JWPR
Journal of World's Poultry Research

2021, Scienceline Publication

J. World Poult. Res. 11(1): 129-135, March 25, 2021

Research Paper, PII: S2322455X2100017-11

DOI: https://dx.doi.org/10.36380/jwpr.2021.17

Effects of Red and Blue Light during the Incubation of Turkey Eggs on Hatchability Performance and Expression Pattern of Some Myogenic Regulatory Genes

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Received: 20 Jan. 2021
Accepted: 07 Mar. 2021

ABSTRACT

The present study aimed to investigate the effects of different light colors on hatching potential traits, including egg weight loss, scientific and commercial hatchability, mortality percentages, hatching wight as well as mRNA expression levels of some muscle growth marker genes (*Myogenin*, *MyoD1,* and *FGF2*) of pectoralis muscle in hatched and non-hatched non pipped Black Bronze turkey chicks. A total of 1500 hatching Black Bronze turkey eggs were assigned equally to three incubation treatment groups, namely dark (control group), red, and blue LED light (treated groups) for 25 days of the incubation period. Results indicated that colored lighting stimuli (red and blue) significantly affected hatching capability. This issue could also affect the expression of muscle growth marker genes in hatched and non-hatched non pipped turkey chicks. Incubation of turkey eggs under red or blue LED light showed an insignificant effect on mortality percentages. It can be concluded that the use of a red or blue light system during turkey eggs' incubation could improve hatchability via upregulating the expression of muscle growth marker genes.

Keywords: Hatchability, Incubation, Light color, Marker Gene expression, Turkey

INTRODUCTION

The success of artificial incubation of avian eggs relies mainly on the environmental conditions inside the incubator. Temperature (Noiva et al., 2014), humidity (Bruzual et al., 2000), ventilation (Okur et al., 2018), and egg turning (Elibol and Brake, 2006) are the four main factors controlling the hatchability percentage. Many studies suggested that monitoring the light during the incubation period has a crucial role in the achievement of successful incubation of avian eggs (Fairchild and Christensen, 2000; Archer, 2015; Huth and Archer, 2015). Introducing light during incubation improved hatchability (Shafey, 2004; Archer and Mench, 2014) and embryonic growth rate (Cooper et al., 2011), as well as reducing early and late embryonic mortality (Shafey and Al-Mohsen, 2002), and post-hatch stress (Archer and Mench, 2014). Furthermore, the light during incubation stimulated opsin expression in photoreceptors (Rozenboim et al., 2013), increased growth and differentiation of myoblast, and myofiber synchronization (Halevy et al., 2006). Moreover, avian scientists have revealed that the impact of light on the embryonic development of different birds' species depends on the color (Tong et al., 2015). Generally, the light plays a crucial role in various scientific aspects. Light is electromagnetic radiation, classified into invisible and visible radiation. Invisible radiation has wavelengths too large or too small for the biological limitations of our discernment. However, the visible light is separated into different wavelengths ranged from violet color (the shortest wavelength) to red color (the longest wavelength). Yang et al. (2016) categorized the light spectrum according to the wavelength into long (red at 610-760 nm, yellow at 580-590 nm light) and short (green at 510-530 nm, and blue at 450-500 nm light).

During embryogenesis, the development of the skeletal muscle depends on myogenic regulatory factors (MRFs) which are a family of basic helix–loop–helix transcription factors important for the proliferation and differentiation of satellite cells (Schultz and McCormick,

