Iranian Society of Animal Science

Journal of Livestock Science and Technologies

Shahid Bahonar University of Kerman

ISSN: 2322-3553 (Print) ISSN: 2322-374X (Online)

Paper type: Original Research

Influence of LED light spectra and photoperiods on broiler breeders production, reproduction, blood metabolites, immunological response, and hepatic sterol regulatory element binding protein-1 (*SREBP1*) relative gene expression

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Received: 10 Feb 2023,
Accepted: 25 Mar 2023,
Published online: 04 May 2023,

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Abstract Lighting is one of the important management factors affecting the health and welfare of birds. The objective of this study was to assess the effects of light-emitting diodes (LEDs) on production, reproduction, blood metabolites, immunology, and hepatic sterol regulatory element binding protein-1 (*SREBP1*) gene expression in broiler breeders. One-day-old Ross 308 female breeder chicks (n=100) were randomly assigned to 20 light-proof pens and reared under five lighting regimens, including green (GL) or white (WL) LED at two exposure photophases (8 or 12 hours) as a 2 × 2 factorial experiment plus a control group (incandescent lamp at 8L:16D duration; CON). The LED color affected the feed intake during the rearing period, being higher in white than in green light; however, green light resulted in more medium and large white follicles on the ovaries, higher mean corpuscular hemoglobin concentration, and plasma cholesterol level. In WL8 lighting, the relative weight of the bursa of Fabricius was higher (P≤0.05) than in CON birds. Plasma cholesterol level in GL12 was higher compared to CON. Conventional and WL12 regimens resulted in lower plasma alkaline phosphatase activity. Twelve hours of GL lighting resulted in higher eggshell weight and strength while it caused a small but significant decrease in egg shape index. Greater numbers of medium white (MWF) and large white (LWF) follicles were recorded in GL12 birds, and there were more small white follicles (SWF) in GL8 group (P≤0.05). The GL12 and CON lighting resulted in higher concentrations of WBC and Hb compared with WL. Hepatic expression of *SREBP1* was higher in WL8 birds. The results indicated that green LED improved egg quality while white LED caused higher relative weight of bursa and hepatic *SREBP1* expression, indicating the LED light may be beneficial to broiler breeder productivity. However, more studies are required to substantiate these findings.

Keywords: LED light; egg quality; endocrinology; follicles; broiler breeder

Introduction

Lighting system greatly impacts on the avian physiological responses, behavior, growth, development, production, and immunity (Coelho et al., 2021). The light-emitting diodes (LEDs) are gradually replacing the conventional incandescent (ICD) and fluorescent lights in poultry houses (Yang

et al., 2016) due to their high energy efficiency, long working life, availability in different peak wavelengths, low electricity consumption, and low rearing cost (Hassan et al., 2013). Although several experiments investigated the effects of LED light spectrum on broiler chickens, studies on the effects of LED on breeders are very limited (Oso et al., 2022).

Fowls possess 4 types of cone cells (Prescott and Wathes, 1999), making them tetrachromatic; therefore, they can detect red, green, blue and also near-UV (380 to 740nm). Parvin et al. (2014) found that in turkeys, constant light resulted in leg disorders and metabolic diseases. Some studies have shown that LED light color may influence performance parameters, including improved egg quality in commercial layers reared under green LED, better broiler finisher performance under blue LED (Er et al., 2007) as well as under stress conditions (Franco et al., 2022). It is known that light information can be converted into biological signals, affecting the neuroendocrine system (Wu et al., 2022). It has been demonstrated that ducks reared under longer light wavelengths were more active and exhibited more frequent feeding and drinking behavior compared to those reared under short wavelengths such as green and blue (Hassan et al., 2013) which may motivate body weight gain as observed in previous studies, while turkeys (Lewis and Morris, 1998) and broilers (Rozenboim et al., 2004) had faster growth when exposed to short-wavelength. These findings indicate differences in the spectral sensitivity between poultry species. Hassan et al. (2013) stated that longer wavelength light penetrates the skin and skull of chickens and stimulates the pineal and pituitary glands, which control the secretion of reproductive hormones like follicle stimulating hormone (FSH), luteinizing hormone (LH), and 17β-estradiol (E2); these hormones stimulate the growth and number of ovarian follicles. A greater understanding of these effects could motivate the industry in taking advantage of light characteristics to improve flock production efficiency (Wei et al., 2020).

The avian pineal gland is a photo-endocrine organ and plays a pivotal role in transducing the light information to physiological processes (Haldar and Bishnupuri, 2001). Environmental factors could change the pineal morphology, for example, great variations in the pinealocyte architecture were observed between nocturnal and diurnal birds (Haldar and Bishnupuri 2001). Bulb spectral output greatly impacts on several stress parameters, such as plasma corticosterone concentration (Huth and Archer, 2015), heterophil/ lymphocyte ratios (Onbaşılar et al., 2009), and immune responses (Xie et al., 2008). It was also reported that green incubation light reduced severe feather pecking and aggressive pecking in brown layers (Özkan et al., 2022). The aim of this study was to determine the effects of two LED light spectra and two lighting duration on growth performance, blood parameters, production characteristics, organ weights, reproductive and immunological attributes, and hepatic sterol regulatory binding protein-1 *(SREBP1)* gene expression in broiler breeders. We were also interested to determine if the response of breeders to these light regimens differed from the birds raised in ICD light commonly practiced in broiler breeder farms.

Materials and methods

Experimental design

One hundred day-old female broiler breeder chicks were placed in 20 light-proof pens, covered with wood shaving, and reared under five lighting regimens, including green (GL) or white (WL) LED at two exposure photophases (8 or 12 hours) as a 2 × 2 factorial experiment plus a control group (ICD lamp at 8L:16D duration; CON), providing 1.3 m^2 of space from 0 to 30 weeks of age (woa), each with feeder and waterer but different light sources. Vaccination was carried out during the rearing period according to the epidemiology of the farm area. The birds were managed according to the guidelines of the Ross Parent Stock Management Handbook (Aviagen 2019). Diets were provided and offered according to the Ross 308 Nutrition Specifications and Performance Objectives (Aviagen, 2021). Experimental procedures were in strict accordance with the recommendations of the Institutional Animal Ethics Committee (IAEC) guidelines for animal care and use. Feed and drinker spaces were sufficient in all treatments. Light bulbs were hung from the ceiling at a height of 120 cm from the ground. Light intensity was measured with a Digital Light Meter (MS6610, Mastech) and kept at the same lux level using three digital dimmers. Fifteen lux was also supplied during the rearing period, as recommended by Ross 308 guidelines. During the first two days of age, 23 h light (80 lux) was provided for adaptation to the new environment; this was gradually decreased to 8 and 12 h within 8 days and kept at 15 lux until 20 weeks of age. The light intensity was increased from 15 lux to 30 lux after 20 woa. The exposure time also increased from 8 to 11 h at 21woa, 12 h at 22 woa and 13 h at 23 woa to the end of the experiment at 30 woa (Figure 1). Prior to commencing the trial, the light spectra were assessed (Figure 2), using Mavospec Base spectral light meter (Gossen Foto- und Lichtmesstechnik GmbH, Nürnberg, Germany).

