weight with BW at 18 woa (liver: $r = 0.719$; $P < 0.001$, ovary: $r = 0.647$; $P = 0.002$), indicating a possible effect of sexual maturation on BW. In retrospect, collection of oviducts from carcasses may have helped identify this earlier maturation as the weight of the oviduct is correlated with the development of the reproductive tract [47]. Overall, the lack of effect on early growth does not agree with results from studies performed on broilers in which green monochromatic lights significantly increased BW [18, 19]. However, our study agrees with a previous report showing no effect of spectrum lighting on layer pullet growth [48].

Following sexual maturity and throughout the laying period, BW was dependent on age ($P < 0.001$). Although an interaction between age and treatment ($P < 0.001$) was observed, this may have arose from 2 significant difference in BW gain observed at 36 woa (GL 2.08 kg $\pm$ 0.03 vs. RL 1.90 kg $\pm$ 0.02) and 56 woa (GL 1.84 kg $\pm$ 0.02 vs. RL 1.98 kg $\pm$ 0.02) as shown in Figure 3. One explanation for these differences may be due to random selection of individuals at each age (no repeated measures), causing variation in the data set rather than BW gain affected by pullet rearing treatment. Nonetheless, no carryover trend on BW was found between the RL and GL pullet rearing treatments.

Effect of Spectrum Lighting on Reproductive Performance

Egg Production and Egg Quality Due to the experimental design and only one replicate per pullet treatment during the laying phase, we were unable to conduct statistical analysis on egg data collected throughout the experiment. Regardless, egg production was calculated on a hen-housed weekly basis for each treatment (Figure 4). Following transfer into the adult barn, hens from the RL treatment appeared to enter lay sooner than hens from GL, with production of 10.7% vs. 5.0% at 19 woa and 48.7% compared to 33.3% at 20 woa, for the RL and GL treatments, respectively (Figure 4A). Lewis et al. [48] reported that although rearing layer

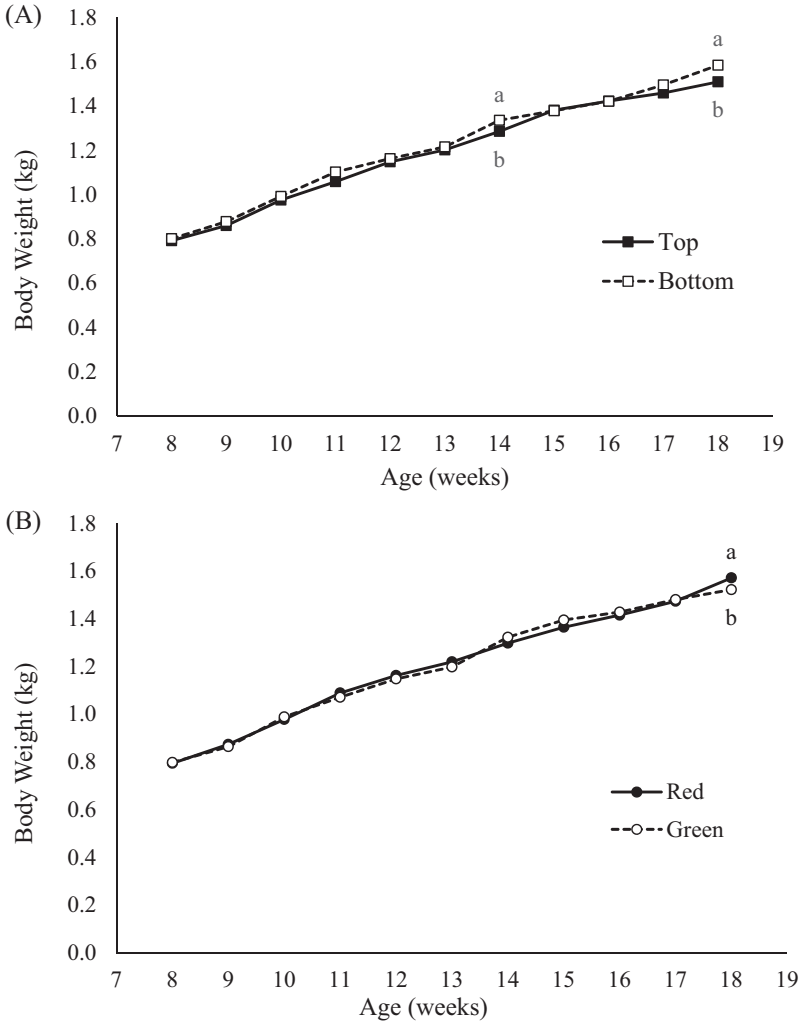

Figure 2. Effect of light spectrum treatment and the story level on body weight (mean ± SEM) during the rearing phase (8 to 18 wk of age). (A) Represents the effect of story level on body weight. (B) Represents the effect of 60% Red or 60% Green LED light on body weight. *P*-values for different sources of variation were as follows: age, $P < 0.001$; story level, $P < 0.001$; treatment, $P = 0.574$; age x treatment, $P = 0.007$; [a,b]Data points lacking the common superscript differ significantly ($P < 0.05$).

pullets under green lamps delayed maturation by 1 d compared to pullet reared under incandescent lights, no other differences in production parameters were observed. Nonetheless, it has been shown that red light advances maturation compared to green light in both layers and broiler breeders [21, 23]. However, most studies that have reported enhanced sexual maturation and subsequent reproductive performance under red light investigated the impact of lighting treatment from photostimulation rather than from the start of the rearing period [21, 23–25].

