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PROOF

RESEARCH PAPER

Exposing Hatching Quail Eggs to High Light Intensity and Its Effect on Post-Hatch Testicular Histology

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Abstract

The objective of this study was to monitor the effect of exposing hatching quail eggs to high light intensity on testicular function Quail eggs were distributed into four groups; dark application during the 18-day incubation period (C), light application during the 18-day incubation period (L), 5 days of light + 13 days of darkness (5L), and 5 days of darkness + 13 days of light (5K) and incubated. After the hatch, 40 chicks were selected from each group and placed in rearing cages. On day 42 after hatching the quails were weighed and their testes were removed and then embedded in paraffin. The blood glucose level of the 5K group was higher than the blood glucose level of the L group, but there was no statistically important difference ($P<0.05$) between the L, C, and 5L groups. The diameters of seminiferous tubules were bigger in the 5L group as compared with the L group. The groups treated with light had bigger tubule diameters than the control group. The number of Sertoli cells was significantly lower in the C group. Incubating quail eggs under five days of darkness and thirteen days of light have a favorable effect on post-hatch male fertility potential.

Introduction

Light is one of the important environmental factors affecting reproduction via the hypothalamic-pituitary-gonadal axis (Olanrewaju et al., 2006). Day length, light intensity, and light colors affect the anatomic, metabolic, and reproductive function of quail (Oishi and Lauber, 1973; Olanrewaju et al., 2006; Deep et al., 2012). Many studies have been conducted to show the impact of light on hatching rate, hatchability, and embryonic mortality (Özkan et al., 2012; Kaya and Aygün, 2019; Maman et al., 2018). But, the data related to the effect of light intensity on reproduction is limited. Therefore, there is a growing trend of studies on the application of different light intensities and colors to increase reproductive performance (Retes et al. 2017). According to a study, exposing the eggs for 12 and 24h/day under 250 lx light intensity caused an increase in hatching rate and hatchability (Riaz et al., 2021). Exposing eggs to light has been reported to relieve stress on embryos (Huth and Archer 2015) and help them hatch. Riaz et al. (2021) have also reported that stress susceptibilities such as heterophil to lymphocyte ratios were significantly lower in eggs exposed 12 h/day under 250 lx light intensity. Therefore, during the incubation, exposing the eggs to light relieves the stress and causes the hatched chicks to start their life with better self-confidence (Archer and Mench 2014).

The effect of exposing hatching quail eggs to high light intensity on blood glucose levels has not been studied in quail. But, some studies were performed to

measure the effect of the lightning regime on blood glucose levels in broiler breed hens. Broiler eggs were incubated totally under darkness, 12 h of lightness and 12 h of darkness, and 24 h of lightness. It was reported that blood glucose concentration (mmol/L) was increased by 12 h and 24 h lightness as compared to eggs incubated in darkness. The glucose concentration in the 24 h lighting group was also significantly high over the 12 h lighting group (Yameen et al., 2020).

The effects of light intensity on reproductive performance and hormone concentrations have been largely studied in poultry. But, not enough studies have been performed on quail, especially on the performance of male quail. In a study, male quail were exposed to six different types of lamps (incandescent, white fluorescent, or blue, white, red, or green light-emitting diode (LED). It was reported that testicular weight was higher in compact fluorescent and red LED bulbs as compared to other groups. In white LED groups, the area of the seminiferous tubules was higher, while no differences were seen in sperm concentration, motility, or fertility rates (Retes et al. 2017). Retes and his colleagues (2017) performed this study on one day old male Japanese quails after hatch. But, we do not know the impact of light on incubating eggs and its effect on the reproductive and metabolic function of hatched male quail after the incubation. Therefore, the objective of this study was to monitor the effect of exposing hatching quail eggs to high light intensity on testis volume, the number of Sertoli cells, and blood glucose level.

Materials and Methods

Eggs and Grouping

A total of 660 hatching quail eggs were used in this study, which were purchased from a private farm in Konya. The eggs were randomly distributed into four groups (C, L, 5L, and 5K). Each group consisted of 160 eggs and a separate tray was designated for each group. Experimental groups are displayed in Table 1.

Table 1. The number of incubated eggs and light application, in each group, during the incubation

Groups	The number of incubated eggs	Applications
C	160	18 days of darkness
L	160	18 days of light
5L	160	5 days of light and 13 days of darkness
5K	160	5 days of darkness and 13 days of light

Light Source and Intensity

White Light-emitting diode (LED) bulbs were used as a light source. The white LED was placed approximately 10 cm above the eggs.The light intensity was measured at different points on egg trays, ranging from 5000 to 6000 lux, and it was manually fixed by adjusting the distance to the LED.

