

## Research Article

# The effect of lighting color on hatching broiler eggs during different storage periods

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**Abstract:** This study evaluated the effects of storage periods (5 and 14 days) and lighting color (white, green, red, and a mix of red and green color) on incubation and some hatching characters. 240 fertile eggs from the Rose 308 broiler breed were purchased and divided into two storage groups of five and fourteen days, each of them divided into five treatments according to the lighting color during incubation. The results showed that the eggs stored for 5 days had the maximum hatching characteristics, but they decreased significantly at 14 days. The percentage of loss in egg weight, shell weight, and relative embryo weight were affected at 5, 7 and 18 days of incubation. There was a significant effect ( $P < 0.05$ ) of storage on the remaining yolk sac after hatching, the weight of the hatched chick, the hatching ratio and the hatching time. The results also indicated that there was an improvement in the use of colored lighting during the incubator on the hatching qualities of eggs stored for broilers for a period of more than 5 days.

**Keywords:** Broiler, Storage periods, Lighting color, Hatchability characters.

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## Introduction

Industrial hatching plays an important role in the renaissance that the poultry industry has been experiencing for a long time. The hatcheries provide the natural hatching needs through heat, humidity, ventilation, and stirring. Romanoff (1960) pointed out that the possibility of improving the economic aspect of industrial hatching by introducing light as an additional environmental factor during the storage and incubation period is conflicting. In large commercial hatcheries, where artificial hatching is used, the lighting was switched on only when the incubator was opened, either for unloading the hatched chicks or for maintenance. There is also little information about the use of different lighting colors

during the storage of hatching eggs and the extent to which this factor affects the rate of hatching in the future. The results of the studies agreed that exposing bird eggs to light during hatching and even before it increased the speed of fetal development.

AigeGil & Murillo-Ferrol (1992) reported an increase in the number and size of pineal intracytoplasmic lipid droplets in embryos exposed to light for 18 days of incubation of broiler eggs, in meat chickens (Coleman et al. 1971), turkeys (Fairchild & Christensen 2000), and quail (Maman et al. 2018). Some studies showed a lack of photostimulation in the hatching rate and the weight of hatched chicks (Zakaria 1989), while others indicated a negative effect on the hatching rate and

the weight of hatched chicks (Rozenboim et al. 2003). In some studies, the use of colored light during egg hatching has led to a positive effect on the hatching rate and the weight of hatched chicks (Cooper et al. 2011, Huth & Archer 2015; Xujie Li et al. 2021). The wavelengths of normal light range from - 400 to 700 nanometers, and the bird is sensitive to light through its eyes via retinal photoreceptors and through photosensitive cells located in the brain called supramolecular photoreceptors (extra-retinal photoreceptors). Lowe & Garwood (1977) pointed out that green light stimulates growth in the early period of growth, while the blue color stimulates growth in the late period at the age of marketing. Bowling et al. (1977) also pointed out that green and blue light significantly increase body weight and that muscle weight gain is associated with the totals in which green and blue light were used and may occur because of an increase in satellite cells in skeletal muscles during the first days of life. The duration of storage and the storage conditions of hatching eggs are two of the most important factors affecting the success of the hatching process of chicken eggs, A negative effect of the length of the storage period on the hatching ratio, hatching weight, and qualitative qualities of hatched chicks from Egyptian Balady eggs were found (Heba 2015), and a negative effect on hatching quality and an increase in the embryonic mortality percentage (Petek et al. 2006; Khan et al. 2013). Therefore, the purpose of this study was to study the impact of the lighting color during the incubation and storage period on some broiler-hatching eggs.

### Materials and methods

This experiment was carried out in the livestock department's poultry field from December 1, 2018, to April 1, 2019, and the investigation included two stages: the first was the storage of eggs hatching meat broilers (240). The fertilized eggs were obtained from the hatches of Barakat al-Rahman Al-Ahli located in the Safwan area south of Basra and kept under storage conditions of 16°C and relative

humidity 78°C for 5 and 14 days. The light was colored for the daylong period (Florence light is white, dark, red, green, and a combination of red and green colors). In the second stage, the eggs were incubated in incubators equipped with colored light LED; the density of the light was 200 Lux; 10cm up the top of the eggs as previously divided. The eggs prepared for hatching were distributed into ten groups. Each group of the experiment included 20 hatching eggs distributed into the following groups: (1) the first group stored 120 eggs of hatching broilers in a room at 16°C and 78% RH. for five days, which were later divided in the incubator into five groups, including the control group of C1: 20 eggs hatched without storage in just darkness; the second control group, D1, hatched 20 eggs under the influence of darkness; the third group, WL5, hatched 20 eggs under the influence of fluorescent light. The fourth group, GL5, consists of 20 eggs hatched under the influence of green light, the fifth group, RL5, consists of 20 eggs hatched under the influence of red light, and the sixth group, RL&GL5, included 20 hatched eggs exposed to red and green light. (2) A second batch of 120 hatching broiler eggs was stored in a room at 16°C and 78% RH, for 14 days. Then they were divided into five incubation groups of control group D2 of 20 eggs hatching under the influence of darkness; the seventh group, WL14, hatched 20 eggs under the influence of white fluorescent light. The eighth group, GL14, contains 20 hatching eggs under the influence of green light; the ninth group, RL14, contains 20 hatching eggs under the influence of red light, and the tenth group, RL&GL14, contains 20 hatching eggs under the influence of red and green light.

Following the expiration of two consecutive storage periods, the incubators were configured in accordance with the experimental parameters to contain the colored emitted light. All the eggs were incubated under the same conditions (37.5°C and 60% RH). The studied measurements were carried out at the ages of 7, 15, and 18 days of incubation, where the hatch window was measured as the number

**Table 1.** The effect of the storage period (17 days) and the lighting color on the weight of the eggshell, the weight loss of the egg, and the relative weight of the embryo, at the seventh day of incubation.

