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Impact of Different Intensities of Green-Red Light During Incubation on Embryonic Index and Hatching Performance in Chicken

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MFR conducted the research and wrote the manuscript. AM, JH and Saima provided necessary support regarding the methodology, and execution of the study by making sure of farm availability and logistics and providing insight in this research topic. All authors read and approved the final manuscript.

Key words
Incubation, Poultry, Light intensity, Hatching, Embryo, Chicken

ABSTRACT

The poultry industry artificially incubates eggs by providing all constituents of natural incubation except light. However, in the last decade, many studies have provided enough proof that photo-stimulation during incubation has a positive impact on embryonic traits. This study was planned to evaluate the impact of different intensities of dichromatic light [combination of Green (520 nm) and Red (630 nm) –GR monochromatic LEDs; Yellowish light (580 nm)] on embryonic index and hatching traits in commercial broilers and layers. For that purpose, 1000 eggs (500 of each genotype) were exposed to 0, 150, 250 and 350 lux of GR dichromatic light during the whole incubation period for 12 h a day. Each treatment was replicated five times having 25 eggs each, and moisture loss along with egg shell temperature was noted daily. Studies indicated that 250 and 350 lux have significantly enhanced embryonic growth from the 8th day of incubation. Similarly, 250 and 350 lux of GR lighting have significantly higher number of hatched chicks at 23 h before hatch-pull. Moreover, significantly higher hatchability percent ($p = 0.0028$), and decreased early and late embryonic mortality percent were found in broilers incubated under 250 lux. In chick quality parameters, heart weight percentage was higher in birds that were incubated in 250 lux lighting. It can be concluded that 250 lux of GR light during incubation can improve embryonic growth, hatch window, hatchability percent and chick quality.

INTRODUCTION

In-ovo photo-stimulation in poultry is one of the significant research areas in the last decade, and it shows that light during incubation can help accelerate embryonic growth by entrainment of circadian rhythms (Riaz *et al*., 2021). This establishment of circadian rhythms at an early stage of life, helps the chicks to perform well in terms of hatching performance and post-hatch performance (Riaz *et al*., 2023). In the natural environment, a broody hen lays and incubates eggs in a darker environment for security reasons, however, still eggs are exposed to light for short periods while foraging for food (Mrosovsky and Sherry, 1980; Rogers, 1995). The wavelength and intensity of light might vary depending on the surroundings, but it is clear that light plays a part in the natural incubation procedure. In commercial hatcheries, lighting sources were avoided because they alter the regulated temperature in incubation machinery, therefore eggs are incubated without light. However, the introduction of light-emitting diodes (LEDs) has made it possible to assess the influence of light on poultry eggs during incubation. Numerous studies have demonstrated the beneficial effects of illumination throughout the incubation stage (Archer, 2018; Wang *et al*., 2020; Riaz *et al*., 2021). Studies found that light entering through the eggshell had a direct effect on the embryo's development acceleration (Ghatpande *et al*., 1995), as illuminated incubation reduced hatch time and increased hatchability percent (Hannah *et al*., 2020).

The formation of circadian rhythms in animals is caused by light, which is a fundamental ecological component. The generation and release of melatonin,

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which is generated by the pineal gland during the dark period, is affected by photo-stimulation (Lewis and Morris, 2006). Furthermore, various colours of light during incubation affect egg and meat-type birds differently. Broiler chickens had a substantial sensitivity to white light during incubation, as it helped to improve the bird's welfare (Yameen *et al*., 2020). Similarly, egg-type birds responded to lighted incubation with considerable effects. Studies discovered that different strains of layer showed significant differences in hatch window response to blue light during incubation (Hannah *et al*., 2020).

In the past studies conducted on lighting during incubation resulted in improved hatchability, decreased hatch window, improved welfare and higher post-hatch performance. But in these studies, however, monochromatic light was the subject of nearly all known experiments, and a specific monochromatic alone demonstrated a favourable reaction towards a particular trait, i.e., decreased hatching time in green light (Hannah *et al*., 2020), or improved welfare in white light (Riaz *et al*., 2021). As a result, it was necessary to evaluate the effects of light colour combinations and to adjust lighting regimes during incubation for better embryonic and post-hatch performance. So, in a series of experiments after selecting a green-red colour combination, this research was planned to see how different intensities of dichromatic light affect embryonic development, hatch window, hatching characteristics, and chick quality measures in broiler and layer chickens throughout the incubation period.