Feed intake, growth and production parameters

Body weight (BW) was measured weekly for determination of the changes in growth (Figure 3). Feed intake (FI) was recorded daily and feed conversion ratio (FCR) was calculated by dividing FI by BW. The incidence of mortality was also recorded daily and FCR was corrected for mortality. Eggs were collected four times a day at 8:00 a.m., 10:00 a.m. 2:00 p.m. and 4:00 p.m. Egg production, egg weight and feed consumption were recorded daily until 30 woa. Accordingly, egg mass (percentage of egg production × egg weight/100) and fe-

ed conversion (g of feed/g of egg mass) were calculated. Egg length (L), and width (W) were measured with a digital vernier caliper to the nearest 0.01 mm, and shell thickness with a vernier micrometer to the nearest 0.001 mm. Egg shape index (ESI) was calculated as: (egg width / egg length) × 100 (Biggins et al., 2018). Egg shells were washed with clean water and dried at room temperature for 48 h. Egg shell weight was measured using a digital scale (Model MH-999, Ming Heng Electronics, China; precision ± 0.01 gram). In order to calculate the percent shell weight, shell weight was divided by egg weight and multiplied by 100 (Mendes et al., 2013). Eggshell breaking strength was measured using an eggshell force gauge (Model-II, Robotmation Co. Ltd., Tokyo, Japan). Albumen height was measured using a standard tripod micrometer (Baxlo Precision, Barcelona, Spain). Haugh unit (HU) score for albumen quality was calculated according to Doyon et al. (1986) as:

$$\text{Haugh unit} = 100 \times \log_{10}(H - 1.7W^{0.37} + 7.6)$$

[H = albumen height in millimeters, W = egg weight in grams]

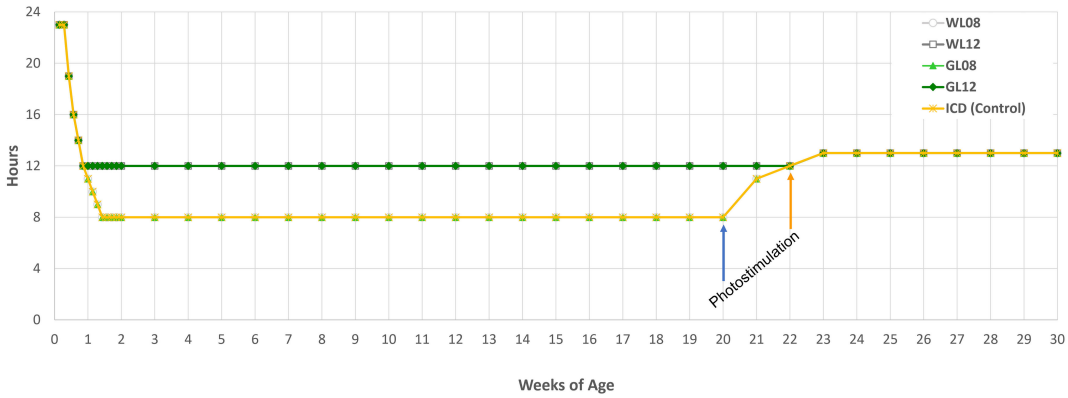
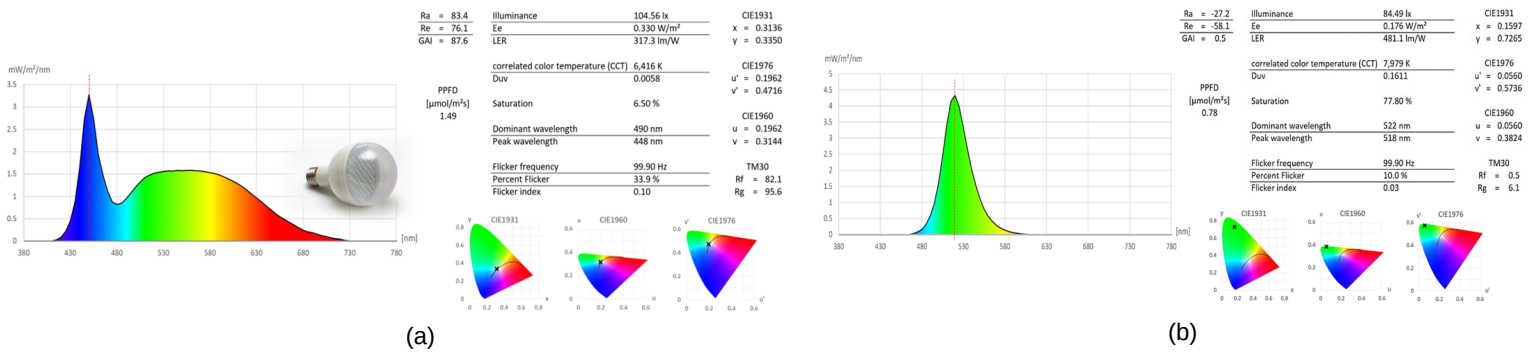
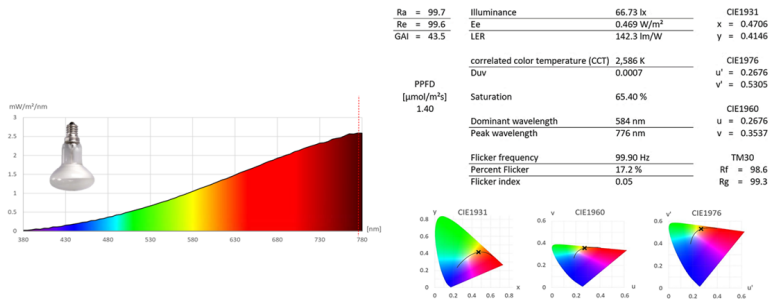


Figure 1. The lighting program during the rearing period of Ross 308 broiler breeders: White (WL) and green lights (GL) were applied for 8 and 12 hours (WL8, WL12, GL8, GL12) vs. ICD (incandescent light).



(a) (b)



(c)

Figure 2. Spectral characteristics of white light (WL) emitting diode (a), green light (GL) emitting diode (b), and incandescent (ICD) lamp (c).

Ovarian weight, follicle classification and plasma reproductive hormones

At 20 woa, within each treatment group, 2 mL blood samples were drawn from the brachial vein of 8 birds into K_3EDTA vacuum tubes between 1 to 3 h into the photoperiod. Blood samples were centrifuged at 3600 × *g* for 10 min and plasma samples were stored at –20°C until further analysis. Concomitant with blood sampling, two birds from each pen were selected randomly and slaughtered. The ovary was weighed and the number and size (diameter) of the follicles were measured using a ×5.5 magnifying loupe. The ovarian follicles were classified as: 1) large yellow follicle (LYF): > 10 mm; 2) small yellow follicle (SYF): 5 to 10 mm; 3) large white follicle (LWF): 3 to 5 mm; 4) medium white follicle (MWF): 1 to 3 mm; 5) small white follicle (SWF): < 1 mm (Renema et al., 1999). Plasma concentrations of LH and FSH were measured using Enzyme-Linked Fluorescent Assay (ELFA) kits for chickens (Shanghai Crystal day Biotech Co., Shanghai, China), according to the manufacturer's instruction. Assays were performed in 96-well plates, and absorbance was measured at 450 nm. Plasma estradiol concentration was measured using a commercial electrochemiluminescence immunoassay (ECLIA) kit (Systems Elecsys e 411; Roche, Mannheim, Germany). The inter-assay coefficients of variation for LH, FSH and estradiol were < 10%; the intra-assay coefficient variation for LH, FSH and estradiol were < 8, < 8 and < 1.8%, respectively. The analytical sensitivities of the assays were 0.3 mIU/mL, 0.1 mIU/mL and 5.0 pg/mL, for LH, FSH and estradiol, respectively.

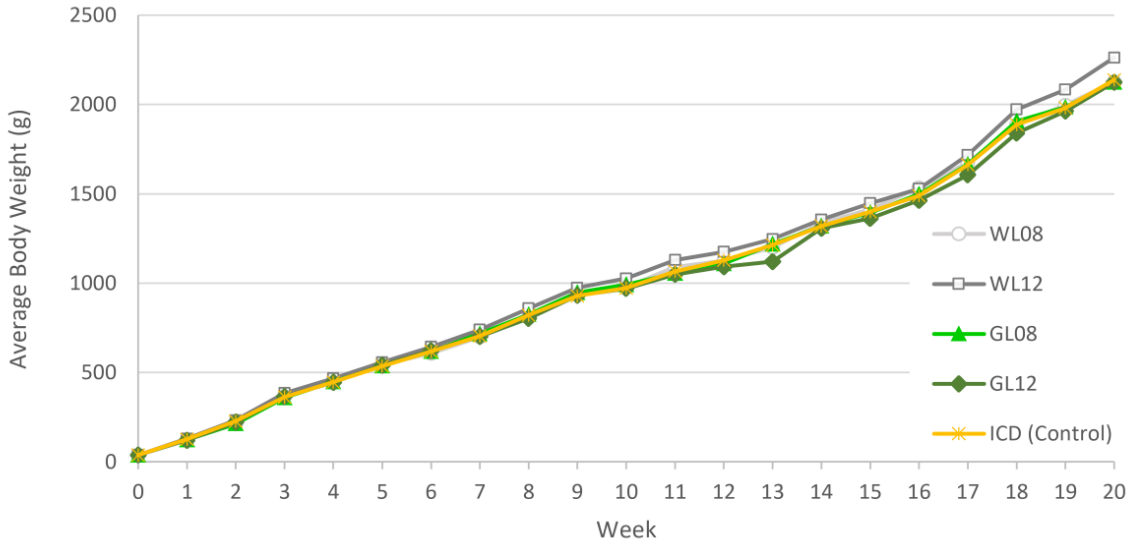


Figure 3. Effect of light spectrum and duration during the rearing period on body weight change in Ross 308 broiler breeders.

