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Effect of LED Light Colour and Stocking Density on Some Hematological and Oxidative Stress Parameters in Japanese Quails

Key words

quail;
LED light;
heterophile/lymphocyte ratio;
oxidative stress;
stocking density

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Abstract: The study evaluated the effects of light-emitting diode (LED) light colour and stocking density on the hematological parameters, oxidative metabolism, and organ weights of quails. Several key management factors that influence the welfare of broilers include light color and stocking density. For this reason, this study aimed to reveal the effect of different light colors and stocking densities on the hematological and oxidative stress parameters in Japanese quails. For this purpose, levels of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) were measured in quails subjected to different light colors and stocking densities, using commercially available ELISA kits. In this study, 720 1-day-old mixed-sex Japanese quails (*Coturnix coturnix japonica*) were randomly assigned to one of six treatments, each having four duplicates of 30 birds. The experiment was designed as a 3 x 2 factorial arrangement of treatments, with three LED light colours (white, blue, and green) and two stocking densities (low = 200 cm^2/bird and high = 100 cm^2/bird). At 42 days of age, 20 quails from each treatment group were randomly selected for some hematological parameter analysis. According to the results obtained, the heterophile/lymphocyte (H/L) ratio was significantly higher in the white LED light colour treatments. However, the effects of stocking density on the H/L ratio were not significant. The oxidative stress indicator MDA was unaffected by the light colour, however, the high stocking density drastically reduced liver CAT and GSH activities. The heart weight was lower in the quail subjected to blue LED light. The heart and liver organ weights were not affected by stocking density. In conclusion, whereas white LED light increases the H/L ratio and the stress situation, it does not affect the oxidative stress indicators. These findings highlighted the need to identify optimum LED light colours for quails in commercial production settings in order to increase flock welfare. More research is needed to understand the effects and find the best colour of LED light for quails at varied stocking densities.

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Introduction

Light intensity, source, and spectrum are vital in modern poultry management (1). The perception of light colour in chickens has a substantial impact on their physiological reactions and stress levels. Exposure to mixed green-blue, blue, or green light resulted in lower heterophile/lymphocyte (H/L) ratios, indicating less stress in broilers (2). Pure blue light was also found to lower the H/L ratio, potentially lowering anxiety and oxidative stress by increasing melatonin release via the pineal gland (3). Green light at 560 nm, on the other hand, encouraged muscle growth and reduced oxidative stress during the early period, while blue light at 480 nm was more effective later (4).

The number of birds in a given space, or stocking density, is an important aspect of poultry management, particularly for quails. Stress, resource competition, hostility, and limited

movement can all result from overcrowding. High stocking density raises hematological stress hormones, the H/L ratio, and oxidative stress, while decreasing immunological response (5, 6).

Previous research has looked at how light colour affects stress indicators in chickens (4, 7). However, no prior research has been conducted on the effects of hematological parameters and oxidative stress in quails subjected to high stocking density. As a result, this study was conducted to investigate how white, blue, and green LED light colours, as well as stocking densities of 100 and 200 cm^2 per bird, influence various hematological parameters and oxidative metabolism in Japanese quails from day 1 to day 42 of their growth period.

Material and methods

Experimental Design: All the experimental procedures involved in this study were performed after ethical approval was taken from the Animal Care and Use Committee of Aydin Adnan Menderes University (64583101/2022/96).

This study took place at the Poultry Research Unit of Aydin Adnan Menderes University, Turkey, over six weeks. LED light bulbs (9W power, CT-4277 CATA, Turkey) were placed inside cages. Quails were randomly assigned to one of three LED light color treatments: white (400-770 nm), blue (480 nm), or green (560 nm). The light/dark cycle was set to 24 hours of light and 0 hours of darkness. Each LED colour treatment was evaluated at two different stocking densities: low (200 cm^2/bird) and high (100 cm^2/bird).

A total of 720 1-day-old mixed-sex Japanese quails (*Coturnix coturnix japonica*) chicks were initially weighted individually so that the cages had a similar beginning weight distribution. These chicks were divided into six groups, with different LED light colors (white, blue, and green) and two stocking densities (100 and 200 cm^2/quail). Each group had 20 quails in four replicates, making a total of 80 quails in the study. The quail chicks were raised in cages measuring 25 x 44 x 30 cm and were provided with the same number of heaters, feeders, and drinkers throughout the experiment. The temperature for the first three days was 33 ºC, gradually decreasing by 3 ºC per week until it reached 23 ºC. The relative humidity was maintained at 50-60% throughout the experiment. All quails were fed with balanced diets (0-14 d; 2910 kcal metabolic energy (ME)/kg, 24% crude protein (CP), and 15-42 d; 2900 kcal ME/kg, 22% CP) (8). Feed and water were ensured *ad libitum* throughout the study.

