The Influence of LED Lighting on the Avian Immune System: Histomorphometric Analysis of Lymphoid Organs and Leukocyte Profiles in Broiler Chicks

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Abstract

The purpose of this study is to evaluate the effect of a lighting system based on a combination of green-blue LED lights on body weight, stress, and the morphostructure of lymphoid organs in chickens. A total of 100 one-day-old Ross 308 broiler chicks were randomly assigned to two homogeneous experimental groups (n = 50 chicks/group) based on the type of lighting technology used. The control group (WL) was exposed to white light-emitting diodes (LED) for a period of 42 days, while the second group (GBL) was exposed to a lighting regimen based on a controlled LED spectrum combination as follows: on days 1–14 exclusively green light, on days 15–28 green and blue light and on days 35–42, exclusively blue light. Our results demonstrated that the combination of blue-green LED lights significantly increased the final body weight (BW) of the chicks and reduced stress by significantly lowering the heterophiles H(%) and the heterophiles to lymphocites (H/L) ratio. Furthermore, the GBL group exhibited better structural development of the lymphoid organs, with significantly higher organ indices for the bursa, thymus, and spleen, a higher cortex/medulla (C/M) ratio in both follicles and lobules, and a larger surface area for the lymphoid sheaths (PALS and PELS) and lymphoid nodules (LN) in the spleen. Nevertheless, these results suggest that combined green-blue LED light has an immunostimulatory effect on lymphoid organs, reflected in better health of the chicks.

Keywords: chicken welfare, lymphocyte, LED light

Introduction

Light is a very important environmental factor that can influence several factors, such as immunity (Horodincu & Solcan, 2023; Xie et al., 2011, p. 201), the endocrine system (Galan et al., 2025), growth performance (Abdel-Moneim et al., 2024), and behavior (Remonato Franco et al., 2022). The use of LED lights in poultry houses has become a highly sought-after lighting source in recent decades, offering numerous benefits related to energy efficiency but primarily due to the wide range of monochromatic light colors it can provide (Parvin et al., 2014). Previous studies have shown that monochromatic green and blue lights, compared to monochromatic red light, improve growth performance by enhancing the feed conversion ratio (FCR) in broiler chickens (Soliman & El-Sabrout, 2020). Furthermore, the combination of green and blue light increased final body weight and stimulated muscle mass growth (Karakaya et al., 2009). The impact of light on production has been the main focus in poultry research, and few studies have also examined its effect on the immune system. However, when assessing animal welfare, health is an important parameter to consider (Fraser, 2008). Therefore, it is essential to understand the effects of combined monochromatic lights on lymphoid organs and the health of poultry.

Due to a complex visual system and the photosensitive nature of the pineal gland in birds, the immune system of broiler chicks may be particularly sensitive to light (Y. Yang et al., 2016). The thymus, bursa of Fabricius, and spleen are key lymphoid organs in chickens and are responsible for the development of T lymphocytes and B lymphocytes, antibody production, and the cellular immune response (Ceccopieri & Madej, 2024; Liu et al., 2013). Previous studies have shown that green light promotes the proliferation of B lymphocytes in the bursa (Li et al., 2015) and of T lymphocytes in the thymus and spleen (Chen et al., 2016; Guo et al., 2017). However, the effect and mechanism by which light influences lymphocytes remain unclear. Melatonin is part of the neuroendocrine-immunoregulatory system; it is the primary hormone synthesized in the pineal gland (Carrillo-Vico et al., 2013) and plays a role in modulating physiological processes related to the immune system.

In line with the above, this study investigated the effect of a lighting system based on a combination of green and blue LED lights on body weight, stress, and the morphostructure of lymphoid organs in 42-day-old broiler chicks.

