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Effects of Timing and Duration of LED Light Supply during Incubation on Embryonic Development and Hatching Parameters of Sasso Eggs

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ABSTRACT

Light has been used as a management tool during incubation, but with varying results. The study was to evaluate the effect of egg exposure to LED light at different phases and for different durations on embryo development, hatching and hematological parameters. Five hundred Sasso broiler eggs were assigned to 4 treatments: eggs incubated in the dark throughout incubation (TA), eggs exposed to light from days 1-21 (TB), days 7-21 (TC) and days 14-21 (TD) of incubation. The eggs were photo-exposed using a 6,500k cool LED at 788 clux intensity. The result shows that TB, TC and TD had decreased ($P<0.05$) average hatch time, internal piping duration, external piping and hatch duration compared to TA. TA eggs recorded a wider hatch window compared to other treatments and the narrowest was TB, comparable to TC and TD. Hematological parameters were not affected at hatch, but the heterophil/lymphocyte ratio was significantly lower ($P<0.05$) in all photo-incubation treatments when compared to TA. Hatching events in slow-growing broiler birds can be accelerated by pre-hatch light stimulation, moreover, photo-incubation from the first day of incubation narrowed the spread of hatch. Photo-incubating eggs from days 1-21 will enhance hatch synchronization.

Key words: Light, LED, Incubation, Photo-Incubation, Embryo Development, Hatching Event, Broiler.

INTRODUCTION

Light is a critical environmental tool in effective hatchery management. The successful development of an embryo during incubation is dependent on the egg's internal and external factors and the ability of the embryo to respond and adjust to the incubator's environmental conditions (Tong et al. 2013). Environmental variables that may influence embryonic development include temperature, humidity, partial pressure of oxygen and carbon dioxide (ventilation), egg turning (Christensen 1995) and light (Fairchild and Christensen 2000). Chicken embryos are sensitive to photo-incubation but in commercial poultry production, eggs are practically incubated in partial or complete darkness (Mench et al. 2008). Photo-incubation during incubation has been demonstrated to be a vital technique that could be used to increase the rate of embryonic development, hatching events and even post-hatch performance (Farghly and Mahrose 2012; Ozkan et al. 2012; Archer 2015, 2017; Dishon 2018). The incubation period has a critical effect on post-hatch life as variation in developmental rate can influence the expression of other traits throughout life (Blount et al. 2006). Hatch time is critical in broiler production (Hannah et al. 2019; Li et al. (2021)) as a late hatch often results in poor post-hatch performance (Lerner 1996). Incubation duration and time of hatch are regulated by embryonic physiological parameters and vocalization behavior (Tong et al. 2013). Hatch synchronization is an environmentally dependent event and adaptive phenomenon (Rumpf and Tzschentke 2010). The progression of physiological processes that occurs during the hatching can predetermine hatch time.

Light-emitting diodes (LED) is a climate-friendly monochromatic light currently being used in the poultry industry (Oso et al. 2022a, b). Due to its low or no heat emission and less energy utilization, the use of LED bulbs for embryonic photo-incubation has been embraced over

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conventional bulbs such as incandescent (ICD) and compact fluorescent light (CFL). Generally, one of the major effects of photo-incubation is the reduction in incubation duration and hatch synchronization (Mench et al. 2008). A shorter hatch window can improve animal welfare and homogeneity of the flock because an increase in the spread of hatch often results in the delay of first access to feed and water (Tong et al. 2013) and this delay has a negative influence on post-hatch growth (Van de Ven et al. 2009). Light stimulation has also been reported to improve chick quality. In the hatchery, day-old chick quality is often judged by qualitative traits such as abnormalities and contamination. A high-quality day-old chick is a potential guarantee for improved broiler production (Tona et al. 2003). The chick quality largely determines the survival and growth performance of chicks in the first few days of life (Christensen 2001). The end of day 7 post-hatch is often considered the real production starting point where chicks' performance at that time is recognized as an indication of chick quality (Tona et al. 2003).