Criteria	Control	D1	WL5	GL5	RL5	RL5&GL5
EWL%	7.842±0.02 <sup>a</sup>	6.201±0.07 <sup>b</sup>	6.001±0.01 <sup>b</sup>	7.185±0.06 <sup>a</sup>	7.443±0.03 <sup>a</sup>	6.344±0.0 <sup>b</sup>
ESW g	7.011±0.05 <sup>a</sup>	6.793±0.05 <sup>b</sup>	6.880±0.02 <sup>b</sup>	6.995±0.01 <sup>ab</sup>	6.693±0.02 <sup>b</sup>	6.831±0.09 <sup>b</sup>
RMW%	3.892±0.00 <sup>a</sup>	3.206±0.00 <sup>bc</sup>	3.054±0.00 <sup>b</sup>	3.775±0.00 <sup>ab</sup>	3.166±0.01 <sup>c</sup>	3.499±0.04 <sup>b</sup>
<sup>1</sup> LC effect<	-	NS	NS	NS	NS	NS
<sup>2</sup> SP effect<	-	*	*	*	*	*
<sup>3</sup> SP×LC	-	*	*	*	*	*

Control treatment, fresh egg incubated in darkness: D1 eggs stored for 5 days in dark incubation. ,WL 5, eggs Stored for 5 days and incubated under the influence of fluorescent, GL5 eggs Stored for 5 days and incubated under the influence of green light (RL5), eggs Stored for 5 days and incubated under the influence of red light (RL5&GL5), eggs were stored for 5 days and incubated under the influence of green and red light. The relative embryo weight RMW %, Egg Shell weight ESW, Egg weight loss percentage EWL. <sup>a, b</sup> means that within a single column, with different letters differ significantly P (0<0.05). \* Significant difference in light color on these items at (P<0.05). <sup>1</sup> LC, Lighting color, <sup>2</sup> SP storage period, <sup>3</sup> SP×LC Storage period ×lighting color

of hatched chicks per tray, and recorded at 3-hour intervals from 454 h to 512 h of incubation to calculate the spread of hatch. Chicks were considered hatched when they completely emerged and were free from the eggshell. Then the weight of the shell WS, yolk weight YW, and the relative embryo weight REW at day 18, the weight of residual yolk (WRSY) were calculated. About 50% of the hatchlings were sampled and weighed at three hours following hatching (emerging from the eggshell) using a two-digit sensitive scale. A sorter a medical anatomy kit was used to separate the egg components and measure the weight of the yolk and eggshell and the weight of the embryo at different stages.

The relative weight of the developing embryo at different age stages was measured by the equation (Hamburger & Hamilton 1951) of Relative embryo weight = developing fetus weight / egg weight before incubation multiplied by 100. The age groups studied determined the percentage of embryo deaths as the following formula: Relative loss of egg weight = egg weight at zero incubation - the weight of the egg at a certain age of the incubation period. Hatchability was calculated based on total fertile eggs.

## Results and discussion

### Effect of the lighting color and storage period (5 days) on the relative weight loss of eggs, eggshell

**weight, and relative weight of the embryo on the seventeenth day of incubation:** Table 1 indicates the effect of the lighting color and the storage period on the loss of egg weight, the eggshell weight, and the relative weight of the embryo in broiler hatching eggs. The results show a significant effect ( $P>0.05$ ) of color lighting on the relative weight loss of eggs, eggshell weight, and the relative embryo weight in all study groups. Therefore, green light, GL, had the highest loss weight of the egg (7.185%) and the relative embryo weight of the eggs (3.775 %) compared with control darkness stored group C (7.842%), gm (3.892%). There was a significant ( $P<0.05$ ) influence of the interference between the color of lighting and the period of storage of eggs on the studied hatching qualities at 7 days of incubation. However, the statistical analysis did not show any significant effect ( $P>0.05$ ) of the interference between the lighting color and the storage period. There were differences ( $P<0.05$ ) between the colored light treatments, where the green light GL had the highest results.

The results of this study were similar to the results of research conducted by Wang et al. (2014), who confirmed an important effect of green light color on stimulating embryonic development in the egg during the incubation period. However, the red or dark color used in the incubators did not affect the

**Table 2.** The effect of the storage period (5) days and the color of the light on the weight of the egg shell, the weight loss of the egg, and the relative weight of the embryo, at fifteen days of incubation.

Criteria	Control	D1	WL5	GL5	RL5	RL5&GL5
EWL%	9.592±0.02 <sup>a</sup>	7.251±0.03 <sup>c</sup>	8.401±0.06 <sup>bc</sup>	8.889±0.08 <sup>b</sup>	8.335±0.04 <sup>ab</sup>	8.303±0.02 <sup>b</sup>
ESW g	6.214±0.03 <sup>a</sup>	6.100±0.07 <sup>b</sup>	6.010±0.05 <sup>b</sup>	6.003±0.17 <sup>b</sup>	6.233±0.11 <sup>ab</sup>	6.335±0.12 <sup>c</sup>
WM%	24.665±0.22 <sup>a</sup>	22.372±0.38 <sup>c</sup>	23.831±0.97 <sup>b</sup>	24.257±0.62 <sup>a</sup>	23.711±0.37 <sup>b</sup>	23.857±0.46 <sup>b</sup>
YW g	18.354±0.44 <sup>a</sup>	17.581±0.59 <sup>ab</sup>	17.598±0.36 <sup>ab</sup>	17.176±0.53 <sup>ab</sup>	17.932±0.51 <sup>a</sup>	17.958±0.46 <sup>a</sup>
<sup>1</sup> LC.effect<	-	NS	NS	NS	NS	NS
<sup>2</sup> SP.effect<	-	*	*	*	*	*
<sup>3</sup> SP×LC	-	*	*	*	*	*

Control treatment, fresh egg incubated in darkness: D1 eggs stored for 5 days in dark incubation. ,WL 5, eggs Stored for 5 days and incubated under the influence of fluorescent, GL5 eggs Stored for 5 days and incubated under the influence of green light (RL5), eggs Stored for 5 days and incubated under the influence of red light (RL5&GL5), eggs were stored for 5 days and incubated under the influence of green and red light. The relative embryo weight RMW %, Egg Shell weight ESW, Egg weight loss percentage EWL.<sup>a, b</sup> means that within a single column, with different letters differ significantly P (0<0.05). \* Significant difference in light color on these items at (P<0.05). <sup>1</sup> LC, Lighting color, <sup>2</sup> SP storage period, <sup>3</sup>SP×LC Storage period ×lighting color.