Blood cell count and hemoglobin content

At 20 woa, about 2 mL blood specimens were collected in K_3EDTA vacuum tubes from two birds in each pen. Each sample was coded and prepared for manual microscopic and automated count. For the relative microscopic differential white blood cell (leukocyte) count (WBC), blood smears were prepared and stained with Giemsa Wright's stain. One hundred cells were counted, including heterophils, lymphocytes, monocytes, basophils, and eosinophils. The WBC and red blood cell count (RBC) were determined by hemocytometer. Hemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) content, and mean corpuscular hemoglobin concentration (MCHC) were analyzed by an automated veterinary hematology analyzer (Nihon Kohden, Celltac α, MEK 6450k, Tokyo, Japan) and the results confirmed by standard manual technique using microhematocrit capillary tubes (Hillgartner et al., 1995).

Other blood parameters

At 20 woa, about 2 mL of the brachial vein from two birds in each pen were drawn into clot activator gel-containing collection tubes (Vacutest® Kima srl, Arzergrande, Italy). Serum samples were prepared after centrifugation at 3600 × g for 10 min, transferred to 1.5-mL microtubes, and stored at -20°C for subsequent analysis. Plasma concentrations of glucose (Glu), total protein (TP), triglycerides (TG), cholesterol (Chol), calcium (Ca), phosphorus (P) and plasmatic activity of alkaline phosphatase (EC 3.1.3.1; ALP), alanine transaminase (EC 2.6.1.2; ALT) and aspartate transaminase (EC 2.6.1.1; AST) were measured by Roche Cobas e 411 Chemistry Autoanalyzer system (Systems Cobas e 411;

Roche-Hitachi Diagnostics) using commercial electrochemiluminescence immunoassay (ECLIA) diagnostic kits (Roche Diagnostics, Mannheim Germany). Intra-assay and inter-assay coefficients of variation for all tests were less than 4.8% and 7.1%, respectively, and assay sensitivities were as following: Glu: 0.011 mmol/L, TP: 0.04 g/L, TG: 0.01 mmol/L, Chol: 0.01 mmol/L, Ca: 0.02 mmol/L, P: 0.01 mmol/L, ALP: 0.5 U/L, AST: 0.5 U/L, ALT: 0.05 U/L, T4: 0.51 nmol/L and T3: 0.3 nmol/L. Control serum (Randox Laboratories Ltd., Ardmore, UK) was used for controlling the measurement accuracy.

Weight of internal organs

At 20 woa, two birds from each pen were randomly selected from each treatment and slaughtered using a sharp knife. The relative weights of crop, proventriculus, gizzard, duodenum, jejunum, ileum, cecum, liver, pancreas, spleen, the bursa of Fabricius, as well as the length of small intestine were recorded.

Immunological attributes

Humoral immunity assessment- SRBC test

Evaluation of antibody titer against sheep red blood cell (SRBC) is considered as a practical, easy, and cost-effective way to measure and estimate the overall humoral immune response which can be easily performed on a large number of samples. A 7% red blood cell (RBC) suspension in sterile phosphate-buffered saline (PBS) was prepared using freshly harvested aseptically collected defibrinated sheep blood (Razi Vaccine and Serum Research Institute, Iran). Two birds from each pen were selected and 1 mL of 7% suspension was injected into the breast muscle. Seven days after injection, blood was drawn from the wing vein under the armpit from broilers at 9 and 19 weeks of the breeding period. Serum samples were incubated at 56°C for 30 min to neutralize the complement system and prevent it from interfering with the anti-SRBC antibody. The primary and secondary titrations were then evaluated as described by Qureshi and Havenstein (1994). Fifty µL of PBS solution were transferred to the wells of the first row of a micro-titer plate containing the bird serum, and 50 µL of 0.01 M 2-mercaptoethanol (2-ME) were added to the wells of the first row (containing bird serum) of the second plate. The plates were placed in an incubator for 30 minutes at 37°C. Then, 50 µL of PBS solution were added to each well in the second to the last row (88 remaining wells) of both plates. By adding 2-ME, IgM is eliminated and the observed titer only indicates the amount of IgG. The IgM titer was calculated from the difference between IgG titer and total Ig titer. The complete dilution of agglutination was recorded and Ig concentration was calculated based on $\log_2$ of the highest dilution of serum causing complete inhibition of the antigen (Cheema et al., 2003).

Cellular immunity assessment- cutaneous basophil hypersensitivity (CBH) response

At 14 weeks of the breeding period, two birds from each pen were randomly selected and marked with different colors. The third right toe of each bird was measured before injection and then 0.1 mL of 1 mg/mL phytohemagglutinin P (PHA-P) solution (Bahar-Afshan, Tehran, Iran) was dermally injected between the 3rd and 4th digits of the right foot. The left foot served as control and was injected with 100 µL of sterile PBS. The thickness of the interdigital membrane was measured at 12 and 24 h post-injection using a pressure sensitive micrometer. The proliferation of T cells was measured (Aslam et al., 1998) as: [(thickness of right toe web post-injection − thickness of right toe web pre-injection) − (thickness of left toe web post-injection − thickness of left toe web pre-injection)].

Titer against Newcastle disease virus (NDV) and avian influenza (AI)

One week after vaccination against NDV and AI at 33 days of age, two birds were randomly selected from each pen and blood samples were obtained from the brachial vein in vacutainers without anticoagulant. After clotting, blood samples were centrifuged at 1200 ×*g* for 15 min at 4°C, and sera were frozen at -20°C for further analysis. The hemagglutination inhibition (HI) test against NDV, and AI-H_9N_2 were performed. The hemagglutination (HA) units for both standard viral antigens (NDV [B1 Type, LaSota Strain] and AI [H9N2], Pasouflu and Pasoucastle, Pasouk Knowledge-based Company, Iran) were 8 $\log_2$. To detect the HI titers for NDV and AI (H_9N_2), 0.025 mL serum sample was serially diluted double-fold in phosphate buffer saline (PBS) across a plastic U-bottomed microliter plate. Four hemagglutinin units (HAU) of virus/ antigen were added in the same quantity to each well, incubated for 60 min at 4°C in a refrigerator, and 0.025 mL of 1.0% (v/v) chicken red blood cells (RBCs) was added to each well. After being gently mixed, the RBCs were allowed to settle to a distinct button for approximately 30 min at room temperature. Positive and negative control sera were run in 2 rows. The HI titer was the highest dilution of serum causing complete inhibition of 4 HAU of antigen. The titers of HI were regarded as positive if there was inhibition at a serum dilution of 1/16 (4 $\log_2$ when expressed as the reciprocal) or more against 4 HAU of antigen (Marquardt et al., 1985).