Hematological Parameters: At the end of the experiment, five quails from each replicate cage (20 birds/group; a total of 120 quails) were randomly selected and slaughtered by decapitation. In EDTA-coated tubes, were collected 1 mL of blood from each quail. It was stained with a blood smear on glass slides using May-Grünwald and Giemsa stain to analyze peripheral blood leukocyte populations. The cells were then enumerated and identified as heterophils, eosinophils, basophils, lymphocytes, and monocytes. The proportional proportions of each cell type were estimated based on the total number of leukocyte cells collected, and the H/L ratio was calculated using the Gross and Siegel method established in 1983 (9).

A total of 48 quails, two quails per replicate, were slaughtered by decapitation at the end of the experiment. The heart and liver were individually weighed, and the weights were noted. Tissue samples from the heart and liver were collected to assess oxidative metabolism, including measurements of MDA, superoxide dismutase (SOD), catalase (CAT), and GSH. To do this, the samples were first homogenized in cold 150 mM PBS (pH 7.4) at 2,000 rpm for 2 min by a tissue homogenizer (IKA WERKE Yellowline OST Basic S2 Analog Overhead Stirrer, Athy, Ireland). Obtained tissue homogenate supernatants were stored at -80 °C (NU 9668E, Nuaire, Japan) until spectrophotometric analyzes. MDA assay was performed as described by Ohkawa et al. (1979) (10). SOD activity was determined as described earlier by Sun et al. (1988) (11). The determination of CAT activity was done according to a modified method by Luck (1965) (12). GSH activity was also measured as described by Tietze (1969) (13). The assays were performed with the use of a spectrophotometer (Shimadzu UV-1601, Duisburg, Germany).

Statistical Analysis: The data were analyzed using the SPSS 22.0 (Statistical Package for the Social Sciences for Windows, IBM Corp., Armonk, NY, US). Data were tested for normality using Shapiro-Wilk's test. Using Levene's test, the assumption of homogeneity of variances was verified. Analysis of variance was performed with the GLM (Univariate General Linear Model) procedure to reveal the effects of some hematological parameters and the oxidative metabolism data (MDA, SOD, CAT, and GSH activity). The assumption of homogeneity of variances was evaluated using Levene's test. Hematological and oxidative stress parameter measurements were subjected to analyzes using a general linear model procedure, and means were compared using the least square difference (LSD) method. The experimental model for the design was defined as follows:

$$Yijk= \mu+\alpha i+\beta j+(\alpha\beta)ij+ Eijk$$

Where Yijk= the observed value, μ=the overall mean, αi=the effect of light colour (white, blue, and green), βj= the effect of stocking density (100 and 200 cm2/quail), (αβ)ij= the interaction between light colour and stocking density, and Eijk= the test error per observation. The partial effects of LED light color and stocking density for each factor were analyzed with the Least Squares Means Test and multiple comparisons were performed with a Duncan Test, with $P < 0.05$ indicating statistical significance. The correlations

between hematological parameters were calculated using Person's correlation coefficients.

Results

Table 1 displays the hematological parameters of quails in various light colour and stocking density treatments. The white LED light group had the greatest heterophile, eosinophil, monocyte counts, and H/L ratio (%28.30, %10.63, %9.78, and 0.88, respectively), with significant differences ($P < 0.01$, $P < 0.001$, $P < 0.05$, and $P < 0.05$, respectively). Hematological values did not differ significantly between stocking density groups. The combination of white LED light and a stocking density of 100 cm^2/bird resulted in the greatest heterophile percentage (30.95), which was statistically significant. On the lymphocyte percentage, there was also a significant interaction between LED light colour and stocking density.