In the current study, a peak production of 92% was reached at approximately 22 woa for both treatments, with no further noticeable differences in production. By the end of the production cycle, hens originating from the RL rearing treatment laid approximately 3 more eggs per hen compared to birds from GL on a hen-housed basis (Table 1). Since all hens were maintained under the same RL in the adult barn, any impact of spectrum lighting during the pullet stage appeared to be on sexual maturation with minimal if any carry-over to the adult stage. This is in line

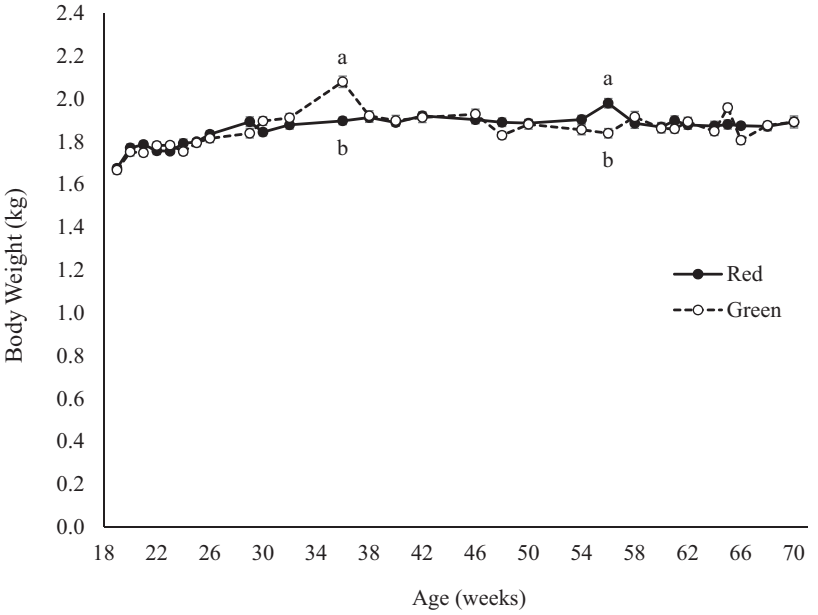

Figure 3. Effect of light treatment (60% Red or 60% Green LED light) during pullet rearing on body weight throughout the adult phase from 19 to 70 wk of age (mean ± SEM). *P*-values for different sources of variation were as follows: age, $P < 0.001$; treatment, $P = 0.555$; age x treatment, $P < 0.001$. [a,b]Data points lacking the common superscript differ significantly ($P < 0.05$).

with Lewis et al. [48] and Harrison et al. [49], who reported that no significant differences in egg production were observed between rearing light treatments (blue, green, red, or white lights) when all birds were switched to the same white incandescent light at photostimulation. Nonetheless, egg production maintained above 87% beyond 70 woa for both RL and GL treatments, which appeared to have a higher egg production rate and better laying persistency compared to published data for this strain (Table 2) [50, 51]. However, since in the adult barn all hens were subjected to the RL, no control treatment was implemented and statistical comparisons to other light source cannot be performed. Thus, although we speculate the high production observed under our experimental conditions was in part due to the RL, we cannot rule out any additional environmental factor.

The cumulative number of cracked eggs collected throughout the trial is shown in Figure 5. From 19 to 70 woa, the cumulative number of cracks was higher in birds raised under GL compared to RL, totaling 8.9 and 7.8 cracked eggs per 1,000 hens, respectively. This difference between treatment was primarily observed at 33 woa in birds exposed to GL, with weekly totals increasing from 0.15 eggs to 0.45 eggs per 1,000 hens, whereas birds exposed to RL did not display this same increase.

Relative Organ Weights and Ovarian Follicles No differences in relative liver or ovary weights were observed between treatments (Table 3). However, organ weights were dependent on age ($P < 0.001$), where after transfer to the adult barn, relative liver weights significantly increased throughout the production period up to 69 woa. As hens sexually mature, elevated levels of E_2 stimulate hepatocyte lipoprotein production in the liver from generic very low-density lipoprotein (**VLDL**) to yolk targeted VLDL (**VLDLy**) production [52]. Thus, an increase in liver size as hens age is likely the result of a continuous stimulation to synthesize egg yolk components [53–55]. Furthermore, as hens age, egg size and weight typically increase resulting from an increase in yolk yield compared to eggs produced from younger flocks [56–58]. While the liver continuously produces lipoproteins, production will drop through the end of the laying period, causing a longer interval between ovulations. This results in a

