Egypt. Poult. Sci. Vol. (44) (IV) : (517-526)(2024) **(2309-1265)**

Egyptian Poultry Science Journal

http://www.epsj.journals.ekb.eg/

ISSN: 1110-5623 (Print) – 2090-0570 (Online)

IMPACT OF CHROMATIC LIGHT ON DEVELOPMENT AND HATCHING OF ALEXANDRIA CHICKEN`S EMBRYOS

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Received: 30 /09/2024 Accepted: 30 /12 /2024

ABSTRACT: Numerous studies have shown the effects of light wave length on the performance of birds in experimental assays; however, we chose to assess the effects of various color lights using light-emitting diodes (LED) on Alexandria chicks during the incubation period to investigate their effects on the morphological, developmental, and hatching traits in addition to the concentration of growth hormone during the hatching period. The experiment was conducted in three groups, totaling 1800 eggs, with the eggs being distributed equally among the groups: the first group of eggs was incubated under white light for 12 hours followed by 12 hours of LED lighting, the second group of eggs was exposed to green light (G2) for 12 hours followed by 12 hours of darkness, and the third group of eggs was incubated for 24 hours of complete darkness. From the obtained results, white and green light had significant increase in morphological traits during incubation period especially at 12^{th} and 18^{th}days. On the other hand, at hatch (21^{th} day) green group showed highly significant difference for values of Wing length (4.78^{a} cm) and Hind limblength (7.74^{a} cm) compared with white and dark groups. Moreover, Compared to the green and dark groups, the white group's head height (2.14 cm) was the highest. additionally, as compared to chicks exposed to darkness during incubation, Alexandria chicks showed a notable improvement in their scientific hatchability. The findings also showed that the unhatched egg characteristic was significantly lower in the green group (2.7%) than in the dark group (6.3%), and that there were no unhatched eggs in the white group. Additionally, the early embryonic mortality percentages of Alexandrian eggs are decreased by green and white light. Growth hormone levels during the development of embryos were discovered to be highly significant; on the 12^{th} day, embryos exposed to green light were reported to have the highest level (0.796 ng/ml); on the 18^{th} day, however, the highest level was observed in embryos exposed to white light (0.520 ng/ml). Previous findings indicate that white or green.

Keywords: chicken`s embryos-chromatic light- led lighting- Alexandria chicks

INTRODUCTION

Light management is a highly effective method in chicken production. Breeders can enhance productivity and decrease mortality by using light-emitting diodes (LED), which consume less energy and have a longer life than fluorescent or typical incandescent lighting (Gongruttananun 2011).Chicken eggs are frequently housed commercially in semi- or complete darkness, yet under natural conditions, avian embryos would undoubtedly receive some light stimulation during development (Rogers 1996).

Exposing eggs to light can increase the embryo's growth (Shafey 2004) and decrease time of incubation (Fairchild and Christensen 2000). However, numerous studies have shown the importance of exposing embryos to light (Shafey and Al-Mohsen2002 , Archer *et a*l 2009). There is evidence that exposing embryos to light during incubation can have an effect on hatchability (Cooper, 1972; Shafey and Al-Mohsen, 2002; Shafey, 2004; Archer and Mench, 2014a and 2014b; Archer, 2015a) and decrease incubation time (Ghatpande *et al*., 1995; Fairchild and Christensen, 2000; Shafey and Al-Mohsen, 2002; Tong *et al.*, 2015). Light exposure has been seen to improve animal welfare posthatch.

Archer (2016) discovered that two different forms of white light boosted hatching and were similarly filtered into the red frequency range. Hluchý et al. (2012) observed that monochromatic illumination during broiler egg incubation resulted in higher hatchability for red light compared to blue light, with white light having the highest overall hatchability. Archer (2016) discovered that red light increased hatchability in white layer eggs but not in broiler eggs. Previous studies found that exposing chicken (Rozenboim *et al*., 2004; Zhang *et al.,* 2016) and turkey (Rozenboim *et al.,* 2003) embryos to green light during incubation resulted in chicks with higher BWs and breast muscle weights There has been little research into how exposing avian embryos to different wavelengths of light affects morphological features and growth hormone, therefore we carried out an experiment to investigate this.

