

REGULAR ARTICLE

Wavelength lighting variation on egg quality and serum glucose

Aline Mirella Fernandes¹, Fernanda Paes de Oliveira¹, Diogo de Lucca Sartori², Silvana Gomes Gonzalez³, Douglas D'Alessandro Salgado², Marcos Martinez do Vale⁴, Danilo Florentino Pereira⁵

¹Graduate Program in Agribusiness and Development, School of Science and Engineering, São Paulo State University - UNESP, Tupã, SP, Brazil.

²Department of Biosystems Engineering, School of Science and Engineering, São Paulo State University - UNESP, Tupã, SP, Brazil.

³University Center of Adamantina – UNIFAI, Adamantina, SP, Brazil.

⁴Department of Animal Science, Federal University of Paraná, Curitiba, PR, Brazil.

⁵Department of Management, Development and Technology, School of Science and Engineering, São Paulo State University - UNESP, Tupã, SP, Brazil.

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Statements and Declarations

Data availability

All data will be shared if requested.

Institutional Review Board Statement

The study is approved by the Ethics Committee on the Use of Animals (CEUA) of the School of Sciences and Engineering (CEUA Protocol n°. 02/2020).

Conflicts of interest

The authors declare no conflict of interest.

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Author contribution

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Abstract

This observational study verified the effects of monochromatic lighting for three monochromatic light spectra on the production and quality of laying hen eggs and blood parameters, as probable indicators of environmental stress. *Lohmann*[®]29-week-old birds were divided into groups of 20 animals, housed in three experimental houses with different lighting treatments (blue, green and red), and monitored for 90 days. Were analysed 4,443 eggs, and the values of the following measurements were extracted: egg weight (g), albumen height (mm), shell resistance (kgf/cm²), shell thickness (mm), Haugh unit, specific gravity (g/cm³), and egg shape. Blood samples were collected from the birds at the beginning and at the end of the study period. The levels of total plasma protein and the heterophile/lymphocyte ratio remained within the normal range. Laying hens housed under blue and red lighting had higher egg production (per hen) and showed better egg quality results. Red lighting stood out for providing significantly better shell resistance than other treatments. Glucose differed between treatments, with a drop in blood glucose levels as the environment light wavelength increased. The quality of the eggs was affected differently by the sources of monochromatic lighting. Correct lighting management promotes better health for birds and increased egg production and quality.

Keywords

Blood cell count; Light-emitting-diode; Performance; Precision Livestock Farming; Animal Welfare



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Introduction

Light is an exogenous physical environmental factor that causes great interference in poultry production, influencing the body in matters of physiology, behavioral functions, immune system, and growth rate (Olanrewaju et al., 2019).

Bird eyesight is much more sensitive, when compared to that of humans, and that is due to the fact that birds can see wavelengths below 400 nm (Er et al., 2007). Besides, birds have proportionally larger eyes, consisting of two photoreceptor cells in the retina, which would be the rods, responsible for facilitating eyesight in low light environments and during the night, and cones, that contribute to seeing during the day (Mendes et al., 2010).

Birds have light receptors in their brain that play an important role in biological and physiological body functions (Wyse & Hazlerigg, 2009). Thus, exposure to different light

sources can alter the physiological state, changing the concentration of various biological components and hormones such as glucocorticoids (Leprould et al., 2001). Blood tests along with other biochemical assessments have been used to assess the health status of animals, especially those that have undergone some change in routine, which promotes stress (Olanrewaju et al., 2017).

The light reflected in the retina reaches associated areas of the bird brain, consisting of the hypothalamus, photoreceptors, and the pineal gland, which emits a hormonal message. The gonadotropin-releasing hormone (GnRH) is secreted and sent to the pituitary Gonadotrophs, that produce luteinizing hormones (LH) and follicle stimulating hormones (FSH), secreting them into the circulatory system (Mendes et al., 2010).

It is worth mentioning that the transorbital pathway is not the main pathway for the perception of light regarding light

* Corresponding author

E-mail address: danilo.florentino@unesp.br (D.F. Pereira).

stimulus, especially for reproduction in birds. Rather, that would be the transcranial pathway, as demonstrated by Baxter et al. (2014), who studied the same lineage of chickens. The author found that there was no significant difference in development between blind and normally sighted birds housed under red and white light.