MATERIALS AND METHODS

This study was carried out at the Avian Research and Training Centre, University of Veterinary and Animal Science, Lahore. A total of 1000 eggs from two chicken genotypes (Broiler: Ross 308 44 weeks old; Layer: Bovans White 43 weeks old) were procured from commercial breeding farms and put on egg trays with the pointed end down. The eggs were distributed using the randomized complete block design (RCBD) and incubated for 12 h each day under four different lighting regimes. The first treatment was control, in which eggs were kept in full darkness (D); while during the second, third, and fourth treatments eggs were incubated, respectively, under 150, 250, and 350 lux of green red (GR) dichromatic light. These illumination patterns were maintained throughout the incubation phase (21 days).

LED strip lights (SMD strip light Lahore, Pakistan; 4 mm, 220 volts; green and red colour; 108 LEDs/meter) were procured to supply eggs with varied intensities of GR lighting stimulation. To deliver 150, 250, and 350 lux of dichromatic GR light, these strip lights were connected with cardboard and set at a distance of 10, 20, and 30 cm over the trays housing eggs in the incubator machine. The intensity was measured using the UNI-T 383 lux meter.

Daily, eggshell temperature, egg moisture loss (Fig. 1), and embryo development were measured during the incubation period. The hatch window was determined by counting the number of chicks that had hatched at 33, 23, 13, and 6 h before the hatch (completion of 21 days of incubation). Furthermore, hatching parameters including hatchability, viable hatch, embryonic mortality, and chick quality attributes were analyzed once the incubation time was completed.

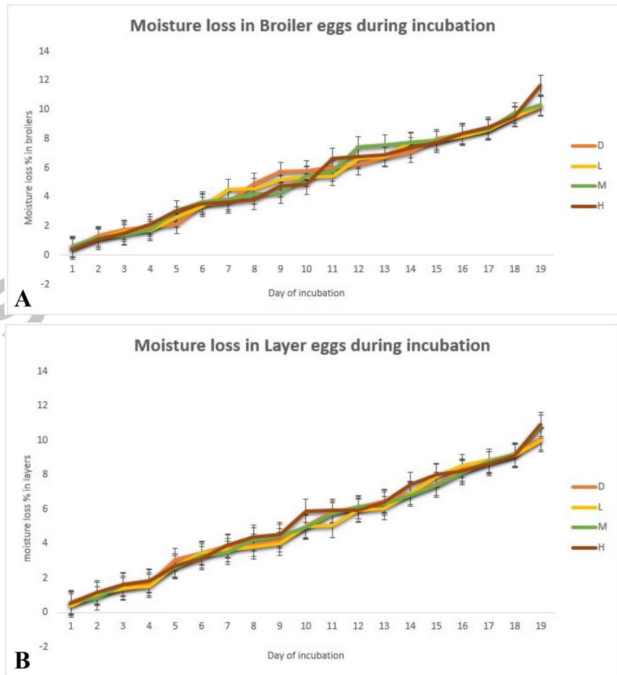

Fig. 1. Moisture loss % in broiler (A) and layer (B) breeder eggs during incubation. D, dark; L, 150 lux; M, 250 lux; H, 350 lux.

Incubation protocol

The eggs were incubated in Victoria Inc. (Quaglie I-36 and H-24, Italy) at a temperature of 37.6 °C and 55–65 % humidity in the setter and at 36.6 °C and 65–75 % humidity in the hatcher after the eggs were transferred at the end of the 18th day. Every hour, the eggs were automatically turned. In the incubator, a partition was made with black cloth to incubate eggs in complete dark, and then eggs that needed to incubate at 150, 250 and 350 lux were placed in an incubator trolley where eggs with 150 lux were on the upper side, eggs with 250 lux in the mid, and the eggs with

350 lux were in the lower portion of egg trolley. A Lux meter (UNI-T 383; Made in China) was used to measure lux on the surface of eggs. To eliminate the heating influence of light during incubation, the temperature of the eggshell was recorded using a digital temperature sensor at four separate points throughout the process.

Embryonic growth

During the incubation phase, many stages of embryo development were observed (8-19 days; these days were selected because from the 8th day embryo gets measurable size and after the 19th day chicks start to hatch). Three eggs were retrieved from each treatment and split open to examine the embryonic developmental stage. The following formula was used to determine embryonic growth (embryo index).

$$\text{Embryo index} = \frac{\text{embryo weight (g)}}{\text{egg weight at 1st day of incubation (g)}} \times 100$$

(Raj *et al*., 2004)

Hatching traits

The following hatching traits were followed: (i) Moisture loss: It was calculated by weighing marked eggs (initial weight noted) daily from the 1st to the 18th day of incubation and the weight change was moisture loss.

$$\text{Moisture loss (g)} = \text{Initial weight (g)} - \text{Final weight (g)}$$

$$\text{Moisture loss (\%)} = \frac{\text{Moisture loss (g)}}{\text{Initial weight (g)}} \times 100$$

(ii) Hatch window: Counting hatched chicks at particular h (33, 23, 13, 6, and 0) before hatch pull was used to determine the Hatch window. The proportion of hatched chicks at a certain hour is then multiplied by the total number of hatched chicks in that treatment (Riaz *et al*., 2021).

