Poultry Studies, 22(2), xx-xx
http://doi.org/10.34233/jpr.1595820

EARLY VIEW
RESEARCH PAPER

POULTRY STUDIES

The Effect of Applying Different Coloured Lighting to Partridge (*Alectoris Chukar*) Eggs in Incubators on Hatching Results and Responses of Open Field Test

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Article History
Received: Oct 08, 2024
Accepted: Nov 01, 2024
First Online: Dec 03, 2024

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Keywords
Monochromatic light
Photoperiod
İncubation
Poultry
Behaviours

Abstract

The aim of this study is to ascertain the impact of dark and two distinct monochromatic lighting applications during incubation on the hatching characteristics of Chukar partridge (*Alectoris chukar*) eggs, as well as certain open field test characteristics following hatching. A total of 300 hatching eggs collected three consecutive days from partridges that mated by chance and were not subjected to selection were used in the study. All eggs were placed equally in same incubators and applied dark, blue, and green illumination. The highest hatchability of fertile eggs and hatchability of total eggs were found to be 89.7% and 87.9% in the blue group ($P<0.05$). It was determined that the reason for this situation was that embryonic deaths in the late period were higher in the group without illumination and in the groups with green monochromatic illumination. In addition, the effects of the lighting applications on the open field test performed after hatching on the characteristics such as movement, jumping and calling were found to be insignificant. As a result, it was determined that the incubation results obtained from the incubator illuminated with blue colour had a better effect than the other groups.

Introduction

Wildlife is damaged due to reasons such as global temperature change, wild animal trade, poaching and pesticides. According to the data of International Union for Conservation of Nature (IUCN), there are more than 157000 species on the IUCN red list and more than 44000 of them are in danger of extinction (IUCN, 2024). One of the ways to prevent this extinction in wildlife is to ensure that species produced and grown under intensive conditions are released into their wild environments and continue to survive. Protecting wildlife is important for protecting our genetic resources. Partridge, one of the birds of wildlife, especially unconscious hunting activities are one of the most important factors that reduce the number of this species. Every year, thousands of partridges raised in enterprises within the Ministry and Universities are released into nature after reaching a certain size. Considering that the production period for partridges is only 3-4 months and that only 20-30 eggs are produced per partridge, eliminating egg losses during incubation will be important for both the farmer and the country's economy (Şengül *et. al.,* 2016). When Türkiye's incubator enterprises were examined, it was determined that there was no lighting in the incubators and the eggs were incubated in a dark environment.

Studies have shown that different lighting colours positively affect hatching results. Zhang *et al*. (2014), a total of 880 chicken eggs with an average weight of 68 g were exposed to green (560 nm) monochromatic light in the incubator. The embryo that developed in the dark environment and the embryo exposed to green light were also examined at the molecular level. According to these results, it was observed that green light increased post-hatching muscle development by accelerating cellular division. In a different study, the effects of white light, green light and blue-green combinations on the embryo in the incubator were investigated. Green or green-blue light combinations have been shown to have an effect on growth performance and carcass development. LED light sources and their intensity are important in terms of energy consumption, cultivation costs and raising healthy individuals. In addition, the use of such lights provides calmer individuals (Gonngruttananun, 2014). In a different study conducted on brown layers (261 eggs) in the Aegean region, it was aimed to investigate the effect of white and green LED light application during incubation (21 days) on the production performance, behaviour and physiological stress and feather pecking behaviour of laying hens. It has been determined that green LED light application does not negatively affect egg production and may help reduce pecking behaviour, stress and anxiety (Dayıoğlu, 2018).

As a result of comparative statistical analysis of the data of 55 different experimental research articles, considering the effects of monochromatic green light stimulation on embryonic development, chick quality and hatching characteristics in broiler chickens; It was determined that green light stimulation did not affect chick weight and hatching performance, but significantly reduced hatching time (Tainika, 2019).