Hepatic sterol regulatory binding protein-1 (SREBP1) relative gene expression

At 20 woa, liver samples were prepared from two birds per treatment, frozen in liquid nitrogen and stored at −70°C for subsequent extraction of total RNA (Gilardi et al., 2014). Total RNA was isolated from the tissue samples using the Sambio™ RNA Extraction kit according to the manufacturer's protocol. The RNA

concentration was estimated by spectrophotometer using A260 and A280 measurements in a photometer (BioTek. EPOCH Microplate reader). The sequences of primers are presented in Table 1. Data were collected by recording SYBR incorporation into amplified DNA. Fluorescent data were used to derive the PCR cycle at a threshold (Ct). Analyses were performed in duplicate. Results were expressed relative to β-actin (control gene) and fold changes were expressed as $2^{-\Delta\Delta Ct}$ Method according to Livak protocol. The $2^{-\Delta\Delta Ct}$ method may be used to calculate the relative changes in gene expression determined from real-time quantitative PCR experiment (Livak and Schmittgen 2001).

Table 1. The PCR primer sequences for hepatic sterol regulatory binding protein-1 (*SREBP1*) and *β-actin* genes

Gene	Orientation	Primer sequence (5′⟶ 3′)	Annealing temperature (℃)	Amplicon size (bp)	Accession number
SREBP1	Forward Reverse	CCTCTGTGCCTTTGTCTTCCTC ACTCAGCCATGATGCTTCTTCC	56.9	128	NM_204126 XM_425238
β-actin	Forward Reverse	GTGCGTGACATCAAGGAGAAGC CCACAGGACTCCATACCCAAG	55.7	194	NM_205518

Statistical analysis

The study was designed to evaluate the effects of two LED light sources, green (GL) or white (WL), at two exposure photophases (8 or 12 hours), as a 2 × 2 factorial experiment plus a control (ICD lamp at 8L:16D duration; CON) group, arranged in a completely randomized design. The Shapiro-Wilk's and Levene's tests were performed to verify the normality and homoscedasticity of the data. The proportional and percentage data that did not show residual normality were submitted to arcsine square-root transformation prior to statistical analysis. Data on antibody titers were analyzed after logarithmic transformation (Log_{10} (x+1)). The values obtained were submitted to the analysis of variance (ANOVA), in SAS® Studio 3.8, on SAS® OnDemand for Academics release 9.04.01M6P110720 18 (accessed 15 January 2022; https://welcome.oda.sas.com/home; SAS Institute Inc., Cary, NC, USA). The mixed models (MIXED) procedure was used to analyze the data with the pens and samples (birds ID) as the random effects including the two-way interaction (light color × exposure duration). Subsequently, the non-significant (P>0.05) interaction term was omitted from the final statistical model. Performance-related variables (body weight, feed intake, FCR, egg production, egg weight and egg mass) were analyzed as repeated measures with week as the repeated variable. Multiple comparisons among least-squares means in factorial analysis were performed using the protected Fisher's LSD test when the probability of the overall F-test was ≤0.05. Effects were considered to be statistically significant when P≤0.05 and P≤0.1 as the indicator of trend. The Dunnett's test was used for comparison of individual treatment means with the control.

Results

Table 2 shows the characteristics that were not significantly affected by the treatments.

Main effect of LED light color

Feed intake during the rearing period was lower in green *vs*. white light (-26.5 g; P≤0.01). There were more medium and large white follicles on the ovaries and greater levels of MCHC and plasma cholesterol under green light (Table 3).

Main effect of duration of LED light

Providing 12 h light resulted in greater feed intake during the rearing period, heavier shell weight and strength (P≤0.05), greater number of medium and large white follicles on the ovaries, and higher plasma cholesterol concentration (P≤0.01) (Table 4).

Interaction between LED light color and duration

Eight hours of green light caused a reduction in the shell strength (P≤0.01) with no difference between other treatments. Twelve hours of green light increased (P≤0.05) the plasma cholesterol level compared with CON group (1.4 mmol/L). The length of jejunum did not differ (P>0.05) between GL8, GL12 and WL12 (Table 5); however, it was shorter in WL8 as compared with GL8 group (P≤0.05).

Comparison of the control (incandescent lamp for 8h) with WL8, WL12, GL8 and GL12 groups

The GL12 birds tended (P=0.10) to produce heavier eggs (about 4.2 g heavier) than CON ones (Table 6). Compared with the GL12, control broilers recorded lighter egg shell weight, lower shell strength, higher egg-shape index, fewer numbers of medium and large white follicles, and lower concentration of plasma cholesterol (Table 6). There were fewer small white follicles in control birds compared with GL8 group. There were more white blood cells and higher hemoglobin concentration in CON birds than in WL8 and WL12 broilers. The control plasma ALP was lower than WL8, -

GL8, and GL12 broilers. The relative weight of the bursa of Fabricius was higher in WL8 as compared to CON birds.

Table 2. The traits in broiler breeders that were not significantly influenced by the lighting system

Traits not responding to lighting system	Treatment					SEM
	White LED Light, 8h	White LED Light, 12h	Green LED Light, 8h	Green LED Light, 12h	ICD Lamp, 8h (Control Group)	
Production and reproduction traits						
Body weight, end of rearing period (g)	2126.60	2264.70	2145.00	2124.70	2137.90	54.03
FCR, rearing period	3.85	3.63	3.81	3.86	3.83	0.09
Egg production (24 to 30 woa) (%)	51.41	48.32	48.73	51.54	54.85	4.27
Egg mass (g/d)	26.20	24.80	23.90	27.80	27.20	2.25
Haugh unit index	79.71	84.64	86.55	79.51	75.87	7.40
SWF	0.00	0.33	0.33	0.00	0.00	0.21
Blood parametrs						
FSH (mIU/mL)	0.24	0.79	0.15	0.75	0.15	0.35
LH (mIU/mL)	0.44	0.94	0.13	0.78	0.16	0.43
Estradiol (pg/mL)	108.5	193.5	179.3	135.5	235.5	44.58
MCH (pg)	45.75	46.33	45.75	46.50	46.50	1.03
RBC (×106/mm3)	2.82	2.71	2.86	2.98	3.01	0.15
Glucose (mg/dL)	223.50	240.50	222.00	227.50	222.50	11.89
Total Protein (g/dL)	3.950	3.950	3.733	4.100	3.850	0.29
Triglyceride (mg/dL)	36.50	47.50	60.0	54.50	42.50	9.97
Calcium (mg/dL)	10.30	10.75	10.83	10.90	10.70	0.35
Phosphorus (mg/dL)	5.200	5.250	5.533	5.550	4.950	0.66
AST (IU/L)	279.50	259.00	274.33	283.00	334.00	36.47
ALT (IU/L)	0.750	1.250	1.000	1.000	1.500	0.36
T_4 (µg/dL)	1.00	1.00	1.00	1.30	1.00	0.18
T_3 (nm/L)	0.62	1.00	0.91	1.27	0.80	0.32
Organ weight (g/kg BW)						
Ovarian relative weight	0.40	0.43	0.46	0.43	0.37	0.048
Proventriculus	3.55	3.23	3.23	3.27	3.81	0.245
Gizzard	24.1	22.90	24.36	25.16	25.52	1.74
Duodenum	14.87	12.76	12.92	14.60	13.81	1.47
Jejunum	28.34	32.32	30.93	32.07	31.47	2.51
Ileum	30.18	30.46	29.70	36.53	33.95	3.60
Cecum	8.85	7.20	8.79	8.48	9.46	0.10
Liver	13.93	14.14	13.54	13.90	15.32	0.58
Spleen	0.92	0.91	0.85	0.81	0.83	0.09
Immunologic response						
IgG titer (Log_2)	2.50	2.25	3.25	2.37	3.00	0.37
IgM titer (Log_2)	3.63	3.63	3.38	3.25	3.64	0.31
Total Ig titer (Log_2)	6.13	5.88	6.50	5.63	6.63	0.46
NDV titer (Log_2)	5.13	5.25	4.88	5.00	4.75	0.33
AI titer (Log_2)	3.75	3.75	4.00	4.00	3.50	0.41
CBH (mm)	0.54	0.65	0.61	0.45	0.48	0.12

AI: Avian influenza, ALT: Alanine transaminase, AST: Aspartate transaminase, CBH: Cutaneous basophil hypersensitivity, FCR: Feed conversion ratio, FSH: Follicle stimulating hormone, Ig: Immunoglobulin, LH: Luteinizing hormone, MCH: Mean corpuscular hemoglobin, NDV: Newcastle disease virus, RBC: Red blood cells, T_3: Triiodothyronine, T_4: Thyroxine, SYF: Small yellow follicle

Table 3. Effect of light spectrum on Ross 308 broiler breeders during the rearing and production periods

Measurement[1]	LED Light Color		SEM
	White	Green	
Feed intake during rearing period (g)	8206.6	8179.2**	9.44
Medium white follicles	1.83	4.0**	0.77
Large white follicles	1.50	3.83*	0.83
MCHC (g/dL)	35.40	36.75*	0.26
Blood cholesterol (mmol/L)	3.0	3.4**	0.07

1. Only the characteristics with significant F values for LED light color are reported in the table.
*, ** significant difference between white and green at *P≤0.05; **P≤0.01, respectively.
SEM: Standard error of the mean; MCHC: mean corpuscular hemoglobin concentration.