For Er et al. (2007), when it comes to laying hens, some colors can be more stimulating than others, since the visible spectrum emitted by the light source can influence egg production and quality. In this way, the light stimulus must be controlled and gradually increased, as the effect of lighting is associated with the development of sexual maturity for these birds. Correct and controlled direct exposure to light is essential for the reduction of physiological stress, and it influences feed consumption (Freitas et al., 2005), improves egg production (Barros et al. 2020), as well as egg quality parameters (Liet et al., 2014; Er et al., 2007).

As for the form of supply and artificial lighting systems, changes have been taking place in recent years with new lamps being used in poultry production. The purpose of those changes is to enhance the luminous efficiency and reduce lighting costs. In this way, the LED type lamp has shown positive results in terms of providing illuminance and, also, lasting longer than other light sources that were traditionally used in poultry farming (Barros et al., 2020).

Systems with LED-type lighting provide heavier eggs and higher albumen height compared to fluorescent lighting (Long et al. 2016). This productive gain is due to the wave spectrum emitted by LED lamps (Rozemboim et al., 1998).

The effects of specific wavelengths emitted by lamps have been described in several studies. Li et al. (2014) found that different wavelengths of monochromatic lighting affected egg production and quality. Archer (2019) found lower stress levels in birds housed under increased red wavelength. Huber-Eicher et al. (2013) found that red light (640 nm) accelerates the sexual development of laying hens and that the productive performance of birds was superior in this range of light spectrum, compared to white and green lights (520 nm). Hassan et al. (2014) found greater production of eggs from laying hens subjected to a combination of red and green lights, and red monochromatic light, with better quality of the shell under green light treatments.

Stressful stimuli directly affect physiological activities, compromising animal productivity. Among them, the blood system represents an important indicator of physiological responses, measuring immunological aspects, such as the heterophile/lymphocyte ratio (Mehaisen et al., 2019). According to Sobotik et al. (2020), the

heterophile/lymphocyte ratio in laying hens correlates positively with animals subjected to stressful conditions, where the number of heterophiles increase in response to the level of stress. Nawab et al. (2018) also highlights the verification of glucose concentration, as stress alters the metabolic function, inducing glucose production due to the increase in corticosteroids to maintain homeostasis during the presence of stressful stimuli.

In view of the evidence that different wavelengths affect the production and quality of laying hen eggs, this work evaluated the lighting effects of three different monochromatic LED lamps (blue, green, and red) on quality, as well as in the heterophile/lymphocyte (H/L) ratio, the levels of total plasma protein and plasma glucose.

Materials and methods

The present observational study was carried out in the experimental area of the School of Science and Engineering of the Sao Paulo State University, campus of Tupã, Brazil, located at Latitude: 21° 56' 18" South and Longitude: 50° 30' 50" West. The experiments took place between June 10th and September 8th, 2020, so during the local winter, with the first seven days being dedicated to the adaptation of the hens, making three cycles of 28-day production. This study was conducted according to the guidelines of the Brazilian National Council for the Control of Animal Experimentation (CONCEA) and approved by the Sao Paulo State University's Animal Ethics Committee (protocol number 02/2020, CEUA-UNESP).

Description of Birds and Experimental Houses

At the beginning of the study, 60 *Lohmann*[®] hens (average body weight 1.476 g ±98g), with 29 weeks old, were used. The maintenance management of the birds during the study was similar to that of the farm where the hens originated: daily supplies of 110 g / poultry once in the morning (Table 1), with access to water *ad libitum* through nipple drinkers, and a 17-hour light period in which the lights were on at 4:00 and off at 21:00.