This study aimed to examine the effects of different coloured lighting (dark, blue and green) applied to partridge eggs in the incubator on hatching results and chick behaviour. In addition to high hatching efficiency, the hatching time of the chicks being close to each other (mass hatching) is also an important criterion for a business. In addition, an open field test was applied to chicks that had completed hatching and dried out.

Materials and Methods

The study was carried out in the summer of 2022 at the Livestock Facilities of Akdeniz University Faculty of Agriculture, Department of Animal Science, and partridge (*Alectoris chukar*) was used as animal material. Animal material was taken from Coşkunlar pheasant and partridge production farm, recommended by the 5th Regional Directorate of Nature Conservation and National Parks. A total of 300 hatching eggs obtained from a breeding partridge herd in 3 days were used in the experiment. Hatching eggs were brought to Akdeniz University Faculty of Agriculture Department of Animal Husbandry Facilities after a 300 km journey under suitable conditions. The eggs were placed in the incubator without any storage process, by measuring their width, length and weight. Three eggs were broken during the transfer. For this reason, trial groups were created with 99 eggs each.

The averages of hatching egg characteristics are presented in Table 1. The average egg weight was found to be similar as 40.96 g in the dark group and 40.75 g and 40.61 g in the green and blue lighting groups. The similar averages indicate that the eggs were successfully randomized. The width of hatching eggs is 21.37-20.99-21.30 mm in the dark, green and blue groups, respectively. The length of the eggs falling in the dark and blue treatment groups was 31.28 and 31.17 mm, respectively, and they were higher than the length of the eggs falling in the green light group (30.85 mm). The shape index averages of the eggs belonging to the groups are 67.17 and 68.29, and the averages are similar to each other.

In the study, a VGS 490 brand (54×67×150) cm incubator with a capacity of 840 partridge eggs, a combined development and hatching compartment, full environmental control and automatic cooling and heating motor was used. This machine has 10 shelves. Each shelf has a separate motor and individual hatching basket. After the incubation room and incubation machines are cleaned and disinfected, blue and green monochromatic (light intensity average of 200 lux) LED lighting mechanisms were prepared inside the incubators. The eggs in the groups were randomly placed in 3 different machines that were identical to each other.

1. Dark group (Control): There was no lighting in the incubator.

2. Green group: The incubator was illuminated with green light (~560 nm wavelength).

3. Blue group: The incubator was illuminated with blue light (~480 nm wavelength).

The temperature and humidity in the incubators were adjusted to 37.5 ± 0.1 °C - 55% during the development of the embryo and 37.2 ± 0.1 °C - 70% in the hatch (last 3 days). The shelves were rotated automatically every hour. As a result of the 24-day incubation period, hatched chicks, dead embryos under the shell and infertile eggs were calculated. In order to reveal differences in embryonic deaths in the experimental groups, macroscopic examination was performed on the eggs. In this process, embryos that were determined to die between days 0-14 of incubation were recorded as early stage, and embryos that were determined to die between days 15-24 were recorded as late stage.

- Hatchability of total eggs (%): (number of chicks hatched/number of total eggs) × 100
- Hatchability of fertile eggs (%): (number of hatched chicks/numbers of fertilized eggs) × 100
- Fertility: (number of fertilized eggs/number of eggs placed in the machine) × 100

- Hatching window: The periods between the first chick hatched and the last chick hatched were calculated (Kızılaslan and Şimşek 2019).

Open field test (chick's first call and first movement time, first defecation time, total number of squares navigated, total number of jumping movements) was applied by randomly taking 30 samples from chicks that had hatched and dried in each group. In this test, an all-white wooden box containing 25 (5×5) squares of size (25×25) cm was used. For the open field test, 10 min of song and behaviour recording was made after the chick was released into the square in the middle of the box (Rodenburg *et al*., 2003). These video recordings were watched three times to determine the chick's first call and first movement time, first defecation time, total number of frames visited, total number of calls and total number of jumping movements.