1994). Satellite cells are the primary donors to muscle growth and regeneration (Relaix, 2006; Biressi and Rando, 2010). *MRFs* include myogenic factor 5 (*Myf5*), myogenic differentiation 1 (*MyoD1*), *Myogenin*, and *MRF4* (Buckingham and Rigby, 2014; Zammit, 2017). When muscle stem cells (satellite cells) are activated, these *MRF* genes are expressed in a consecutive pattern. During the proliferation of muscle stem cells, the *MyoD* and *Myf5* genes are expressed, and after that, *Myogenin* is expressed as the cells begin to differentiate (Cornelison and Wold, 1997). Fibroblast growth factor 2 (*FGF2*) is a potent regulator of muscle cell proliferation and differentiation (Velleman, 1999). It also plays a serious role in the maintenance of satellite cells' self-renewal by inhibiting their differentiation (Pawlikowski et al., 2017). Furthermore, *FGF2* has shown a possible influence on tissue regeneration and repair (Yun et al., 2010). Until now, few studies have investigated the relationship between hatching potentiality and molecular alterations of muscle growth related genes of turkey embryos incubated under different wavelengths of light. The present study aimed to investigate the effects of red and blue light stimuli during the incubation of Black Bronze turkey eggs on hatchability performance and expression profile of some muscle growth marker genes as a trail to understand the strategy of light color effect on hatching improvement.

MATERIALS AND METHODS

Ethical Approval

All experimental procedures and management conditions used in this study were approved by the local ethics committee of animal use, Faculty of Veterinary Medicine, Alexandria University, Egypt.

Fertile eggs and incubation condition

A total of 1500 hatching Black Bronze turkey eggs were collected from the Research Centre, Mahalet Mousa Station, Kafr El Sheikh Province, and were randomly allotted to three groups. The first group (control) was incubated at a complete dark incubator avoiding penetration of external light by covering incubator windows and door with black sheets, the second and the third ones were incubated under red LED light and blue LED light for the first 25 days of the incubation period. Light intensity was 12W/m^2 at the top surface of the eggs with a wavelength of 610-760 nm for the red light and 450-500 nm for the blue light. Incubator and hatcher were fumigated using formaldehyde gas by mixing of 40 ml formalin 40% and 20 gm potassium permanganate (KMNO4) per three cubic meters. Diluted TH4 solution (2ml/L) was sprayed on the eggs as a disinfectant before the incubation. All groups were incubated at normal incubation conditions (37.5°C, 65 % relative humidity, and turning automatically every three hours by angle ± 45). After 25 days of incubation, the eggs were transferred to the hatcher (37°C, 75% relative humidity, and no turning). During incubation, 100 eggs from each group were individually weighted every week to estimate the weight loss. Candling of the incubated eggs was performed on the seventh day of incubation to check whether the eggs are fertile or not and inspect early mortality percentage. At 14 and 25 days of incubation, eggs were candled again to determine mid, and late embryonic mortality percentages. The hatchability percentage was determined as the number of viable chicks hatched divided by the total eggs set (commercial hatchability) or fertile eggs set (scientific hatchability). The non-hatched eggs were left for an extra one day later hatching in the hatcher to give further chance to hatch. The number of hatched and non-hatched non-pipped eggs were recorded. Newly hatched chicks on the first day were weighted.

Sample collection

The pectoralis muscle samples were collected from hatched and non-hatched non-piped chicks (four samples from each group) then homogenized, immediately snap-frozen in liquid nitrogen, and stored in -80ºC.

Total RNA isolation and cDNA synthesis

Total RNA was isolated from homogenized muscle tissue using the Biozol reagent (Bioflux, Japan) according to the manufacturer's recommendations. The SensiFAST™ cDNA Synthesis Kit (Bioline, United Kingdom) was used for cDNA synthesis according to the manufacturer's instructions. Briefly, 4 µl of total RNA was mixed with 4 µl 5X buffer, 1 µl reverse transcriptase and 11 µl RNase free H_2O. The reaction was carried out as follows: 25°C for 10 min, 42°C for 15 min (reverse transcription), and 4°C holds. The cDNA was tested by amplification of the *ß-actin* gene, and then stored at -20°C.