Materials and Methods

This Animals and Experimental Design

A total of 100 Ross 308 broiler chicks were monitored from one day of age until the end of the experiment at the "Ion Ionescu de la Brad" University of Life Sciences Biobase in Iași, where they were randomly assigned to two homogeneous experimental groups (n = 50 chicks/group), based on the type of lighting technology used (Greentek Lighting DARA, Bucharest, Romania). The first group, which also served as the control group (WL), was exposed to a standard artificial lighting regimen using white light-emitting diodes (λ = 400–760 nm) for 42 days, while the second group (GBL) was exposed to a lighting regimen based on a controlled LED spectrum combination as follows: on days 1–14, exclusively green light (λ = 560 nm); on days 15–28, green and blue light (λ = 480–560 nm); and on days 35–42, exclusively blue light (λ = 480 nm). For both lighting technologies, light

intensity was adjusted weekly to 0.19 W/m² and a photoperiod of L:D = 23:1 on days 0–14 and L:D = 16:8 on days 15–42. The two plots were separated by an opaque black plastic wall to prevent light contamination between the two lighting technologies.

Microclimate conditions were continuously monitored and maintained within the optimal technological limits recommended by the hybrid management guide. The temperature ranged from 32 to 30 °C during the first week of life and then gradually decreased by 2 degrees each week thereafter. Relative humidity was maintained constantly between 60 and 70%. Throughout the entire 42-day period, the birds had ad libitum access to fresh water and standardized compound feeds, adapted to the growth phases (starter, grower, and finisher) in accordance with National Research Council NRC (Dale, 1994).

Sample Collection and Assessment of Somatic Parameters

At 42 days of age, following a 12-hour fasting period, a population of 20 chicks per batch (n = 40 chicks in total), selected completely at random, were weighed individually to determine their final body weight (BW). Subsequently, the birds were slaughtered using standardized methods (cervical dislocation) in accordance with veterinary and public health ethical guidelines. Immediately after slaughter, the lymphoid organs (bursa of Fabricius, thymus, and spleen) were removed in their entirety and weighed using a precision analytical balance. For each bird, the organ index (relative weight of the organ) relative to final body weight was calculated using the formula: Organ index (%) = Absolute organ weight (g) / Bird body weight (g) × 100.

Hematological analyses

Prior to slaughter, blood samples were collected from the wing vein of the same 20 chicks selected from each batch. To evaluate the leukocyte profile, blood smears were stained using the May-Grünwald-Giemsa panoptic method. Differential cell counting was performed under an optical microscope with a 100× immersion objective (Leica DM500 microscope, Wetzlar and Mannheim, Germany), with a minimum of 100 leukocytes identified per slide. The percentages of lymphocytes and heterophils were calculated relative to the total leukocyte count, and the heterophil-to-lymphocyte (H/L) ratio was determined by dividing the absolute count of heterophils by that of lymphocytes; this ratio was used as a biometric indicator of physiological stress.

Histomorphometric Analysis

Tissue samples taken from bursa of Fabricius, the thymus, and the spleen were immediately fixed in Bouin's solution for 24 hours, dehydrated in ascending series of ethyl alcohol, clarified in xylene, and embedded in paraffin blocks. Histological sections 5 µm thick were prepared using a microtome and subsequently stained using the standard hematoxylin-eosin (H&E) method.

Images were captured using a Leica ICC50W Camera, and microscopic examination was performed using QuPath (version 0.5.1). Histomorphometric measurements included: the ratio of the cortical to medullary area (C/M) in both the bursa and the thymus, expressed in µm. In the spleen, the diameters of the periarterial lymphoid sheath (PALS), the perielipsoidal lymphoid sheath (PELS), and the splenic lymphoid nodules (LN) were measured.

Statistical Analysis

Primary data processing and statistical analysis were performed using SPSS (version 22.0 for Windows). Initially, the Shapiro-Wilk test was applied to each dependent variable within each group, revealing significant deviations from the normal distribution. Consequently, the nonparametric Mann-Whitney U test was used exclusively to assess the significance of the differences between WL and GBL. To ensure the biological relevance and zootechnical applicability of the results, the final data were presented in tables as Mean ± SEM (Standard Error of the Mean). The effect size for all nonparametric tests performed was calculated by determining the correlation coefficient r, using the formula: $r = |Z| / \sqrt{N}$. Where |Z| represents the absolute value of the standardized score generated by the Mann-Whitney U test, and N represents the total sample size (N = 40). The threshold for statistical significance was set at $p < 0.05$.

Results

In the present study, the final BW of the chicks in the GBL group averaged 3025.58 ± 44.48 g, which was significantly higher than that of the WL group (P = 0.03). An effect size analysis confirmed the magnitude of the weight differences between groups (r = 0.34), reflecting the strong impact of the light spectrum on somatic development (Table 1).

