Effects of monochromatic light stimuli during embryogenesis on some performance traits, behavior, and fear responses in Japanese quails

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ABSTRACT Lighting is crucial in poultry rearing and the subjects with light intensity, source, and color having been addressed in numerous studies. Numerous studies with monochromatic light from light-emitting diode (LED) bulbs have been reported. In the current study, fertile Japanese quail eggs were exposed to a dark environment (Control) or monochromatic green (560 nm) and blue (480 nm) lighting throughout incubation. There were no significant differences in hatch weight, hatchability, total embryonic mortality, hatch time, growth performance, and slaughter-carcass traits in the study ($P > 0.05$). Furthermore, the lowest mean in terms of early embryonic mortalities (12.37%) was determined in the group treated with green LED lighting ($P < 0.05$), whereas it was discovered that the lowest mean in terms of late embryonic mortalities (13.59%) was in the group treated with blue LED lighting ($P < 0.05$). During the test time, the green LED group showed higher averages in terms of the number of peeps and first defecation time as response to environmental stimuli ($P < 0.05$). The highest mean for jumping (7.6 times) was detected in the group treated with blue LED lighting ($P < 0.05$). In conclusion, it was revealed that the blue and green LED lighting applied to the Japanese quail eggs in incubation had no effects on incubation traits, growth, and slaughter-carcass traits but had positive effects on some behavioral traits.

Key words: monochromatic lighting, embryonic mortality, hatchability, carcass, open-field test

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INTRODUCTION

The effects of the incubation environment can be observed throughout embryonic development and the life span of birds. These environmental factors include temperature, humidity, ventilation (William et al., 1991), air quality (Lourens et al., 2007), sound (Tong et al., 2015), and light (Erwin et al., 1971; Lauber, 1975; Rogers and Krebs, 1996; Özkan et al., 2012b; Archer and Mench, 2014a). The fertilized eggs are generally incubated in the dark in commercial hatcheries and only rarely are lighting programs applied. Sometimes periodic lighting programs are applied in incubators, although rarely. There are some studies on the effects of these applications on embryonic development, hatchability, and post-hatch performance (Özkan et al., 2012a; Zhang et al., 2012; Huth and Archer 2015; Farghly and Mahrose, 2012). Most researchers have determined the application of conventional lighting in incubation to increase the live weight of embryos of various poultry species (Siegel et al., 1969; Cooper, 1972; Walter and Voitle, 1973; Coleman and McNabb, 1975). There are also reports that the application of conventional lighting in incubation positively affects hatchability and shortens the incubation period (Cooper, 1972; Shafey and Al-Mohsen, 2002; Shafey, 2004; Archer and Mench, 2014b; Huth and Archer, 2015). Rosenboim et al. (2003) have determined that some conventional lighting equipment emits additional heat into the incubator. Researchers have suggested that this additional heat may have led to the rapid development of the embryos and that lighting equipment that does not emit heat should be used (Rozenboim et al., 2003). The light-emitting diode (**LED**) lamp that does not emit ambient heat and can use different light wavelengths has recently been preferred for incubation lighting (Huth and Archer, 2015).

Having applied monochromatic lighting to broiler and turkey eggs in incubation by using LED light, Rozenboim et al. (2003, 2004) revealed that green light had positive effects on embryogenesis and post-hatch development for both species. Similarly, Zhang et al. (2012) also reported that the green LED lighting continually applied to broiler eggs in incubation accelerated embryogenesis, increased embryo weight, and post-hatch breast muscle weight. While the studies on lighting in incubation were generally carried out on chickens, few studies have been carried out with other

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species. Embryonic development and post-hatch growth have been the primary traits measured and there have been few studies on behavior and fear responses (Jones et al., 1992; Satterlee et al., 1993). There have been no studies on LED lighting and incubation in quail. There are spots on quail eggs and the subject of the LED light transmission of these spots arouses curiosity. In the studies carried out by Shafey and Al-Mohsen (2002) and Shafey et al. (2005), it was determined that the eggshell color affected the amount of light reaching the embryo, and the light transmission through dark eggshells was reduced. The objective of this study was to study the effects of green (560 nm) and blue (480 nm) monochromatic lighting on incubation and post-hatch growth traits in Japanese quail in addition to selected behavior responses.