Light stimulation can be initiated hours after eggs are laid and the rate and mechanism of photo-acceleration vary with stages of development (Cooper et al. 2011). In poultry, incubation is divided into three developmental stages: the early, mid and late stages of growth (Hamburger and Hamilton 1992). Organs and systems develop during the first two stages while organ and system maturation occur at the late stages (Tong et al. 2013). Cooper et al. (2011) suggested that the metabolic rate of eggs increases during the photoperiod than in the dark phase, thus resulting in a shorter incubation duration. Reduction in incubation duration through photo-incubation has been demonstrated by several authors, using different light sources (El-Sabrout and Khalil 2017), varying intensity (Shafey et al. 2005), different light wavelengths (Archer et al. 2017; Dishon 2017), diverse photo-incubation durations (Archer et al. 2009) varying onset of lighting (Archer and Mench 2014) different species (Farghly and Mahrose 2012; Archer et al. 2017) and strains of poultry birds (Huth and Archer 2015; Wang et al. 2020). The timing of the onset of lighting appears to have a significant influence on embryonic responses too. It has been documented that an embryo responds to light as early as 2 days of incubation and the rate and mechanism of photo-acceleration varies with the stages of development of the embryo (Cooper et al. 2011) and strain of bird (Hannah et al. 2019). A report by Hannah (2019) and Li et al. (2021) showed that light stimulation can result in disruptive hatch synchronization because photo-periodic sensitivity in birds depends on the strain and the timing of the onset of photo-incubation. Disruptive photo-incubation elongates the incubation duration instead of shortening it. Information is scarce on the impact of embryonic photo-incubation on the slow-growing Sasso broiler chickens which is a slow-growing species. There is a need to establish the physiological responses of the birds to photo-incubation initiated at different stages of development.

Therefore, this study aimed to evaluate the embryonic and post-hatch growth performance of the Sasso broiler chicken in response to the timing and duration of photo-incubation.

MATERIALS AND METHODS

Ethical Approval

The animal care guidelines recommended by the Animal Ethics Committee of the University of Lomé in Togo were followed (ref: 008/2021/BC-BPA/FDS-UL).

Experimental Site

The experiment was conducted at the Regional Centre of Excellence in Avian Sciences (CERSA), University of Lomé, Togo.

Experimental Design

Five hundred naked neck strain of Sasso broiler chicken eggs were obtained from 75 weeks old breeders from the research farm of CERSA, University of Lome. The eggs were graded and weighed and eggs with an average weight of 52.5±2.5g were randomly allotted to 4 incubation treatments, 125 eggs per treatment: Conventional incubation in the dark, resulting in 0 day of light exposure (TA); Photo-incubating from day 1-21, resulting in 21 days of light exposure (TB); Photo-incubating from day 7-21, resulting in 14 days of light exposure (TC); Photo-incubating from day 14-21 resulting in 7 days of light (TD). A photoperiod of 12 hours of light and 12 hours of darkness was used. The LED bulb of 6500k Correlated Color Temperature (CCT) with an intensity of 788 clux at the egg level was used. The light intensity was measured using a poultry lux meter. The experiment was conducted using PAS Reform incubators. The temperature was set at 37.7^{o}C and relative humidity of 60% and was monitored using data loggers in the incubators.

Eggs were candled on the 14th day and transferred from the setter to the hatcher on the 18th day. After hatch, chicks were weighed at hatch before transporting to the farm. From day old, chicks were placed in brooding pens according to their incubation treatments. Charcoal was used as the heat source and a 6,500K cool white LED was used as a light source with an intensity of 28 clux photoperiod of 23L: 1D. The birds were brooded in an enclosed facility with temperatures ranging from 30-32^{o}C for the first 6 days before it was gradually reduced to 27-29^{o}C on the 7th day.

Data Collection

Incubation phase parameters

On days 1, 3, 5, 7, 14, 16 and 18, the eggshell temperature was monitored and recorded during the lighting period using a Thermo flash infrared thermometer.

Ten eggs were weighed from each treatment on days 7, 10, 12, 14, 16 and 18 to determine the Six eggs per treatment were randomly selected for breakout on days

$$\text{Egg weight loss (\%)} = \frac{\text{Weight at the beginning of incubation} - \text{current egg weight}}{\text{Weight at the beginning of incubation}} \text{X } 100$$

12, 14, 16, and 18 of incubation for the data collection on embryonic development:

$$\text{Weight of embryo}(\%) = \frac{\text{Weight of embryo}}{\text{Egg weight}} \text{X } 100$$

$$\text{Weight of residual yolk}(\%) = \frac{\text{Weight of residual yolk}}{\text{Egg weight}} \text{X } 100$$

Embryo length, middle toe length, and beak length were measured using a meter rule calibrated in mm.