Increased hatchability and hatch of fertile percentages may be linked to beneficial changes in hatch-related hormones, particularly thyroid and corticosterone, in response to light exposure treatments (Tong *et al.* 2018). Furthermore, this improvement could be attributed to having a positive effect on an embryo's developmental pace, as the study found that light during incubation accelerates embryonic development (Ghatpande *et al.* 1995). Furthermore, it could be attributed to a shift in the melatonin cycle in embryos exposed to light during incubation (Tong *et al.* 2018).

The objective of this work is to study the effect of different wave lengths (colors) of LED light on embryo development during incubation and some chick's traits at hatch. To achieve this goal, the following tasks were performed:

- Study the morphological parameters of chicken embryos of different wave lengths (colors) of LED light during the incubation period.
- Study the biochemical Examination of growth hormone of chicken embryos of different wave lengths (colors) of LED light during the incubation process.
- Study the chick's traits at hatch (hatching &morphological traits) of different wave lengths (colors) of LED light.

MATERIAL AND METHODS

The present study was carried out at the poultry research center, poultry faculty of Agriculture, Alexandria University, and also carried out at Histology and Pathology Laboratory of Medical Technology Center during incubation season. During the month of November2019 to February 2020. All treatments and chicks care and use committee in AU-IACUC, Alexandria University Egypt with the review report number AU08190115207.

A total of 1800 Fertile Alexandria chicken eggs were used. Eggs were weighed, numbered, and placed in commercial incubator located in dark room. Eggs were divided equally into three groups, two light treated groups (white - green)

from the first day of incubation until 18th day, and the control group.

Light treatments were allotted into three trials, first trial eggs were incubated in white LED light (full spectrum), the second trail was in Green LED light (wavelength 510–530 nm) and the third group was incubated in complete darkness. Incubator was outfitted with four bulbs. Two bulbs at each side of incubator located 10 cm above the eggs in the compartment of the light treatment. Light intensity was 12W/m^2 at the top surface of the eggs. Lighting managements (white and Green) were operated by an automatic timer to 12 hours light and 12 hours off (12 L: 12 D) light schedule (**Archer, 2015**). The front windows of incubator were blacked out with cardboard to prevent light intrusion into the machine and treatments were separated by black curtains to eliminate any crossover stimulation. The eggs were moved to the hatch on 18th day. The hatch had no light and the eggs were in complete darkness. After complete hatching process, the non-hatched eggs were broken and examined for fertility, hatchability and embryonic mortality percentages.

Studied traits:

1-Embryo development during incubation:

A. Morphological measurement.

An equal number of eggs were opened in each treatment ,18 Chicks were morphologically examined, weighed and measurements were carried out for two morphometric parameters on ages 4th, 8th, 12th and 18th days of incubation by using sensitive balance and tap measure:

-Body weight

-Body length

B-Growth hormone concentration.

Few embryos form each group were immediately frozen for biochemical investigations. Biochemical studies were carried out on ages 12 and 18 days of incubation. Embryos were carefully removed from the yolk and membranes and they were transferred to a new saline solution for washing then stored in -80 ºC**.** For preparation of 10% weight/volume homogenate, 0.5 g of chick embryo tissue was homogenized in 5 ml (0.0067 M) phosphate buffer (pH 7.4) using homogenizer (Unidrive 1000 D, Germany). The homogenates were centrifuged at 5000 rpm for 20 min and the supernatant was kept frozen at -20°C for the subsequent biochemical assays.

The Growth hormone concentration in all samples was measured using a commercial Elisa kit of anti-chicken IGF-I (USCN Life Science and Technology Company, Wuhan, China; Liu *et al*., 2010), which designed to detect and quantify the level growth hormone in serum, plasma, and cell culture media.

2. Chicks traits at hatch:

A- Hatching traits

-Scientific hatchability percentage.

- Embryonic mortalities.

- Un hatched eggs.

Scientific hatchability percentage (No. of hatched eggs / Total number of fertile eggs)*100, embryonic mortalities of Alexandria chicken eggs .