MATERIALS AND METHODS

The experiment was conducted in the Faculty of Agriculture and the Faculty of Veterinary Medicine at Namik Kemal University. The care and use of animals were in accordance with the laws and regulations of Turkey and approved by the Ethical Committee of Namik Kemal University (Decision Number 02/04-10.27.2015). The study was carried out by using 3 homogeneous automatic incubators. The lighting in these incubators was provided through the LED lights attached to the 2 lateral walls and the back wall of the incubators and 560 nm of wavelengths and 480 nm of wavelengths were used for green and blue lights, respectively (wavelengths were measured quantitatively). The light intensity was measured with a luxmeter and varied between 1,400 and 1,650 lux according to the surface of the eggs. The conditions in the incubators were kept constant except for lighting. In the 3 incubators, a thermal environment was created to attain a temperature of 37.5 ± 0.1°C and 60% relative humidity and automatic rotation was applied hourly. In the last 3 d of incubation (day 15 of incubation), the eggs were taken to the hatching trays and a temperature of 37.2 ± 0.10°C and 70% relative humidity were provided in the incubators. The hatching eggs were obtained from a randombred breeder flock at 48 wk of age. To calculate embryonic mortalities and hatchability, macroscopic examinations were made on 328 hatching eggs in the control group, 204 hatching eggs treated with blue LED lighting, and 204 hatching eggs treated with green LED lighting that remained after eliminating the unfertilized eggs. In m acroscopic examinations, the deaths were determined in 2 ways, early and late embryonic period (Aygun et al., 2012; Aygun and Sert, 2013). Wing bands were attached to all hatched chicks and after the chicks had dried, their individual live weights were measured. The quail chicks, whose wing numbers were attached and which were divided into 3 groups, were individually weighed every week. A total of 10 randomly selected quail chicks from each incubation light source were used for an open-field test determination. A 25 cm × 25 cm square grid was divided into 5 cm × 5 cm individual squares within an entirely white wood box. There are some reactions of birds as a response to environmental changes and fear situations, some of these are vocalization, flight, jumping, and escape (Davis et al., 2008). In this study, we focused on observations of peeping and jumping. For the open-field test, peeps and behaviors were recorded for 10 min starting from the moment individual chicks were placed in the middle of the grid (Rodenburg et al., 2003; Saito et al., 2005). These video recordings were watched 3 times each and the first peep time, the first moving time, and the first defecation time of a chick, the total number of squares traveled, the total number of peeps, and the total number of jumping behaviors were recorded. Quails were grown in mixed sex throughout the treatment. Until sex determination was performed on the 21st day following the hatching of the quails, they had been kept in mixed sex condition in 6-story and heated growing cages containing divisions with dimensions of 96 cm × 43 cm × 21 cm on each story. At these cages, the chicks were kept at 32°C on the first 3 d and the ambient temperature was set to be 27°C at the end of the second week by decreasing the temperature by 1°C every 3 d. The lighting program post hatch was 18 h of light and 6 h of dark in this period and all quails were fed a mash diet containing 24% CP and 12.14 MJ/kg ME. The quail chicks were kept at 6-story fattening cages having divisions with dimensions of 85 cm × 55 cm × 50 cm after the 21st day (Narinc et al., 2013). The quails were again fed the same fattening feed (24% CP and 12.14 MJ/kg ME) between the 21st and 56th days and the quails were treated with 23 h of lighting daily. Tonic immobility (**TI**) is an unlearned response characterized by a catatonic-like state of reduced responsiveness to stimuli, and is elicited by a brief period of physical restraint. Reduced time to come out of TI indicates reduced fearfulness; on the contrary, increasing TI time indicates that the level of fear is higher. At 21 d and 42 d, tonic immobility was measured on 15 quails from each incubation lighting treatment. Briefly, each quail was gently placed onto a cradle in the supine position with its head hanging down and gently held for 15 s until the animal calmed down. The maximum value in the measurement of the tonic immobility time was determined as 5 min (300 s) (Campo and Davilla, 2002). The quails were slaughtered at 56 d of age. Before the slaughter, feed had been removed for 4 h and the slaughter weights of the quails had been determined. Their hot carcass weights including the neck and abdominal fat but excluding the edible inner organs (liver, gizzard, heart) were calculated following the slaughter, wet plucking, and dissection. At this stage, the abdominal fat and the edible inner organs consisting of the heart, the liver, and the empty gizzard were weighed. After the carcasses had been left at +4°C for a day, their cold carcass weights were measured, the carcasses were dissected with breast muscle weights, leg weights, and wing weights determined. By calculating the ratios of the cold carcass, edible inner