Table 1. Effect of LED light spectra on somatic and hematological parameters in broiler chickens at 42 days of age.

	WL	GBL	P value	SEM	r

Somatic indices					
BW (g)	2872.51	3025.58	0.03	44.48	0.34
Bursa index (%)	0.13	0.17	0.03	0.01	0.34
Thymus index (%)	0.34	0.38	0.007	0.01	0.43
Spleen index (%)	0.11	0.12	0.04	0.01	0.33
Hematological indices					
Lymphocytes (%)	48.40	56.80	0.36	3.70	0.15
Heterophils (%)	33.80	23.80	0.02	3.24	0.37
H/L ratio	0.54	0.42	0.002	0.03	0.48

Notes: WL = group of chicks exposed to white light; GBL = group of chicks exposed to green and blue light; SEM = Standard Error of the Mean; BW = final body weight; H/L = heterophils to lymphocytes ratio. Data are presented as Mean ± SEM.

The use of LED lights did not induce stress and did not result in changes in hematological indices, which remained within physiological limits. However, the GBL group (0.42 ± 0.03; P = 0.002; r = 0.48) showed a significant decrease in the percentage of heterophils and the H/L ratio compared to the WL group (Table 1). These results reflect a strong effect of blue-green LED lights on the stress response in broiler chickens. Spearman's correlation analysis revealed a significant negative correlation between the percentage of heterophils and the splenic index (r = −0.417, P = 0. 007) as well as between the H/L ratio and the thymic index (r = -0.654, P < 0.001), demonstrating that the development of lymphoid organs is directly linked to reduced stress, which can be induced by the use of blue-green LED lights (Fig. 1).

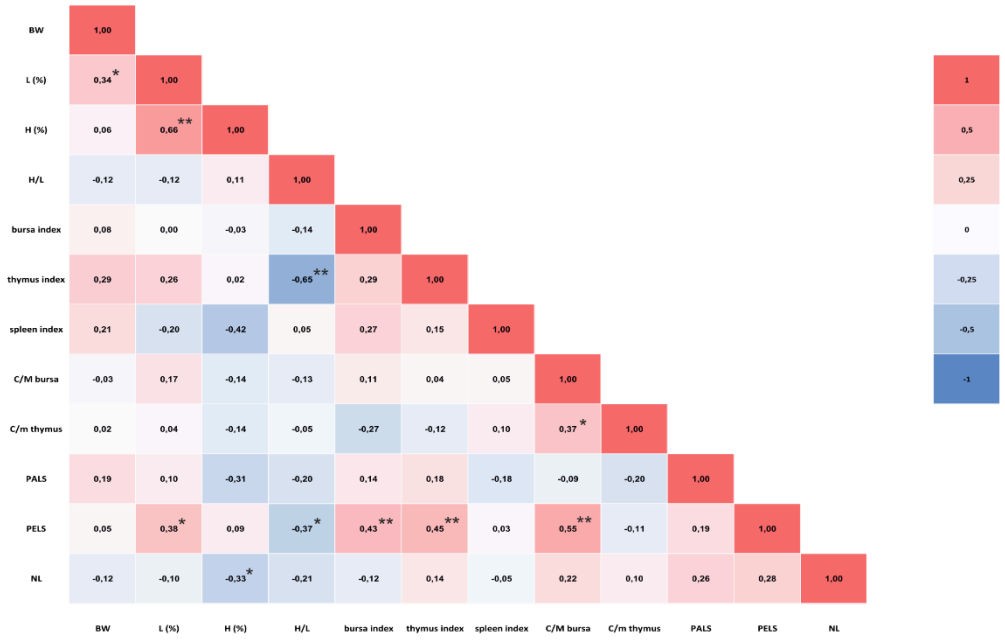

Fig. 1. Heatmap graph of Spearman´s correlation coefficient (r). Correlation is significant at the 0.05 level (*) and 0.01 level (**).