Quantitative real-time PCR

The quantitative real-time PCR was carried out to investigate the expression levels of *Myogenin*, *MyoD1*, and *FGF2* genes as described previously (Abd El Naby and Basha, 2018). The primer sequences designed by using (https://primer3.ut.ee/) are listed in Table 1. The *β*-

actin (housekeeping gene) was used as normalizer. The relative mRNA expression was calculated using the comparative Ct method ($2^{(-\Delta\Delta ct)}$), and the results were reported as the fold change±SD (Rao et al., 2013).

Statistical analysis

The Chi-square test was used to determine the relationship between the light color (dark, red, and blue) during the incubation period and the number of hatched or non-hatched non pipped chicks of Black Bronze turkey ($p \leq 0.05$). Other data were analyzed by one-way ANOVA using SAS (Statistical Analysis System, version 6, 4th Edition, SAS Institute, USA). Data are expressed as mean ± SE and $p \leq 0.05$ were considered significant. Analyses of significant main effects were performed using multiple range comparisons with Duncan multiple range test. However, gene expression data were statistically analyzed by GraphPad Prism software version 6 (GraphPad Prism Software, La Jolla, California, USA) using one-way ANOVA.

Table 1. Primers used for quantitative real-time PCR

Genes	Primer Sequence (5`-3`)	Amplicon size (base pair)
Myogenin (NM_001303170.1)	F: CTCTCTGAGCTGGAAACGGG R: GGTCCACAGTGTTGGAGGAT	86
MyoD1 (NM_001303171.1)	F: CATGGGAAGAGTTCCGTTGT R: GGAAATCCTCTCCACAATGC	63
FGF2 (XM_003205699.3)	F: CTGGCACTGAAATGTGCAAC R: CTTCCGTGACCGGTAAGTGT	84
ß-actin (NM_001303173.1)	F: ATGGCTCCGGTATGTGCAA R: TGTCTTTCTGGCCCATACCAA	120

MyoD1: Myogenic factor D1, *FGF2*: Fibroblast growth factor, and *ß-actin*: Beta actin; F: forward, R: reverse.

RESULTS

The relationship between light color and the number of hatched or non-hatched non-pipped chicks

The statistical analysis showed that 13.85 is the estimated Chi-Square value ($p \leq 0.05$), indicating that the hatching ability of fertile Black Bronze turkey eggs depends on light color during the incubation period.

Effect of different lighting color during the incubation period on the hatching performance

The results showed that weekly weight loss of Black Bronze turkey eggs incubated at dark, red, and blue light had no significant difference at all incubation periods (Table 2). However, the incubated eggs in darkness had the highest significant total weight loss, compared to incubated eggs exposed to red and blue light.

Table 3 showed that the application of colored light significantly increased the scientific and commercial hatchability percentages of turkey eggs. Incubation under red light exhibited the highest scientific and commercial hatchability percentages, while the lowest hatchability was recorded for the dark condition. Additionally, early, mid, and late mortality percentages did not show significant differences between colored incubation or dark management. Furthermore, the hatching weight of turkey chicks showed that eggs exposed to the red light in the incubator had the highest significant hatching weight (50.87 gm) than the other groups.

Effect of different lighting color during the incubation period on the expression profile of *Myogenin*, *MyoD1*, and *FGF2* genes of turkey chicks

The effects of different light colors during the incubation period on mRNA expression levels of muscle growth marker genes in hatched and non-hatched non-pipped chicks are shown in figures 1 and 2. Red and blue light colors induced changes in the expression patterns of *Myogenin, MyoD1,* and *FGF2* genes, compared to control (figures 1 and 2). Hatched chicks exposed to the red light during incubation showed increased *Myogenin* and *MyoD1* expression levels with 14.65 ± 2.27 and 19.01 ± 2.70 folds than in case of blue light exposure (8.5 ± 1.6 and 3.64 ± 1.35 fold) relative to control (figures 1 a and b). The *Myogenin* level was significant ($p \leq 0.05$) upregulated (59.98 ± 0.04 fold) in non-hatched non-pipped chicks were exposed to blue light during the incubation period relative to control (Figure 2a). Its relative expression level also increased in non-hatched non-pipped chicks exposed to red light, but it was less than blue color. Moreover, the *MyoD1* mRNA expression profile showed significant ($p \leq 0.05$) upregulation (38.85 ± 0.96 folds) in non-hatched