Hatching Events

Beginning from 465 until 498 hours of incubation, eggs were observed every 2 hours individually for hatching events such as internal piping, external piping, and chick emergence.

The following parameters were evaluated for each treatment according to Careghi et al. (2005) and Zhong et al. (2018).

$$\text{Internal piping duration} = \text{Time of external piping} - \text{time of internal piping}$$

$$\text{External piping duration} = \text{Time of hatch} - \text{time of external piping}$$

$$\text{Hatch duration} = \text{Time of hatch} - \text{time of internal piping}$$

$$\text{Average hatch time} = \frac{\text{total hatching time of all chicks}}{\text{total number of chicks}}$$

$$\text{Hatch window} = \text{Hatch time of the last chick} - \text{hatch time of the first}$$

Hatch time was defined as hatching time with 100% hatch; early hatch was defined as hatch between 466-474h; mid hatch (474-486h); late hatch (486-498h); peak hatching period (time with the highest number of hatch) (Zhong et al. 2018).

Hatch Performance

The incubators were stopped at 510 hours and unhatched eggs (after 510 hours) were cracked to determine the embryonic mortality and stage of death. Early embryonic (mortality from 0–9th day of incubation); mid embryonic mortality (death from 9th–18th day of incubation) and late embryonic mortality (from 18th-21st days of incubation); Total embryonic mortality (early + mid + late mortality) and hatchability were expressed as the percentage of fertile eggs.

At hatch, chicks were weighed and tagged for identification. After recording the time of hatch and hatching weight, the chicks were placed in another hatching basket and returned to the hatcher. After 510 hours when all incubators stopped, all hatching baskets were pulled out from the hatcher, the chicks were reweighed individually and chick weights at pull were recorded.

Chick weight loss was calculated as;

$$\text{Weight at hatch} = \frac{\text{Weight at pull}}{\text{Chick weight at hatch}} \text{X}100$$

The chicks were assessed for chick quality according to Tona's scoring method (Tona et al. 2003).

Hematological Parameters

At hatch, blood samples were collected, from 6 birds per treatment, into EDTA bottles and analyzed for the following parameters: Hemoglobin/Red blood cell (RBC); Leucocyte/White blood cells (WBC); Hematocrit; heterophils; lymphocyte using an automatic analyzer. Heterophil/lymphocyte ratios were calculated thereafter.

Organ weights at day old

Heart, liver and spleen of chicks at day old were weighed and recorded.

Statistical Analysis

The data were subjected to one-way analysis of variance (ANOVA) using Minitab 17 (2017) and separation of means was done using the Tukey test of the software.

RESULTS

Table 1 shows the effect of light exposure at different periods during incubation and the duration of exposure on eggshell temperature compared to incubating under total darkness throughout incubation (TA), exposure to light at any period (TB, TC, TD) had no significant effect on eggshell temperature except at day 16 when the eggshell temperature was significantly higher ($P<0.05$) than those exposed to light. However, varying the period of exposure and duration had no effect ($P>0.05$).

Fig. 1 shows the effect of exposure to light at different periods of incubation for varying durations compared to incubation under darkness. Relative egg weight loss increased as incubation progressed until day 18. However, between treatments, exposure to light at different periods and duration had no significant effect on weight loss compared with incubation under darkness ($P>0.05$).

The effects of incubation under light for different durations at differing periods during incubation on embryonic development parameters are shown in Table 2. 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).

Hatching events (Table 3) were significantly advanced by exposure of eggs during incubation ($P<0.05$). However, the duration of exposure or the timing of the exposure during incubation did not significantly influence ($P>0.05$). Duration of the events except for the hatching window which was significantly shortened in the eggs exposed for 21 days (TB) compared with others (TC and TD)

Fig. 2 shows the spread compartmentalized into the early hatch, mid hatch, late hatch and peak hatch period of each treatment group. All the treatment groups recorded

their peak period of hatch within the mid-hatch period. TB recorded the highest percentage of hatch within that period. Exposure to light initiated early hatch compared to incubation in the dark throughout the incubation period.

Table 4 shows that no treatment effect (P>0.05) was observed for hatchability, total embryonic mortality, early embryonic mortality, mid embryonic mortality and late embryonic mortality, chick weight at hatch, chick weight at pull and chick weight loss.