(iii) Break-out analysis: After the completion of 504 h of incubation, unhatched eggs were broken out for analysis.

(iv) Dead germ (DG), dead in shell (DIS) and infertile egg (INF) (%):

$$\text{DG or DIS or INF (\%)} = \frac{\text{No. of DG or DIS or INF eggs}}{\text{Number of eggs set}} \times 100$$

(Riaz *et al*., 2021)

(v) Hatchability (%): The percentage of number of hatched chicks compared to no. of eggs set for incubation was hatchability %.

$$\text{Hatchability \%} = \frac{\text{No. of chicks hatched}}{\text{No. of eggs set}} \times 100$$

(vi) Hatch of fertile (%): The hatch of fertile was calculated by the following formula.

$$\text{Hatch of fertile \%} = \frac{\text{No. of chicks hatched}}{\text{No. of fertile eggs set}} \times 100$$

(vii) Chick weight (g): Chicks were weighted by using a weighing scale that have an accuracy of up to 0.05 g.

Chick quality

Chick quality characteristics, including red hock, red beak, and naval score, were measured in hatched chicks from various treatments. The approach used in the previous study was used to evaluate the red hock, red beak, and navel scores (1, good; 2, moderate; 3, poor). The body weight, residual yolk weight, heart weight, liver weight, gizzard weight, and intestine weight of 5 chicks per treatment were (Guz *et al*., 2021).

Eggshell temperature

The eggshell temperature was noted daily using 4 temperature sensors (HTC-2, Made in China) placed at different points in the setter machine (Fig. 2).

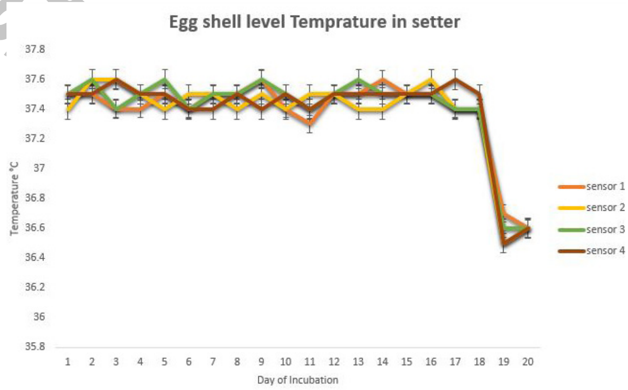

Fig. 2. Egg shell temperature during incubation.

Statistical analysis

Collected data were first tested for homogeneity of variance to verify normality and then subjected to factorial ANOVA using the GLM procedure in SAS software (Version 9.1). Significant treatment means were separated through Duncan's multiple range test considering $P \leq 0.05$ (Duncan, 1955).

RESULTS

Embryo index

In Table I, within the genotype, the embryo index showed a significant difference ($P \leq 0.05$) from the 8th day to the 12th day of incubation. Then towards the end of the incubation period from the 15th to 18th day, a significantly better ($P \leq 0.05$) embryo index was noted in broilers compared to layers. While, within the different lighting intensities embryo index was significantly affected ($p \leq 0.05$) by 250 lux of GR LEDs during incubation starting from the 8th day to the 19th day, except the 9th and 15th day where the difference was not significant ($P \geq 0.05$). In the interaction, results showed significance ($P \leq 0.05$) during the 8th to 19th day of incubation, except the 9th and 13th day ($P \geq 0.05$); where 250 and 350 lux of GR lighting of LED in broilers and layers showed enhanced embryonic growth compared with the other treatments.

Hatch window

In Table II, within the genotype, the hatch window was significant ($P \leq 0.05$) in broilers at 13 h before hatch pull. While, within the different lighting combinations hatch window was significantly affected ($P \leq 0.05$) at 33, 23, 13 and 6 h before hatch pull, where 250 lux of GR combination of LEDs showed better results followed by 350 lux. In the interaction, results showed significance ($P \leq 0.05$) at 23, 13 and 6 h before hatch pull, where 350 lux showed a significantly higher % of hatched chicks at 23 h before hatch pull ($P \leq 0.05$), but then matched up by 250 lux at 13 and 6 h before hatch pull ($P \leq 0.05$) in both genotypes.

Table I. Effect of different intensities of green-red light on embryo index in broilers and layers from 8th to 19th day of incubation.