Histomorphometric analysis of lymphoid organs at 42 days of age revealed profound structural changes induced by the light spectrum. The group exposed to the combination of green and blue LEDs showed a higher C/M ratio both in the bursa of Fabricius (0.67 ± 0.02; P = 0.008) and in the thymus (0.98 ± 0.03; P = 0.007), with an effect size ranging from 0.42 to 0.43 (Fig. 2). Furthermore, a positive correlation was observed between the C/M ratio in the bursa and the C/M ratio in the thymus (r = 0.373, P = 0.018) (Fig. 1). All these changes underscore the

immunostimulatory effect of blue-green LED light on T and B lymphocyte populations, ultimately leading to superior development of the follicular and lobular cortical zones, which are essential for lymphopoiesis.

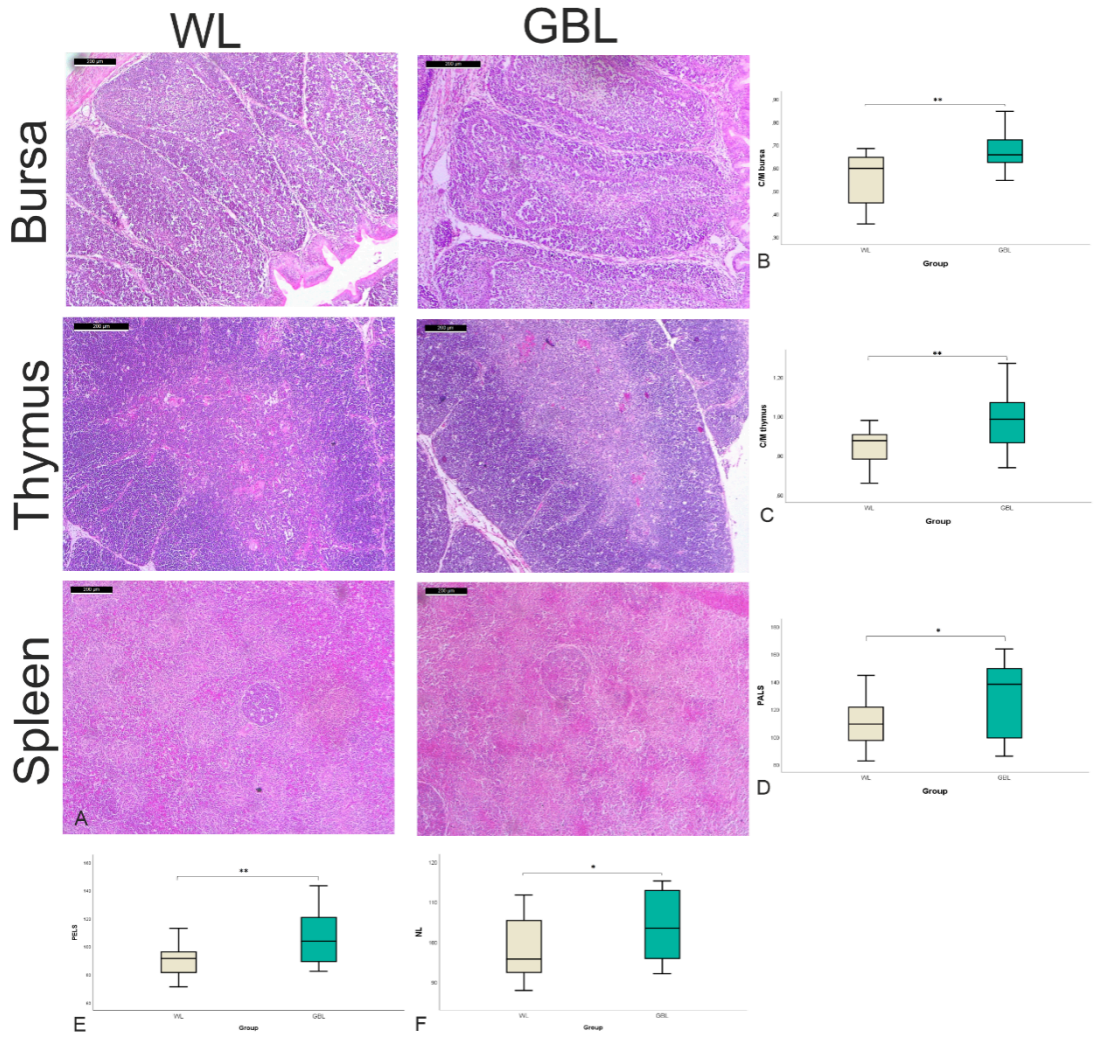

Fig. 2. The effect of led light spectra on the development of lymphoid organs in 42-day-old broiler chicks. (A) Impact on the morphology of lymphoid organs; (B) Cortex/medulla ratio in the bursa; (C) Cortex/medulla ratio in the thymus; (D) PALS diameter in the spleen; (E) PELS diameter in the spleen; (F) NL diameter in the spleen. Data are presented as Mean ± SEM. The threshold for statistical significance was set at $p < 0.05$ (*) or $p < 0.01$ (**).