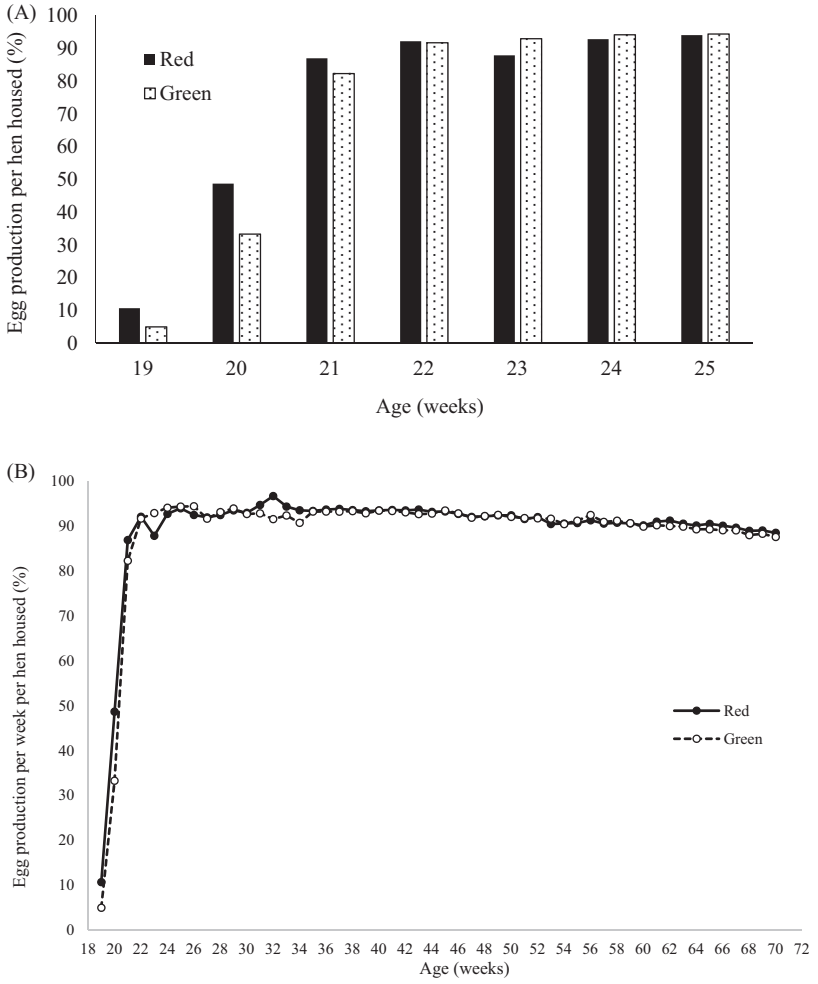

Figure 4. Effect of light treatment during pullet rearing on egg production. (A) Represents the comparison of 60% Red or 60% Green LED on egg production rate from 19 to 25 wk of age. (B) Represents the egg production of 60% Red or 60% Green LED from 19 to 70 wk of age.

Table 1. Effect of 60% Red and 60% Green LED Light Treatments During Pullet Rearing on Cumulative Egg Production at 20, 25, 45, 60, and 70 wk of Age.

Age	Treatment (number of eggs per hen housed)		
(weeks)	Red	Green	Differences
20	4.2	2.7	1.5
25	35.9	34.6	1.3
45	167.0	164.7	2.3
60	263.0	260.9	2.1
70	327.2	324.1	3.1

longer accumulation of egg yolk lipoprotein deposition, increasing egg size [49, 56, 57]. As expected, the relative ovary weight without hierarchal follicles increased as birds aged ($P < 0.001$), especially following sexual maturation, although no difference was observed between treatments. Interestingly, the largest significant increase was observed between 41 and 69 woa, well past the peak of lay. This increase in relative ovary weight was associated with a significant increase ($P < 0.001$) in the number of small follicles (1 to 4 mm), with the greatest number occurring at 69 woa (Table 4). Additionally, the number of medium (5 to 8 mm) follicles was significantly greater in hens at 25, 41, and 69 woa, compared to those at 18 woa ($P < 0.001$). Similarly, the number of large (≥9 mm) follicles were greatest in birds at 25 and 69 woa, with those at 41 woa displaying a significantly lower

Table 2. Comparison of Egg Production Rate Between Birds Reared Under 60% Red, 60% Green LED Light and Lohmann Brown From the Management Guideline and Published Data.

	Egg production rate (%)			
Treatment	20 to 30 woa	31 to 45 woa	46 to 50 woa	22 to 58 woa
60% Red	87.8	93.9	92.4	92.7
60% Green	86.8	92.9	92.3	92.6
Lohmann Brown-Lite Target	76.7	93.0	90.4	90.2
Singh et al. [48]	92.3	88.4	76.4	
Bozkurt et al. [49]				81.0

woa, weeks of age.