development of the embryo in the egg in their study. Rozenboim et al. (2004) showed that exposure to the green light at various stages of incubation and at irregular intervals of 15 minutes on and 15 minutes off has a substantial impact on the weight gain of the growing embryo relative to the weight of the egg. El Sabry & Essa (2017) showed that using continuous cold light for 12 hours of light and 12 hours of darkness during the incubation period of Cobb broiler hatching eggs had no effect on egg weight loss or hatching qualities. Also, they suggest that intermittent lighting with short periods of light and dark stimulates muscle growth in both embryos and broiler chicks post-hatching. Monochromatic green light seems superior to other wavelengths in the stimulation of muscle growth. The pineal on chromosome 3, which is the main secretory organ for the melatonin hormone and one of the candidates to explain how illuminated incubation influences embryonic development, plays a critical function in entrainment in addition to building the embryonic visual system (Yalcin et al. 2022).

**Effect of the storage period of five days and lighting color on weight loss of egg, the weight of the shell, and relative weight of embryo on 15 days of incubation:** According to Table 2, lighting color in incubation had not a significant effect ( $P>0.05$ ) on

the weight loss of the egg, the weight of the eggshell and the relative weight of embryo of hatching eggs. The storage period of 5 days also had an impact on the characteristics of hatching eggs at 15 days of incubation. The darkness control group C had the highest percentage of weight loss recorded at 9.59%, followed by the group of the green light GL at 8.88%, and the lowest percentage of weight loss recorded in-group of the darkness negative control D1 at 7.251%. This indicates that the amount of light absorption was greater in the lighting groups compared with the control darkness group un-stored, and the darkness group stored D1, showing that the absorption reactions of calcium bicarbonate in the eggshell led to a decrease in its weight. The green light color group GL5 was the best among the light treatments studied, while the control group had the highest percentage of weight loss in the egg, and the green light color group recorded approximate values of 6.003 and 6.214g, respectively. The weight of the growing embryo inside the egg on day 14 of incubation showed that the green light has the highest weight among the color light groups (24.257g), however, in other experimental lighting color groups WL5, GL5, and RL5+ GL5, the weights were 23.831, 23.711, and 23.857%, respectively, but the positive control group (C) was the highest (24.665%). There

**Table 3.** The effect of the storage period (five days) and the color of the light on the weight of the eggshell, the weight loss of the egg, and the relative weight of the embryo, at eighteen days of incubation.

Criteria	Control	D1	WL5	GL5	RL5	RL5+GL5
EWL %	12.634±0.0 <sup>a</sup>	10.498±0.01 <sup>c</sup>	11.749±0.0 <sup>b</sup>	11.93±0.10 <sup>ab</sup>	11.032±0.03 <sup>bc</sup>	11.255±0.06 <sup>bc</sup>
ESW g	6.500±0.05 <sup>a</sup>	5.071±0.11 <sup>c</sup>	5.311±0.3 <sup>b</sup>	5.501±0.06 <sup>b</sup>	5.351±0.01 <sup>ab</sup>	5.211±0.06 <sup>b</sup>
RMW%	33.586±0.14 <sup>a</sup>	28.014±0.21 <sup>d</sup>	29.021±0.16 <sup>b</sup>	32.818±0.14 <sup>ab</sup>	29.407±0.11 <sup>b</sup>	29.425±0.012 <sup>c</sup>
YW g	13.524±0.16 <sup>a</sup>	12.321±0.1 <sup>c</sup>	12.553±0.25 <sup>b</sup>	12.598±0.22 <sup>b</sup>	12.587±0.21 <sup>b</sup>	12.549±0.12 <sup>b</sup>
<sup>1</sup> LC.effe<	-	NS	NS	NS	NS	NS
<sup>2</sup> SP.effec<	-	NS	NS	NS	NS	NS
<sup>3</sup> SP×LC	-	*	*	*	*	*

Control treatment C, fresh egg incubated in darkness: D1 eggs stored for 5 days in dark incubation. WL 5, eggs stored for 5 days and incubated under the influence of fluorescent GL 5, eggs stored for 5 days and incubated under the influence of green light RL5, eggs stored for 5 days and incubated under the influence of red light RL5&GL5, eggs stored for 5 days and incubated under the influence of green and red lighting color Egg Shell weight ESW, The relative embryo weight RMW %, Egg weight loss percentage EWL. Yolk weight YW, <sup>a, b</sup> means that within a single column, with different letters differ significantly ( $P<0.05$ ). \* Significant difference in light color on these items at ( $P<0.05$ ).

is no significant effect ( $P>0.05$ ) or difference between groups of lighting color on yolk weight; therefore, there is a significant effect ( $P<0.05$ ) of storage period on these traits. In this study, the interference between the color of the lighting and the storage period also has a significant impact ( $P<0.05$ ) on the studied qualities.

The findings of Dishon et al. (2017, 2018) supported the existence of a strong lighting effect on hatching characteristics in the early days of incubation. This can be explained by the large amount of growth hormone found in the blood of the embryo in the egg and its exposure to the green light from age 14 until the time of hatching. The development of the embryo was positively influenced. These results revealed that a period of light throughout incubation might stimulate rosin melatonin secretion and regulate the manufacture of growth hormone. However, when the light exposure was continuous, excluding the external period of chicken embryos, an increase in the embryo's overall weight and the weight of its tissues was also observed. Another study hypothesized that muscle growth may be dependent on exposure to light, but also related to biological clock cycles that were dragged by the light period, as the explanation for the increase in embryo weights and the decrease in the weight of the egg's shell. on the second day of embryonic age, the neurons of the optic vesicles

begin to differentiate, and by the fourth day, the relationship between the retinal ganglion cells and the optic chiasma is complete (Rogers 1995). By the embryonic age of fourteen, embryonic eye development is finished, and the expression of light-sensing proteins (opsins) in sensory neurons, which react to various light wavelengths, is observed (Perez et al. 2019). Bruhn & Cepko (1996) reported that on the 18th day of incubation, the chicken embryo's visual system is ready for use (Rogers 1995). The amount of light that can penetrate eggshells and reach the embryo can vary depending on the wavelength and intensity of the light. In the present work, the light intensity may have a significant impact on the fatal division of the neural summit's median epidermis at the preliminary phase of the embryo's gestation (Yu et al. 2018). By using 200-300 lux illumination, an increased embryo weight was observed (Özkan et al. 2012).