Table 4. Effect of LED light duration on Ross 308 broiler breeders during the rearing and production periods

Measurement[1]	Light duration		SEM
	8h	12h	
Feed intake during rearing period (g)	8179.5	8206.0*	9.44
Egg-shell weight (g)	6.23	6.70*	0.20
Shell strength (N/cm2)	38.03	41.56*	1.43
Medium white follicles (n)	1.16	5.00**	0.77
Large white follicles (n)	0.83	4.50**	0.83
Blood cholesterol (mmol/L)	3.10	3.35**	0.07

1. Only the characteristics with significant F values for LED light duration are reported in the table.
*, ** significant difference between light durations at *P≤0.05; **P≤0.01, respectively.
SEM: Standard error of the mean.

Table 5. Interaction effects of LED light color and duration on Ross 308 broiler breeders during the rearing and production periods

Measurement[1]	Color and duration				SEM	P-value
	White		Green			
	8h	12h	8h	12h		
Shell strength (N/cm^2)	40.76^{a}	39.51^{ab}	35.30^{b}	43.62^{a}	1.43	**
Blood cholesterol (mmol/L)	2.95^{b}	3.05^{b}	3.15^{b}	3.65^{a}	0.07	*
Jejunal length (cm)	54.90^{b}	69.93^{ab}	73.17^{a}	64.57^{ab}	5.24	*

1. Only the characteristics with significant F values for interaction effect are reported in the table.
SEM: Standard error of the mean.
a,b within rows, means with common superscript(s) are not different (P>0.05).
*P≤0.05; **P≤0.01 for interaction effects between light color and duration.

Hepatic gene expression of sterol regulatory element binding protein-1 (SREBP1)

The finding of qPCR investigation indicated that the relative hepatic gene expression of *SREBP1* was highest in WL8, medium in CON and GL8, and lowest in WL12 and GL12 broilers (Figure 4).

Discussion

Main effect of LED light color

Feed intake during the rearing period was lower in green *vs*. white light. In another study with broiler chickens, feed intake was significantly higher under white LED (Mendes et al., 2013) as observed in the present study.- The difference in feed intake may be due to the spectral sensitivity of the chickens. The greater number of medium (MWF) and large (LWF) white follicles in GL birds may be due to increased hepatic production of insulin growth factor-1 (IGF-1) as reported by Li et al. (2016). There was no effect of the lighting regimen on the number of small yellow follicles (Table 2) while no large yellow follicles (LYF) were observed at 20 woa. In another study, there was no significant difference in the number of SYF between GL and WL in laying hens at 22 woa (Hassan et al., 2013). Greater levels of MCHC and plasma cholesterol were observed under green light. Unlike our study, (Hassan et al., 2013) did not find any significant difference in cholesterol level in Hy-line Brown pullets between treatments. These contradictory findings might be due to differences in photoperiod and chicken -

strains. It has been suggested that strain variation in cholesterol concentrations might be related to the inherent genetic differences (Chowdhury et al., 2002).

Table 6. Effects of incandescent and LED light color on several performance characteristics in Ross 308 broiler breeders during the rearing and production periods

Measurement[1]	LED Light				SEM	ICD Lamp, 8h (Control group)	SEM	Dunnett's Test, P-value			
	White		Green					CON *vs.* WL8	CON *vs.* WL12	CON *vs.* GL8	CON *vs.* GL12
	8h	12h	8h	12h							
Egg weight (g)	50.97	51.35	49.04	53.89	1.01	49.65	1.06	NS	NS	NS	0.10
Egg-shell weight (g)	6.37	6.46	6.10	6.94	0.20	5.59	0.26	NS	NS	NS	*
Shell strength (N/cm^2)	40.76	39.51	35.30	43.62	1.43	35.17	1.58	NS	NS	NS	*
Egg shape index	76.36	77.10	76.51	75.82	0.36	76.89	0.29	NS	NS	NS	**
Small white follicles	333.33	166.67	433.33	266.67	44.09	216.67	40.14	NS	NS	*	NS
Medium white follicles	0.33	3.33	2.00	6.67	0.76	1.33	0.91	NS	NS	NS	**
Large white follicles	0.33	2.67	1.33	6.33	0.83	0.33	0.76	NS	NS	NS	***
White blood cells ($\times 10^3$/mm^3)	223.20	222.37	224.78	228.90	1.39	229.68	1.55	*	*	NS	NS
Hemoglobin (g/dL)	12.95	12.57	13.13	13.83	0.25	14.00	0.39	*	*	NS	NS
Blood cholesterol (mmol/L)	2.95	3.05	3.15	3.65	0.07	2.89	0.10	NS	NS	NS	**
Alkaline phosphatase (U/L)	1011.0	824.0	1006.3	1066.5	48.51	692.00	48.56	*	NS	**	**
Bursa of Fabricius (g/kg body	1.08	0. 80	0. 47	0. 59	0.189	0.41	0.183	*	NS	NS	NS
Ileal length (cm)	57.53	69.83	70.27	72.63	5.36	74.70	5.50	0.10	NS	NS	NS

1. Only the characteristics with significant F values are reported in the table.
ICD, incandescent; CON, control; SEM, Standard error of the mean; NS, non-significant; *P≤0.05; **P≤0.01; ***P≤0.001

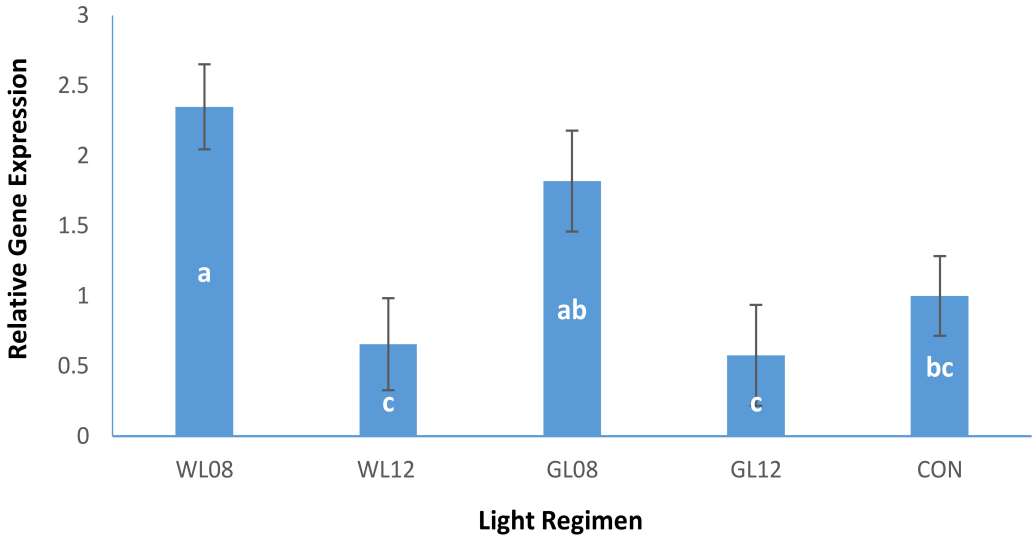


Figure 4. Effect of light spectrum and duration during the rearing period on the relative hepatic sterol regulatory binding protein-1 gene expression in Ross 308 broiler breeders.