The lymphoid tissue in the spleen develops under the influence of the primary lymphoid organs, and its structural compartments are populated by lymphocytes originating from the thymus and bursa; thus, PALS consists predominantly of T lymphocytes, PELS of B lymphocytes, and LNs of both types of lymphocytes. The use of combined green-blue LED light also influenced the morphology of the spleen, resulting in significantly larger diameters for PALS (126.38 ± 5.33; P = 0.04), PELS (106.24 ± 3.48; P = 0.007), and LN (103.83 ± 1.87; P = 0.03) compared to the WL group, with an effect size ranging from 0.33 to 0.43 (Fig. 2). These results suggest a direct immunostimulatory effect of the light spectrum used. This hypothesis is further supported by the positive correlation between PELS and the bursa index (r = 0.432, P = 0.005), PELS and the thymus index (r = 0.451, P = 0.003), PELS and the C/M ratio in the bursa (r = 0.549, P < 0.001), as well as the negative correlation with the H/L ratio (r = -0.367, P = 0.02) (Fig. 1). All these results underscore the fact that the white pulp in the spleen depends on the proper development of the bursa and thymus, as well as on the rate of lymphocyte proliferation in the cortical zone and on stress reduction in the birds, all of which are governed by the immunostimulatory effect of blue-green LED light on 42-day-old chicks.

Discussion

The development of lymphoid tissue can be influenced by various factors such as radiation and light (Archer, 2019; González Maglio et al., 2016), medications (G. Yang et al., 2015), or stress (Gao et al., 2018). In this study, we investigated the effects of a lighting regimen based on a controlled combination of blue-green LED spectra on body weight, physiological stress, and the morphology of lymphoid organs in 42-day-old chicks.

In chicks, although the lymphoid organs begin their morphological development during the embryonic stage, the immune system is not fully functional immediately after hatching (Madej et al., 2013), requiring at least 3 weeks to develop and become immunologically competent (Fellah et al., 2014). Thus, we use immune organ indices as a crucial marker for determining the immune status of birds (Heckert et al., 2002). Our results showed that the combination of green-blue LED lights stimulated the development of lymphoid organs by significantly increasing the organ index for the bursa, thymus, and spleen at 42 days of age. In birds, the primary lymphoid organs (the bursa and the thymus) consist of structural units comprising two distinct zones: the cortical and the medullary zones. In the thymus, the cortical zone is responsible for the proliferation and maturation of thymocytes, which eventually migrate to the medulla following a gradual process of negative selection. In bursa of Fabricius, the proliferation, maturation, and migration of B lymphocytes occur in the opposite order in the two compartments (Nagy et al., 2022). Histomorphometric analysis of lymphoid organs at 42 days of age revealed profound structural changes induced by the light spectrum. The group exposed to the combination of green and blue LEDs exhibited a higher C/M ratio in both the bursa and the thymus ($P < 0.05$). Physiologically, at the time of slaughter, industrial broilers tend to exhibit a reduction in the C/M ratio as a result of early involution triggered by end-of-cycle stress. The maintenance of a well-developed cortical zone in the bursa follicles and thymic lobules of the chicks in the experimental group demonstrates active lymphopoiesis and a low rate of cellular apoptosis. This histological integrity directly explains the relatively higher mass of the immune organs observed in this group.