B -Morphological Traits at hatch

Chicks were morphologically examined, weighed and measurements were carried out for six morphometric parameters on 75 chicks from each treatment by using tap measure:

1-Head height

2-Crown –rump (body length)

3-Wing length

4-Body weight

5-Hind limblength

6-Head circumference

Statistical analysis:

Duncan's multiple range test (Duncan's ,1955) was used to test differences within means of treatments while level of significances was set typically at minimum ($P <\_ 0.05$) .

The statistical model for analysis data was as following:

$Y_{ij} = \mu + T_i + e_{ij}$

Where:

Y_{ij} = the observation.

μ = the overall mean .

T_i = The effect of light groups.

e_{ij} = Random error.

RESULTS AND DISCUSSION

1-Embryo development during incubation:

A. Morphological measurements:

Body weight

Table (1) Determine the body weight of embryos incubated in various light colors (white-green-dark) . at different ages. The results were significant in all treatments during incubation, with the exception of the 8th day, when no significant difference was seen between treatments.

On the other hand, embryos incubated under white light had a significantly higher body weight than those incubated under green or darkness on the fourth day, while embryos incubated under both white and green lights had a significantly higher body weight than those incubated under dark conditions on the12thday.

Finally, the body weight of embryos incubated under green lights on the 18th day differed significantly from the white and dark groups. This result is in good agreement with the result of (Shaefy and Mohsen, 2002), who reported that continuous stimulation with green light enhances embryo development and body weight.

Body length

The effect of various color lights on embryos body length during incubation at different periods of age are presented in Table (2).

The results demonstrated that the embryos that incubated under white and green lights at 4th, 12thand 18th days had higher significant differences in the values of body length than the embryos that incubated under dark condition.

Meanwhile, There was no significant observed between treatments on embryos body length on 8th day.

Oso *et al* (2023) reported the effects of incubation under light for different durations at differing periods during incubation on embryonic development parameters are showed that Exposure of eggs to light during incubation had no significant effect on the development of all the parameters measured compared to incubating under darkness throughout incubation. The timing of exposure to light influenced embryo length development. Eggs exposed for 21 days (TB) had a higher length compared to those exposed for 7 days (TD).

B- Growth hormone concentration:

Table (3) shows the growth hormone levels of Alexandria embryos that incubated under different light colors at 12th and 18th days.

The results showed a highly significant difference between all treatments. Green light treatment resulted in a highly significant difference (0.796 ng/ml) in growth hormone levels at the 12th day of incubation compared to the white and dark groups, whereas white light treatment had the same effect (0.520ng/ml) on growth hormone levels at the 18th day as the green and dark groups. These findings suggested that incubation under varied color lights resulted in an increase in growth hormone release, which improved the growth and development of birds.

It has been suggested that *in-ovo* green light photostimulation might act centrally by activating neuroendocrine agents, such as melatonin, it was found to be higher, when chicks were exposed to GL photostimulation (Zhang *et al*., 2016). This raising might increase GHRH mRNA gene expression in the hypothalamus (Zhang *et al*., 2016).

1- Chicks traits at hatch:

A- Hatching traits:

Fertility and hatchability:

The fertility and scientific hatchability percentage of Alexandria chicks incubated in different light colors are presented in Table (4) and Figures (1& 2).

It was clear that, higher significant differences in scientific hatchability of Alexandria eggs treated with white and green lights (94.14 %, 92.43%) compared to those exposed to dark condition (86.84%).

The same results were recorded by Shafey and Al-Mohsen (2002) observed that eggs incubated under green light had significantly higher hatchability than those incubated under the dark treatment.

The White light treatments produced more viable eggs than the Green and Dark treatments, however it has been discovered that Green light did not improve hatchability in his investigation, implying that the wavelength of light is responsible for increased hatchability. (Archer (2017). This findings, however, contradicted Bowling *et al* ., (1981) found that white light incubation reduced the hatchability of White Leghorn eggs.

Embryonic mortalities and unhatched eggs :

Early and late embryonic mortalities percentages of Alexandria eggs in different light colors are presented in Tables (4) and Figure (3).

The dark group showed the highest significant differences for early embryonic mortality percentages (5.7 %) compared with white (3.7%) and green (3.2%) while for late embryonic mortality the dark group expressed the lowest significant difference (1%) relative to white (2.1%) and green group (1.6 %).