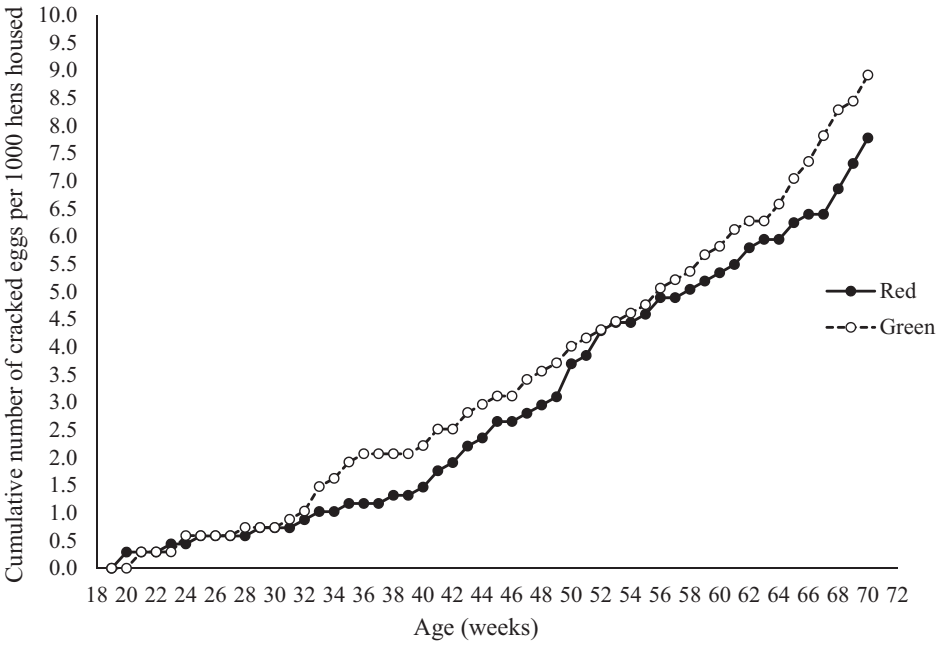

Figure 5. Effect of light treatment (60% Red or 60% Green LED light) during pullet rearing on cumulative number of cracked eggs collected from 19 to 70 wk of age per 1,000 hens housed.

subset, and 18 woa containing the lowest number of follicles within this group out of all ages ($P < 0.001$). Regardless, no difference was observed between hens from GL and RL. In avian species, FSH is known to trigger growth and maturation of pre-hierarchical follicles, especially small white follicles, and stimulate proliferation and differentiation of granulosa cells [59, 60]. It has been reported that growth of pre-hierarchal follicles (<8 mm) continues until ovulation, thus increasing ovarian weight observed in the current study is speculated to be due to an increasing number of follicles, as well as growth and maturation of pre-hierarchical follicles over time. Furthermore, it can be suggested that laying persistency, referred to here as egg production maintained above 87% at 70 woa for birds exposed to RL and GL vs. 80% for Lohmann Brown-Lite [28], was supported by the maintained ovarian activity observed at 69 woa, indicated via ovarian weight and follicular development at this time.

Estradiol No significant difference was observed in plasma levels of E_2 between birds exposed to RL and GL ($P = 0.076$; Figure 6). Unfortunately, plasma samples collected at 18 woa were lost before E_2 levels could be quantified. This age corresponded to sexual maturation and thus, the elevation in E_2 typically seen during that time [61] could not be captured. However, regardless of pullet treatment, E_2 concentration

Table 3. Effect of Age, Light Treatment (60% Red or 60% Green LED Light) During Pullet Rearing, and Age and Light Treatment Interaction on Relative Liver and Ovary Weight (Mean ± SEM).

			Liver				Ovary[3]			
			Weight		Percentage[2]		Weight		Percentage[2]	
	Age	Treatment	(g)	SEM	(%)	SEM	(g)	SEM	(%)	SEM
Age[1]	7		22.5[c]	0.4	3.35[a]	0.05	0.3[c]	0.01	0.05[c]	0.002
	11		23.6[c]	0.8	2.22[c]	0.06	0.4[c]	0.02	0.04[c]	0.002
	18		20.1[c]	0.7	1.33[d]	0.03	0.9[c]	0.09	0.06[c]	0.005
	25		39.5[b]	1.1	2.13[c]	0.05	7.1[b]	0.42	0.39[b]	0.023
	41		38.7[b]	0.7	2.10[c]	0.03	6.8[b]	0.33	0.37[b]	0.017
	69		47.2[a]	1.4	2.53[b]	0.06	11.1[a]	0.41	0.60[a]	0.023
Treatment		Red	32.0	1.4	2.27	0.08	4.6	0.57	0.26	0.03
		Green	31.9	1.5	2.28	0.08	4.4	0.56	0.24	0.03
Age	7	Red	23.0	0.6	3.33	0.07	0.3	0.02	0.04	0.003
×		Green	22.1	0.5	3.37	0.09	0.3	0.02	0.05	0.004
Treatment	11	Red	23.8	1.0	2.26	0.06	0.4	0.05	0.04	0.004
		Green	23.4	1.3	2.20	0.10	0.4	0.03	0.04	0.003
	18	Red	20.6	1.3	1.33	0.05	1.1	0.15	0.07	0.008
		Green	19.5	0.7	1.33	0.04	0.7	0.07	0.05	0.005
	25	Red	39.2	1.7	2.08	0.07	7.4	0.63	0.40	0.036
		Green	39.7	1.4	2.17	0.07	6.9	0.57	0.38	0.031
	41	Red	38.0	0.8	2.12	0.03	6.6	0.42	0.37	0.021
		Green	39.3	1.2	2.08	0.06	7.0	0.53	0.37	0.029
	69	Red	47.1	2.0	2.53	0.10	11.5	0.53	0.62	0.027
		Green	47.3	1.9	2.53	0.07	10.8	0.65	0.58	0.037
Source of variation			*P*-value							
Age			**<0.001**		**<0.001**		**<0.001**		**<0.001**	
Treatment			0.928		0.927		0.404		0.389	
Age × treatment			0.950		0.914		0.790		0.933	

[1]Age, in weeks.

[2]Percentage = organ weight/body weight × 100%.

[3]Ovary weight recorded without pre-ovulatory follicles (F1 to F6).