Table 1. The least-square means and statistical analysis results for hatch weight, hatchability, and embryonic mortality traits of the treatment groups.

Characteristics	Control (n = 328)	Blue (n = 204)	Green (n = 204)	*P*
Hatch weight, g	8.12 ± 0.04	8.17 ± 0.04	8.13 ± 0.04	0.655
Hatchability, %	70.82 ± 0.76	69.02 ± 0.76	70.43 ± 0.76	0.702
Early embryonic mortality, %	14.07 ± 0.26^{b}	17.39 ± 0.27^{a}	12.37 ± 0.25^{c}	0.001*
Late embryonic mortality, %	15.11 ± 0.19^{b}	13.59 ± 0.18^{c}	17.2 ± 0.19^{a}	0.005*
Total embryonic mortality, %	29.18 ± 0.48	30.98 ± 0.48	29.57 ± 0.48	0.702

$^{a–c}$Means within a row with no common superscript differ significantly ($P < 0.05$). Results are presented as mean ±SE.

organs, abdominal fat, breast, leg, and wing weights to the slaughter weight, phenotypic values were obtained for the cold carcass ratio, the ratio of edible inner organs, the abdominal fat ratio, the breast ratio, the breast muscle ratio, the leg ratio, and the wing ratio, respectively.

The generalized linear mixed effect model with logit functions was employed at the 0.05 significance level in the statistical analysis of the binomial data about the embryonic mortalities and hatchability observed in the treatment groups and the differences among the groups were analyzed with the Tukey–Kramer method— a multiple comparison test suitable for this method. An analysis of variance was applied to compare the data of the quails concerning the open-field test, their tonic immobility times, and the phenotypic values for the slaughter-carcass traits in terms of the treatment groups and the groups were compared with Duncan's test—a multiple comparison test suitable for this method—for the variables for which statistically significant differences were found at the 0.05 significance level. The technique of profile analysis, one of the methods of multivariate analysis of variance (MANOVA), was employed to test the difference in terms of the growth examples among the groups according to the weekly values of individual live weights. In the study, the SAS program was used in all statistical analyses and the procedures of GLIMMIX and GLM in the program were utilized in the analyses concerned.