The results also revealed that, the green group expressed higher significant in unhatched eggs trait (2.7%) than dark group (6.3%). While in white group there is no unhatched eggs observed.

Significant differences for early and late embryonic mortality percentages among different light colors were recorded by (Shafey and Al-Mohsen, 2002). However non-significant differences among different light colors were recorded (Archer, 2017).

B-Morphological Traits.

Table (5) elucidate morphological traits of embryos that incubated under different light colors (white-green dark) at hatch (21st day of age).

The results clarified improvement in body weight trait by using white and green lights during incubation but without significant recorded between the three group. However green group showed highly significant difference for values of wing length and hind-Limb compared with white and dark groups. Moreover, white group achieved the highest value of head hight in the comparison with green and dark groups.

On the other hand birds that incubated under white and green lights showed higher significant differences for crown. Ru than those subjected to dark condition. According to the researches done by Hashemian *et al* (2024) The body weight of hatched chicks at 24D was higher than that in the 12L:12D green lighting group; however, indices of skeletal growth (the beak and the digit) and embryonic development (crown-rump length) did not change significantly (P>0.05, Table 5). On the other hand, Tong *et al.* (2015) reported that the use of light treatment resulted in longer crown rump, beak, and digit lengths in hatchlings. This discrepancy could be related to the light's duration and intensity in this and the current studies.

Table (1): Effect of different color lights on body weight (BW) at different period of age during incubation period for Alexandria chicks.

Age / Treatments	Embryo BW4 (gm)	Embryo BW8 (gm)	Embryo BW12 (gm)	Embryo BW18 (gm)
White light	0.09^{a}	1.28	5.17^{a}	21.69^{ab}
Green light	0.06^{b}	1.18	5.16^{a}	23.88^{a}
Dark	0.05^{b}	1.18	4.57^{b}	20.02^{b}
MSE	0.01	0.11	0.32	2.10
P value	0.0001	0.165	0.003	0.010

Means carrying different litters within the same column are significantly different (P < 0.05)

Table (2): Effect of different color lights on embryo body length (BL) at different period of age during incubation for Alexandria chicks.

Age / Treatments	Embryo BL4 (cm)	Embryo BL8 (cm)	Embryo BL12 (cm)	Embryo BL18 (cm)
White light	0.81[a]	1.92	4.07[a]	6.14[a]
Green light	0.80[a]	1.87	4.07[a]	6.17[a]
Dark	0.51[b]	1.78	3.30[b]	5.24[b]
MSE	0.11	0.13	0.38	0.37
P value	0.0002	0.173	0.001	0.0002

Means carrying different litters within the same column are significantly different (P < 0.05).

Table (3): Effect of different color lights on growth hormone (ng/ml) levels at the 12th and 18th day of incubation.

Items / Treatment	AT 12th day	AT 18th day
White light	0.470[b]	0.520[a]
Green light	0.796[a]	0.460[b]
Dark	0.5000[b]	0.243[c]
MSE	0.0686	0.0815
P value	0.0001	0.0001

Means carrying different litters within the same column are significantly different (P < 0.05)

Table (4): Effect of different color lights on fertility, scientific hatchability, embryonic mortality (Dead 1&2) and unhatched eggs.

Traits	Treatments			MSE	P- value
	White light	Green light	dark		
Total egg set (eggs)	600	600	600	-	-
No of fertile eggs (eggs)	564	555	570	-	-
No. of chicks (chicks)	531	513	495	-	-
Fertility(%)	94.00	92.50	95.00	3.74	0.634
Scientific hatchability(%)	94.14[a]	92.43[a]	86.84[b]	3.13	0.032
Dead 1 (%)	3.72[b]	3.24[b]	5.78[a]	1.52	0.021
Dead 2 (%)	2.12	1.62	1.05	1.03	0.216
Unhatch egg (%)	0	2.70	6.31	-	-

Means carrying different litters within the same column are significantly different (P < 0.05)

Table (5): Effect of different color lights on morphological traits of Alexandria chicks at 21th day of incubation (at hatch).