[a,b,c,d]LSMEANS within a column and treatment group lacking a common superscript differ significantly ($P < 0.05$).

increased ($P < 0.001$) once birds entered production (25 woa) and remained elevated throughout the reproductive cycle. It has been reported that as hens reach the end of lay, the ovary and oviduct regress, along with decreased liver weight and E_2 concentrations [54, 62]. Conversely, our results showed a continuous increase in ovary weight, liver weight, and small follicle numbers until 69 woa. Stimulating laying hens with red light has been shown to result in elevated E_2 concentration [23, 54], produced primarily from the small white follicles [5]. As E_2 concentration was maintained in the current study, we hypothesize that continuous recruitment of small follicles resulted in continuous synthesis of E_2, allowing the hens to maintain a high level of egg production until 70 woa compared to published performance for this strain [50, 51]. This is possibly supported by the observed increase in this follicular pool under RL in the adult barn. Additionally, injecting FSH to older hens that had ceased egg production increased serum E_2 concentrations, which in turn was associated with an increased number of small and large yellow follicles [63]. However, it is worth noting that intensive selection of layers for extended persistency has resulted in laying cycles lasting past 100 woa [5], most likely changing the follicular dynamics and associated hormonal profiles. For example, we recently showed that commercial laying hens with sustained laying persistency displayed another rise in E_2 at 52 woa after the initial peak at sexual maturation [64]. Furthermore, as discussed previously, the use of long wavelengths such as red light on hens has been reported to increase E_2 concentration after photostimulation, which was associated with earlier age at first egg, higher sustained peak production,

Table 4. Effect of Age, Light Treatment (60% Red or 60% Green LED Light) During Pullet Rearing, and Age and Light Treatment Interaction on Number of Small (1–4 mm), Medium (5–8 mm), and Large (≥9 mm) Follicles (Mean ± SEM).

			Follicle count (n)					
	Age	Treatment	Small (1–4 mm)	SEM	Medium (5–8 mm)	SEM	Large[2] (≥9 mm)	SEM
Age[1]	18		24.0[d]	4.6	2.6[b]	0.4	0.2[c]	0.1
	25		314.6[c]	10.6	10.4[a]	0.7	1.6[a]	0.2
	41		860.0[b]	60.4	10.6[a]	0.9	0.8[b]	0.1
	69		1031.8[a]	50.6	10.6[a]	0.6	1.3[a]	0.1
Treatment		Red	566.7	71.0	8.9	0.7	1.0	0.1
		Green	548.5	70.4	8.2	0.7	0.9	0.1
Age	18	Red	23.9	6.9	3.0	0.5	0.4	0.2
×		Green	24.1	6.4	2.3	0.6	0	0
Treatment	25	Red	330.2	15.4	10.4	1.1	1.5	0.3
		Green	298.9	13.6	10.3	1.1	1.6	0.2
	41	Red	918.5	81.7	10.8	1.4	0.8	0.1
		Green	801.5	89.4	10.3	1.1	0.7	0.2
	69	Red	994.2	86.7	11.2	1.0	1.4	0.2
		Green	1069.3	54.4	10.0	0.6	1.1	0.1
Source of variation			*P*-value					
Age			**<0.001**		**<0.001**		**<0.001**	
Treatment			0.650		0.349		0.164	
Age × treatment			0.404		0.950		0.501	

[1]Age, in weeks.

[2]Hierarchal follicles (F1 to F6) not included.

[a,b,c,d]LSMEANS within a column and treatment group lacking a common superscript differ significantly ($P < 0.05$).

and cumulative egg number [21, 23–25]. Thus, we hypothesize that in our current study, persistent egg production with sustained ovary and liver weight, follicle number, and E_2 concentration in both RL and GL rearing treatment was possibly supported by the combination of genetic selection and exposure to RL throughout the adult phase. However, as discussed earlier, no true controlled treatment was available in the current study to compare the effect of RL on the production performance in the adult barn. Thus, at this stage, exposing hens under RL in the adult barn is one possible cause for the high production level observed. Furthermore, the interaction between genetic selection and the light spectrum effect has yet to be demonstrated and further investigation is required.

Bone Characteristics There was no effect of pullet lighting treatment on bone density, cross-sectional area, or mineral content of the femur total content, trabecular space, and cortical tissues (Table 5). However, each bone characteristic was dependent on age ($P < 0.05$), except for total bone cross-sectional area. Bone density and mineral content were highest at 69 woa compared to all other ages. Furthermore, these parameters were also significantly higher at 41 woa compared to 18 woa, regardless of light treatment. An increase in mineral content as hens aged was previously reported, although bones became brittle due to changes in collagen matrix rather than bone loss [16, 65, 66]. As hens age, the amount of medullary bone increases, even as structural bone decreases [42, 67]. However, a significant decrease in cortical bone cross-sectional area and mineral content was not observed in our study. Other studies have also shown that the total mineral content of the femur, measured as femur ash, is highest at the initiation of lay and lowest at the end [68]. Meanwhile, trabecular space bone density and mineral content significantly increased with age ($P < 0.05$), likely as a result of increased medullary bone tissue, therefore contributing to the greatest density observed at 69 woa. However, the trabecular space cross-sectional area was greatest at 18 woa. Changes in the trabecular space bone measures are reflective of the increase in medullary bone and decrease