In the study, hypothesis tests were analyzed with one-way ANOVA, and 95% probability value was accepted as the acceptance limit of H0 hypothesis. Statistical analyses of the study were performed using SPSS 26 software.

Results and Discussion

Incubations results of the groups

The hatching results, early and late embryonic mortality detected in the experimental groups are summarized in Table 2. As can be seen from the relevant table, the fertility rate in the control, green and blue groups is 90.9%-88.9% and 99.0%, respectively; differences between means were insignificant. Early embryonic death was found to be similar at 4.4% in the control (dark) group, 5.3% in the green group and 4.1% in the blue group.

Differences between groups were found to be significant (P<0.05) in terms of late embryonic period deaths. The rate of late embryonic deaths detected in the blue group (6.2%) was lower than the rates detected in control (dark) and green light applications (17.8%-18.1%) (P<0.05). Total embryonic mortality rate was also significantly (P<0.05) affected by blue light application. The lowest embryonic mortality rate (10.3%) was average for the blue group. Hatchability of fertile eggs and hatchability characteristics were also significantly affected by lighting (P<0.05 for both). The highest hatchability of fertile eggs was found to be 89.7% in the blue group. The highest hatchability of total eggs was measured as 87.9% in the blue group.

When the hatching window of the incubation results is examined, at the end of the 24-day incubation period, hatching occurs on the 22^{nd} - 24^{th} day in the dark group, and on the 24^{th} - 28^{th} day in the green group and hatching in the blue group were collected between the 22^{nd} and 25^{th} days. It was determined that chick hatching in the dark group (control) started 2 days before the predicted day and lasted a total of 48 hours. In the green light group, chicks started hatching on the predicted day and the total hatching window lasted 98 hours. In the blue group, hatching started 2 days ago and the last chick hatching was recorded at 75 hours.

Behavioural test

The results of the open field test used to reveal the anxiety level of the animals are given in Table 3. Among the behaviours taken into account in the open field test, only the time of first defecation (seconds) was significantly (P<0.05) affected by the lighting application. The average time to first defecation was found to be similar in the dark and green groups (157.00 sec and 106.66 sec). The average determined in the blue lighting group (81.09 sec) is similar to the average in the green group, but lower than the average in the dark group (P<0.05).

Discussions

It is known that lighting practices positively affect the embryo before incubation and chick development afterwards (Karen *et al*., 2017; El-Sabrout and Khalil, 2017). Many studies have been conducted to reveal the best lighting colour, light intensity and duration in incubators (Yıldırım *et al.,* 2008; Yu *et al*., 2018; Wang *et al.,* 2020; Li *et al.,* 2021).

In this study, which investigated the effects of monochromatic blue and green light application in incubators on hatching results and behaviour, the highest hatchability of fertile eggs and hatchability of total partridge eggs was found in the blue group (89.7% and 87.9%) (P<0.05) (Table 2). Zhang *et al*. (2014), in a study they conducted on chicken eggs, determined that green light accelerated cell division and increased muscle development after hatching (Shavey and Al-mohsen 2002). Tainika (2019) conducted a study on the comparative statistical analysis of the data of 55 different experimental research articles, considering the effects of monochromatic green light stimulation on embryonic development, chick quality and hatching characteristics in broiler chickens, and found that green light stimulation did not affect chick weight and hatching performance, but reduced the incubation period. The hatching window of partridge eggs in the dark group spanned a shorter period (48 hours) than the hatching window of chicks in incubators illuminated with blue and green light and started 2 days earlier than expected. The hatching window for the eggs in the blue group also started 2 days earlier than expected but was spread over a 75-hour period. Blue light application reduced embryo mortality and increased hatchability compared to dark and green light applications. Studies have shown that such illumination studies conducted with eggs from different species are also effective in the results (Shavey *et al.,* 2004; Cooper *et al*., 2011).