Genotype	intensity	Eight	Nine	Ten	Eleven	Twelve	Thirteen	Fourteen	Fifteen	Sixteen	Seventeen	Eighteen	Nineteen
Broiler		2.62^{a} ± 0.08	3.81 ± 0.05	5.04^{b} ± 0.08	7.79^{a} ± 0.10	12.33^{a} ± 0.30	16.73 ± 0.24	21.45 ± 0.25	30.55^{a} ± 0.39	34.44^{a} ± 0.31	36.92^{a} ± 0.31	43.58^{a} ± 0.49	62.23 ± 0.38
Layer		2.45^{b} ± 0.06	3.81 ± 0.04	5.29^{a} ± 0.10	7.32^{b} ± 0.09	11.31^{b} ± 0.12	16.41 ± 0.15	21.32 ± 0.23	26.50^{b} ± 0.39	32.48^{b} ± 0.43	35.96^{b} ± 0.40	42.26^{b} ± 0.53	62.21 ± 0.31
	p-value	0.0137	0.9562	0.0138	0.0002	<.0001	0.2214	0.5482	<.0001	<.0001	0.0257	0.0117	0.9609
	D	2.20^{b} ± 0.07	3.69^{b} ± 0.07	4.85^{c} ± 0.10	7.21^{c} ± 0.13	10.95^{c} ± 0.12	16.01^{b} ± 0.13	20.64^{c} ± 0.28	27.44 ± 1.22	31.72^{b} ± 0.73	35.05^{b} ± 0.62	40.76^{c} ± 0.74	61.05^{c} ± 0.38
	L	2.54^{a} ± 0.08	3.75^{ab} ± 0.04	5.06^{bc} ± 0.07	7.45^{bc} ± 0.13	11.44^{b} ± 0.23	16.35^{ab} ± 0.30	21.02^{bc} ± 0.26	28.55 ± 0.85	33.72^{a} ± 0.45	36.33^{a} ± 0.35	42.71^{b} ± 0.41	61.75^{bc} ± 0.35
	M	2.68^{a} ± 0.07	3.89^{a} ± 0.05	5.44^{a} ± 0.12	7.83^{a} ± 0.18	12.45^{a} ± 0.38	17.09^{a} ± 0.29	22.29^{a} ± 0.15	29.29 ± 1.11	34.14^{a} ± 0.45	37.32^{a} ± 0.31	44.35^{a} ± 0.45	63.26^{a} ± 0.38
	H	2.71^{a} ± 0.08	3.91^{a} ± 0.05	5.32^{ab} ± 0.10	7.74^{ab} ± 0.11	12.45^{a} ± 0.37	16.83^{a} ± 0.22	21.60^{b} ± 0.19	28.82 ± 0.92	34.25^{a} ± 0.45	37.07^{a} ± 0.29	43.86^{ab} ± 0.44	62.81^{ab} ± 0.24
	p-value	<.0001	0.0541	0.0010	0.0015	<.0001	0.0325	0.0004	0.1390	0.0001	0.0034	0.0003	0.0025
Broiler	D	2.24^{cd} ± 0.11	3.72 ± 0.13	4.78^{c} ± 0.16	7.45^{cd} ± 0.08	11.07^{cd} ± 0.20	16.05 ± 0.11	20.97^{cd} ± 0.53	29.78^{a} ± 1.28	33.08^{c} ± 0.62	35.87^{a} ± 0.70	41.90^{c} ± 1.16	61.00^{c} ± 0.57
	L	2.61^{ab} ± 0.07	3.74 ± 0.07	4.97^{bc} ± 0.05	7.69^{abc} ± 0.09	11.76^{b} ± 0.35	16.58 ± 0.64	21.09^{bcd} ± 0.52	30.34^{a} ± 0.58	34.70^{ab} ± 0.14	36.69^{a} ± 0.62	43.1^{abc} ± 0.77	61.54^{bc} ± 0.66
	M	2.75^{ab} ± 0.12	3.90 ± 0.11	5.32^{ab} ± 0.19	8.09^{a} ± 0.26	13.26^{a} ± 0.17	17.49 ± 0.34	22.48^{a} ± 0.16	31.36^{a} ± 0.84	34.98^{a} ± 0.30	37.71^{a} ± 0.46	45.00^{a} ± 0.48	63.58^{a} ± 0.50
	H	2.86^{a} ± 0.06	3.89 ± 0.08	5.11^{bc} ± 0.06	7.95^{ab} ± 0.10	13.22^{a} ± 0.26	16.79 ± 0.49	21.28^{bcd} ± 0.26	30.72^{a} ± 0.25	34.99^{a} ± 0.56	37.40^{a} ± 0.21	44.29^{ab} ± 0.57	62.79^{ab} ± 0.42
Layer	D	2.15^{d} ± 0.11	3.66 ± 0.06	4.91^{bc} ± 0.15	6.97^{e} ± 0.14	10.83^{d} ± 0.13	15.97 ± 0.25	20.31^{d} ± 0.09	25.09^{b} ± 0.50	30.35^{d} ± 0.67	34.22^{b} ± 0.68	39.63^{d} ± 0.30	61.10^{c} ± 0.62
	L	2.48^{bc} ± 0.05	3.76 ± 0.06	5.16^{abc} ± 0.10	7.21^{de} ± 0.12	11.12^{bcd} ± 0.17	16.12 ± 0.03	20.96^{cd} ± 0.25	26.76^{b} ± 0.16	32.73^{c} ± 0.14	35.98^{a} ± 0.34	42.28^{bc} ± 0.24	61.96^{abc} ± 0.36
	M	2.61^{ab} ± 0.09	3.88 ± 0.05	5.56^{a} ± 0.15	7.57^{bcd} ± 0.14	11.63^{bc} ± 0.11	16.69 ± 0.39	22.09^{ab} ± 0.21	27.22^{b} ± 1.06	33.30^{bc} ± 0.48	36.92^{a} ± 0.35	43.69^{abc} ± 0.60	62.94^{ab} ± 0.60
	H	2.56^{b} ± 0.05	3.93 ± 0.07	5.52^{a} ± 0.07	7.53^{bcd} ± 0.08	11.67^{bc} ± 0.15	16.87 ± 0.08	21.93^{abc} ± 0.07	26.92^{b} ± 0.72	33.51^{bc} ± 0.36	36.74^{a} ± 0.53	43.44^{abc} ± 0.69	62.84^{ab} ± 0.35
	p-value	0.0003	0.2630	0.0033	0.0007	<.0001	0.1026	0.0026	0.0002	<.0001	0.0105	0.0011	0.0220