Main effect of LED light duration

In the present study, 8 h light resulted in lower feed intake (26.5 g), shell weight and shell strength. Brake et al. (1989) also reported short photoperiods (8 or 9 h) resulted in lower egg specific gravity, shell percentage, egg weight, and eggshell weight in broiler breeders. Decreased egg shell quality might be due to less calcium

deposition in short photoperiods. Lower serum cholesterol concentration under the shorter photoperiod in the present experiment supports the findings of Kim et al. (2022). The findings of the fewer medium and large white follicles in 8-h illumination may be due to inadequate conditioning of the hypothalamus and pituitary gland to secrete GnRH and gonadotropins which were not measured in our experiment.

Interaction between LED light color and duration

There was a significant interaction between LED light color and duration on shell strength, cholesterol level and jejunal length. Jejunum is the main site of absorption in the small intestine (Thompson and Applegate, 2006) and the lower length of jejunum in WL8 birds might imply smaller surface area for nutrient absorption. However, it is unlikely that WL8 lighting did actually reduce the length of jejunum; this was more likely due to chance differences in bird allocation to treatments. The lowest egg shell strength in GL8 birds is in accordance with lower feed intake under GL (Table 3) and shorter duration of lighting (Table 4) which indicates lower calcium intake. Our data showed that despite lower feed intake, green light increased serum cholesterol concentration especially at longer durations. Ibrahim et al. (2012) also showed that green light group had significantly higher cholesterol level than the birds exposed to yellow light. It is suggested elevated serum glucose, cholesterol and triglyceride levels are indicators of stress (Thaxton et al., 2000). However, in the present study, glucose and triglyceride levels were not affected by the lighting systems (Table 2). Therefore, the likely mechanism of this finding needs to be clarified.

Comparison of the control (incandescent lamp for 8h) with WL8, WL12, GL8 and GL12 groups

Egg shape index impacts on the internal and external egg quality traits (Duman et al., 2016) but the published data in the literature are not fully consistent. Alasahan and Copur (2016) reported that egg shape index was correlated with the early embryonic mortality, hatchability and chick carcass yield in Japanese quails. Egg shape index in GL12 was reduced slightly ($P<0.05$) compared to the control treatment but it is unlikely that such small decrements (about 1% point) is biologically important in terms of the egg quality and subsequent embryonic development. Studying the effect of light on egg quality in laying hens, Er et al. (2007) did not calculate the egg shape index but reported no significant difference in egg length and width between the ICD and green lights. They also found that eggshell strength was greater and the eggshell was thicker in GL than in ICD from 21 to 45 wk. According to Er et al. (2007), the egg quality in GL was the best as also observed in the present study. Egg shell weight was heavier in GL12 birds compared with CON group. Yue et al. (2019) showed that under green light, pineal melatonin could promote the expression of pituitary-specific transcription factor-1 (Pit-1), thereby increasing growth hormone (GH) secretion and growth in chickens. Growth hormone receptors were identified in the shell gland of the chicken oviduct (Ni et al., 2007), and Hrabia et al. (2013) showed the differential mRNA expression and protein localization of GH receptors in the infundibulum, magnum, isthmus, vagina and shell gland of laying hens; indicating that the chicken oviduct is a GH-responsive organ. Injection of GH to hens increased the shell quality of the eggs laid near the end of the laying period (Donoghue et al., 1990). The higher relative weight of the bursa of Fabricius in WL8 as compared to CON birds may imply better immunity. Mohamed et al. (2017) reported that broilers reared under blue (BL) and green light had lower bursa relative weight compared with white light group. Tabeekh (2016) found no differences in RBC number between WL and GL in broilers and layers, while WBC count was higher in WL which contradicts our findings. This contradiction might be due to the effect of light duration which was different (24 h *vs.* 12 or 8 h in the present study) and chicken strains. It has been shown that antioxidative enzyme (superoxide dismutase and glutathione peroxidase) activities increased in GL and BL in broilers (Ke et al., 2011). On the other hand, antioxidant supplementations like thyme and ginger were capable of improving the packed cell volume, hemoglobin and total leukocytic count in broilers (Saleh et al., 2014).

Hepatic gene expression of sterol regulatory element binding protein-1 (SREBP1)

The *SREBP1* activity, a circadian transcription factor, is regulated by the availability of nutrients through the insulin signaling pathway which is fundamental in controlling many physiological processes, including the sleep-wake activity, neurotransmitter production, lipid synthesis and several of other metabolic functions (Gilardi et al., 2014). In the present study, the relative *SREBP-1* gene expression was 2.34 times higher in 8 h *vs.* 12 h light exposure. In birds, lipid accumulation in the extra-hepatic tissues results to a large extent from the combined effects of hepatic lipogenesis and lipoprotein production; therefore, plasma triglyceride levels are dependent on the level of hepatic lipogenesis (Huang et al., 2008). However, in our study blood level of triglycerides was not affected by the lighting system while cholesterol level was higher in GL12 birds which showed lower expression of *SREBP-1* compared to GL8. On the other hand, it is believed that the levels of *SREBP-1* are significantly elevated in obese patients and in animal models of obesity (Ruiz et al., 2014), which may be related to more feed intake observed in birds reared under white light.

Other parameters

Body weight and feed conversion ratio (FCR) through rearing and production periods, and also egg production in broiler breeder hens were similar between the conventional and LED lighting regimens. Similar results were observed in Pekin duck reared under different LED lights (House et al., 2021). It has been argued that the green light is capable of improving the expression of arylalkylamine N-acetyltransferase (AANAT) mRNA that is involved in the day/night rhythmic production of melatonin (MEL) in the pinealocytes and retinal cells by modification of serotonin release (Jin et al., 2011). Qin et al. (2021) reported that MEL has dual effects on growth hormone secretion from the adenohypophysis, by acting both directly and indirectly; MEL can directly promote the secretion of adenohypophyseal GH through Mel1b and Mel1c receptors, or indirectly by inhibiting somatostatin secretion from the hypothalamus under green light. Jie et al. (2022) found that green light inhibited the broiler growth (Arbor Acres, AA). There may be differences amongst broiler age and genetics in growth response to various lights (Olanrewaju et al., 2006). Egg production and total egg number were not significantly affected by the light sources, suggesting that lighting may not be the primary trigger for the beginning of lay and instead, internal factors such as body weight might activate the reproductive axis (Baxter and Bédécarrats, 2019). The Haugh unit values demonstrate better egg quality (Mellor et al., 1975). In the present study, the Haugh unit was not different among treatments; a finding that is in line with Hassan et al. (2013) and Su et al. (2021) in laying hens and Brown Tsaiya ducks, respectively. Light color and duration failed to affect the relative ovarian weight, plasma concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH) and 17β - estradiol (E2). The data on the ovarian weight is in line with the findings of Hassan et al. (2013). However, the LH and FSH concentrations in 12 h-lighting system showed small but nonsignificant increases compared to 8 h groups in pre-laying period (Table 2). This is consistent with Lewis et al. (2005) who concluded that plasma LH and FSH concentrations have minimal value for predicting the degree of photorefractoriness, the age at sexual maturity, or subsequent egg production in broiler breeders.

Our results showed that AST and ALT were not affected by treatments (Table 2) which is in agreement with the findings in layer hens reared under white and green lights (Yenilmez et al., 2021), indicating that color of light is not likely to have a harmful effect on the liver function. Higher serum activity of AST and ALT may indicate functional liver damage in broilers (Alyileili et al., 2020). Thyroid hormones are important factors that determine the level of hepatic lipogenesis in birds (Hillgartner et al., 1995). According to Rozenboim et al. (2004), there was no significant difference in blood concentrations of triiodothyronine (T3) and thyroxine (T4) between light treatments. In agreement with Yang et al. (2016), spleen and liver weights at 20 woa were not affected by the lighting system (Table 2). Antibodies, such as immunoglobulins (Ig), are important components of the humoral immune system in protecting the body against bacteria and viruses (Onbaşılar et al., 2009). Xie et al. (2008b) found that the effect of different lights on the immune system was dependent on the stage of growth in broilers and photoperiod beside the light wavelength plays an important role in immunoregulation. In the present study, the immunoglobulin titer was not affected by treatments (Table 2). It is suggested that melatonin production might be the reason of these variations since melatonin has a pleiotropic role in the mammalian and avian immune systems (Kliger et al., 2000).