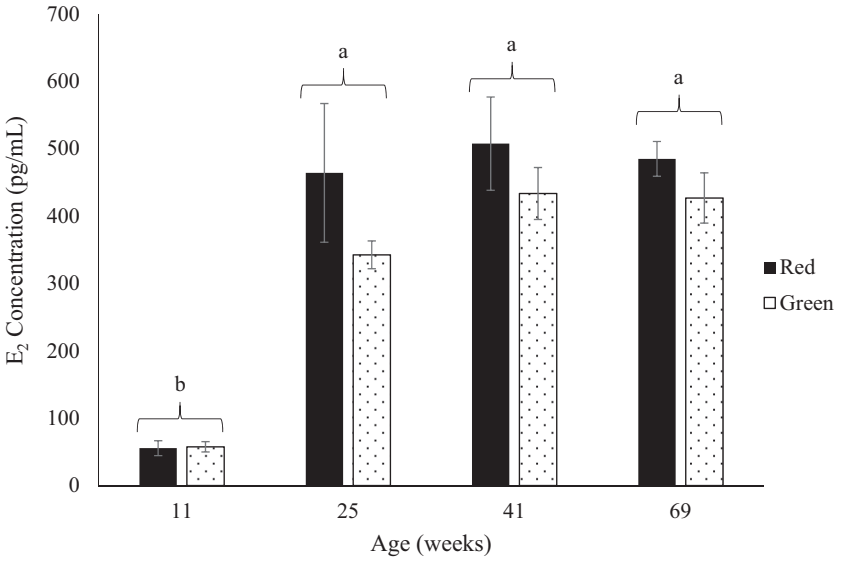

Figure 6. Effect of light treatment (60% Red or 60% Green LED light) during pullet rearing on estradiol (E_2) concentration (mean ± SEM). *P*-values for different sources of variation were as follows: age, $P < 0.001$; treatment, $P = 0.076$; age x treatment, $P = 0.661$. [a,b]Data points lacking the common superscript differ significantly between ages ($P < 0.05$).

in trabecular bone with age [42]. Therefore, the increase in trabecular space bone mineral content with age is likely due primarily to the increased medullary bone reserves masking losses in trabecular bone with age in the laying hen. Our results are in line with other studies [67, 69]. The positive correlations shown in Table 6 between the trabecular density and mineral content and ovary weight, liver weight, as well as E_2 concentration, suggest that the sampled hens were in production at the time of euthanasia, with the medullary bone content increasing throughout the cycle to maintain egg production [42, 67]. Furthermore, cortical bone density at 18 woa was significantly greater than at 25 woa ($P < 0.001$), and both cortical area and mineral content were greatest at 69 woa ($P < 0.05$). The deposition of cortical bone ceases when E_2 concentration rises, during the period of reproductive activation and sexual maturation, in favor of medullary bone reserves for eggshell formation [5, 8, 17]. This translates into increased cross-sectional area of bone in the trabecular space. Additionally, the hydroxylation ability of the liver and kidney to convert dietary vitamin D_3 into its active form progressively deteriorates as hens get older, and hence production of $1,25(OH)_2D_3$ decreases [70]. Thus, older hens are capable of producing sufficient amount of $1,25(OH)_2D_3$ to maintain egg production and shell quality, but not for tibia strength and weight, leading to endocortical thinning, bone fragility, and susceptibility to fracture [16, 69, 70]. According to Burnbrae Farms' standard management program, all hens were supplemented with additional calcium in the diet at the later stages of lay (65 woa) [33] to support eggshell quality and bird health, which could have minimized the loss of bone mineral.

Calcium No treatment effect nor interaction between age and treatment was observed. More specifically, calcium concentration at 69 woa was higher than 11 and 41 woa ($P < 0.001$; Figure 7). Periods of eggshell formation can be detected through elevated plasma calcium levels, as this mineral is transported through the blood [71]. As expected, plasma calcium at 11 woa was relatively low, since pullets had not yet initiated the laying cycle. However, the declined levels observed at 41 woa, which while inconsistent with previous studies, corresponded with the slight elevation in the number of cracked eggs around 40 woa, potentially explaining the decline in shell quality at this time. E_2 is responsible

Table 5. Effect of Age, Light Treatment (60% Red or 60% Green LED Light) During Pullet Rearing, and Age and Light Treatment Interaction on Femur Bone Parameters Determined by Bone Quantitative Computed Tomography (Mean ± SEM).