chicks exposed to red light during the incubation period relative to control (Figure 2b) and increased (5.86 ± 1.33 folds) in case of blue light. Meanwhile, the *FGF2* gene revealed different expression patterns in both hatched and non-hatched non-pipped chicks. However, their relative expression levels showed a significant ($p \leq 0.05$) increase with 7.00 ± 0.45-fold in hatched chicks exposed to blue light (Figure 1c), and 5.24 ± 1.1 fold in the non-hatch non-pipped chicks exposed to red light during incubation relative to control (Figure 2c).

Table 2. Effect of the different lighting colors during incubation on egg weight loss of Black Bronze turkey eggs

Incubation period (Day)	Egg weight loss in different light conditions (g)		
	Dark	Red	Blue
1-7	1.117 ± 0.256	1.422 ± 0.079	1.300 ± 0.199
7-14	4.298 ± 0.334	2.653 ± 0.226	1.714 ± 0.256
14-21	4.941 ± 0.333	2.869 ± 0.295	2.984 ± 0.388
21-25	5.399 ± 0.297	4.395 ± 0.411	4.983 ± 0.444
Total	15.872 ± 0.541[a]	11.554 ± 0.585[b]	10.179 ± 0.442[b]

Means within the same row with different superscripts are significantly different ($p \leq 0.05$).

Table 3. Effect of the different lighting colors on hatchability, mortality percentages and hatching weight of Black Bronze turkey eggs

Variable	Light group	Dark	Red	Blue
Hatchability (%)	Scientific	80.58 ± 0.932[c]	90.43 ± 0.785[a]	87.73 ± 0.543[b]
	Commercial	69.24 ± 0.88[b]	76.67 ± 0.97[a]	73.45 ± 1.23[a]
Mortality (%)	Early	1.50 ± 0.005	2.43 ± 0.010	1.00 ± 0.033
	Mid	3.00 ± 0.002	4.55 ± 0.001	2.30 ± 0.003
	Late	18.36 ± 0.024	20.12 ± 0.096	22.88 ± 0.176
Hatching weight (gm)	Day old	46.05 ± 0.44[b]	50.87 ± 0.17[a]	45.43 ± 0.56[b]

Means within the same row with different superscripts are significantly different ($p \leq 0.05$).