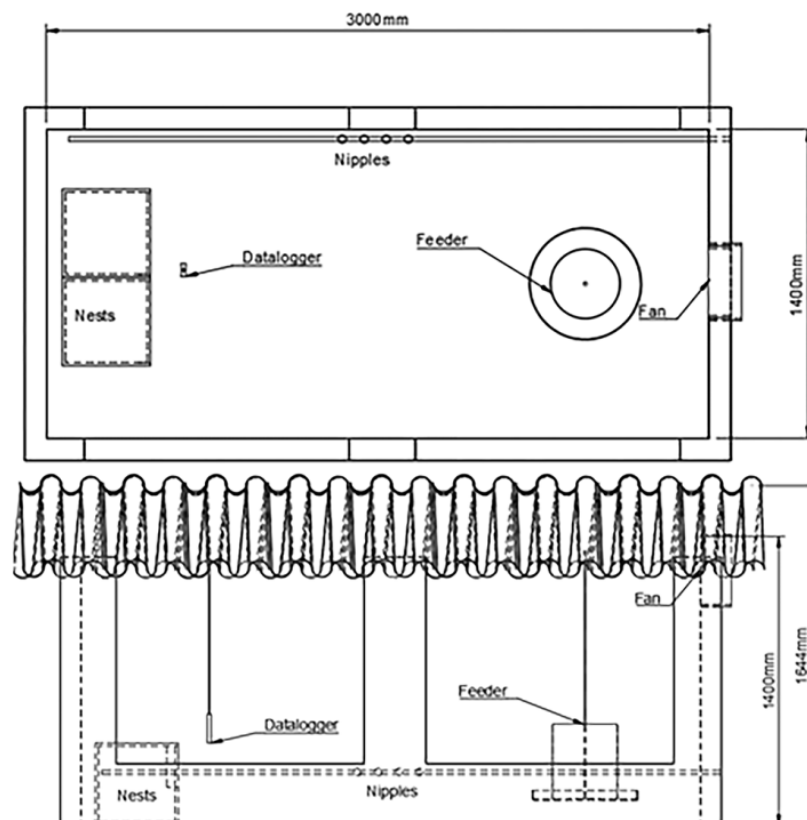
The poultries were randomly divided into groups of 20 individuals and housed in three experimental aviaries. Each one had two 40 x 40 x 40 cm box-type nests, a pendulum-type feeder, a four-spout nipple drinker, a 15 cm high shavings bed, and a 30 cm diameter exhaust fan to control ventilation and internal air quality. The facilities were adapted with the total blockade of the sides, in order to completely isolate the internal environment from the external income of natural light (Figure 1).

Table 1. Basic diet ingredients and calculated nutritional composition opted in all treatments.

Ingredient (g/kg, as-fed basis)		Calculated nutritional composition	
Corn	616.0	Metabolizable energy, Kcal/kg	2850.00
Soybean meal	256.0	Crude protein, %	17.40
Meat and bone meal	15.0	Ether extract, %	3.83
Limestone	94.0	Linoleic acid, %	1.26
Common salt	4.0	Crude fiber, %	3.63
Calcium phosphate	7.0	Calcium, %	4.15
DL-Methionine	0.5	Available Phosphorus, %	0.46
L-Lysine	1.2	Sodium, %	0.16
L-Threonine	0.2	Chlorine, %	0.25
Choline	0.5	Lysine, %	0.85
Adsorbent	1.0	Methionine, %	0.37
Lysolecithin (50%)	0.4	Methionine + cysteine, %	0.60
Mineral and Vitamin mixture ¹	4.0	Tryptophan, %	0.19
Tecnase ²	0.2	Threonine, %	0.56
		Arginine, %	0.87
		Isoleucine, %	0.64
		Valine, %	0.75

¹ Containing: vitamin A 1,333,330 IU/kg, vitamin D3 383,330 IU/kg, vitamin E 2,500 IU/kg, vitamin K3 166.6 mg/kg, vitamin B1 33.3 mg/kg, vitamin B2 500 mg/kg, vitamin B6 283.3 mg/kg, vitamin B12 1.67 g/kg, niacin 3.33 g/kg, pantothenic acid 1.07 g/kg, folic acid 83.3 mg/kg, biotin 2.5 mg/kg, iron 5.0 g/kg, copper 1.5 g/kg, manganese 10 g/kg, chelated manganese 3.0 g/kg, zinc 10 g/kg, chelated zinc 3.0 g/kg, iodine 166.6 mg/kg, selenium 41.6 mg/kg, phytase 100 FTU/g, canthaxanthin 333.3 mg/kg, beta-apo-8 carotenol 666.7 mg/kg and zinc bacitracin 4.67 g/kg.