RESULTS AND DISCUSSION

The means for hatch weight, hatchability, and embryonic mortality concerning the treatment groups kept in a dark environment (control) or exposed to blue and green LED lighting during embryonic development are presented in Table 1. No statistical differences in hatch weight, hatchability, and total embryonic mortality were observed among the experimental groups ($P > 0.05$). There are some studies which report that different LED lighting applications to hen eggs in incubation did not affect the traits of hatch weight and hatchability (Özkan et al., 2012a; b; Zhang et al., 2012). Similarly, Rosenboim et al. (2003), who applied green and white monochromatic lighting to turkey eggs throughout incubation, reported that there were no differences in hatch weight and hatchability between the chicks incubated in a dark environment and those treated with monochromatic lighting. The findings determined in this study were found in agreement with the results reported by Rosenboim et al. (2003), Özkan et al. (2012a,b), and Zhang et al. (2012). On the contrary, Huth and Archer (2015), who applied LED lighting and darkness to broiler breeder eggs in incubation, reported that the chicks obtained from the dark environment were heavier. In the study by Huth and Archer (2015), it was discovered that the application of LED lighting to layer breeder eggs in incubation had no effect on hatchability. Having applied conventional lighting to Japanese quail eggs in incubation, Farghly and Mahrose (2012) reported that this application had a positive effect on hatchability. Findings similar to the results determined in this study in terms of total embryonic mortalities were also reported by Huth and Archer (2015) and Archer (2015), who provided the broiler breeder eggs with brown eggshells and the layer breeder eggs with white eggshells with LED lighting and dark conditions. Having incubated the Japanese quail eggs with white, spotted violet, and spotted brown eggshells under conventional light and dark conditions, Farghly et al. (2015) similarly reported that embryonic mortalities were unaffected by lighting. In the study, the lowest mean in terms of early embryonic mortalities (12.37%) was determined in the group treated with green LED lighting ($P < 0.05$), whereas it was established that the lowest mean in terms of late embryonic mortalities (13.59%) was in the group treated with blue LED lighting ($P < 0.05$). No study on alternate green and blue LED lighting in the incubation period is available in the literature. It may be recommended to investigate the effect of applying green light in the first half of the period of embryogenesis of Japanese quails and blue light in the second half of it on embryonic mortalities in the following studies.

The effects of the monochromatic lighting programs at different wavelengths applied in incubation on the hatch times of the chicks are presented in Figure 1. At 400 h of incubation, hatched chicks in the blue, green, and control light treatments were found as 0.76, 13.91, and 6.54%, respectively and this increased to 17.56, 63.48, and 81.78% at 410 h. All hatched chicks were obtained at the 428th hour in the control group, at the 434th hour in the green light group, and at the 438th hour in the blue light group ($P > 0.05$).

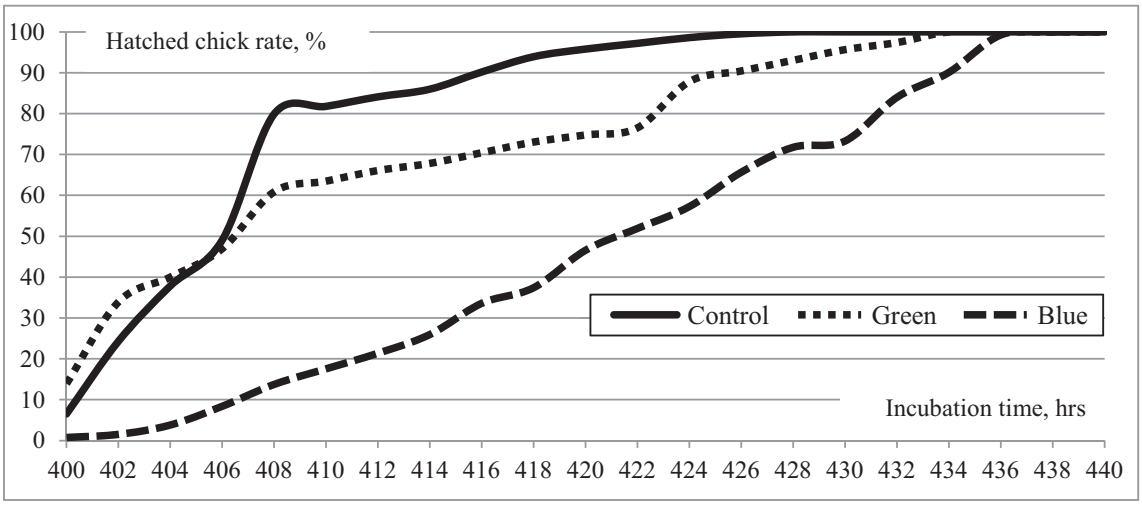

Figure 1. Distribution of hatched chick rates by treatment group in the last 40 h of incubation.