**Effect of the storage period of five days and lighting color on egg weight loss, the weight of eggshell, the relative weight of embryo, and the weight of yolk at 18 days of incubation:** Table 3 shows the results of the storage period (5 days) effect and the color of the lighting on the eggs' hatching qualities in broilers, which reveal the loss of egg weight, the weight of the eggshell, the relative weight of the embryo in the egg and yolk weight at 18 days of hatching. There is no significant influence ( $P>0.05$ ) of the lighting color

during the incubation period on the qualities of hatching eggs stored for five days and studied at the age of eighteen days. The weight loss of fertile eggs increased during their storage period prior to incubation; the largest weight loss (2.60%) was observed in eggs kept for 8 days. The results revealed that interference between colored illumination and the 5-day storage had a significant impact ( $P<0.05$ ) on the weight of the shell and the weights of the embryo at 18 days, with the highest values recorded at green light group GL being 11.93%, 5.001 g, and 28.717%, respectively.

The results also revealed that the percentage of egg weight loss increased in the light treatment group compared to the dark and control groups (C), as 11.749, 11.930, 11.032, 11.255%, for WL5, GL5, RL5, and GL+RL5, respectively. The green-light egg group had the highest relative percentage weight of the egg embryo. The weights loss of the egg increase with the number of storage day. The weight loss of the eggs during storage was higher than that reported by Reijrink et al. (2009) for broiler breeder eggs stored at 16 to 18°C and unspecified relative humidity and by González-Redondo (2010) for red-legged partridge eggs stored at room temperature (15°C) and 80% RH. The weight loss of the eggs during storage in the current study followed the expected pattern. There was a negative effect of storage for 6, and 8 days on the weight loss of eggs in incubation for Fayumi eggs (Khan et al. 2013). Prolonged storage duration has detrimental effects on embryo growth, and other important hatching characteristics, reducing chick quality at hatching (Reijrink et al. 2010). Eggs held for longer periods lose weight more quickly because of water loss (Cherian et al. 1990), but may also result from internal egg transition reactions.

Ovomucin-lysozyme complex dissociates during storage, resulting in the liquefaction of the albumen and the transformation of ovalbumin into S-ovalbumin, which is crucial for better incubation outcomes. Through the pores of the eggshell, these processes help water evaporate (Seibel et al. 2005).

However, longer storage durations result in higher shell relative weight (Silversides & Scott 2001). Therefore, expanded egg storage for 12 days enhanced weight loss (Melo et al. 2021). Different LED light colors had an impact on egg quality (Er et al. 2007). The relative weight of the developing embryo in eggs has not been affected using color lighting. The blood IGF-1, which is primarily naturally produced by the liver in conjunction with melatonin (Wang et al. 2014), and upregulation of genes encompassing myogenic regulatory aspects (MYF5, and MYOD), coupled box 7, which preserves elderly skeletal satellite cell integrity, and muscle-specific regulatory factor 4 are linked to light-induced muscle emergence (Bai et al. 2019).

Numerous studies have found that light is crucial for the growth and development of birds because it controls, through the eyes and extra-retinal photoreceptors, the hypothalamic region, which secretes TSH, which activates the synthesis and secretion of thyroid hormones. An organism's biological rhythm can be altered over time by light stimulation, and this has an impact on practically all physiological functions. Continuous mild stress causes significant alterations in the endocrine system, particularly pituitary-gonadal axis dysfunction, which manifests as metabolic disorders, early aging, a shorter life span, and an increase in the incidence of tumors, abnormal body weight, gonadal development, and level and persistence of gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) secretion (Zhang et al. 2019). On the other hand, hens that are exposed to oxidative stress over a longer duration in a dark environment produce many oxygen-free radicals. The normal metabolism of the chicken was disrupted by excessive free radical damage to proteins, nucleic acids, and other biological macromolecules (Fariás et al. 2016). This may also be the case with eggs incubated and stored in dark conditions. Previous research has shown that incubating an embryo with green LED lights from six days before hatching to sixteen and eight days later promotes the growth of

**Table 4.** The effect of the storage period (14 days) and the color of the light on the relative weight of the embryo, the weight of the shell, the weight loss of the egg at the seventh day of incubation.

Criteria	Control	D2	WL14	GL14	RL14	RL14&GL14
EWL%	7.207±0.06 <sup>a</sup>	6.022±0.05 <sup>c</sup>	6.532±0.03 <sup>b</sup>	6.623±0.05 <sup>b</sup>	6.745±0.04 <sup>b</sup>	6.462±0.02 <sup>c</sup>
ESW g	7.723±0.07 <sup>a</sup>	6.174±0.01 <sup>b</sup>	6.823±0.00 <sup>b</sup>	6.811±0.00 <sup>b</sup>	6.839±0.00 <sup>b</sup>	6.824±0.00 <sup>b</sup>
RWM%	4.822±0.04 <sup>a</sup>	3.009±0.01 <sup>c</sup>	3.746±0.03 <sup>b</sup>	3.937±0.02 <sup>a</sup>	3.171±0.01 <sup>c</sup>	3.849±0.01 <sup>b</sup>
<sup>1</sup> LC.effec<	-	NS	NS	NS	NS	NS
<sup>2</sup> SP.effec<	-	*	*	*	*	*
<sup>3</sup> SP×LC	-	*	*	*	*	*

Control treatment, fresh egg incubated in darkness; D2 eggs stored for 14 days in dark incubation. (WL 14) eggs stored for 14 days and incubated under the influence of fluorescent (GL 14) eggs Stored for 14 days and incubated under the influence of green light (RL14), eggs stored for 14 days and incubated under the influence of red light (RL14&GL14), eggs stored for 14 days and incubated under the influence of green and red . The relative embryo weight RMW %, Egg Shell weight ESW, Egg weight loss percentage EWL.<sup>a, b</sup> means that within a single column, with different letters differ significantly  $P (0<0.05)$ . \* Significant difference in light color on these items at ( $P<0.05$ ).

myoblasts (Stojanovic et al. 2014; Kanacki et al. 2017). Pre-incubation egg weight, storage times, embryo weight, and chick weight of several chicken species all have substantial positive connections. (Nahm 2001), The influence of storage was clearly seen in the hatching characteristics of the weight loss, the weight of the embryo, and the weight of the shell during the first and last incubation periods. The results also revealed significant variations in the coefficients and that the colored lighting group outperformed the two dark control groups C and the dark group that had been stored for five days (D\_.