Table 5 shows that light treatment had no significant influence (P>0.05) on overall chick quality, activity, down and appearance (D and A) and retracted yolk. However, there was a significant effect (P<0.05) on the eyes, navel, remaining membrane and remaining yolk. The eye and leg score obtained in TA was significantly lower (P<0.05) compared to other treatments. TB recorded a significantly higher (P<0.05) navel score compared to the other treatments. Significantly lower (P<0.05) remaining membrane was recorded in TD in contrast to TB and TC. Chicks in the TA group recorded a significantly lower (P<0.05) remaining yolk score.

Treatments had no significant influence (P>0.05) on all hematological parameters measured except heterophil/ lymphocyte where significantly higher (P<0.05) values were obtained in TA birds compared to other treatment groups. Heart, liver and spleen weights were not significantly influenced (P>0.05) by the treatment.

DISCUSSION

The data from this study showed that incubating a hatching egg with light in the incubator had no significant influence on the developmental trajectory of the embryo but can enhance hatching events that result in an early hatch. Hatching outcomes in terms of hatchability, mortality, chick quality, and chick weight were not influenced by incubation under lighting. Further, the timing and duration of the application lighting proved inconsequential in benefits derived in the advancement of hatching/incubation duration.

Differences in the onset of photo-incubation did not influence eggshell temperature during the incubation phase in the present study, however, a slight increase was observed in the dark incubated eggs (TA) on day 16 compared to those that were photo-stimulated.

Sufficient water loss must occur for the proper formation of an air sac which is essential for embryonic respiration. Egg weight loss usually occurs as a result of moisture loss through the shell. There was no significant effect of variation in the timing and duration of light exposure on egg weight loss in this study. This is similar to the findings of El Sabry and Essa (2017) who incubated chicken eggs with LED from embryonic day 0 to 18 and reported that egg weight loss was not significantly different when compared to those incubated in the dark.

Table 1: Effect of timing and duration of light exposure on eggshell temperature during incubation

	Day of photo-incubation					
Age of Embryo	TA 0	TB 21	TC 14	TD 7	SE	P-Value
Day 1	37.22	37.34	37.36	37.41	0.09	0.179
Day 3	37.19	37.19	37.44	37.34	0.24	0.681
Day 5	37.37	37.21	37.49	37.14	0.19	0.276
Day 7	37.35	37.28	37.70	37.51	0.13	0.012
Day 14	37.59	37.51	37.47	37.87	0.19	0.243
Day 16	38.16a	37.73b	37.39b	37.37b	0.17	0.000
Day 18	38.15	38.23	38.04	38.04	0.27	0.882
Average eggshell temperature	37.48	37.47	37.44	37.38	0.144	0.882

Different letters indicate significant differences between means within rows (P<0.05). TA=Dark incubation, TB=Exposed to light from day 1-21, TC=Exposed to light from day 7-21, TD=Exposed to light from day 14-21.

Table 2: Effect of timing and duration of exposure to light on developmental parameters during incubation

		Duration of photo-incubation					
Age of Embryo	Parameters	TA 0	TB 21	TC 14	TD 7	SE	P-Value
Day 12	Embryonic weight (%)	8.21	8.62	8.38	8.03	0.58	0.784
	Residual yolk Sac (%)	43.57	41.77	41.76	33.03	43.57	0.401
	Beak length (mm)	1.92	2.08	2.42	1.83	0.22	0.073
	Middle toe length (mm)	7.48	7.83	8.50	7.17	0.66	0.251
	Embryo length (mm)	71.83	69.50	70.83	71.33	3.06	0.884
Day 14	Embryonic weight (%)	17.58	19.07	19.08	18.60	0.91	0.338
	Residual yolk Sac (%)	33.43	34.98	33.43	34.85	2.02	0.085
	Beak length (mm)	3.42	3.50	3.58	3.58	0.31	0.938
	Middle toe length (mm)	11.58	9.83	10.67	8.50	1.46	0.219
	Embryo length (mm)	101.00	101.50	100.67	101.83	1.75	0.616
Day 16	Embryonic weight (%)	28.18	29.38	29.12	28.78	1.63	0.895
	Residual yolk Sac (%)	27.30	24.60	23.28	27.34	1.68	0.060
	Beak length (mm)	4.55	4.42	5.00	4.83	0.27	0.163
	Middle toe length (mm)	17.33	17.17	17.500	18.17	0.49	0.223
	Embryo length (mm)	123.17^{ab}	128.00^{a}	127.17^{ab}	121.17^{b}	1.89	0.013
Day 18	Embryonic weight (%)	41.42	44.17	45.17	44.30	1.90	0.105
	Residual yolk Sac (%)	24.91	23.25	23.20	23.52	3.51	0.965
	Beak length (mm)	8.25	7.92	8.25	8.58	0.40	0.373
	Middle toe length (mm)	19.83	20.33	20.66	20.50	0.70	0.663
	Embryo length (mm)	144.50	151.83	148.67	147.67	4.26	0.411