Table 2. Open-field responses of the treatment groups.

Criteria	Control (n = 10)	Blue (n = 10)	Green (n = 10)	*P*
Freezing time, s	127.30 ± 33.64	204.00 ± 33.64	229.80 ± 33.64	0.100
Jumps, no	3.90 ± 1.51^{a,b}	7.60 ± 1.50^{a}	0.50 ± 1.51^{b}	0.010*
Vocalizations, no	286.10 ± 49.22^{b}	424.40 ± 49.22^{a,b}	496.80 ± 49.22^{a}	0.017*
Sectors entered, no	218.70 ± 38.76	284.80 ± 38.76	163.40 ± 38.76	0.104
First peep time, s	9.88 ± 2.63	4.78 ± 2.63	6.74 ± 2.63	0.396
First defecation time, s	238.00 ± 126.83^{a,b}	156.44 ± 59.79^{b}	457.67 ± 73.23^{a}	0.021*
First moving time, s	5.33 ± 1.71	8.62 ± 1.71	4.35 ± 1.71	0.200

$^{a–c}$Means within a row with no common superscript differ significantly ($P < 0.05$). Results are presented as mean ± SE.

Nevertheless, it is known that the conventional lighting equipment used in these studies emitted additional heat into the incubator and that the incubation time was shortened accordingly. Having applied fluorescent lighting to broiler breeder eggs in incubation, Özkan et al. (2012a) reported that there was no difference between the hatch times of the embryos treated with the dark and light in the study, where the eggshell temperatures were not different either. The results obtained about the incubation time in our study were found in agreement with the findings reported by Rosenboim et al. (2003), Özkan et al. (2012a), and Zhang et al. (2012). Farghly et al. (2015), who reported similar results, incubated Japanese quail eggs with white eggshells under incandescent light and dark conditions in incubation. The researchers reported that the mean incubation time of the group treated with light was 396.60 h, whereas the mean of the group treated with the dark was 397.10 h and that there was no difference between the groups.

The physiological and behavioral development of chickens in the post-incubation period displays rapid changes day by day; therefore, the open-field test used to examine their responses to a new situation at the very beginning of life is essential (Balážová and Baranyiová, 2010). An open-field test is a widely employed method of evaluating the reactions to a new environment in both mammals and birds. Some stress occurs in a bird exposed to a new situation and environment that offer hardly any stimuli in an open-field arena (Balážová and Baranyiová, 2010). The capability to cope with this situation reflects the stability of the nervous system and the degree of individual excitability. Open-field responses reflect the tendencies to communicate with flocks or breeder parents and to minimize the risks of hunters. Horizontal and vertical locomotor activities and vocalization are the most addressed behaviors during an open-field test. The means for the first peep time, the first moving time and the first defecation time of the chicks, the total number of squares traveled, the total number of peeps, and the total number of jumping movements in the open-field test and the results of the analysis of variance are provided in Table 2. According to the results of the open-field test, no statistically significant differences in the means for the criteria of tonic immobility time, the number of squares traveled, the first peep time, and the first moving time were found among the experimental groups ($P > 0.05$). Besides, higher means in terms of the total number of peeps and the first defecation time (496.8 times and 457.67 s, respectively) were obtained from the chicks treated with green light in incubation ($P < 0.05$), whereas the highest mean for jumping (7.6 times) was determined in the chicks obtained from the eggs treated with blue light in incubation ($P < 0.05$). According to the results of the fear level tests performed on the 21st and 42nd days of the experiment, the mean TI times of

Table 3. Mean values for the weekly body weights of the treatment groups and statistical analysis results.