**Effect of storage period of 14 days and lighting color on relative weight of embryo, weight of shell, and weight loss of egg on 7th day of incubation:** Table 4 shows the impact of the 14-day storage time, and the color of the illumination on incubation duration on the hatching characteristics at the age of 7 days, the weight of the loss in the incubated eggs, the weight of the eggshell, and the relative weight of the embryo. According to the results, the storage periods had a negative impact ( $P>0.05$ ) on these above-mentioned metrics, as shown by the decrease in weight loss, shell weight, and relative embryo weight in the lighting groups compared to the dark and non-stored control group (D2). The incubation duration of the stored lighting groups was 14 days compared to the dark group D2 which had the same length, suggesting that lighting may have a role in minimizing the detrimental storage impact. According to the results, there is a significant effect ( $P<0.05$ ) of interference

between the storage and color lighting.

In a study conducted by Abdulateef et al. (2021), the exposure of various light colors' influence on embryonic growth showed a significant increase in embryo weight between RL, BL, and GL (33.55, 33.93, and 34.70 g, respectively) and D after 19 days of incubation (30.00g). Moreover, there was a considerable increase in body weight ( $P<0.01$ ). The length of egg storage have an impact on embryo weight throughout incubation in agreement with previous finding (Christensen et al. 2002; Yalcin & Siegel, 2003; Bakst et al. 2016). Hamidu et al. (2011) showed a reduced embryo weight with 21 days of storage compared to 4 days of storage from day 4 of incubation onward. Eggs from Lohmann light-brown chickens aged between 24 and 29 weeks (Jin et al. 2011) have increased in yolk weight (+0.93 g; 1.14%) after the same amount of storage period. The decline in egg weight and the movement of water from the albumen to the yolk can be used to explain the rise in the yolk percentage.

**Effect of the storage period of 14 days and color of light on the weight of shell, weight loss of egg, the relative weight of embryo, and weight of yolk at fifteen days of incubation:** Table 5 shows the impact of lighting color during the incubation and storage period on the percentage of weight loss in the egg, the relative weight of the developing embryo, and the weight of the yolk at fifteen days of incubation. The results show a significant effect ( $P<0.05$ ) of colored lighting (WL, GL, RL, GL and RL) on the treatment

**Table 5.** The effect of the storage period (14) days and the color of the lighting on the weight of the shell, the weight loss of the egg, and the relative weight of the embryo, at fifteen days of incubation.

Criteria	Control	D2	WL5	GL5	RL5	RL5&GL5
EWL%	10.694±0.27 <sup>a</sup>	5.473±0.12 <sup>d</sup>	9.385±0.54 <sup>b</sup>	9.652±0.46 <sup>b</sup>	8.736±0.42 <sup>c</sup>	8.204±0.24 <sup>c</sup>
ESW g	6.004±0.37 <sup>a</sup>	6.000±0.38 <sup>a</sup>	6.020±0.33 <sup>a</sup>	6.001±0.23 <sup>a</sup>	6.100±0.41 <sup>a</sup>	6.011±0.44 <sup>a</sup>
WM%	23.749±0.31 <sup>a</sup>	19.013±0.31 <sup>d</sup>	20.213±0.22 <sup>c</sup>	21.01±0.26 <sup>b</sup>	21.05±0.36 <sup>b</sup>	20.936±0.52 <sup>c</sup>
YW g	18.265±0.66 <sup>b</sup>	19.298±0.64 <sup>a</sup>	19.746±0.47 <sup>a</sup>	18.012±0.41 <sup>b</sup>	19.854±0.45 <sup>a</sup>	19.879±0.64 <sup>a</sup>
LC effect <	-	*	NS	*	*	*
SP effect <	-	*	*	*	*	*
LC×SP effect<	-	*	*	*	*	*

Control treatment, fresh egg incubated in darkness; D2 eggs stored for 14 days in dark incubation. (WL 14) eggs stored for 14 days and incubated under the influence of fluorescent (GL 14) eggs Stored for 14 days and incubated under the influence of green light (RL14), eggs stored for 14 days and incubated under the influence of red light (RL14&GL14), eggs stored for 14 days and incubated under the influence of green and red . The relative embryo weight RMW %, Egg Shell weight ESW, Egg weight loss percentage EWL, yolk weight YW. <sup>a, b</sup> means that within a single column, with different letters differ significantly ( $P<0.05$ )

of eggs incubated under colored lighting at the fourteen-day storage period, and the green GL, and red color RL were the highest in terms of the weight of the developing embryo compared to the negative control group D2. The relative embryo weights in the green and red color groups were the highest.

The lowest percentage of relative egg weight loss was shown in the group of eggs incubated under green and red lighting (GL and RL). The yolk weight increases with increasing storage period, and this naturally affects the yolk weight in the hatching eggs, where an increase was observed in the negative colored and dark lighting group D2 stored for fourteen days compared with the positive control of dark and non-stored. There are no significant differences ( $P>0.05$ ) in the weight of the eggshell, and this may be due to the slight effect on the shell components due to storage or lighting (Stepińska et al. 2017). Both the length of the storage period and the laying season had an effect on the amount of water lost by evaporation from hatching eggs. Reis et al. (1997) hypothesized that eggs with more advanced embryonic development up to 18 days of incubation resulted in increased metabolic heat production, which may raise embryo temperature, and hence water vapor pressure, inside the egg. Furthermore, at the same incubation stage, more advanced embryos produced more metabolic water (Ar & Rahn 1980), which may potentially contribute

to increased water loss. There is also a significant effect ( $P<0.05$ ) of the storage period on all of the quality parameters at the age of fifteen from the incubation period, and a significant effect of the interference ( $P<0.05$ ) between colored lighting and the storage period on the qualities of broiler hatching eggs. By increasing storage days, the weight of the yolk increased due to the sprouting of water from the albumin to the yolk (Barbosa et al. 2004).