Different letters indicate significant differences between means within rows (P<0.05). TA=Dark incubation, TB=Exposed to light from day 1-21, TC=Exposed to light from day 7-21, TD=Exposed to light from day 14-21.

Table 3: Effect of timing and duration of exposure to light during incubation on hatching events of chicken eggs

Parameters	TA	TB	TC	TD	SE	P-Value
Average hatch time (h)	482.13a	476.47b	477.47b	476.27b	1.18	<0.001
Internal Piping duration (h)	14.83a	11.000b	11.27b	12.27b	0.98	<0.001
External piping duration (h)	16.03a	12.33b	12.87b	11.93b	1.03	<0.001
Hatch duration (h)	30.87a	23.33b	24.13b	24.20b	1.30	<0.001
Hatch Window (h)	26.00a	22.00c	24.00b	24.00b	0.41	<0.001

Different letters indicate significant differences between means within rows (P<0.05). TA=Dark incubation, TB=Exposed to light from day 1-21, TC=Exposed to light from day 7-21, TD=Exposed to light from day 14-21.

Table 4: Effects of exposure of hatching eggs to light at different timing and duration during incubation on hatchability, embryonic mortality and chick weight

Parameters	TA	TB	TC	TD	SE	P-Value
Hatchability (%)	92.26	95.45	93.00	93.65	2.82	0.711
Total Embryo mortality (%)	7.80	4.55	7.07	6.35	2.40	0.592
Early Embryo mortality (%)	1.00	2.27	2.08	2.38	1.74	0.850
Mid Embryo mortality (%)	2.62	3.03	2.41	1.59	2.06	0.900
Late Embryo mortality (%)	3.89	0.00	2.78	3.17	1.49	0.123
Chick weight at hatch	43.97	44.90	44.14	43.20	0.61	0.100
Chick weight at pull	43.95	44.90	44.13	43.20	0.65	0.099
Chick weight loss (%)	7.98	8.21	8.61	8.73	0.53	0.460

TA=Dark incubation, TB=Exposed to light from day 1-21, TC=Exposed to light from day 7-21, TD=Exposed to light from day 14-21.

Table 5: Effects of exposure of hatching eggs to LED light at different times and duration on the quality of hatched chicks

Parameters	TA	TB	TC	TD	SE	P-Value
Overall chick quality	97.04	97.19	97.93	97.06	0.84	0.989
Activity	6.000	5.9032	6.000	5.898	0.03	0.526
D and A	10.00	9.94	9.97	9.97	0.01	0.532
Retracted yolk	12.00	12.00	11.82	12.07	0.12	0.320
Eyes	15.53b	16.00a	16.00a	16.00a	0.12	0.005
Legs	15.19b	16.00a	16.00a	16.00a	0.20	0.002
Navel	10.870b	13.81a	10.94b	11.32b	0.69	0.000
Remaining membrane	11.59ab	11.68a	11.636a	11.05b	0.15	0.019
Remaining yolk	14.71b	15.23ab	15.58a	15.59a	0.21	0.004

Different letters indicate significant differences between means within rows (P<0.05). TA=Dark incubation, TB=Exposed to light from day 1-21, TC=Exposed to light from day 7-21, TD=Exposed to light from day 14-21, D and A – Down and Appearance.

Table 6: Effects of exposure of hatching eggs to LED light at different times and duration during incubation on hematological parameters and organ weights of hatched chicks at one-day-old.