Time, d	Control (n = 93)	Blue (n = 95)	Green (n = 82)	MANOVA *P*
Hatch	8.14 ± 0.07	8.17 ± 0.09	8.11 ± 0.08	0.565
7	18.56 ± 0.35	18.73 ± 0.50	18.49 ± 0.51	0.933
14	46.82 ± 0.67	48.58 ± 0.89	46.73 ± 0.87	0.212
21	75.10 ± 1.25	77.80 ± 1.49	74.09 ± 1.27	0.146
28	104.08 ± 1.47	107.72 ± 2.06	103.89 ± 1.80	0.247
35	135.82 ± 1.74	137.09 ± 2.97	134.77 ± 2.59	0.804
42	154.59 ± 1.84	162.72 ± 3.10	156.72 ± 3.05	0.093
Wilk's Lambda (General *P*-value for profile analysis)				0.103

MANOVA, multivariate analysis of variance.

Table 4. The least-square means and statistical analysis results for the carcass traits of the treatment groups.

Characteristics	Control (n = 54)	Blue (n = 52)	Green (n = 48)	*P*
Cold carcass, g	110.20 ± 2.75	110.93 ± 2.62	109.22 ± 3.29	0.920
Cold carcass, %	62.31 ± 0.86	61.87 ± 0.84	63.76 ± 1.02	0.349
Edible inner organs, g	11.22 ± 0.36	10.33 ± 0.35	11.02 ± 0.43	0.191
Edible inner organs, %	6.24 ± 0.17	5.79 ± 0.17	6.37 ± 0.20	0.056
Abdominal fat, g	0.77 ± 0.11	0.72 ± 0.12	0.89 ± 0.13	0.598
Abdominal fat, %	0.43 ± 0.06	0.39 ± 0.06	0.51 ± 0.06	0.362
Leg, g	24.07 ± 0.59	23.63 ± 0.61	23.99 ± 0.71	0.861
Leg, %	21.83 ± 0.21	21.88 ± 0.22	21.99 ± 0.26	0.895
Wing, g	9.19 ± 0.38	9.86 ± 0.39	9.67 ± 0.46	0.449
Wing, %	8.35 ± 0.44	9.35 ± 0.45	9.07 ± 0.53	0.270
Breast, g	39.32 ± 1.08	38.02 ± 1.11	39.80 ± 1.30	0.540
Breast, %	35.77 ± 0.54	34.95 ± 0.55	36.47 ± 0.64	0.198
Breast muscle, g	29.56 ± 0.81	27.68 ± 0.83	27.76 ± 0.96	0.146
Breast muscle, %	26.88 ± 0.45	25.16 ± 0.47	25.43 ± 0.54	0.072

the quails in the control group, the green lighting group, and the blue lighting group were found as 26.80, 38.40, and 37.58 s as well as 39.13, 45.99, 53.97 s, respectively (data not shown in a table). No statistically significant difference was found among the TI means of the groups in either measurement ($P > 0.05$).

The weekly mean live weights of the quails in the treatment groups and the results of the statistical analysis are presented in Table 3. In the profile analysis, where the overall growth performances of the groups were compared, it was discovered that the profiles of the groups were parallel according to Wilk's Lambda significance level ($P > 0.05$). When the results of the MANOVA concerning the weekly mean live weights are examined, it is seen that there are no differences among the mean live weights of the experimental groups by weeks ($P < 0.05$ for all). The weekly mean live weights obtained from our study were found in agreement with the results of many studies (Omidiwura et al., 2016; Bagh et al., 2016).

The means and the statistical analysis results regarding the slaughter-carcass traits of the experimental groups are provided in Table 4. No statistically significant differences in the means for the cold carcass, edible inner organ, abdominal fat, breast, leg, wing, and breast muscle weights and ratios of the quails were found among the experimental groups ($P > 0.05$ for all). The means determined for the slaughter-carcass traits in the study were found in agreement with the results reported by Toelle et al. (1991) and Khaldari et al. (2010).

In conclusion, it was revealed that the blue and green LED lighting applied to the eggs of Japanese quails in incubation had no effects on incubation traits, growth, and slaughter-carcass traits but had positive effects on some behavioral traits. It is envisaged that the alternate repetition of blue and green lighting in incubation in similar studies will have positive effects on incubation traits. It might be stated that more studies should be carried out on this subject.

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