Conclusion

In summary, we did not find any adverse effects of LED lighting system on the broiler breeder performance, blood metabolites and hepatic function. The overall egg production was not influenced by the experimental LED; however, egg quality was improved by using green LED light. A relatively higher hepatic SREBP1 gene expression and heavier relative weight of the bursa of Fabricius were observed in eight hours of white LED light which may suggest a better immune response. Therefore, specific LED lighting system, as energy saving source of light, may be used in lieu of the conventional light in commercial broiler breeder houses, when more detailed information is available by further experimentation.

Acknowledgments

We would like to acknowledge the Zarbal GP Complex for donating one-day-old broiler breeder chicks, and Noor Gostaran Alborz Company for providing the LED lights.

References

Alasahan, S., Copur, A.G., 2016. Hatching characteristics and growth performance of eggs with different egg shapes. *Brazilian Journal of Poultry Science* 18, 01-08.

Alyileili, S.R., El-Tarabily, K.A., Belal, I.E., Ibrahim, W.H., Sulaiman, M., Hussein, A.S., 2020. Effect of Trichoderma reesei degraded date pits on antioxidant enzyme activities and biochemical responses of broiler chickens. *Frontiers in Veterinary Science* 7, 338.

Aslam, S.M., Garlich, J.D., Qureshi, M.A., 1998. Vitamin D deficiency alters the immune responses of broiler chicks. Poultry Science 77, 842-849.

Aviagen. 2019. Ross Parentstock Management Handbook, (Aviagen).

Aviagen. 2021. Ross 308 Parentstock: Nutrition Specifications, (Aviagen).

Baxter, M. and Bédécarrats, G.Y., 2019. Evaluation of the impact of light source on reproductive parameters in laying hens housed in individual cages. *The Journal of Poultry Science* 56, 148-158.

Biggins, J.D., Thompson, J.E., Birkhead, T.R., 2018. Accurately quantifying the shape of birds' eggs. *Ecology and Evolution* 8, 9728-9738.

Brake, J., Garlich, J.D., Baughman, G.R., 1989. Effects of lighting program during the growing period and dietary fat during the laying period on broiler breeder performance. *Poultry Science* 68, 1185-1192.

Cheema, M.A., Qureshi, M.A., Havenstein, G.B., 2003. A comparison of the immune response of a 2001 commercial broiler with a 1957 randombred broiler strain when fed representative 1957 and 2001 broiler diets. *Poultry Science* 82, 1519-1529.

Chowdhury, S.R., Chowdhury, S.D., Smith, T.K., 2002. Effects of dietary garlic on cholesterol metabolism in laying hens. *Poultry Science* 81, 1856-1862.

Coelho, L.M., Leão, A.P.A., Bernardes, L.F., Alves, V.V., Martins, B.G., Peixoto, J.V., Pereira, L.J., Fassani, É.J., Alvarenga, R.R., Zangeronimo, M.G., 2021. Reproductive aspects of Japanese quails (Coturnix coturnix japonica) hatched from eggs incubated under different light colors. *Theriogenology* 170, 67-76.

Donoghue, D.J., Campbell, R.M., Scanes, C.G., 1990. Research Note: Effect of biosynthetic chicken growth hormone on egg production in White Leghorn hens. *Poultry Science* 69, 1818-1821.

Doyon, G., Bernier-Cardou, M., Hamilton, R.M.G., Castaigne, F. and Randall, C.J., 1986. Egg quality. 2. Albumen quality of eggs from five commercial strains of White Leghorn hens during one year of lay. *Poultry Science* 65, 63-66.

Duman, M., Sekeroglu, A., Yildirim, A., Eleroglu, H., Camci, O., 2016. Relation between egg shape index and egg quality characteristics. *European Poultry Science* 80, 1-9.

Er, D., Wang, Z., Cao, J., Chen, Y., 2007. Effect of monochromatic light on the egg quality of laying hens. *Journal of Applied Poultry Research* 16, 605-612.

Franco, B.R., Shynkaruk, T., Crowe, T., Fancher, B., French, N., Gillingham, S., Schwean-Lardner, K., 2022. Light color and the commercial broiler: effect on behavior, fear, and stress. *Poultry Science* 101, 102052-102060.

Gilardi, F., Migliavacca, E., Naldi, A., Baruchet, M., Canella, D., Le Martelot, G., Guex, N., Desvergne, B., Consortium, C., 2014. Genome-wide analysis of SREBP1 activity around the clock reveals its combined dependency on nutrient and circadian signals. *PLoS Genetics* 10, e1004155.

Haldar, C., Bishnupuri, K.S., 2001. Comparative view of pineal gland morphology of nocturnal and diurnal birds of tropical origin. *Microscopy Research and Technique* 53, 25-32.

Hillgartner, F.B., Salati, L.M., Goodridge, A.G., 1995. Physiological and molecular mechanisms involved in nutritional regulation of fatty acid synthesis. *Physiological Reviews* 75, 47-76.

House, G.M., Sobotik, E.B., Nelson, J.R., Archer, G.S., 2021. A comparison of white/red and white/blue LED light fixtures on Pekin duck production, stress and behaviour. *British Poultry Science* 62, 467-473.

Hrabia, A., Leśniak, A., Sechman, A., 2013. In vitro effects of TCDD, PCB126 and PCB153 on estrogen receptors, caspases and metalloproteinase-2 mRNA expression in the chicken shell gland. *Folia Biologica (Kraków)* 61, 277-282.

Huang, J., Yang, D., Gao, S., Wang, T., 2008. Effects of soy-lecithin on lipid metabolism and hepatic expression of lipogenic genes in broiler chickens. *Livestock Science* 118, 53-60.

Huth, J.C., Archer, G.S., 2015. Comparison of two LED light bulbs to a dimmable CFL and their effects on broiler chicken growth, stress, and fear. *Poultry Science* 94, 2027-2036.

Ibrahim, S.A., El Kholya, S.Z., El-Far, A.H., Mahrous, U.E., 2012. Influence of lighting color on behavior, productive traits and blood parameters of Japanese quail (Coturnix Coturnix Japonica). *World Academy of Science, Engineering and Technology* 67, 1234-1244.

Jie, D., Zhang, Z., He, J., Zhou, Y., Zhu, G., 2022. Effects of different LED light colors on growth performance and harmful gas emission of broilers breeding in a digital rearing chamber. *International Journal of Agricultural and Biological Engineering* 15, 71-78.

Ke, Y.Y., Liu, W.J., Wang, Z.X., Chen, Y.X., 2011. Effects of monochromatic light on quality properties and antioxidation of meat in broilers. *Poultry Science* 90, 2632-2637.

Kim, H.J., Son, J., Jeon, J.J., Kim, H.S., Yun, Y.S., Kang, H.K., Hong, E.C., Kim, J.H., 2022. Effects of Photoperiod on the Performance, Blood Profile, Welfare Parameters, and Carcass Characteristics in Broiler Chickens. *Animals* 12, 2290.

Kliger, C.A., Gehad, A.E., Hulet, R.M., Roush, W.B., Lillehoj, H.S., Mashaly, M.M., 2000. Effects of photoperiod and melatonin on lymphocyte activities in male broiler chickens. *Poultry Science* 79, 18-25.

Lewis, P.D., Morris, T.R., 1998. Responses of domestic poultry to various light sources. *World's Poultry Science Journal* 54, 7-25.