**Effect of the storage period of 14 days and color of light on the weight of shell, weight loss of egg, and relative weight of embryo on 18 days of incubation:** Table 6 shows the effect of the storage period and color lighting on the weight loss of the egg, the weight of the eggshell, the relative weight of the embryo, and the weight of the yolk at eighteen days of incubation. The results showed that the storage period at 14 days had a negative significant effect ( $P>0.05$ ) on the hatching qualities studied at eighteen days of incubation, as evidenced by the high percentage of loss in the weight of hatching eggs in the color lighting groups. The results revealed a significant ( $P<0.05$ ) decrease in the weight of the shell and the relative embryo weight in the color light treatments compared to the positive control, but the best values were observed in the green-light and red-light groups, while the lowest values were in the dark control treatment. A higher yolk weight was also observed in the eggs hatching the storage and light



**Table 6.** The effect of the storage period (14) days and the color of the light on the weight of the shell, the weight loss of the egg, and the relative weight of the embryo at eighteen days of incubation.

Criteria	Control	D1	WL5	GL5	RL5	RL5&GL5
EWL%	9.00 ±0.11 <sup>d</sup>	11.687 ±0.9 <sup>b</sup>	10.995 ±0.4 <sup>b</sup>	12.453 ±0.4 <sup>a</sup>	11.534 ±0.6 <sup>b</sup>	10.13 ±0.5 <sup>c</sup>
ESW g	7.010±0.1 <sup>a</sup>	5.235±0.1 <sup>c</sup>	6.251±0.4 <sup>b</sup>	6.072±0.5 <sup>b</sup>	5.031±0.7 <sup>c</sup>	5.231±0.4 <sup>c</sup>
WM%	26.974±0.11 <sup>a</sup>	17.00±0.16 <sup>e</sup>	19.622±0.15 <sup>d</sup>	21.833±0.12 <sup>b</sup>	20.593±0.21 <sup>c</sup>	20.001±0.9 <sup>c</sup>
YW g	18.466±0.45 <sup>c</sup>	19.778±0.21 <sup>ab</sup>	19.922±0.64 <sup>a</sup>	17.002±0.32 <sup>d</sup>	20.811±0.42 <sup>a</sup>	18.804±0.41 <sup>c</sup>
LC effect <	*	NS	*	*	*	*
SP effect <	-	*	*	*	*	*
LC×SPeffect<	-	*	*	*	*	*

Control treatment, fresh egg incubated in darkness: D2 eggs stored for 14 days in dark incubation. (WL 14) eggs stored for 14 days and incubated under the influence of fluorescent (GL 14) eggs Stored for 14 days and incubated under the influence of green light (RL14), eggs stored for 14 days and incubated under the influence of red light (RL14&GL14), eggs stored for 14 days and incubated under the influence of green and red . The relative embryo weight RMW %, Egg Shell weight ESW, Egg weight loss percentage EWL, yolk weight YW. <sup>a, b</sup> means that within a single column, with different letters differ significantly ( $P < 0.05$ ) \* Significant difference in light color on these items at ( $P < 0.05$ ) NS refer to Not significant

treatments compared to the dark and non-stored control group. These results are consistent with the findings of Alsobayel et al. (2013) which confirmed that a 14-day storage period has a negative effect ( $P \leq 0.05$ ) on egg weight loss and embryo weight in broiler eggs Rose 308, where fresh eggs have the highest weight of developing embryos and the percentage of egg weight loss increases as the storage period increases. Reijrink et al. (2010) showed similar findings for eggs held for four and fourteen days before incubation, however the egg weight loss, during storage was substantially higher for eggs stored for 14 days. Yu et al. (2018) discovered that low-intensity green LED light (50lux) enhanced embryo development during incubation. When the green light was adjusted at 150 or 300lux, no improvement in embryo weight to the original egg weight was observed. The intensity in our study was around 200 lux at the egg level, which may be too high, when exposed to fluorescent light at 200 to 300lux, however, embryo weight rose (Özkan et al. 2012). There is a significant effect ( $P < 0.05$ ) of the interference between the storage period and the color of the lighting during the hatching period on the studied productive qualities at this age in our study.

**Effect of the storage period and the color of the lighting on the hatchability rate, the weight of the**

**chicks', remaining yolk sac, hatch time, and total embryotic mortality:** Table 7 represents the effect both the storage period (5 and 14 days) and lighting color on the hatching ratio, the weight of the hatched chick, the proportion of remaining yolk, the hatching time and the total embryotic mortality of broilers Rose 308. The results showed no significant effect ( $P > 0.05$ ) of the storage period (5 days) on the hatching rate, weight of the hatched chick, proportion of the remaining yolk, or hatching time. The results showed a significant effect ( $P < 0.05$ ) of lighting color on the above mentioned characters. The highest value of the hatching percentage was recorded for the green light (GL) hatching egg group (94.55%) and the lowest value for control groups C and D1 (92.04 and 91.48%, respectively). There were also significant differences ( $P < 0.05$ ) between the different lighting totals in the hatching ratio.

The green group also recorded the highest weight of hatched chicks (38.52) than the control and D1 groups, which were 37.12 and 36.22, respectively. The proportion of yolk sac residues was the lowest in the colored lighting groups (9.10) inside the incubator than the control and D1 groups (10.15 and 10.41%, respectively). There were also significant differences ( $P < 0.05$ ) between the colored lighting aggregates. The results also showed a significant

**Table 7.** Effect of the storage period and the color of the lighting on the hatchability rate %, weight of the chicks' g, Remain yolk sac % and hatch time Hr. and total embryonic mortality %.