			Age (weeks)								
			18	SEM	25	SEM	41	SEM	69	SEM	*P*-value
Total bone density (mg/cm^3)	Age		328.9^{c}	8.7	376.3^{b,c}	24.5	423.9^{b}	17.5	491.4^{a}	11.8	**<0.001**
	Age × treatment	Red	338.1	15.1	429.7	40.2	408.7	12.9	503.4	15.8	0.853
		Green	319.7	8.7	322.9	19.0	439.1	33.9	479.5	18.3	
Total bone area (mm^2)	Age		59.8	0.9	61.2	1.6	60.9	1.1	63.2	1.1	0.672
	Age × treatment	Red	59.1	1.6	59.9	2.5	60.2	0.8	62.5	1.7	0.996
		Green	60.5	0.9	62.6	2.1	61.6	2.1	64.0	1.6	
Total bone mineral content[1] (mg/mm)	Age		19.6^{c}	0.5	22.5^{b,c}	0.9	25.8^{b}	1.1	31.0^{a}	0.7	**<0.001**
	Age × treatment	Red	19.9	0.9	25.0	1.1	24.6	0.6	31.4	0.9	0.560
		Green	19.3	0.5	20.0	0.9	27.0	2.1	30.6	1.2	
Trabecular space[2] density (mg/cm^3)	Age		27.1^{d}	7.1	75.1^{c}	7.9	155.7^{b}	15.3	216.9^{a}	11.8	**0.030**
	Age × treatment	Red	23.6	10.5	101.3	10.1	141.8	8.9	237.1	15.3	0.988
		Green	30.7	9.9	48.9	3.0	169.6	30.3	196.6	17.1	
Trabecular space area (mm^2)	Age		41.4^{a}	0.9	39.9^{a,b}	2.4	39.1^{a,b}	1.3	38.6^{b}	1.3	**0.003**
	Age × Treatment	Red	40.2	1.5	35.9	4.0	40.8	1.7	39.0	1.9	0.531
		Green	42.5	0.8	44.0	2.4	37.4	1.9	38.3	1.9	
Trabecular space mineral content (mg/cm^3)	Age		1.1^{d}	0.3	2.9^{c}	0.3	5.9^{b}	0.5	8.2^{a}	0.4	**0.001**
	Age × treatment	Red	0.9	0.4	3.6	0.5	5.8	0.4	9.1	0.3	0.788
		Green	1.3	0.4	2.1	0.1	6.1	1.0	7.4	0.6	
Cortical density (mg/cm^3)	Age		1026.4^{a}	0.4	971.7^{b}	0.9	989.1^{a,b}	0.9	1014.8^{a,b}	0.5	**<0.001**
	Age × treatment	Red	1034.8	8.5	955.0	13.7	1027.7	3.1	1015.2	8.8	0.859
		Green	1018.0	7.5	988.3	11.9	950.5	20.7	1014.4	18.9	
Cortical area (mm^2)	Age		18.3^{b}	0.4	19.7^{b}	0.9	19.3^{b}	0.9	21.2^{a}	0.5	**<0.001**
	Age × treatment	Red	18.8	0.6	21.4	1.6	17.8	0.5	21.1	0.3	0.708
		Green	17.9	0.4	18.0	0.8	20.8	1.6	21.2	1.0	
Cortical mineral content (mg/cm^3)	Age		18.8^{b}	0.4	19.1^{b}	0.8	18.9^{b}	0.6	21.4^{a}	0.4	**<0.001**
	Age × treatment	Red	19.4	0.6	20.3	1.5	18.3	0.5	21.5	0.4	0.777
		Green	18.2	0.3	17.8	0.8	19.6	1.2	21.4	0.7	

[1]Calculated by multiplying the respective bone cross-sectional area by bone mineral density to yield the amount of mineral (mg) contained in 1-mm-thick x-ray scan.

[2]Included all bone contained within the cortical shell, and included both trabecular and medullary bone.

P-values for treatment source of variation: total bone density, $P = 0.247$; total bone area, $P = 0.142$; total bone mineral content, $P = 0.667$; trabecular space density, $P = 0.438$; trabecular space area, $P = 0.351$; trabecular space mineral content, $P = 0.471$; cortical density, $P = 0.457$; cortical area, $P = 0.949$; cortical mineral content, $P = 0.753$.

[a,b,c]LSMEANS within a row and age/treatment group lacking a common superscript differ significantly ($P < 0.05$).

Table 6. Correlation Coefficients Between Organ Weight, Estradiol Concentration, and Bone Parameters of Lohmann Brown-Lite Hens.

	Liver	Estradiol	Trabecular space[1] density	Trabecular space area	Trabecular mineral content	Cortical density	Cortical area	Cortical mineral density
Ovary weight[4]	0.82[2]	−0.02	0.70	−0.25	0.66	−0.18	0.29	0.29
	<0.001[3]	0.91	**<0.001**	**0.03**	**<0.001**	0.12	**0.01**	**0.01**
Liver weight[3]		0.01	0.69	−0.13	0.67	−0.22	0.20	0.18
		0.97	**<0.001**	0.23	**<0.001**	**0.05**	0.07	0.11
Estradiol			0.25	0.004	0.27	−0.03	0.20	0.18
			0.05	0.97	**0.04**	0.80	0.07	0.11

[1]Included all bone contained within the cortical shell, and included both trabecular and medullary bone.

[2]Pearson product-moment correlation coefficients.

[3]Probability (*P*-value).

[4]Relative to body weight, organ weight/body weight × 100%.

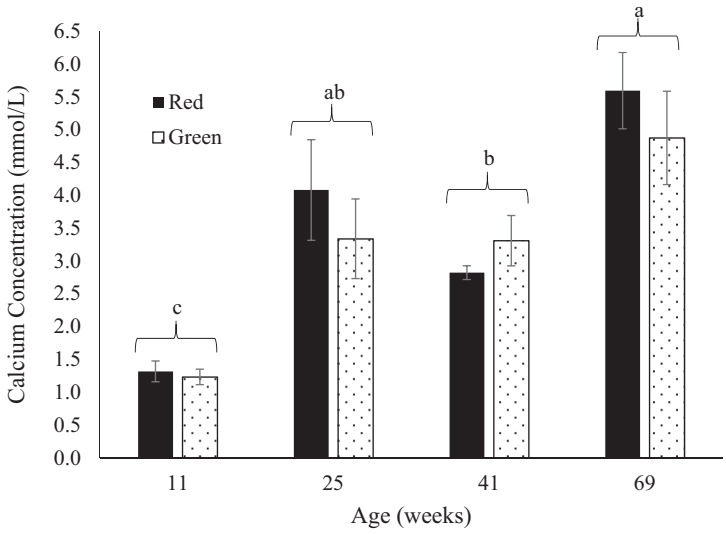

Figure 7. Effect of light treatment (60% Red or 60% Green LED light) during pullet rearing on calcium concentration (mean ± SEM). *P*-values for different sources of variation were as follows: age, $P < 0.001$; treatment, $P = 0.482$; age x treatment, $P = 0.496$. [a–c]Data points lacking the common superscript differ significantly between ages ($P < 0.05$).