The spleen, as a secondary lymphoid organ, is populated by both T lymphocytes and B lymphocytes, and the organ index is directly correlated with immune status (John, 1994, p. 199). The area of lymphoid follicles and lymphoid nodules are crucial histological markers for splenic stimulation (Madej et al., 2015). Our results showed that the combination of green-blue LED lights increased the PALS ($P = 0.04$), PELS ($P = 0.007$), and LN ($P = 0.03$) diameters compared to the WL group. Similar to our results, green light increased the thymus index (Xiong et al., 2019), bursa index (Y. Zhang et al., 2021), and spleen index in broiler chickens (Xiong et al., 2020). In addition, the combination of monochromatic green and blue lights improved immune function by increasing serum melatonin levels (Xiong et al., 2020; Z. Zhang et al., 2014). In this regard, our research is limited because we did not measure serum melatonin levels; however, we believe that the combination of green and blue LED light may have modulated melatonin secretion in the pineal gland, and that this melatonin led to lymphocyte proliferation and improved morphological development of lymphoid organs. These results suggest a direct immunostimulatory effect of the light spectrum used, which supports the well-established influence of the light signal emitted by green and blue LED light on the development of lymphoid organs and the immune system in broiler chickens (Horodincu & Solcan, 2023). Melatonin plays a very important role in light-induced effects on the immune system (Haldar & Ahmad, 2010). Numerous recent studies have demonstrated that monochromatic green light induces proliferation of B lymphocytes in the bursa (Li et al., 2015) and of T lymphocytes in the thymus (Chen et al., 2016) through melatonin secretion. All of these improvements disappeared following pinealectomy; however, upon administration of exogenous melatonin, the proliferative effect on T and B lymphocytes in the spleen was restored (Xiong et al., 2020).

According to the scientific literature (Mehaisen et al., 2017), atrophy of the lymphoid organs is a direct consequence of chronic stress induced by microclimate factors, mediated through the hypothalamic-pituitary-adrenal axis. In our study, the maintenance of a higher mass of lymphoid organs in the GBL group may indicate a reduction in corticosterone secretion under the influence of green and blue light. Once again, our study is limited because we did not measure serum corticosterone levels; however, this hypothesis is further supported by the close correlation with structural histomorphometric parameters, with the GBL group exhibiting greater development of the follicular and lobular cortical zones—which are essential for lymphopoiesis—as well as greater development of the lymphoid sheaths and lymphoid nodules in the spleen, which are responsible for the general immune response. The increase in the level of adrenal-secreted corticosterone associated with an increase in the H/L ratio is often interpreted as an indicator of a state of stress (Hefnawy et al., 2024). In the present study, we performed a leukocyte analysis and observed that the combination of blue-green LED lights significantly reduced the percentage of heterophils H(%) ($P = 0.02$) and the H/L ratio ($p = 0.002$), making it a suitable lighting method for promoting health and reducing stress in broilers. These results are consistent with those obtained by Mohamed (R. Mohamed et al., 2017) and Xie (Xie et al., 2008, 2011) and are likely due to the calming effect of blue light (Prayitno et al., 1997), which reduces physical activity (R. A. Mohamed et al., 2014).

Therefore, rearing the chicks under a combination of green-blue LED lights induced the development and activity of lymphoid organs, as well as hematological parameters that reflect better health and growth performance. Our

results showed that, compared to the WL group, the GBL group had a significantly higher average final weight (P = 0.03). The same results were obtained using blue light during the final growth period, with a significantly higher final weight compared to white light (R. Mohamed et al., 2017, 2020). These results are consistent with previous studies recommending the use of LED lighting systems in poultry production without negative effects on the weight and health of chicks (Riber, 2015). Future studies are needed to explain how melatonin is synthesized in the pineal gland under the influence of lights of different spectra, as well as the influence of this hormone on growth performance, behavior, and the endocrine and immune systems.

Conclusions

Our results demonstrated that, compared to monochromatic white LED light, the combination of blue-green LED lights significantly increased the final body weight of the chicks and reduced stress by significantly lowering the H/L ratio in the blood. Furthermore, the GBL group exhibited better structural development of the lymphoid organs, with significantly higher organ indices for the bursa, thymus, and spleen, a higher C/M ratio in both follicles and lobules, and a larger surface area for the lymphoid sheaths (PALS and PELS) and lymphoid nodules in the spleen. Nevertheless, these results suggest that combined green-blue LED light has an immunostimulatory effect on lymphoid organs, reflected in better health status among the chicks.

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Conflicts of Interest

The authors declare no conflicts of interest.

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