Items / Treatment	B.W (gm)	Crown Rump (cm)	Wing length (cm)	Head circumference (cm)	Head length (cm)	Hind limb (cm)
White light	38.24	10.96 a	4.47 b	6.22	2.14 a	7.13 b
Light Green	39.20	10.97 a	4.78 a	6.09	2.01 b	7.74 a
Dark	37.08	9.78 b	4.79 a	6.23	1.94 b	6.56 c
MSE	3.05	1.07	0.42	0.36	0.12	0.54
P value	0.44	0.0003	0.011	0.17	0.0001	0.0001

Means carrying different litters within the same column are significantly different ($P < 0.05$).

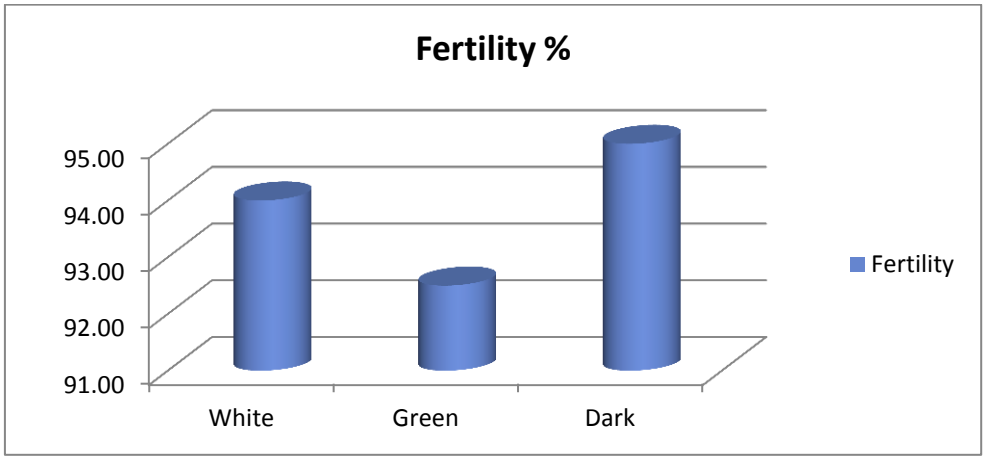

Figure (1): Effect of different color lights on fertility Percentage.