Superscripts on different means within the column differ significantly at $p \leq 0.05$. D, dark; L, 150 lux; M, 250 lux; H, 350 lux.

Table II. Effect of different intensities of green-red light on hatch window in broilers and layers.

Genotype	Light intensity	No. of hours before hatch pull (504 h)				
		33	**23**	**13**	**6**	**0**
Broiler		17.16 ± 1.25	44.23 ± 1.85	70.04^{b} ± 2.17	93.17 ± 1.60	100 ± 0.00
Layer		14.56 ± 1.99	47.12 ± 2.17	77.40^{a} ± 1.94	93.76 ± 1.64	100 ± 0.00
	p-value	0.2343	0.2552	0.0074	0.7698	-
	D	12.09 ± 2.43	39.18^{b} ± 2.40	68.23^{b} ± 2.83	87.81^{c} ± 2.89	100 ± 0.00
	L	18.54 ± 2.22	43.24^{ab} ± 3.13	71.44^{b} ± 3.63	91.43^{bc} ± 1.67	100 ± 0.00
	M	19.19 ± 2.59	49.71^{a} ± 1.93	81.24^{a} ± 2.42	98.26^{a} ± 1.16	100 ± 0.00
	H	13.62 ± 1.56	50.56^{a} ± 2.48	73.96^{ab} ± 2.13	96.35^{ab} ± 1.50	100 ± 0.00
	p-value	0.0612	0.0077	0.0084	0.0033	-
Broiler	D	17.45 ± 2.12	40.36^{ab} ± 1.45	67.45^{cd} ± 4.55	88.73^{bc} ± 4.42	100 ± 0.00
	L	18.18 ± 2.87	38.18^{b} ± 3.40	65.45^{d} ± 4.45	90.91^{abc} ± 2.87	100 ± 0.00
	M	18.03 ± 3.00	48.33^{ab} ± 2.63	78.79^{abc} ± 4.18	96.52^{ab} ± 2.14	100 ± 0.00
	H	14.97 ± 2.48	50.03^{a} ± 4.34	68.45^{bcd} ± 2.17	96.52^{ab} ± 2.14	100 ± 0.00
Layer	D	6.72 ± 2.77	38.00^{b} ± 4.80	69.00^{bcd} ± 3.87	86.89^{c} ± 4.19	100 ± 0.00
	L	18.90 ± 3.72	48.30^{ab} ± 4.43	77.42^{abc} ± 4.64	91.96^{abc} ± 2.04	100 ± 0.00
	M	20.34 ± 4.52	51.09^{a} ± 2.97	83.70^{a} ± 2.44	100.00^{a} ± 0.00	100 ± 0.00
	H	12.26 ± 1.97	51.09^{a} ± 2.97	79.47^{ab} ± 0.77	96.18^{ab} ± 2.34	100 ± 0.00
	p-value	0.0654	0.0260	0.0061	0.0332	-

Superscripts on different means within the column differ significantly at $p \leq 0.05$. D, dark; L, 150 lux; M, 250 lux; H, 350 lux.