Parameter	TA	TB	TC	TD	SEM	P-VALUE
WBC ($x10^3$/uL)	313.3	173.3	180.0	153.3	53.5	0.061
RBC ($x10^6$/uL)	2.52	2.51	2.92	2.32	0.21	0.098
HGB (g/dl)	11.77	12.57	11.87	11.30	0.42	0.088
Hematocrit (%)	35.97	37.87	36.97	34.23	1.59	0.207
Heterophils (%)	22.67	11.0	13.17	12.33	3.65	0.045
Lymphocyte (%)	47.33	41.33	46.66	46.67	2.61	0.168
Hetero/Lymph	0.47a	0.26b	0.27b	0.26b	0.06	0.018
Heart (g)	0.28	0.28	0.28	0.28	0.02	0.995
Liver (g)	0.94	0.97	1.02	0.95	0.06	0.623
Spleen (g)	0.02	0.02	0.03	0.02	0.00	0.004

Different letters indicate significant differences between means within rows (P<0.05). TA=Dark incubation, TB=Exposed to light from day 1-21, TC=Exposed to light from day 7-21, TD=Exposed to light from day 14-21.

The rate of embryonic development can be measured using relative embryonic weight, embryonic length, beak length, middle toe length, and relative residual yolk. In this study, embryonic length was significantly higher in TB compared to TD on day 16, whilst all the other embryonic developmental parameters observed were not significantly influenced by the presence or duration of light on days 12, 14, 16 and day 18 of incubation. This aligns with the findings of Dishon et al. (2017; 2018) who highlighted that light treatment had no significant effect on embryo body weights of turkey eggs. On the contrary, Ozokan et al. (2012) reported an increase in embryo weight and a corresponding decrease in the residual yolk of eggs exposed to light from the first day of incubation until the end, compared to other treatments. The discrepancy might be due to the lighting schedule, the strain of chickens or varying light intensities used by the authors. Fairchild et al. (2000) suggested that non-continuous lighting could minimize the acceleration of embryonic development compared to continuous lighting. While Hannah et al. (2019) reported that the response of chickens varied based on strain and that disruptive synchronization can occur. Cooper et al. (2011) observed marked differences in developmental rates of embryos under high intensity of 1100 lux and above when compared to those incubated in the dark.

The presence of light during incubation shortened the average hatch time (hatching time of all chicks/ number of

chicks) when compared to the control group, although the average hatch time did not differ significantly within light treatments. This result aligns with the findings of El Sabry and Essa (2017) who incubated with LED from 0 to 18 days in fast-growing broilers and reported that the total incubation period was significantly lower in LED incubated birds when compared to those incubated in the dark. However, the report of Ozokan et al. (2012) contradicts our findings. The authors indicated that the incubation time of eggs exposed to lighting from the first day of incubation until the end did not differ from those incubated in the dark.

Internal piping duration, external piping duration and hatch duration were significantly reduced under light treatment in the present study. The Hatch window, which is defined by the hatching time of the last chick minus the hatching time of the first chick, was influenced by the presence of light and the timing of the application. The hatch window or spread of hatch was significantly narrower in light stimulated treatments compared to those incubated in darkness. Interestingly, the hatch window was 2 hours shorter in TB compared to TC and TD. This agrees with Hannah et al. (2019) who reported that all strains of chickens used in their study responded positively to synchronized hatching of 12D to 12L when the lighting was initiated early during incubation. However, our findings contradict the report of El Sabry and Essa (2017) who incubated with LED from 0 to 18 days and reported that the hatch window was not significantly different when compared to those incubated in the dark. Under the same schedule of light application, Hannah et al. (2019) stated that some strains had a narrower hatch window compared to others. The authors inferred those different strains of birds respond differently to varying lighting schedules. The findings in the current study suggest that exposure of Sasso eggs to light from the first day of incubation is more favorable for a narrower hatch window when compared to an application at the later stages of development.

A higher early hatch was observed in TD compared to other treatments, while a higher rate of the mid hatch was obtained in TB and a higher rate of the late hatch in TA. This further explains the rate of hatch synchronization. Hatch synchronization appeared better in TB as a high quantity of chicks hatched around the same period. Although the early hatch rate seemed faster in TD, it is worthy of note that the percentage of late hatches in TB was lower when compared to TD. This suggests that the high rate of early hatch does not directly imply a narrower spread of hatch nor tighter hatch window. The early hatch rate was faster in photo-exposed treatments. Hannah et al. (2019) observed that the trend of hatch rate response to varying periods of photo-incubation was strain-dependent.