Temperature and Humidity

For the first 14 days of incubation, the temperature and humidity within the incubator were set to 37.5 °C and 55-60% respectively. For the last three days of incubation, the temperature, and humidity were adjusted to 37.2 °C and 75%.

Chick Rearing and Feeding

After the hatch, 40 chicks were randomly selected from each group and placed in rearing cages. Each rearing cage consisted of 5-layer plastic structures and each layer had a width of 60 cm and a length of 120 cm (60x120). Stocking density was adjusted to ten centimeters area per chick. During the 42 days of the growing period, chicks were fed with a diet providing 24% crude protein and 2900 kcal/kg metabolic energy. In the first week, the ambient temperature is set at 30-33 °C. Every week, the temperature was reduced by 3 °C until it reached 21 °C. The lightning program is set to continuous light for the entire rearing period.

Measurements of Blood Glucose Levels

After the ten-day growing period, six chicks were randomly selected from each group and weighted by using precision balances (Radwag, USA). Blood glucose levels were measured using a blood glucose meter (IME-DC, Germany). A drop of blood, from the wing, was placed on the test strip of the blood glucose meter, and the blood glucose level was measured.

Measurement of Body Weight, Testes Weight, and Its Fixation

Quails were weighed on day 42 after hatching using a balance, with 0.01 sensitivity and then slaughtered. After the slaughter, their testes were carefully removed and weighed. Testes weights were plotted in g and expressed as a percentage of bodyweights. Dissected testes were kept in 10% formalin for tissue examination.

Measurements of Diameter of Seminiferous Tubules and the Number of Sertoli Cells

Tissue samples were fixed in 10% formalin for 24 hours and dehydrated with graded alcohol then embedded in paraffin. Embedded tissues were cut at 5 µm thickness and placed on glass slides and stained with hematoxylin and eosin solutions by using standard paraffin-embedding methods.

The diameters of seminiferous tubules and the number of Sertoli cells were differentiated (Figure 1) and measured by using an image processing and analysis system (ZEN 2012 SP2).

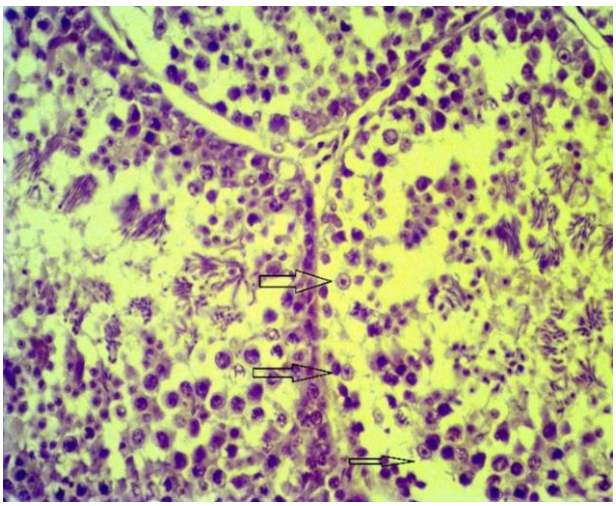

Figure 1. Photomicrograph of Sertoli cells, indicated with arrows, differentiated by a big white nucleus and dark small nucleolus

Statistical Analysis

For the statistical analysis of the data, initial assumptions regarding parametric tests were questioned. For this purpose, the Kolmogorov-Smirnov and Levene tests were used to determine normality and variance homogeneity, respectively. After meeting the parametric test assumptions for all variables, the hypothesis tests were conducted using variance analysis. When the difference between groups was found to be statistically significant, Tukey's multiple comparison test was used to identify its cause. All statistical analyses employed a significance level of 0.05 and were conducted using Minitab software.

Results

The data relating to body weights, testicular weights (g and %), and blood glucose levels in the C, L, 5L, and 5K groups were displayed in Table 2. The C, L, 5L, and 5K groups had testes weights of 5.86 g, 4.71 g, 5.62 g, and 5.94 g, respectively. But, no statistically significant differences between the groups were seen. Testis weights, as a percentage of bodyweight, were determined as 3.37%, 2.89%, 3.17%, and 3.46% in the C, L, 5L, and 5K groups, respectively, with no statistically significant variations between the groups. The blood glucose level of the 5K group (313 mg/dL) was higher than the blood glucose level of the L group (272 mg/dL), but there was no statistically important difference ($P<0.05$) between the L group, C (291 mg/dL) and 5L (289 mg/dL) groups. The differences in blood glucose levels between the 5K group (313 mg/dL), group C (291 mg/dL), and group 5L (289 mg/dL) were also not statistically important (Table 2).