Table III. Effect of different intensities of green-red on hatching traits in broilers and layers.

Genotype	Light Intensity	Hatch %	HOF %	DIS %	DG %	IFE %
Broiler		83.46^{a} ± 1.01	87.24^{a} ± 1.68	6.15^{b} ± 1.06	6.15^{b} ± 0.90	4.23^{b} ± 0.88
Layer		74.62^{b} ± 1.49	81.15^{b} ± 1.35	8.85^{a} ± 1.01	8.46^{a} ± 0.95	8.08^{a} ± 0.88
	p-value	<.0001	0.0021	0.0383	0.0302	0.0069
	D	76.15 ± 2.13	81.74 ± 1.22	8.46^{ab} ± 0.77	8.46b^{a} ± 0.77	6.92 ± 1.79
	L	79.23 ± 2.58	83.72 ± 2.49	4.62^{b} ± 1.70	10.77^{a} ± 1.26	5.38 ± 1.18
	M	81.54 ± 2.05	86.90 ± 1.82	5.38^{bc} ± 1.18	6.92^{b} ± 0.77	6.15 ± 1.54
	H	79.23 ± 2.31	84.42 ± 2.25	11.54^{a} ± 1.28	3.08^{c} ± 1.26	6.15 ± 1.03
	p-value	0.2421	0.2677	0.0015	<.0001	0.8802
Broiler	D	80.00^{abcd} ± 1.88	83.85 ± 0.31	7.69^{abc} ± 0.00	7.69^{b} ± 0.00	4.62 ± 1.88
	L	84.62^{ab} ± 0.00	87.44 ± 1.73	3.08^{c} ± 1.88	9.23^{ab} ± 1.54	3.08 ± 1.88
	M	86.15^{a} ± 1.54	90.51 ± 2.85	3.08^{c} ± 1.88	6.15^{b} ± 1.54	4.62 ± 1.88
	H	83.08^{abc} ± 2.88	87.18 ± 3.05	10.77^{ab} ± 1.88	1.54^{e} ± 1.54	4.62 ± 1.88
Layer	D	72.31^{d} ± 3.08	79.63 ± 2.09	9.23^{ab} ± 1.54	9.23^{ab} ± 1.54	9.23 ± 2.88
	L	73.85^{d} ± 3.92	80.00 ± 4.25	6.15^{bc} ± 2.88	12.31^{a} ± 1.88	7.69 ± 0.00
	M	76.92^{bcd} ± 2.43	83.29 ± 0.44	7.69^{abc} ± 0.00	7.69^{b} ± 0.00	7.69 ± 2.43
	H	75.38^{cd} ± 2.88	81.67 ± 3.12	12.31^{a} ± 1.88	4.62^{bc} ± 1.88	7.69 ± 0.00
	p-value	0.0028	0.0536	0.0059	0.0005	0.2672

Superscripts on different means within the column differ significantly at $p \leq 0.05$. D, dark; L, 150 lux; M, 250 lux; H, 350 lux. Hatch, hatchability; HOF, hatch of fertile; DIS, dead in shell; DG, dead germ; IFE, infertile egg.

Hatching traits

In Table III, within the genotype, hatch traits showed significant results ($P \leq 0.05$) in broilers in terms of hatchability %, hatch of fertile %, dead germ %, dead in shell % and infertile eggs. While, within the different light intensities hatchability and hatch of fertile % showed non-significant results ($P \geq 0.05$), but dead germs and dead in shell % were significantly higher ($P \leq 0.05$) in Dark and 150 and 350 lux of dichromatic GR light respectively. The interaction results showed significant ($P \leq 0.05$) improvement in hatchability in broiler eggs that were incubated under 250 lux of GR light followed by 150 and 350 lux respectively. However, breakout analysis showed that dead germs were significantly higher ($P \leq 0.05$) in layer eggs that were incubated under lower intensity (150 lux) of GR of LED light; and dead in shell % was significantly higher ($P \leq 0.05$) in eggs that were incubated at higher intensity (350 lux) in both genotypes.

Chick quality

In Table IV, within the genotype, chick quality attributes of live weight, unabsorbed yolk weight, heart weight, liver weight and intestinal weight showed significant differences ($P \leq 0.05$). The chick quality attributes of

Table IV. Effect of different intensities of green-red light on chick quality attributes in broilers and layers.