for stimulating medullary bone formation and vitamin D_3 activity, increasing blood calcium levels for eggshell formation [5, 8, 16, 17]. Maintained elevations of E_2 may have supported laying persistency in this flock; however, recommended calcium and vitamin D supplementation according to management guidelines may not have been provided early enough to fully support production performance, indicated by the rise in number of cracked eggs and decrease in plasma calcium around 40 woa. Furthermore, mortality (Figure 8) appeared to rise around 60 woa. While we were unable to provide a definitive answer, we speculate that if the cause of mortality was associated with increased production performance, identifying the timing of E_2 elevation may help determine better management practices. Providing the appropriate amount of calcium and vitamin D supplementation to further support egg production, egg quality and bird health will help optimize the production performance. Additionally, the rise in calcium level at 69 woa was in line with the additional calcium and vitamin D supplementation provided according to the Burnbrae Farms' nutritional program [33].

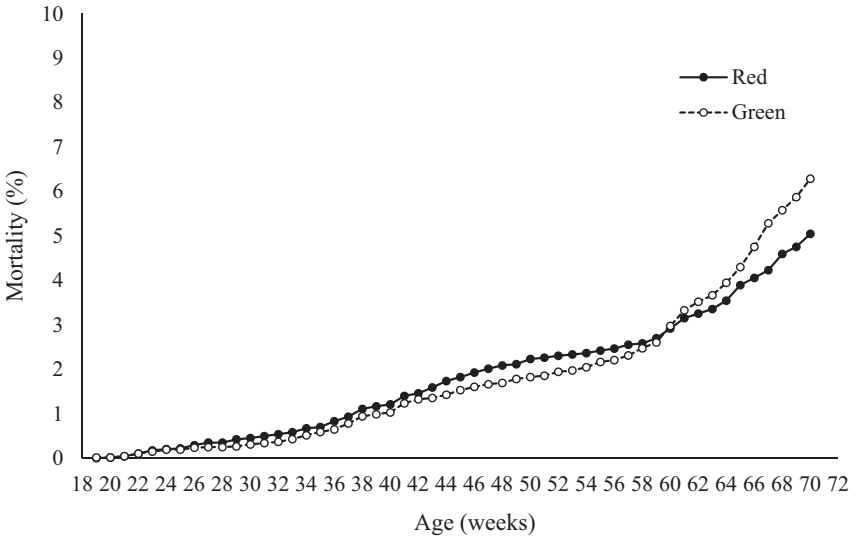

Figure 8. Effect of light treatment (60% Red or 60% Green LED light) during pullet rearing on mortality rate during the adult phase from 19 to 70 wk of age.

CONCLUSIONS AND APPLICATIONS

1. Spectrum lighting during the rearing period did not affect growth in layer pullets; however, rearing pullets under RL possibly accelerated sexual maturation as observed from higher egg production rate and heavier BW at initiation of lay.
2. No carry-over effect of LED light treatments was observed from rearing to the active laying stage on the production performance of hens.
3. The higher than expected production performance observed during the mid-lay cycle may require an advancement in the timing of calcium and vitamin D supplementation to synchronize calcium with E_2 profiles and reduce the occurrence of cracked eggs and possible calcium deficiency.

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34. Ethanol, Commercial Alcohols, Brampton, ON, Canada. Diluted with distilled water.

35. At 11, 25, 41 and 69 woa, approximately 2 ml of blood was collected from each bird, by venipuncture of the branchial vein, and placed in a sodium heparin blood vacutainer. Blood samples were centrifuged at 1,800 x *g* for 15 minutes at 4°C to recover blood plasma, and stored at -20°C until hormone extraction and enzyme immunoassay.

36. Plasma samples were diluted with cold ethanol at 5:1 (ethanol: plasma) ratio, vortexed for 2 minutes, centrifuged at 900 x *g* for 5 minutes at 20°C, and then placed in the -80°C freezer for 5 minutes. Once the organic phase recovered, the supernatant for each sample was decanted carefully into smaller glass tubes, and dried in hot bath at 35°C under an air flow. The samples were then reconstituted in half the original volume with Trizma assay buffer (20 m*M* Trizma, 0.3 *M*NaCl, 0.1% BSA; pH 7.5), and stored at -20°C until assayed.

37. Arbor Assays Interactive Assay Solutions, Detect X 17β-Estradiol Enzyme Immunoassay kit, K030-H1. Ann Arbor, MI, USA. The inter-assay coefficient of variation was 15.5%.

38. Immunochemistry analyzer, Cobas c311. Roche, Hitachi, USA.

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