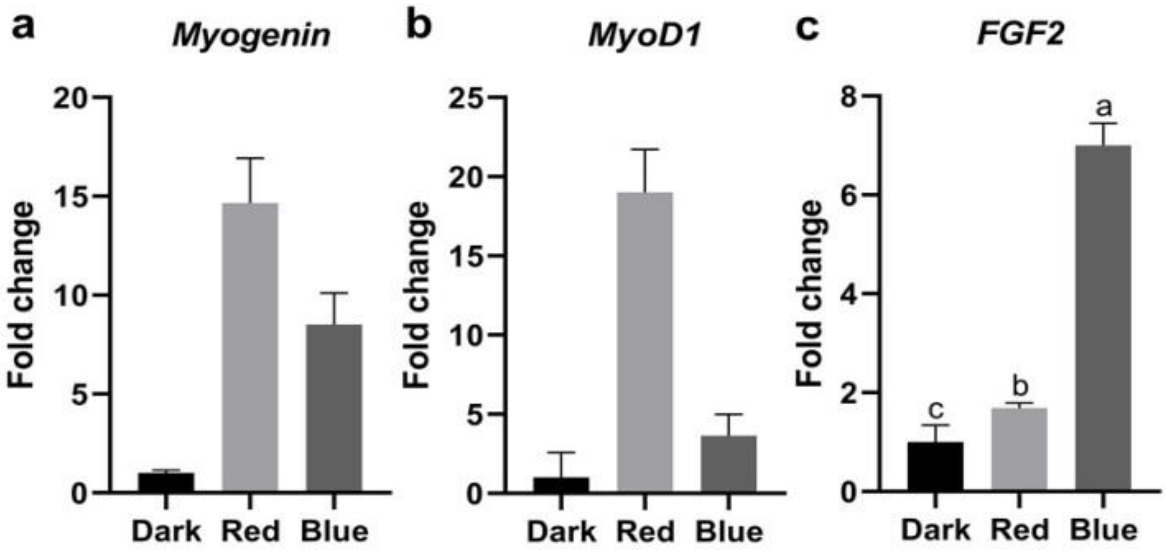

Figure 1. The relative expression levels of *Myogenin, MyoD1,* and *FGF2* genes in hatched turkey chicks with different light color treatments during egg incubation. Different letters in columns mean significant differences ($p \leq 0.05$).

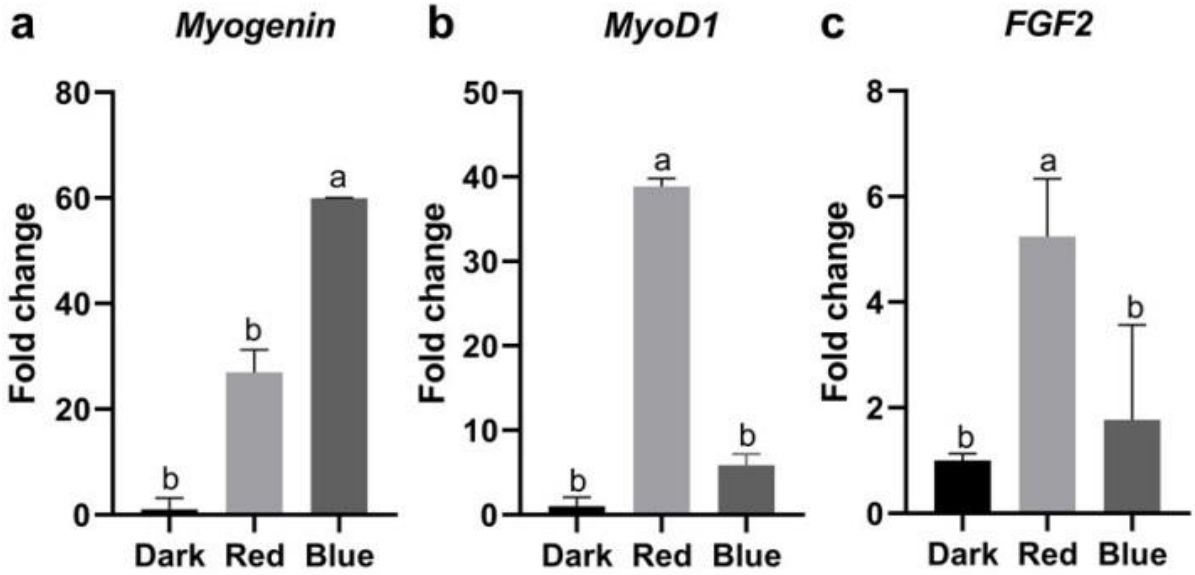

Figure 2. The relative expression levels of *Myogenin*, *MyoD1*, and *FGF2* genes in non-hatched non-pipped turkey chicks with different light color treatments during egg incubation. Different letters in columns mean significant differences ($p \leq 0.05$).