² Containing: alpha galactosidase 100 µg, amylase 600 µg, beta mannanase 800 µg, beta glucanase 500 µg, protease 3000 µg and xylanase 10000 µg.

**Figure 1.** Aviary layout used for housing birds during the monitoring period under monochrome lighting.

Each facility received a different monochromatic LED lighting system, blue, green, or red (length of dominant wave of 460.3, 533.0, 621.6 nm respectively for the colors). The number of lamps in each aviary was calculated so that all experimental houses had an illuminance of 100 lux.

Each light source has a different light spectrum and an illuminance of 100 lux was defined inside the experimental houses. Thus, a lumino-technical calculation was carried out to determine the number of lamps in each treatment.

Monitoring of the Thermal Environment

To monitor the thermal environment, a HOBO® datalogger was installed in each shed, model U12-012, positioned close to the centre of the installation and at the height of the hens. The dataloggers recorded the temperature and relative humidity of the air at 5 min intervals throughout the study period.

Considering the situation of complete confinement of the birds in the completely enclosed installations, the exhaust fans fulfilled the function of guaranteeing a minimum ventilation for the exchange of air with the external environment.

From the temperature and relative humidity data, the Temperature and Humidity Index (THI) was calculated for each internal environment using Equation 1, which was used for birds by Biaggioni et al. (2008).

$$THI = 0.8 \times TBS + \frac{ARH \times (DBT - 14.3)}{100} + 46.3 \quad (1)$$

Where: DBT = dry bulb temperature (°C);
ARH = air relative humidity (%).

For laying hens, temperatures above 28° C and THI above 78 are considered situations in which the birds are outside the thermal comfort zone and, therefore, characterizing stress by heat (Biaggioni et al., 2008). On the other hand, temperatures and THI below 15° C and 59, respectively, are considered cold stress.

Measurement of Egg Quality

Eggs were collected twice a day and properly identified. Every day, all eggs were subjected to shell and internal quality analysis, registering the following variables: egg weight, albumen height, eggshell resistance, eggshell thickness and Haugh unit, using Nabel's DET-6000® equipment. The specific gravity (SG) was determined by immersing the eggs in containers with saline solutions (NaCl), with densities ranging from 1.060 to 1.100 g / mL in intervals of 0.005.

In order to analyse the shape of the egg, a digital image was taken of all eggs collected during the experiment, using a Sony digital camera, model HDR-XR160, installed on a tripod, positioning the camera lens at 90° angle down where the egg was placed on a black background. The measures of area and perimeter of the shape of the eggs were obtained using the Egg Shape software, developed in a Matlab® environment. Figure 2 shows an example of an egg analysed computationally.

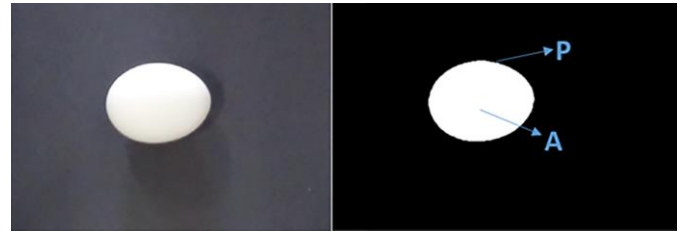


Figure 2. Demonstration of the digital image captured from an egg and processing on the screen of the Egg Shape software.

From the area and perimeter measurements, the shape coefficient (SC) was calculated using Equation 2.

$$SC = \frac{P^2}{4 \times \pi \times A} \quad (2)$$

Where: P = egg image perimeter in pixels;
A = egg image area in pixels.

The shape coefficient (SC) described in Equation 2 approaches the value 1 (one) when the analysed shape approaches a circle. In this way, it is possible to easily check thought only this single measure if the eggs are more elongated or spherical.

Blood Analysis

Blood samples were collected from all poultries on the first and last days of the experimental period. Collections were performed in the afternoon, through a puncture of the femoral vein on the posterior face of the tibia, with the aid of a 20 x 0.55 mm needle and syringe of 3 mL. The collected samples were placed in EDTA tubes (0.5 mL) and immediately refrigerated for further laboratory evaluation.