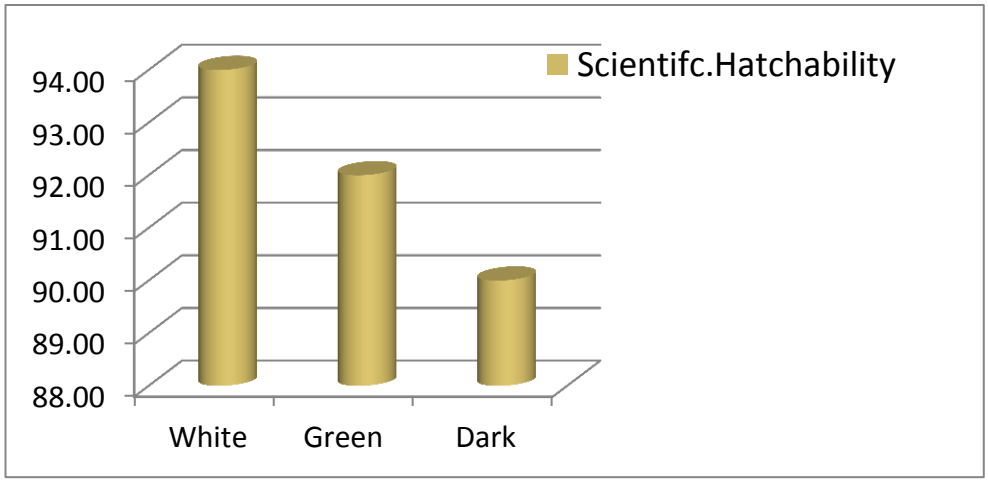

Fig (2): Effect of different color lights on Scientific Hatchability

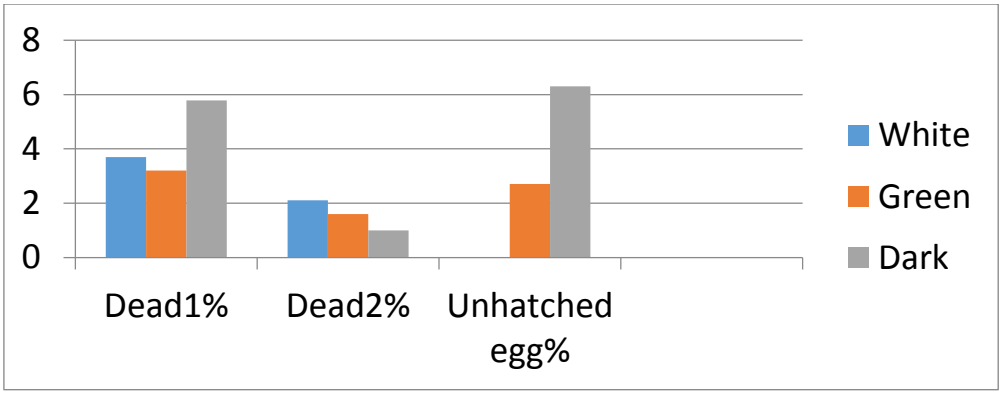

Figure (3): Effect of different color lights on embryonic mortalities and unhatched eggs of Alexandria chicks

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الملخص العربى

تأثير التحفيز الضوئي على أداء وتطورأجنة الدجاج الأسكندرانى أثناء فتره التفريخ

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أظهرت العديد من الدراسات تأثير الطول الموجى للضوء على أداء الطيور في الفحص التجريبي ، وكان الهدف من هذه التجربة تقييم تأثيرات أضواء الألوان المختلفة باستخدام لمبات الليد (LED) على دجاج الإسكندرانى خلال فترة التفريخ (في مراحل عمرية تم تحديدها فى اليوم 4و8و12) للتحقق من تأثيرها على تطور الأجنة و الصفات المورفولوجية و صفات الفقس بجانب تركيز هرمون النمو خلال فترة التفريخ داخل المفرخة.

أجريت التجربة على ثلاث مجموعات بإجمالي عدد1800 بيضة. تم توزيع البيض بالتساوي على ثلاث مجموعات. تم تحضين مجموعة البيض الأولى(G1) تحت الضوء الأبيض لمدة 12 ساعة من إضاءة (LED) ثم 12 ساعة من الاظلام ، مجموعة البيض الثانية تم تحضينها تحت الضوء الأخضر (G2) 12 ساعة من إضاءة (LED) ثم 12 ساعة من الاظلام بينما مجموعة البيض الثالثة (G3) حضنت لمدة 24 ساعة كاملة تحت ظروف الاظلام .

من أهم النتائج التي تم الحصول عليها ، كان للضوء الأبيض والأخضر زيادة معنوية في الصفات المورفولوجية خلال فترة التفريخ خاصة في اليومين الثاني عشر والثامن عشر من التفريخ . من ناحية أخرى ، أظهرت (مجموعة الضوء الأخضر) عند الفقس (اليوم الحادي والعشرين) فرقاً معنوياً عالياً لقيم طول الجناح (4.78 سم) وطول الطرف الخلفي (7.74 سم) مقارنة بمجموعتين الضوء الابيض والاظلام . علاوة على ذلك ، حققت مجموعة الضوء الأبيض أعلى قيمة لصفة ارتفاع الرأس (2.14 سم) مقارنة بمجموعتين الضوء الاخضر والاظلام .

أظهرت النتائج أيضا تحسن معنوى فى نسبة الفقس العلمية لكتاكيت الإسكندرية بالمقارنة مع تلك التي تعرضت للظلام أثناء فترة التفريخ ، ولوحظ من النتائج أنخفاض نسب النفوق المبكر للأجنة فى مجموعتين الضوء الأبيض و الأخضر مقارنتا بمجموعة الأظلام. كما أظهرت النتائج معنوية كبيرة لمستوى هرمون النمو أثناء نمو الأجنة. وتم الوصول الى أعلى مستوى للهرمون عند أجنة اليوم الثاني عشر التي تعرضت للضوء الأخضر (0.796 نانوجرام / مل) ، ومع ذلك ، كان الضوء الأبيض في اليوم الثامن عشر هو الأعلى (0.520 نانوجرام / مل). من النتائج السابقة ، يمكن أن نستنتج أن الضوء الأبيض أو الأخضر خلال فترة التفريخ من المحتمل أن يحسن من التطور والنمو وقابلية الفقس لأجنة دجاج اسكندرية.