DISCUSSION

Avian embryogenesis is strongly influenced by the light wavelength and color (Shafey and Al-Mohsen, 2002; Rozenboim et al., 2013). The present study investigated the impact of colored light of different wavelengths on the hatchability performance of Black Bronze turkey. The results showed that using red or blue light in the first 25 days of incubation of Black Bronze turkey eggs improved scientific and commercial hatchability percentages. Incubation of Black Bronze turkey eggs in red light resulted in the highest improvement of hatchability percentages as well as hatching weight. However, weekly egg weight loss, mortality percentages (Early, mid, and late) were not significantly affected by colored light during incubation. These results are compatible with a study conducted by Archer (2015) indicating that red light is the success key of hatchability for broiler or layer eggs. Moreover, Archer (2017) ensured that the incubation of broiler eggs under red or white light could improve hatchery efficiency in comparison with the green light. El-Komy et al. (2017) reported increased hatchability and decreased mortality percentages for quail and Cobb 500 broiler breeder's eggs exposed to red light. Contrary to findings of the current study, providing 30 lx green LED light during the incubation of Arbor Acres fertile broiler eggs had no detrimental effect on the development of eyes, heart, and liver of embryos (Zhang et al., 2016). Santos (2019) also found no impact of red or blue light on the hatching behavior of Lohmann White and Brown eggs during incubation.

In this study, both light color stimuli (red and blue) upregulated *Myogenin*, *MyoD1*, and *FGF2* in the embryonic stage and improved hatching capability compared to the group incubated in the dark condition. The *Myogenin* gene was upregulated in non-hatched and non-pipped chick's muscle at the red light, however, this increase was less when compared to the exposure to blue color light, which revealed a significant increase compared to non-hatched non-pipped chicks in a dark condition. The *MyoD1* mRNA levels were significantly upregulated in the group exposed to red light during incubation in non-hatched and non-pipped chicks and also increased in hatched chicks. Otherwise, the *Myogenin* expression level in hatched chicks exposed to red light during incubation was more than the case of using blue light. As several studies reported, *MyoD1* is responsible for the activation and proliferation of skeletal satellite cells, however, *myogenin* plays a pivotal role in myoblast differentiation (Yablonka-Reuveni and Paterson, 2001; Cao et al., 2006). Furthermore, Zhang et al. (2014) suggested that the use of green light during incubation until hatching enhanced proliferation and differentiation of skeletal muscle satellite cells in the late embryonic stage and newly hatched chicks. This enhancement was a result of an increase in *MyoD1* expression level on day 17 of the embryo until day 3 of hatched chicks as well as an increase in *Myogenin* expression level on the first day to the fifth day after

hatch. Additionally, Halevy et al. (2006) showed that *in ovo* green light illumination has a stimulatory effect on skeletal muscle development in chicks during late embryogenesis and post-hatch period through enhanced proliferation and differentiation of adult myoblasts. regarding broiler chicken, a recent study reported that monochromatic green light stimulation during incubation increased the mRNA expressions of *Pax7* 18.77%, *MyoD*, 10.85%, *Myf5*, 13.48%, and *Myogenin*, 17.79%, which performed the satellite cell myogenic program (Bai et al., 2019).

Fibroblast growth factor 2 is a vital regulator of muscle cell proliferation and differentiation (Pawlikowski et al., 2017). the findings of the current study indicated that red light significantly increased *FGF2* mRNA expression level during incubation in non-hatched non-pipped chicks while in hatched chicks, blue light showed a significant increase in *FGF2* mRNA expression level. Based on the results of this study, it could be suggested that the red or blue light spectrum during incubation of turkey eggs is better than darkness. Furthermore, myoblast growth may be affected by light color through its influence on myogenic regulatory genes and consequently on hatching performance.

CONCLUSION

The findings suggest that using different LED light colors (red and blue) during the incubation period may affect myogenesis through its effect on the expression profiles of muscle growth marker genes. However, there should be future studies addressing the effect of light color on the intracellular events, such as the expression of *Myogenin MyoD1* and *FGF2*.

DECLARATIONS

Authors' contributions

Walaa Slouma Hamouda Abd El Naby and Heba Abdo Basha designed the plan of methodology, performed the experimental work, and wrote the main draft of the manuscript. Samya Erian Ibrahim, Magda Ismail Abo-Samaha, collected the samples and analyzed the data. All authors critically interpreted the data, revised the manuscript, and approved the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

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