Data showing the impact of light intensity on tubule diameter and Sertoli cell number were displayed in Table 3 and Figure 2. The diameters of seminiferous tubules were greater in the 5K group than the diameter of tubules in other groups ($P<0.05$, Table 3, Figure 2). The diameters of seminiferous tubules were significantly smaller in the C group as compared to the others ($P<0.05$). The diameters of seminiferous tubules were bigger in the 5L group as compared with the L group ($P<0.05$, Table 3, Figure 2). The groups treated with high light had bigger tubule diameters than the control group ($P<0.05$). The number of Sertoli cells was significantly lower in the C group than the number of Sertoli cells in the other groups ($P<0.05$, Table 1).

Discussion

Light intensity during incubation, as well as light colour, affect the bodyweight of birds. In a study, quail eggs were incubated under white LEDs, green LEDs, red LEDs, and darkness (control). Growth rates, after the hatch, were higher ($P<0.05$) in birds from eggs incubated under dark and white LED (Coelho et al., 2021). In another study, the positive effect of continuous lightning during incubation on body weight at hatching was reported by Farghly and Mahrose (2012). According to their report, birds produced from eggs exposed to light during the incubation period had significantly higher ($P\leq0.05$) post-hatch daily weight gain (Farghly and Mahrose 2012). In another study, the effect of different light sources on the post-hatch bodyweight of female Japanese quail was also studied. It was reported that; the lowest body weight score was obtained under compact fluorescent light (Bobadilla-Mendez et al., 2016). In another study, the effect of different types of lambs on the bodyweight of post-hatch male Japanese quail (Coturnix coturnix japonica) was studied. Higher body weight scores were observed with incandescent lam 35 days after hatching (Retes et al., 2017). In this study, the light intensity did not affect the body and testes weights 42 days after hatching. Here, we use the light intensity of 5000 to 6000 lux during the incubation and we measured body weight at 42 days of age. This might be the reason why we could not see significant differences.

Exposure of quails to different light intensities and colors can influence testiscular development. According to a study, higher testicular weights were observed in quails reared under a compact fluorescent bulb at 35 days of age. But at 57 days of age, the highest testicular weights were observed in quails reared under white LED (Retes et al., 2017). According to another study, conducted on three weeks old sexually immature male Japanese quails, testicular volume and seminiferous tubule diameters were significantly higher in quails

Table 2. Body and testes weights and blood glucose levels in experimental groups

Group	Bodyweight (42 d; g)	Testes weight, g	Testes weight (%)	Glucose (mg/dL)
C	173.48	5.86	3.37	291[ab]
L	162.79	4.71	2.89	272[b]
5L	176.78	5.62	3.17	289[ab]
5K	171.78	5.94	3.46	313[a]
SEM	5.932	0.407	0.202	9.230
P	0.418	0.150	0.217	0.042

[a-b]Data with different superscripts in the same column are statistically different (P<0.05)

Table 3. The diameters of seminiferous tubules and the number of Sertoli cells in testes of quails in different experimental groups

Groups	Diameters of seminiferous tubules (µm)	Count of Sertoli cell
C	86.10[d]	3.60[b]
L	119.19[c]	4.57[a]
5L	152.70[b]	4.67[a]
5K	169.91[a]	5.07[a]
SEM	3.68	0.16
P	0.001	0.012

[a-d] Data with different superscripts in the same column are statistically different (P<0.05)