Criteria	Control	D1	WL5	GL5	RL5	RL5&GL5
<sup>1</sup> Hatchability%	92.04±0.18 <sup>ab</sup>	91.48±0.21 <sup>c</sup>	92.55±0.2 <sup>b</sup>	94.77±0.01 <sup>a</sup>	93.70±0.21 <sup>ab</sup>	92.38±0.15 <sup>b</sup>
WOC g	37.12±0.23 <sup>b</sup>	36.22±0.42 <sup>bc</sup>	37.93±0.46 <sup>b</sup>	38.52±0.43 <sup>a</sup>	37.97±0.43 <sup>b</sup>	37.58±0.33 <sup>b</sup>
<sup>2</sup> RYS%	10.15±0.02 <sup>a</sup>	10.41±0.02 <sup>b</sup>	9.31±0.07 <sup>a</sup>	9.10±0.08 <sup>a</sup>	9.22±0.10 <sup>a</sup>	9.12±0.04 <sup>a</sup>
hatch time Hr.	521±0.86 <sup>c</sup>	526±0.92 <sup>cd</sup>	515±0.57 <sup>b</sup>	510±0.78 <sup>a</sup>	513±0.82 <sup>a</sup>	512±0.85 <sup>a</sup>
<sup>3</sup> T. E. M. %	20.26±0.02 <sup>a</sup>	19.24±0.04 <sup>b</sup>	15.62±0.07 <sup>e</sup>	15.21±0.02 <sup>c</sup>	18.16±0.05 <sup>c</sup>	16.37±0.05 <sup>d</sup>
L C effect<		*	*	*	*	*
SP 5D. effect<		NS	NS	NS	NS	NS
LC×SP effect<		*	*	*	*	*
Criteria	Control	D2	WL5	GL5	RL5	RL5&GL5
Hatchability %	90.04±0.85 <sup>a</sup>	63.63±0.5 <sup>d</sup>	64.22±0.65 <sup>b</sup>	67.08±0.66 <sup>b</sup>	62.49±0.78 <sup>c</sup>	64.01±0.76 <sup>b</sup>
WOC g	38.74±0.45 <sup>a</sup>	34.85±0.86 <sup>bc</sup>	34.28±0.73 <sup>c</sup>	35.52±0.64 <sup>b</sup>	35.56±0.68 <sup>ab</sup>	35.72±0.62 <sup>ab</sup>
RYS%	8.42±0.02 <sup>b</sup>	9.01±0.07 <sup>c</sup>	8.77±0.01 <sup>b</sup>	8.94±0.09 <sup>b</sup>	7.83±0.04 <sup>a</sup>	7.22±0.07 <sup>a</sup>
hatch time Hr.	516±0.45 <sup>a</sup>	580±0.67 <sup>b</sup>	520±0.41 <sup>a</sup>	533±0.042 <sup>b</sup>	524±0.85 <sup>a</sup>	518±0.55 <sup>a</sup>
<sup>3</sup> T. E. M. %	21.37±0.26 <sup>d</sup>	25.04±0.25 <sup>a</sup>	22.87±0.18 <sup>c</sup>	22.64±0.25 <sup>c</sup>	22.54±0.26 <sup>c</sup>	22.05±0.11 <sup>b</sup>
L C effect<		NS	NS	NS	NS	NS
SP 14 D.effect<		*	*	*	*	*
LC×SP effect<		*	*	*	*	*

<sup>1</sup>Hatchability of fertile eggs, %, <sup>2</sup>Remain yolk sac % after 3 hours of hatching, <sup>3</sup>Total embryonic mortality%.

effect ( $P<0.05$ ) of the color of the lighting on the period and time of hatching of the chicks. The lowest hatching time was in the green color group GL, the red color group RL, and the green, red color group RL5&GL5, compared with the control group C and D1 (Table 7). There is a significant effect ( $P<0.05$ ) of the interference between the color of the lighting in the incubator and the storage period on the measured qualities.

There was no significant effect ( $P>0.05$ ) of light color on the hatching rate of eggs that had already been stored for 14 days before incubation, but a superiority was observed as the highest hatching rate in the color lighting group was for the green color group compared to the control group D2 (Table 7). There were also significant differences ( $P>0.05$ ) between the color lighting totals; color lighting did not significantly affect ( $P>0.05$ ) on the weight of hatched chicks or the proportion of yolk residue or hatching time. However, there was a significant negative effect ( $P>0.05$ ) of the storage period before incubation on the hatching rate, the weight of the

hatched chick, the remains of the yolk sac, and the hatching time.

The results revealed no significant effect ( $P>0.05$ ) of storage period on the percentage of total fetal deaths, whereas the data revealed that colored lighting had a significant effect, with the control group having the highest percentage of total embryo mortality compared to the totals of colored lighting at a storage period of 5 days. The greatest reported rates were in the control group was 20.26%, whereas the lowest reported rates were in the green and white light groups was 15.62 and 15.21% respectively. The table also confirmed that there are important differences between the aggregates exposed to colored lighting, as well as showing that there is an important interference between the storage period and colored lighting at 5 storage days. The results confirmed that there is a significant negative effect ( $P>0.05$ ) of storing eggs for 14 days on the percentage of total embryonic losses of broiler hatching eggs, where the dark control group D2 recorded the highest percentage of losses of 25.04

compared to the control group C, while there was no significant effect of colored lighting on the percentage of embryonic losses of the totals, as the table showed that there were no significant differences ( $P>0.05$ ) between the colored totals, but a clear intervention was shown by the results between the storage period of 14 days and the colored lighting used in the incubation of broiler.

In the broiler hatchery industry, storing eggs at a low temperature prior to incubation is routine practice; however, extended storage (more than 7 days) increase early embryonic death and lower chick quality and performance. Hatchability rates at 12°C were considerably higher than at 18°C after 7 days of storage, and chick quality remains proper (Pokhrel et al. 2018). Early mortality elevated earlier and steeper with longer egg storage in older breeders compared to younger breeders, whereas in 2- and 5-day stored eggs no effect of breeder age was found. Hatchability of viable eggs in 19-day stored eggs was lower (Nasri et al. 2020). Extended egg storage (>7 days) has been linked to decreased egg quality metrics (Schmidt et al. 2009) increased embryonic abnormalities and mortality, reduced hatchability (Decuypere et al. 2007) and inferior hatchling quality (Tona et al. 2003). Aside from the impacts of storage time, a greater breeder age has a detrimental impact on fertility (Zakaria et al. 2009), egg quality, early and late embryonic mortality (Joseph & Moran 2005) and hatchling weight (Yildirim 2005). Short egg storage (5 days) results in increased hatchability than 19-day egg storage. Pokhrel et al. (2018) found that in young breeders, hatchability was higher after 7 days of storage than after 0 days of storage and decreased with prolonged storage duration (21 to 28 days).