Table 1: Influences of LED light color and stocking density on some hematological parameters of quails[1]

Treatment main effects	Hematological Parameters						
	n	Heterophile (%)	Lymphocyte (%)	Eosinophil (%)	Basophil (%)	Monocyte (%)	H/L
Expected mean (μ)	120	25.38	55.13	7.77	3.52	8.20	0.62
LED light treatment							
White	40	28.30^{a}	48.70^{b}	10.63^{a}	2.60^{b}	9.78^{a}	0.88^{a}
Green	40	20.33^{b}	61.10^{a}	6.75^{b}	3.73^{a,b}	8.10^{a,b}	0.41^{b}
Blue	40	27.53^{a}	55.60^{a}	5.95^{b}	4.23^{a}	6.73^{b}	0.57^{a,b}
Stocking density							
200 cm^2/bird	60	23.90	57.07	7.25	3.82	7.98	0.63
100 cm^2/bird	60	26.87	53.20	8.30	3.22	8.42	0.61
Pooled SEM[2]		1.12	1.22	0.44	0.23	0.40	0.07
LED color x stocking density							
White - 200 cm^2/bird	20	25.65^{a}	52.65^{b,c}	9.75	2.15	9.80	0.94
White - 100 cm^2/bird	20	30.95^{a}	44.75^{c}	11.50	3.05	9.75	0.81
Green - 200 cm^2/bird	20	15.15^{b}	66.50^{a}	6.25	4.15	7.95	0.26
Green - 100 cm^2/bird	20	25.50^{a}	55.70^{b}	7.25	3.30	8.25	0.56
Blue - 200 cm^2/bird	20	30.90^{a}	52.05^{b,c}	5.75	5.15	6.20	0.68
Blue - 100 cm^2/bird	20	24.15^{a}	59.15^{a,b}	6.15	3.30	7.25	0.47
Pooled SEM[3]		2.74	3.00	1.09	0.57	0.99	0.18
Significance of main effects				***P* value**			
LED light color		0.007	< 0.001	< 0.001	0.017	0.010	0.034
Stocking density (SD)		0.188	0.117	0.239	0.203	0.592	0.941
LED light color x SD		0.007	0.007	0.824	0.057	0.850	0.319

n: The total number of quails in the group. H/L: Heterophile/ Lymphocyte ratio. [a, b, c]: Means with different superscript letters in the same column differ ($P < 0.05$). [1]: Data presented as the least square means, [2]: Pooled SEM for main effects, [3]: Pooled SEM for interaction effect.

Table 2: Effect of LED light color and stocking density on oxidative stress parameters and weights of the heart and liver organs of quails

Treatment main effects		MDA (nmol/mg protein)		SOD (U/mg protein)		CAT (k/mg protein)		GSH (mg/g protein)		Organ weights (g)	
	n	Heart	Liver	Heart	Liver	Heart	Liver	Heart	Liver	Heart	Liver
Expected mean (μ)	48	21.48	2.99	1.09	0.29	0.10	2.74	29.65	22.37	1.80	4.62
LED light treatment											
White	16	23.28	2.93	1.07	0.30	0.09	2.63	29.43	21.93	1.94[a]	4.70
Green	16	20.06	2.92	1.07	0.28	0.11	2.83	31.48	22.79	1.77[a,b]	4.33
Blue	16	21.09	3.12	1.12	0.27	0.10	2.75	28.04	22.40	1.71[b]	4.81
Stocking density											
200 cm^2/bird	24	23.56	2.98	1.11	0.29	0.09	3.08	29.57	24.29	1.86	4.68
100 cm^2/bird	24	19.40	3.00	1.07	0.28	0.11	2.39	29.73	20.46	1.75	4.55
Pooled SEM[1]		1.32	0.16	0.06	0.02	0.01	0.12	1.10	0.80	0.04	0.25
Significance of main effects						***P* value**					
LED light color		0.598	0.851	0.907	0.741	0.253	0.807	0.447	0.907	0.042	0.719
Stocking density (SD)		0.122	0.953	0.727	0.626	0.026	0.008	0.942	0.021	0.126	0.799
LED light color x SD		0.333	0.977	0.989	0.240	0.207	0.568	0.135	0.233	0.554	0.478

MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase; GSH: Glutathione. n: The number of pens. [a,b]: Means with different superscript letters in the same column differ ($P<0.05$). [1]: Pooled SEM for main effects.

Table 3: Pearson correlation coefficients and correlation significance among hematological parameters of quails

	Heterophile	Lymphocyte	Eosinophil	Basophil	Monocyte	H/L
Heterophile	—					
Lymphocyte	-0.879**	—				
Eosinophil	0.058	-0.391**	—			
Basophil	-0.051	-0.012	-0.236**	—		
Monocyte	-0.074	-0.248**	0.091	-0.133	—	
H/L	0.784**	-0.727**	0.087	-0.087	0.045	—

H/L: Heterophile/ Lymphocyte ratio; **: $P<0.01$.