Hatchability was not influenced by light treatment in this study. Interestingly, both LED photo-incubation (El-Sabrout and Khalil 2017) and the period of application of light had no significant effect on hatchability (Ozokan et al. 2012, Archer et al. 2014b, Hannah et al. 2019). Early, mid, late and total embryonic mortality was not significantly influenced by light treatments. This aligns with the report that LED photo exposure had no significant effect on early dead, mid-dead and late dead embryos (Huth and Archer 2015; El-Sabrout and Khalil 2017; Wang et al. 2020). Hannah et al. (2019) reported that under the same photo-incubation condition, mortality percentage was significantly influenced by strains.

Chick weights, both at hatch and pull, were not significantly influenced by light treatment. Our results align with that of Archer (2015) and Ozokan et al (2012) who expressed that the total number of days of egg's exposure to light did not have a significant influence on the chick's weight. This contradicts the findings of El - Sabrout and Khalil (2017) who stated that LED light improved chick weight over dark. On the contrary, Huth and Archer (2015) reported a significantly lower chick weight in LED light-exposed chicks when compared to those incubated in the dark. These variations might be attributed to the differences in the lighting schedule (photoperiod) used by the authors, as El-Sabrout (2017) had highlighted that photo-incubation with LED at 24 hours of light increased chick weight at hatch over 12 hours of light and 12 hours of darkness. In this study, chick weight at pull and chick weight loss was not significantly influenced. This suggests that the presence of light in the hatcher had no dehydrating effect on chicks.

Good chick quality is important because poor chick quality can result in high FCR, low weight gain and low market weight (Lerner 1996). Overall chick, chick activity, down and appearance and retracted yolk were not significantly influenced by light treatment in the present study. This is in congruence to an earlier finding where light manipulation during incubation had no significant effect on chick overall activity level (Archer and Mench 2014) and proportion of dirty chicks (Archer 2015). On the contrary, Huth and Archer (2015) reported that LED incubation improved chick quality, and chick activeness, and had chicks with no defects. In this study, eyes and legs scores were significantly improved under light treatments compared to those in the dark. TB had the best score for healed navel and the remaining membrane was significantly lower in TB and TC compared to others whilst the remaining yolk was significantly higher in TC and TD compared to TA birds. Archer (2015) reported that the proportion of chicks with unhealed navel was significantly lower in birds incubated for 21 days or the first 18 days, compared to those incubated in the dark while leg abnormality remained unaffected.

The hematological responses of chicks to the timing and duration of light photo-incubation revealed that white blood cell, RBC, HGB, hematocrit, heterophils, and lymphocyte counts were not significantly influenced by light treatment. However, the heterophil and lymphocyte ratio was significantly influenced. The presence of light significantly lowered the heterophil/lymphocyte ratio although the effects did not significantly differ when the timing of application was considered. This implies that light reduces the stress level of the chicks as indicated by the heterophil/lymphocyte ratio. It is a reliable stress indicator in poultry and further confirms the assertion of Archer and Mench (2014b). Photo-incubation is a potential tool for reducing stressors for newly hatched chicks.

Organ weights at hatch were not significantly influenced by light treatment. Dishon et al. (2017, 2018),

who obtained a similar result, stated that liver weight was not influenced by varying the timing of photo-incubation. Also, Kaya and Aygun (2019) reported that in quail, incubation lighting had no significant influence on the heart and liver. On the contrary, Fairchild et al. (2000) who exposed turkey eggs to light with ICD reported that heart and liver weight increased with an increase in hatching time. The variations might be attributed to the differences in the light source and the breeds of the poultry bird used.

Data Availability Statement

All necessary data are embedded in the body of the manuscript.

Declaration of Interest

The authors declare no conflicts of interest.

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Author's Contribution

Oluwadamilola Moyin OSO: Experimental design, data collection, analysis and interpretation, drafting of the article. Kossi METOWOGO: Supervision and approval of experimental critical revision of the manuscript. Benjamin ADJEI-MENSAH: Assistance in data collection, analysis and interpretation. Oyegunle Emmanuel OKE: Language and critical revision of the manuscript. Clement GBONGBON: Assistance in data collection. Okanlawon Mohammed ONAGBESAN: Language and critical revision of the manuscript. K. Tona: Supervision, approval of the experimental design, critical revision of the manuscript and final approval of the version to be published.

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