For the measurement of plasma glucose, an AccuChek Active® glucose kit was used at the time of collection, using a blood drop analysed by the glucometer.

For differential leukocyte count, blood smears were individually stained with the rapid panoptic kit. Differential count data were obtained manually by counting a total of 100 leukocytes per slide for differentiation into heterophils and lymphocytes. The heterophile/lymphocyte relationships were calculated by dividing heterophiles and lymphocytes. To evaluate total plasma proteins, blood samples were centrifuged in a micro-hematocrit centrifuge and the plasma of each tube was checked by refractometry in blood with EDTA anticoagulant.

Design and Statistical Analysis

The experimental design was entirely casualized with three treatments (light source) and the evaluated response parameter was the egg quality (egg weight, albumen height, shell resistance, shell thickness, Haugh unit, specific gravity and Shape coefficient). The experimental period was divided in 1, 2 and 3; correlated respectively to 29th to 32th, 33th to 35th and 36th to 39th week of age. When the poultries were housed at 29th week of age and in the end of the experimental period (39th week of age) there was compared the heterophile/lymphocyte relationships and the plasmatic concentration of glucose and plasma proteins between the treatments. The comparisons were made between quality variables between treatments in

the periods. For this purpose, exploratory data analysis and confirmatory analysis were performed using distribution test of Anderson-Darling, analysis of variance and the Tukey test for each variable at 5% significance.

Results and discussion

All poultry houses had the thermal environment mostly within the thermoneutrality zone in the three production cycles throughout the experiment with average THI of 68.5 classified as comfort by Biaggioni et al. (2008). It was considered that, for analysis of egg quality, there was no interference from the thermal environment in the measurements performed. In the present study, throughout the experiment, all poultry houses

had the thermal environment mostly within the thermoneutrality zone.

During this study, a total of 4,443 eggs were produced and analysed. The different light wave length treatments between 29th to 39th weeks of age affect the egg weight, eggshell strength and thickness, specific gravity and shape coefficient ($p < 0.05$; Table 2). The eggs weight and shape coefficient were negatively affected under blue wave length in comparison with the green and red light. The eggshell strength was affected differently in the wave lengths and improved in the colour red and decreasing in the blue and green. The eggshell thickness and specific gravity were decreased in the green wave lengths.

Table 2. Egg quality response of laying hens in terms of egg weight, albumen height, Haugh unit, eggs hell strength and thickness, specific gravity, and shape coefficient, for blue, Green and red monochromatic lighting treatments between 29th to 39th weeks of age.

Variable	Treatment	Means	±SE
Eggweight (g)	Blue	57.8 ^B	0.1
	Green	58.6 ^A	0.1
	Red	58.4 ^A	0.1
Albumenheight (mm)	Blue	9.15	0.07
	Green	9.34	0.07
	Red	9.21	0.07
Haugh Unit	Blue	94.48	0.30
	Green	95.05	0.32
	Red	94.44	0.33
Eggshellstrength (Kgf)	Blue	4.653 ^B	0.023
	Green	4.554 ^C	0.026
	Red	4.833 ^A	0.026
Eggshellthickness (mm)	Blue	0.388 ^A	0.001
	Green	0.383 ^B	0.001
	Red	0.386 ^A	0.001
Specificgravity (g / cm ³)	Blue	1090.0 ^A	0.1
	Green	1089.3 ^B	0.1
	Red	1090.3 ^A	0.1
Shape coefficient	Blue	1.025 ^A	0.001
	Green	1.024 ^B	0.001
	Red	1.023 ^B	0.001

Uppercase letters indicate differences between the treatments at 5% significance of the Tukey test for each variable. SE = Standard error.

The different light source affected the egg quality in different ways along the production cycles 1 (29th to 32th), 2 (33th to 35th) and 3 (36th to 39th weeks of age). In all cycles, the blue light promote lighter eggs that statistically differ with the green in first, with the red in second and with the Green and red in the third cycle. After three weeks in red and six in the Green light the treatments improve the egg weight when compared with the blue light. The albumen high was reduced ($p < 0,05$) in blue and green and was stable in the red light along the cycles ($p > 0,05$). Red light provided eggshell strength values higher than the other two treatments in all production

cycles and was verified a drop in the resistance and specific gravity of the eggshell over the production cycles ($p < 0,05$; Table 3).