According to the findings, storage length has the highest effect on early embryonic development. Once the embryo has passed the key developmental period, it will continue to develop normally. This shows that the key stage for embryonic survival after lengthy storage is early embryogenesis, which occurs within the first three days of incubation. This may be due to

the considerable changes that occur during storage to both the embryo and its surrounding environment the yolk and albumen (Pokhrel et al., 2018). Schmidt et al. (2009) also pointed out that the greatest impact of increased storage is attributable to early mortality (0 to 6 days) at 40.32%, followed by late mortality (18 to 21 days) at 37.61% after 14 days of storage.

Christine et al. (1976) found hatchability of fertilized eggs was 19.5% lower in eggs held for 14 days, and more embryos survived between 4 and 18 days than in not stored eggs when compared to incubated eggs that were not stored. The embryonic development of eggs incubated for 42 hours after being stored for 7 or 5.3 and 12.2 hours, respectively delayed 14 days. Storage at 16.5°C compared with 10°C decreased both hatchability of fertile eggs and chick weight at hatch (Ruiz & Lunam 2002). The storage of eggs for 14 days resulted in a 134-hour delay in the mean overall incubation time; In addition, according to Christine et al. (1976), the hatchability of viable eggs was reduced by 19.5% in eggs stored for 14 days, and more embryos died between 4 and 18 days of incubation than in un-stored eggs. Chicks from the 4-day storage period also hatched before chicks from the 14-day storage period; they did so after 486.3 hours of incubation, whereas chicks from the 14-d storage period (Reijrink et al. 2010). The storage duration has a substantial impact on chick weight at hatch. In comparison to eggs kept for 7 and 14 days, which had identical chick weight, fresh eggs had a considerably ( $P\leq 0.05$ ) greater chick weight. Romao et al. (2008) observed that quail eggs had higher hatchability until 10 days of storage and that eggs supplied for storage had a lower weight loss throughout incubation. The findings of Rozenboim et al. (2004) imply that green light stimulation promotes growth and development in chicks. Moreover, when this stimulus is given during incubation, the best effect is obtained. There were no significant differences in hatch weight, hatchability, or overall embryonic mortality between the experimental groups ( $P>0.05$ ). According to previous works (Özkan et al. 2012a, b; Zhang et al.

2012), varied LED lights applications to hen eggs in incubation had no effect on hatch weight and hatchability.

El-Sabrouh & Khalil (2017) showed that the use of lighting during the incubation period has no effect on hatching rate. The hatchability percentage of eggs had no significant effect ( $P>0.05$ ). This result does not agree with the results of Pandian et al. (2013) which found that light had insignificant effect on chick weight. Khalil (2009) revealed that chicks hatched under light regime incubation appeared to be more active than those hatched under dark incubation. The results of the study conducted by Ayeni et al. (2020) indicated that the storage of broiler hatching eggs for different periods (1, 4, 7, 10, and 13 days) has an impact on the hatching qualities, hatching ratio, and weight of the hatched chick. The reduction of hatchability percentage and the number of chicks produced with storage times longer than day 4 as seen in this study may be caused by albumen degradation and water loss. In addition, stored eggs for 13 days diminish the hatching by 92 to 78%. This finding was consistent with Ewonetu's (2016), which examined how long eggs are stored for in related to weight loss, hatchability, and chick development performance. Ewonetu (2016) also found that eggs are less likely to hatch when they are stored for an extended length of time. Yassin et al. (2008) showed that storage times longer than 7 days significantly affect hatchability. An extended egg storage time may result in a decrease in chick weight (Ewonetu 2016). According to Fasanko (2007), the lengthy storage of the eggs delayed the growth and metabolism of the embryo after incubation, which slowed the rate of development and resulted in a decline in the weight of newly hatched chicks in the long-stored eggs. According to Heba (2015) and Van de Ven (2004), the albumen's higher pH and decreased viscosity were the primary factors in the reduced hatchability of long-stored eggs. Schmidt et al. (2009) and Romao et al. (2008) pointed out that a protracted storage period may be harmful to the quality of hatching eggs and decrease hatchability.

Khan et al. (2014) revealed that storage of eggs for varied periods had a detrimental influence on the hatching rate in Rhode Island Red (RIR) chickens. Hatchability rates at 12°C were higher than at 18°C after 7 days of storage (Pokhrel et al. 2018).

Chicks hatching under white and blue lights weighed more ( $P<0.05$ ). During the first week after hatching, providing photoperiodic blue and white light increase broiler production characteristics (Li et al. 2021). Early research employed incandescent and fluorescent lighting, which had a favorable effect on rapid embryo growth and decreased incubation time to hatch (Archer et al., 2009; Cooper et al. 2011). Eggs incubated in the light have quicker embryo development (Shafey & Al-Mohsen 2002; Cooper et al. 2011), higher hatchability (Archer 2017; Archer et al. 2017), and improved chick quality at hatch (Archer 2017; Archer et al. 2017). According to Huth & Archer (2015), layer breeder eggs incubated under different lights has no impact on their capacity to hatch. Based on the previous works, the light stimulation during incubation decrease or no effect on hatchability or body weight at hatch (Archer et al. 2009; Ozkan et al. 2012; Archer & Mench, 2014a; Zhang et al. 2016; Sabuncuoglu et al. 2018). Farghly & Mahrose (2012) showed that this application improved hatchability. Huth & Archer (2015) and Archer (2015), who gave broiler breeder eggs with brown eggshells and layer breeder eggs with white eggshells with LED lighting and dark surroundings, found identical results in total embryonic mortalities.

## Conclusion

This study indicated that the use of colored lights can improve hatching results and that storage for 15 days can lead to a decrease in hatching rate and hatching quality.

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