It has been determined that different lighting applied to incubators not only positively affects the development of the embryo, but also affects stress factors (Archer and Mench, 2013). In this study, an open field test was applied to chicks that completed their development and hatched in environments with different colours. When Table 3 was examined, it was determined that the time to first defecation was shorter in the blue group. It is thought that this creates negative stress for the animal. In a different study, the relationship between the number of defecations and timidity in rats was examined. And it was concluded that taking animals from their accustomed environments and placing them in a new environment (separating from the group or herd and leaving them in an open area) causes anxiety (Çalışkan *et al*., 2017).

As a result, it is thought that a more detailed investigation of the effects of different lighting applications on stress and productivity in partridges will be economically important for businesses.

Ethical statement

This research was approved by Akdeniz University, Experimental Animals Application and Research Center local ethics committee for animal experiments.

Funding information

This study was supported by The Scientific and Technological Research Council of Turkey (Tübitak) within the scope of University Students Research Projects Support Program. Project number: 2209/A

Note: The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Table 1. Egg external quality characteristics*

Egg characteristics	Dark	Green	Blue	P
Weight, g	40.96 ± 0.28	40.75 ± 0.28	40.61 ± 0.28	0.689
Width, mm	21.37 ± 0.14	20.99 ± 0.14	21.30 ± 0.14	0.144
Length, mm	31.28 ± 0.08	30.85 ± 0.08	31.17 ± 0.08	0.082
Shape index %	68.27 ± 0.34	67.17 ± 0.34	68.29 ± 0.34	0.763

*The table gives averages values with standard error

Table 2. Fertility, hatchability of set eggs, hatchability of fertile eggs, and embryonic mortality according to light coloured*

Hatchability (%)	Dark	Green	Blue	P
Fertility**	90.9 ± 2.6	88.9 ± 2.6	99.0 ± 2.6	0.054
Early embryonic mortality	4.4 ± 2.2	5.3 ± 2.2	4.1 ± 2.1	0.922
Late embryonic mortality	17.8 ± 3.6^{a}	18.1 ± 3.5^{a}	6.2 ± 3.5^{b}	0.025
Total embryonic mortality	22.2 ± 44.1^{a}	23.4 ± 4.0^{a}	10.3 ± 3.9^{b}	0.036
Hatchability of fertile eggs***	77.8 ± 4.1^{b}	76.6 ± 4.0^{b}	89.7 ± 3.9^{a}	0.036
Hatchability of total eggs****	70.7 ± 4.2^{b}	72.7 ± 4.2^{b}	87.9 ± 4.2^{a}	0.007

^{a,b} Means with different superscript letters in rows differ significantly (P < 0.05)

*The table gives averages values with standard error

** Fertility (%) = (number of fertilized eggs/number of eggs set) ×100

***Hatchability of fertile eggs (%) = (number of hatched chicks/total number of fertile eggs) ×100

**** Hatchability of set eggs (%) = (number of hatched chicks/total number of set eggs) ×100

Table 3. Effect of different light applications on responses of open field test*

	Dark	Green	Blue	P
First movement time, sn	35.16 ± 7.37	33.76 ± 7.88	19.78 ± 7.59	0.288
First call time, sn	20.4 ± 4.32	23.66 ± 4.9	19.69 ± 4.68	0.824
First defecation time, sn	157.00 ± 21.6^{a}	106.66 ± 25.92^{ab}	81.09 ± 19.14^{b}	0.044
First jump time, sn	104.16 ± 17.5	67.37 ± 21.43	123.61 ± 23.78	0.200
Total number of frames visited pieces	67.4 ± 12.9	62.88 ± 13.41	75.25 ± 12.67	0.793
Total number jumps, pieces	8.25 ± 1.68	8.00 ± 2.06	6.23 ± 2.29	0.766
Total call number, pieces	23.07 ± 1.83	17.42 ± 2.07	18.26 ± 1.98	0.086

^{a,b} Means with different superscript letters in in rows differ significantly (P < 0.05)

*The table gives averages values with standard error