Genotype	Light intensity	Live weight	Unabsorbed yolk wt.	Heart wt.	Liver wt.	Gizzard wt.	Intestine wt.	Navel score	Hock score	Beak score
Broiler		45.04^{a} ± 0.02	3.93^{a} ± 0.06	0.54^{a} ± 0.01	4.29^{a} ± 0.03	5.60 ± 0.03	5.68^{b} ± 0.3	1.25 ± 0.10	1.05 ± 0.05	1.25 ± 0.10
Layer		35.82^{b} ± 0.02	3.04^{b} ± 0.03	0.49^{b} ± 0.01	3.95^{b} ± 0.03	5.54 ± 0.03	5.92^{a} ± 0.03	1.45 ± 0.11	1.10 ± 0.07	1.10 ± 0.07
	p-value	<.0001	<.0001	<.0001	<.0001	0.2816	<.0001	0.2260	0.5316	0.2296
	D	40.42 ± 1.52	3.71^{a} ± 0.18	0.49^{c} ± 0.01	4.05 ± 0.07	5.58 ± 0.05	5.82 ± 0.05	1.50 ± 0.17	1.30^{a} ± 0.15	1.40 ± 0.16
	L	40.41 ± 1.53	3.49^{b} ± 0.16	0.50^{bc} ± 0.01	4.13 ± 0.07	5.53 ± 0.05	5.75 ± 0.06	1.30 ± 0.15	1.00^{b} ± 0.00	1.10 ± 0.10
	M	40.48 ± 1.54	3.37^{b} ± 0.14	0.53^{ab} ± 0.01	4.16 ± 0.06	5.60 ± 0.05	5.81 ± 0.06	1.30 ± 0.15	1.00^{b} ± 0.00	1.10 ± 0.10
	H	40.42 ± 1.56	3.37^{b} ± 0.14	0.54^{a} ± 0.01	4.16 ± 0.07	5.57 ± 0.04	5.82 ± 0.06	1.30 ± 0.15	1.00^{b} ± 0.00	1.10 ± 0.10
	p-value	0.4622	<.0001	0.0029	0.3192	0.7817	0.6310	0.7674	0.0239	0.2333
Broiler	D	44.97^{a} ± 0.04	4.24^{a} ± 0.05	0.51^{cd} ± 0.02	4.23^{a} ± 0.06	5.62 ± 0.07	5.72^{bc} ± 0.08	1.40 ± 0.24	1.20 ± 0.20	1.40 ± 0.24
	L	45.00^{a} ± 0.02	3.94^{b} ± 0.08	0.52^{bc} ± 0.02	4.30^{a} ± 0.05	5.56 ± 0.08	5.63^{c} ± 0.06	1.20 ± 0.20	1.00 ± 0.00	1.20 ± 0.20
	M	45.10^{a} ± 0.06	3.74^{b} ± 0.12	0.56^{ab} ± 0.01	4.31^{a} ± 0.06	5.62 ± 0.07	5.66^{c} ± 0.04	1.20 ± 0.20	1.00 ± 0.00	1.20 ± 0.20
	H	45.10^{a} ± 0.05	3.79^{b} ± 0.06	0.58^{a} ± 0.01	4.33^{a} ± 0.06	5.59 ± 0.07	5.70^{bc} ± 0.05	1.20 ± 0.20	1.00 ± 0.00	1.20 ± 0.20
Layer	D	35.86^{b} ± 0.05	3.17^{c} ± 0.06	0.48^{d} ± 0.01	3.87^{b} ± 0.07	5.55 ± 0.07	5.91^{a} ± 0.04	1.60 ± 0.24	1.40 ± 0.24	1.40 ± 0.24
	L	35.82^{b} ± 0.04	3.05^{cd} ± 0.07	0.48^{cd} ± 0.01	3.96^{b} ± 0.09	5.50 ± 0.08	5.88^{ab} ± 0.07	1.40 ± 0.24	1.00 ± 0.00	1.00 ± 0.00
	M	35.86^{b} ± 0.05	3.01^{cd} ± 0.06	0.50^{cd} ± 0.01	4.00^{b} ± 0.04	5.57 ± 0.07	5.96^{a} ± 0.05	1.40 ± 0.24	1.00 ± 0.00	1.00 ± 0.00
	H	35.75^{b} ± 0.05	2.95^{d} ± 0.04	0.51^{cd} ± 0.01	3.98^{b} ± 0.05	5.55 ± 0.04	5.94^{a} ± 0.08	1.40 ± 0.24	1.00 ± 0.00	1.00 ± 0.00
	p-value	<.0001	<.0001	<.0001	<.0001	0.9304	0.0005	0.9066	0.1277	0.4981

Superscripts on different means within the column differ significantly at $p \leq 0.05$. Heart, liver, gizzard and intestine wt. percentage of bird live wt. D, dark; L, 150 lux; M, 250 lux; H, 350 lux.

unabsorbed yolk weight and heart weight were significantly improved in chicks that were incubated under 250 and 350 lux of GR light. However, liver weight %, intestinal weight, gizzard weight, beak score, hock score and navel score were not affected by different lighting intensities ($P \geq 0.05$). In the interaction, results showed a significant difference ($P \leq 0.05$) where lighting treatment in broiler showed significant ($P \leq 0.05$) results in chick quality attributes of unabsorbed yolk weight, heart weight, liver weight and intestinal weight compared with control treatment.