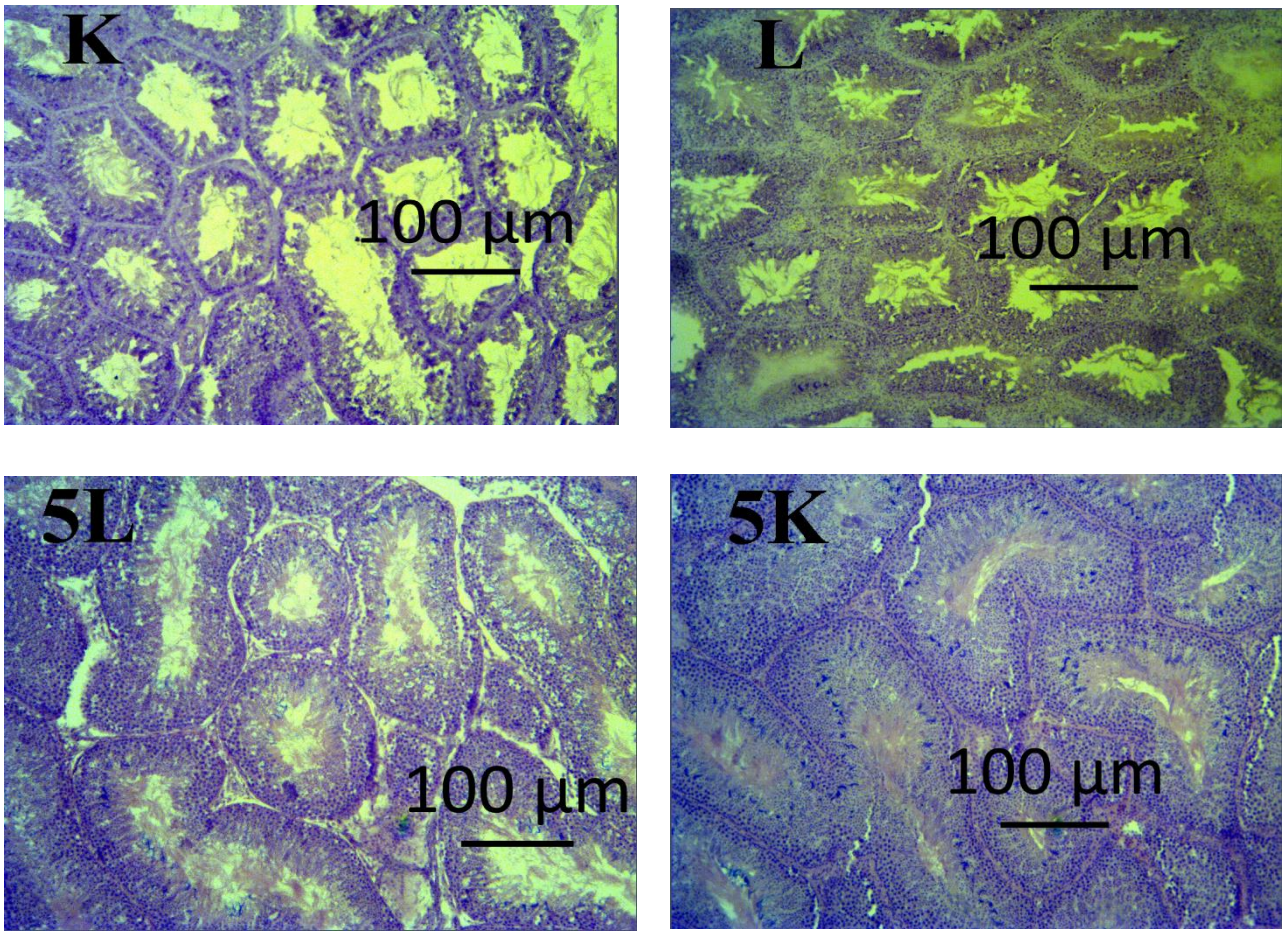

Figure 2. Photomicrograph of seminiferous tubules. The smallest diameters of seminiferous tubules were measured in the control group (K). Lightning increased the diameter of seminiferous tubules as compared with that of the control group. The biggest diameters of tubules were measured in the 5K group as compared with other groups.

exposed to 100 lux white fluorescent light as compared with quails exposed to 30 lux white or blue LEDs (Yadav and Chaturvedi, 2015). According to Coelho et al. (2021), no difference in the anatomical and histological characteristics of the testes due to the light treatment during the incubation except for the diameter of the seminiferous tubules, which was greater ($P < 0.05$) in the dark and in the white LED groups. According to Retes et al. (2017), semen volume and sperm concentrations were not statistically different due to the light treatment, except for sperm motility, which was higher ($P < 0.05$) in birds from eggs incubated in different colors of light.

According to our results, exposing the incubating eggs to 5000-6000 lux white LED increased post-hatch seminiferous tubule diameter and the number of Sertoli cells at 42 days of age as compared to that of chicks hatched from eggs incubated in the dark. Even though we used different intensities of light, our results are partly in parallel with the results of Coelho et al. (2021). In a study, broiler eggs were incubated under darkness, under 12 h of lightness, and under 24 h of lightness. Blood glucose concentrations, post-hatch, were significantly increased under 12 and 24 h of lightness as compared with eggs incubated in the dark (Yameen et al., 2020). Our results show that incubation of the egg under light significantly increased post-hatch blood glucose concentration as compared with the post-hatch blood concentrations of chicks hatched from eggs incubated in darkness. Even though the species of animal, application, and intensity of lightning are different our results are rare partly in agreement with the results of Yameen et al. (2020). In this study, our expectation was to see increases in the diameter of seminiferous tubules and related increases in the number of Sertoli cells by light application. This expectation was partly met as the diameter of seminiferous tubules increased by light application as compared with the control group. But more research is required to monitor the effect of light application during the incubation period on the hatchability of eggs produced during the breeding period.

Conclusion

Incubating quail eggs under five days of darkness and thirteen days of light have a favourable effect on post-hatch male fertility potential.

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