The eggshell strength was increased in the red light when compared to the green light. There is an increase in egg weight and a drop in the quality of the eggshell and internal parameters of the eggs with the advance of the production cycles. In the red lighting, better results were obtained for eggshell strength and egg weight. Laying hens under green light had lower egg quality parameters, when compared to the green and blue light. The analysis of the egg images allowed

for the evaluation of the shape coefficient accurately, being possible to verify significant differences ($p < 0.05$).

In comparison between the colours blue, green and red as monochromatic lighting sources for laying hens between 29th do 39th week of age, the egg quality was affected differently, indicating that further studies should be carried out to increase knowledge about the effects of different wavelengths on hens and their egg production.

The averages values of the serum levels of total plasma proteins, blood serum glucose, and the

heterophile/lymphocyte ratio (6.47 g/dL, 192.4 mg/dL, and 1.49, respectively) between the treatments at the beginning of the experiment were equal ($p > 0.05$). The normal range results at the beginning of the study indicate the equalization and standardized condition on the start of the experiment. After 90 days of exposure to treatments only the serum levels of glucose showed differences ($p < 0.05$; Table 3). There was a decrease in blood glucose levels as there was an increase in light wavelength. The glucose levels of birds housed under red light also differed from the levels of birds housed under blue.

Table 3. Mean blood parameters values of blood serum glucose (mg/dL), total plasma proteins (g/dL) and heterophilic/lymphocyteratio, after 90 days of exposure of the poultries to blue, green, and red lighting treatments.

Parameter	Treatment	Mean	±SE
Plasma glucose	Blue	219.1 ^a	3.4
	Green	213.9 ^{ab}	3.6
	Red	204.5 ^b	2.8
H / L ration	Blue	0.663	0.075
	Green	0.487	0.032
	Red	0.612	0.052
Plasma protein	Blue	7.845	0.300
	Green	7.740	0.239
	Red	7.995	0.239

Means of variables in the same column with different letters differ by the Tukey test at 5% significance.

The monochromatic lighting source blue, green and red for laying hens between 29th do 39th week of age affect the egg quality (Table 2), corroborating with Li et al. (2014) that observed hens with 19 to 63 weeks of age and in thermoneutral conditions, when exposed to red light, laid eggs heavier than the ones laid by chickens exposed to white, blue, or green light. In contrast, Hassan et al. (2014) show that, for Hy-line Brown chickens, between 19 and 52 weeks of age, the heavier eggs were produced under blue and green lighting treatments, when compared to red.

Er et al. (2007) found that the weight of the egg was affected by the light wavelength, where they observed that the eggs under the green lighting treatment had an improvement in internal quality.

All evaluated eggs showed a Haugh unit greater than 90 (Table 2). This result corroborates the USDA Egg-Grading Manual (USDA, 2000), which recommends values above 72 for eggs of excellent quality. Long et al. (2016) also establish values greater than 88 as an ideal value for fresh eggs.

Red lighting also promoted eggs with better specific gravity. Rosa and Avila (2000) found eggs with specific gravity ranging from 1.075 to 1.090 g / mL for chickens between 35 and 55 weeks old, while birds with 56 weeks of age and older produce a higher proportion of eggs with inferior shell quality and specific gravity less than 1.074 g / ml. Specific gravity is directly associated with shell quality and inversely associated with egg quality.

According to the USDA Egg-Grading Manual (USDA, 2000), the normal shape of the egg is considered elliptical (oval). Round or very elongated eggs have a bad appearance and do not fit properly in the packaging used to send the

product to the market. In addition, they are easier to break during transport, when compared to normal shaped eggs. Based on the values of the shape coefficient (Table 2), it was found that birds exposed to blue light produced eggs with a more elongated shape than in relation to the other treatments.

When the shape coefficient is observed, it appears that the eggs have become more elongated over time (Table 3). However, it can be considered that the difference is not noticeable to the human eye. Er et al. (2007) also found that eggs placed under blue light had a more elongated shape when compared to eggs produced under red light, corroborating the results found.