Lewis, P.D., Ciacciariello, M., Ciccone, N.A., Sharp, P.J., Gous, R.M., 2005. Lighting regimens and plasma LH and FSH in broiler breeders. *British Poultry Science* 46, 349-353.

Li, S., Cao, J., Wang, Z., Dong, Y., Wang, W., Chen, Y., 2016. Melatonin Mediates Monochromatic Light-induced Insulin-like Growth Factor 1 Secretion of Chick Liver: Involvement of Membrane Receptors. *Photochemistry and Photobiology* 92, 595-603.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25, 402-408.

Marquardt, W.W., Snyder, D.B., Savage, P.K., Kadavil, S.K., Yancey, F.S., 1985. Antibody response to Newcastle disease virus given by two different routes as measured by ELISA and hemagglutination-inhibition test and associated tracheal immunity. Avian Diseases 29, 71-79.

Mellor, D.B., Gardner, F.A., Miller, M.M., 1975. Interior Quality Decline in Eggs Cartoned and Sealed in Plastic Bags. *Poultry Science* 54, 1998-2001.

Mendes, A.S., Paixão, S.J., Restelatto, R., Morello, G.M., de Moura, D.J., Possenti, J.C., 2013. Performance and preference of broiler chickens exposed to different lighting sources. *Journal of Applied Poultry Research* 22, 62-70.

Mohamed, R.A., El-Kholya, S.Z., Shukry, M., El-Kassas, S., El Saidy, N.R., 2017. Manipulation of broiler growth performance, physiological and fear responses using three monochromatic LED lights. *Alexandria Journal for Veterinary Sciences* 53, 57-62.

Ni, Y., Zhu, Q., Zhou, Z., Grossmann, R., Chen, J., Zhao, R., 2007. Effect of dietary daidzein on egg production, shell quality, and gene expression of ER-α, GH-R, and IGF-IR in shell glands of laying hens. *Journal of Agricultural and Food Chemistry* 55, 6997-7001.

Olanrewaju, H.A., Thaxton, J.P., Dozier, W.A., Purswell, J., Roush, W.B., Branton, S.L., 2006. A review of lighting programs for broiler production. *International Journal of Poultry Science* 5, 301-308.

Onbaşılar, E.E., Yalçın, S., Torlak, E., Özdemir, P., 2009. Effects of early feed restriction on live performance, carcass characteristics, meat and liver composition, some blood parameters, heterophil-lymphocyte ratio, antibody production and tonic immobility duration. *Tropical Animal Health and Production* 41, 1513-1519.

Oso, O.M., Metowogo, K., Oke, O.E., Tona, K., 2022. Influence of LED bulb on reproductive and production performance of different poultry species: a review. *World's Poultry Science Journal* 78, 515-529.

Özkan, S., Yalçın, S., Bayraktar, Ö.H., Bilgen, G., Dayıoğlu, M., Bolhuis, J.E., Rodenburg, T.B., 2022. Effects of incubation lighting with green or white light on brown layers: hatching performance, feather pecking and hypothalamic expressions of genes related with photoreception, serotonin, and stress systems. *Poultry Science* 101, 102114.

Parvin, R., Mushtaq, M.M.H., Kim, M.J., Choi, H.C., 2014. Light emitting diode (LED) as a source of monochromatic light: a novel lighting approach for behaviour, physiology and welfare of poultry. *World's Poultry Science Journal 70*, 543-556.

Prescott, N.B., Wathes, C.M., 1999. Spectral sensitivity of the domestic fowl (Gallus g. domesticus). *British Poultry Science* 40, 332-339.

Qin, X., Liu, X., Yan, X., Long, M., Wang, Z., Dong, Y., Chen, Y., Cao, J., 2021. Melatonin mediates monochromatic light-induced expression of somatostatin in the hypothalamus and pituitary of chicks. *Poultry Science* 100, 101285.

Renema, R.A., Robinson, F.E., Proudman, J.A., Newcombe, M. and McKay, R.I., 1999. Effects of body weight and feed allocation during sexual maturation in broiler breeder hens. 2. Ovarian morphology and plasma hormone profiles. Poultry Science 78, 629-639.

Rozenboim, I., Biran, I., Chaiseha, Y., Yahav, S., Rosenstrauch, A., Sklan, D., Halevy, O., 2004. The effect of a green and blue monochromatic light combination on broiler growth and development. *Poultry Science* 83, 842-845.

Ruiz, R., Jideonwo, V., Ahn, M., Surendran, S., Tagliabracci, V.S., Hou, Y., Gamble, A., Kerner, J., Irimia-Dominguez, J.M., Puchowicz, M.A., DePaoli-Roach, A., 2014. Sterol regulatory element-binding protein-1 (SREBP-1) is required to regulate glycogen synthesis and gluconeogenic gene expression in mouse liver. *Journal of Biological Chemistry* 289, 5510-5517.

Saleh, N., Allam, T., El-Latif, A.A., Ghazy, E., 2014. The effects of dietary supplementation of different levels of thyme (Thymus vulgaris) and ginger (Zingiber officinale) essential oils on performance, hematological, biochemical and immunological parameters of broiler chickens. *Global Veterinaria* 12, 736-744.

Seo, H.S., Kang, M., Yoon, R.H., Roh, J.H., Wei, B., Ryu, K.S., Cha, S.Y., Jang, H.K., 2015. Effects of various LED light colors on growth and immune response in broilers. *The Journal of Poultry Science* 53, 76-81.

Su, C.H., Cheng, C.H., Lin, J.H., Liu, H.C., Yu, Y.T., Lin, C.C., Chen, W.J., 2021. The effect of different colored light emitting diode illumination on egg laying performance, egg qualities, blood hormone levels and behavior patterns in Brown Tsaiya duck. *Animal Bioscience* 34, 1870-1878.

Tabeekh, M.A.A., 2016. An investigation on the effect of light color and stocking density on some blood parameters of broilers and layers. *Donnish Journal of Agriculture Research* 3, 8-12.

Thaxton, J.P., Puvadolpirod, S., 2000. Model of physiological stress in chickens 5. Quantitative evaluation. *Poultry Science* 79, 391-395.

Thompson, K.L., Applegate, T.J., 2006. Feed withdrawal alters small-intestinal morphology and mucus of broilers. *Poultry Science* 85, 1535-1540.

Wei, Y., Zheng, W., Li, B., Tong, Q., Shi, H., 2020. Effects of a two-phase mixed color lighting program using light-emitting diode lights on layer chickens during brooding and rearing periods. *Poultry Science* 99, 4695-4703.

Wu, Y., Huang, J., Quan, S., Yang, Y., 2022. Light regimen on health and growth of broilers: an update review. *Poultry Science* 101, 101545-550.

Xie, D., Li, J., Wang, Z.X., Cao, J., Li, T.T., Chen, J.L., Chen, Y.X., 2011. Effects of monochromatic light on mucosal mechanical and immunological barriers in the small intestine of broilers. *Poultry Science* 90, 2697-2704.

Xie, D., Wang, Z.X., Dong, Y.L., Cao, J., Wang, J.F., Chen, J.L., Chen, Y.X., 2008. Effects of monochromatic light on immune response of broilers. *Poultry Science* 87, 1535-1539.

Yang, Y.F., Jiang, J.S., Pan, J.M., Ying, Y.B., Wang, X.S., Zhang, M.L., Lu, M.S., Chen, X.H., 2016. The relationship of spectral sensitivity with growth and reproductive response in avian breeders (*Gallus gallus*). *Scientific Reports* 6, 1-9.

Yenilmez, F.A.T.M.A., Atay, A., Serbester, U., Celik, L., 2021. Effects of monochromatic light on performance, egg quality, yolk cholesterol and blood biochemical profile of laying hens. JAPS: *Journal of Animal and Plant Sciences* 31, 46-52.

Yue, L., Qin, X., Liu, X., Wang, Z., Dong, Y., Chen, Y., Cao, J., 2019. Melatonin receptor Mel1b-and Mel1c-mediated green light induced the secretion of growth hormone in anterior pituitary of chicks. *Photochemistry and Photobiology* 95, 1387-1394.