The colour of the LED light did not influence MDA (nmol/mg protein) (23.28 for heart and 2.93 for liver in white light; 20.06 for heart and 2.92 for liver in green light; 21.09 for heart and 3.12 for liver in blue light), SOD (U/mg protein) (1.07 for heart and 0.30 for liver in white light; 1.07 for heart and 0.28 for liver in green light; 1.17 for heart and 0.27 for liver in blue light), CAT (k/mg protein) (0.09 for heart and 2.63 for liver in white light; 0.11 for heart and 2.83 for liver in green light; 0.10 for heart and 2.75 for liver in blue light), or GSH (mg/g protein) (29.43 for heart and 21.93 for liver in white light; 31.48 for heart and 22.79 for liver in green light; 28.04 for heart and 22.40 for liver in blue light) activities

(Table 2). However, higher stocking density increased CAT activity in cardiac tissue ($P < 0.05$). Furthermore, the 200 cm^2/bird group had significantly higher CAT and GSH activities in liver tissue than the 100 cm^2/bird group ($P < 0.01$ and $P < 0.05$, respectively). In this investigation, there was no relationship between light colour and stocking density for MDA level, SOD, CAT, and GSH activities in quails.

The correlations among hematological parameter values within quails are presented in Table 3. Lymphocytes had a significant negative correlation with heterophile, eosinophil, monocyte, and H/L values (-0.879, -0.391, -0.248, and -0.727, respectively). A negative correlation of r= -0.236 (P<0.01) was observed between eosinophil and basophil groups.

Discussion

The LED light hue had a strong influence on the heterophile, lymphocyte, eosinophil, basophil, and monocyte count in Japanese quails in our current study. Blue and green LED lights, in particular, increased lymphocyte percentage when compared to the effects of white light. Furthermore, green LED lights had a lower heterophile proportion than both blue and white LED lights. Notably, both blue and green light sources reduced the H/L ratio substantially. Similar results were obtained in other studies on other poultry species (1, 3, 7, 14). Because of the medium wavelength (about 560 nm) of the LED light, the lowered H/L ratio seen with green LED light in our study implies its beneficial impact on stress reduction and subsequent increases in welfare. It could be, that green light likely reduces stress in quails by promoting a more balanced immune response, improving circadian rhythms, and inducing a calming effect. This results in a lower H/L ratio, suggesting that green light may have a positive impact on quail welfare, particularly in terms of stress reduction. Similar to the effects reported on lymphocyte percentages and the H/L ratio, the use of blue and green LED lights resulted in lower eosinophil percentages when compared to white light exposure. While the precise role of eosinophils in avian biology is unknown, they are thought to function similarly to mammalian counterparts and are linked to glucocorticoid levels. However, the relationship between higher eosinophil counts (eosinophilia) or decreased eosinophil granulocytes (eosinopenia) and increasing glucocorticoid levels remains unclear (15). Eosinophil numbers often have an inverse connection with stress levels. They may, however, increase as a result of parasite infection or inflammation, making them potentially useful for distinguishing between infection and chronic stress (16). Furthermore, when compared to white light, blue light was found to decrease the number of monocytes while increasing the percentage of basophils. This finding contradicts the expected increase in basophil numbers and decreases in monocyte counts, which is especially noticeable in broiler chickens suffering from acute heat stress. In summary, the observed disparity between expected stress changes and the effects of blue light shows that the influence of light on immune cells may include distinct mechanisms that are not purely stress-driven. It alludes to the complexities of biological responses to various stimuli, as well as the necessity for further research to completely know the specific mechanisms underlying these cellular changes under various environmental situations.

Assessing stress and the balance between antioxidants (SOD, CAT, GSH) and oxidants (MDA-lipid peroxidation) is crucial for biological balance. Stress can increase oxidant production, resulting in a decrease in antioxidants (17). The colour of the LED light did not affect MDA levels, SOD, CAT, or GSH activities in the heart and liver of quails in our investigation. This is the first study to look at the effects of LED light colour on quails reared at densities of 200 and 100 cm^2/bird. Studies have shown that different light colours have different effects on oxidative metabolism in serum and different tissues, especially in broiler chickens (18, 19). Aside from light exposure, a variety of factors influence oxidative stress and antioxidant activity, including food, environmental circumstances, stocking density, and genetic factors. These variables may have interacted differently in our study than in other studies, resulting in different outcomes. Furthermore, various tissues may react differently to light exposure. While our study concentrated on the heart and liver, future studies exploring different tissues or organs may reveal differences in reactivity to LED light colour.