DISCUSSION

In-ovo photo-stimulation helped to improve the embryonic index in broilers and layers throughout the incubation period. These results follow previous studies (Dishon *et al*., 2018; Abdulateef *et al*., 2021), which find that lighted incubation resulted in improved embryonic metabolic activity, moreover, improvement in embryo index in this study may be due to the impact of lighting treatments on the somatotropic axis. Light stimulation during incubation enhances the heart rate which is an important metabolic index, that ultimately accelerates embryonic growth (Abdulateef *et al*., 2021).

Results indicated that the hatch window was significantly affected by various lighting intensities compared with dark treatment. These results are following the previous studies (Tong *et al*., 2018; Hannah *et al*., 2020; Riaz *et al*., 2021). In this study, at 23, 13 and 6 h before hatch pull significantly higher no. of chicks were hatched in eggs that were incubated under lighting treatments. As, at 6 h before hatch pull 98% and 96% of chicks were hatched from 250 lux and 350 lux of lighting treatments, respectively, while in the control environment, only 87% of chicks were hatched at that time. This indicated that this higher percentage of hatched chicks may be due to the impact of light on improving the growth hormone level. This acceleration of embryo growth can also be attributed to increased digestive activity and the usage of egg yolk nutrients. As results show significantly decreased residual yolk was found in chicks from 250 and 350 lux lighting treatments.

Results show that hatching traits are positively affected by 250 lux of lighting treatment followed by 150 and 350 lux. These results are in accordance with the study conduted by Safwan *et al*. (2023) and Ali *et al*. (2023). Moreover, this improvement could be due to a positive impact on the developmental rate of an embryo as the study suggested that light during incubation increases the rate of embryonic development (Ghatpande *et al*., 1995). Further, it may also be due to the change in the rhythm of melatonin in the embryos provided light during incubation (Tong *et al*., 2018).

Certain attributes of chick quality are also affected by the lighting treatments, such as heart and liver weight percentages. This increased weight percentage of these vital organs can be attributed to the accelerated growth of the embryo in response to the lighting during the incubation period (Ghatpande *et al*., 1995).

In a recent study (Riaz *et al*., 2024) it was found that green-red light during incubation has no impact on hatching traits and post-hatch growth performance. However, in that study, it was found that based on the vocalization test birds incubated in green-red light had better adjustability in the novel environment after hatching. On the other hand, the current study suggests that when eggs are provided with different intensities of GR light during incubation, better hatchability and improved embryonic index can be found under 250 lux lighting treatment. This difference can be due to the genetic variability of broiler eggs (cobb-500 vs Ross-308) used in both trials. Moreover, the nutrition status of the embryo can also be a vital dissimilarity in these two experiments. As the

Hormonal changes due to light stimulation

Studies reported that light during incubation stimulates the somatotropic axis, resulting in a cycle of hormonal release. Stimulated by light during incubation, the hypothalamus started to secrete growth hormone-releasing hormone (GHRH) at the early stages of embryonic growth, which forces the pituitary gland to release somatotropin hormone (the growth hormone GH). So, when the concentration of GH increases in the blood, the liver starts to produce Insulin-Like Growth Factor-1 (IGF-1). This series of hormonal secretions stimulates the growth of the embryo during the incubation phase which ultimately affects the hatching traits and chick quality (Dishon *et al*., 2018).

Similarly, light during incubation affected the secretion of melatonin and corticosterone hormone (Tong *et al*., 2018). This lighted incubation caused a rhythmic release of these hormones which help to establish the circadian rhythms during the embryo stage in birds. So, during the light h of incubation metabolism increased in the birds and resulted in the increased consumption of yolk nutrients.

CONCLUSION

Keeping in mind the results of this study which indicated that various light intensities have different impacts on hatching traits and post-hatch performance in chickens; it can be concluded that exposing eggs to 250 lux of GR light can help to increase the hatching performance. This improved hatching performance with not only increase the no. of hatched chicks but also improve the welfare of these chicks.

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Ethical statement and IRB approval

The study was executed according to the rules and regulations of the Animal Ethical Review Committee, the University of Veterinary and Animal Sciences, Lahore, Pakistan (DR/95, 2021).

Statement of conflict of interest

The authors have declared no conflict of interest.

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