Long et al. (2016) portray the decrease in shell resistance as the birds age. The shell is the part of the egg that takes the longest to form, taking an average of 18 to 20 hours to complete the calcification. Its resistance is influenced by the thickness of the shell, size, and shape of the egg (Sapkota et al. 2017). In this study, the thickness of the shells was higher than the reference (0.310 mm) value in all treatments.

As reported by Chen et al. (2015), the greater the force registered by the equipment, the greater the resistance of the shell to breaking. The shell strength was 4.554 Kgf for the green light treatment, 4.653 Kgf for the blue, and 4.833 Kgf for the red treatment. Er et al. (2007) point out that eggs of hens exposed to red and green lights showed greater shell resistance when compared to blue light.

For broilers and chickens that are not laying eggs, the values are less than 3.6 g / dL, being even lower for young chicks. Values above normal may indicate water deficiency, due to dehydration, chronic inflammatory diseases, and immune-mediated diseases. In general, the main factors that

affect the concentrations of total proteins in birds are age, breeding conditions (management), and diseases.

In all treatments, the H / L ratio showed values close to those considered normal (the reference value for well-being is 0.5 according to Macari and Luquetti 2002). These results are in agreement with those obtained by Astuti et al. (2015), who studied broilers under continuous and intermittent monochromatic blue light and found that the H / L ratio did not increase, suggesting that blue light is not related to increased stress in animals. Archer (2019), while studying white and white-with-red LED lamps, found that laying hens under the white-with-red treatment had lower heterophiles/lymphocytes ratios. Hong et al. (2020) also found no differences in the H / L ratio in chickens subjected to incandescent, LED, and fluorescent lamps.

Hassan et al. (2013) evaluated the H / L ratio in laying hens housed under monochromatic light associated with LED lamps in the colours: red (R), green (G), blue (B), and combinations of RG and RGB treatments, as well as white fluorescent light (W), as Control. They found that the H / L ratio was not influenced by monochromatic lighting and, in these conditions, the differential leukocyte count showed that there was no increase in heterophiles in relation to lymphocytes. Those were the same results obtained in this study. Regarding the values in the concentration of plasma glucose, Hassan et al. (2013) found a significant increase in birds exposed to red light, which differs from this study in the way that the increase was observed under blue light, while birds housed under the red lighting treatment showed lower values.

In this study, the average glucose concentrations ranged from 204.5 to 219.1mg / dL, in which the author reports that, in stressful situations, values above 594 mg / dL can be observed. It is worth mentioning that the feeding time occurred in the morning, during which the birds were eating in greater quantity, and the collections were carried out in the afternoon, around 16:00 hours approximately. Yalçinkaya et al. (2012) report that, under stress conditions, blood glucose levels increase significantly.

Significant differences in blood glucose concentration have also been reported by Hong et al. (2020), confirming that glucose can be used as an indicator of the welfare state of laying hens.

Wei et al. (2020) found that serum glucose concentration levels in laying hens increased with the associated treatment of green and blue light, when compared to white and orange-yellow light.

Yang et al. (2016) tested a mixed combination of monochromatic blue and green light, assessing the metabolism of broilers, and found that there was a significant increase in glucose levels when compared to white artificial light. Mohamed et al. (2020) found that there was no significant result in the concentration of glucose in relation to broilers with monochromatic green light, blue light, and a mixed combination of the two. The author observed values such as 237.0 mg / dL for the green treatment, 234.70 mg / dL for the blue one, and 234.0 mg / dL for the mixed combination.

Soliman and Hassan (2019) report that blue LED light is able to significantly improve productive performance, some

biochemical parameters, growth hormones, and significantly reduce intestinal bacterial load, when compared to white and red LED lights.

Conclusions

In this current study it was demonstrated that the wavelengths affect the quality of eggs of laying hens, and the red light provided greater quality of eggshells. Given the relationship between plasma glucose and the observed wavelength, it is recommended that further studies should be made to better assess the effects of monochromatic lighting on the well-being and health of laying hens. However, it is possible to affirm that efficient lighting management can improve egg quality.

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