In the present study, it was determined that high stocking density had no significant effect on the H/L ratio in quails. The lack of a significant difference in H/L ratios between the low and high stocking density groups could be due to a combination of factors such as the birds' adaptation to stocking density, mitigating effects of other environmental variables (such as green light), individual variations in stress response, and the experimental conditions under which the measurements were taken.

Stocking density also did not show a significant effect on oxidative stress in the heart and liver, similar to Cengiz et al. (2015) (20). The high density of 100 cm^2/bird in our study lowered liver CAT and GSH activities, which is similar to Koç Yildirim et al. (2023) (21). However, Sevim et al. (2021) found no variations in SOD and GSH activity in broilers with different stocking densities (22). These diverse consequences illustrate the intricate link that exists between an organism's responses and external circumstances. Understanding this complexity is critical for animal well-being, yet varying research methodologies, housing, and management practices may contribute to inconsistencies in stress research in animals.

Conclusion

Finally, the study discovered that the colour of LED lights impacts numerous hematological parameters and welfare indicators in Japanese quails. Quails exposed to green LED light showed a decreased stress ratio, which could help their well-being. Stocking density had no discernible effect on hematological values. Different LED colours did not affect oxidative stress markers in the heart and liver. Studying how LED color and stocking density interact is crucial for understanding quail stress. More research is needed to improve bird welfare practices.

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Conflict of Interest. The datasets used and/or analyzed during the current study are available from the corresponding author (Evrim DERELİ FİDAN) upon reasonable request. The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Author Contributions. The study's inception and design were contributed to by all authors. Evrim DERELI FIDAN and Ece KOC YILDIRIM prepared the materials, collected data, and analyzed the results. Evrim DERELI FIDAN wrote the first draft of the manuscript, and all contributors provided feedback on prior draughts. The final manuscript was read and approved by all writers.

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Vpliv barve led-svetlobe in gostote živali na nekatere hematološke parametre in oksidativni stres pri japonskih prepelicah

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Izvleček: Študija je ocenjevala učinke barve svetlobe svetlečih diod (LED) in gostote naselitve na hematološke parametre, oksidativno presnovo in težo organov prepelic. Več ključnih dejavnikov upravljanja, ki vplivajo na dobro počutje brojlerjev, vključuje barvo svetlobe in gostoto naselitve. Zato je bil namen te študije ugotoviti učinek različnih barv svetlobe in gostote naselitve na hematološke parametre in parametre oksidativnega stresa pri japonskih prepelicah. V ta namen so bile pri prepelicah, ki so bile izpostavljene različnim barvam svetlobe in gostoti naselitve, izmerjene ravni malondialdehida (MDA), superoksid dismutaze (SOD), katalaze (CAT) in glutationa (GSH) z uporabo komercialno dostopnih kompletov ELISA. V študiji je bilo 720 enodnevnih japonskih prepelic (*Coturnix coturnix japonica*) naključno razvrščenih v eno od šestih obravnav, vsaka s štirimi dvojniki po 30 ptic. Poskus je bil zasnovan kot faktorska razporeditev obdelav 3 x 2 s tremi barvami LED-svetlobe (bela, modra in zelena) in dvema gostotama naselitve (nizka = 200 cm^2/ptico; visoka = 100 cm^2/ptico). Pri starosti 42 dni je bilo naključno izbranih 20 prepelic iz vsake skupine za analizo nekaterih hematoloških parametrov. Glede na dobljene rezultate je bilo razmerje med heterofilci in limfociti (H/L) znatno višje pri prepelicah, izpostavljenih beli LED-svetlobi, vendar vpliv gostote naselitve na razmerje H/L ni bil značilen. Na indikator oksidativnega stresa MDA barva svetlobe ni vplivala, je pa visoka gostota naselitve drastično zmanjšala aktivnosti CAT in GSH v jetrih. Teža srca je bila manjša pri prepelicah, ki so bile izpostavljene modri LED-svetlobi. Gostota naselitve ni vplivala na maso srca in jeter. Zaključimo lahko, da bela LED-svetloba sicer poveča razmerje H/L in stresne razmere, vendar ne vpliva na kazalnike oksidativnega stresa. Te ugotovitve so poudarile potrebo po določitvi optimalnih barv LED-svetlobe za prepelice v komercialnih proizvodnih okoljih, da bi povečali dobrobit jate. Za razumevanje učinkov in iskanje najboljše barve LED-svetlobe za prepelice pri različnih gostotah naselitve je potrebnih več raziskav.

Ključne besede: prepelice; LED-svetloba; razmerje med heterofilci in limfociti; oksidativni stres; gostota naselitve