



Effect of Monochromatic LED Lights on Physiobiochemical and Behavioural Performance of Broiler Chicken

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ABSTRACT

Poultry production is done worldwide and is an important economic activity for providing food security to world population. Among all livestock sectors, the poultry industry is one with the fastest growing industry with advantage of lowest turnover time and better flexibility. Physiology and behaviour of poultry is influenced by several characteristics of light, including intensity, photoperiod, and wavelength. The present study was aimed to find effect of different colour lighting regimen either in solo or in combination on poultry production, meat quality and behaviour with aim to enhance the productivity and well-being of poultry. In the present work a study of 6 weeks duration was conducted in which four hundred and eighty commercial broiler chicks of Cobb-430 strain were randomly distributed in ten treatment groups. There was significant ($P < 0.05$) change observed in various behavioural pattern as well as physiology of birds reared under coloured light with respect to the incandescent and white LED.

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Authors' Contribution

AS carried out the experiment. PL and R contributed to designing of research and data analysis. N does data compilation and paper writing. MG contributed to data interpretation.

Key words

Photoperiod, Feed conversion ratio, Carcass, Biochemical, Hormonal, Behavioural

INTRODUCTION

Understanding environment and managing them is one of important tool in animal production (Lewis and Morris, 1998; Wineland, 2002). Among environmental factors light is a key; sensed by birds through four specific photoreceptor cells. Recent studies had indicated that colour provided through lighting regime may have impact on poultry physiology and production (Olanrewaju *et al.*, 2006). However, understanding the effect of different lighting h regime is missing. The current study was planned to understand effect of monochromatic light on various parameters under 16 and 23 h lighting pattern under tropical climatic condition.

MATERIALS AND METHODS

The proposed work was conducted using 480 Cobb-430 strain broilers divided randomly in 10 groups, each of 5 for two photoperiods (16 h and 23 h) on 7th day till day

42 of experiment. Except for providing monochromatic light all other managerial practices including feed were similar to all group. There were three replicates per treatment, and within each replicate, there were sixteen birds. Groups were named according to the type of light and its duration.

Table I. Different treatment groups.

	Photoperiod	
	16h	23h
White LED	White16	White23
Yellow incandescent light	Incadesent16	Incadesent23
Blue LED	Blue16	Blue23
Green LED	Green16	Green23
Red LED	Red16	Red23

Details of grouping and treatment are given in table I. For future reference treatment groups naming was done. For example, treatment of white LED light for 16 h - White16 and treatment of red light for 23-h-Red23 were used.

Parameters studied

Daily feed and water intake per replicate (three for each group) was recorded throughout the experiment, body weight was measured on weekly basis for each bird and feed

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Abbreviations

FCR, feed conversion ratio; WBC, white blood cells; RBC, red blood cells; SGOT, glutamate oxaloacetate aspartate aminotransferase; SGPT, glutamate pyruvate transaminase; GH, growth hormone.

conversion ratio (FCR) for each replicate was calculated accordingly. Blood was collected on day 35th day from 6 birds of each group and was used for measurement of PCV%, TEC, TLC using hematology analyzer (Melet Schloesing Laboratoires France Model-MS4S). Haemoglobin (Hb) was estimated by cyanmethemoglobin method using drabkin solution (Bankowski, 1942). Plasma glucose (from NaF tube), total protein, cholesterol, triglyceride, SGPT, SGOT was estimated using commercially available kit (ERBA Mannheim, Germany). Plasma was also used to assess Growth hormone, tetraiodothyronine (T_4) and triiodothyronine (T_3) using a commercially available ELISA kit (Krishgen Biosystems, India).

The behaviors of broilers were assessed by closed-circuit television (CCTV) camera in the experimental house. The birds involved in sitting, standing, walking, feeding, drinking, sleeping and pecking were recorded for 60 minutes. Observation was recorded during 11:00 am to 12:00 pm, 07:00 pm to 08:00 pm and 02:00 am to 03:00 am on day 14 and day 35. 6 birds were randomly chosen from each replication for each behavior and individual behavior data averaged. Human approach test (HA Test) where the latency of the first chicken to touch the boots of the observer was observed and recorded as per method described by Giersberg *et al.* (2020).

Carcass traits were studied on day 42nd where six birds from each group were sacrificed for carcass trait analysis as per standard protocol. Carcass was used for analysis of dressed weight of lungs, heart, liver, and gizzard, length of intestine. The length of muscle fibre was determined from the thigh (Biceps femoris) and breast (Pectoralis major) (Duclos *et al.*, 1996).

Statistical analysis

Statistical analysis was performed on the collected

data using one-way ANOVA through the Statistical Package for Social Sciences (SPSS version 29.0), following the principles of a completely randomized design (CRD).

RESULTS

Average feed intake

There was significant difference in the feed intake of birds among different groups across the observation period (Table II). In 16-h lighting regimen feed intake was significantly ($P < 0.05$) higher in red group with respect to incandescent groups at all-time intervals except day 14 under the observation. All other group have shown non-significant ($P > 0.05$) change in feed intake with respect to birds reared under red light except those reared under blue light which had shown lower feed intake up to day 21 and up to day 35 in significant manner. In general feed intake was lowest in incandescent light as compared to other groups. Incandescent light in 23-h lighting regimen had showed non-significant difference in term of cumulative feed intake in comparison to all groups up to day 35. The feed intakes of Incandescent 23 group up to day 42 was on lower side and significantly lower than blue, greencolour lighting group.

Average water intake

The detail of weekly water intake in each group is depicted in Table III. The average water intake during observation period differed significantly ($P < 0.05$) in all treatment groups. In the birds reared under 16 h lighting a significant ($P < 0.05$) higher water intake was observed among treatment group provided with green light in comparison to birds reared under Incandescent lighting during the complete experimental period. The birds reared under red light also have significantly lower water intake in

Table II. Effect of treatment with different colour lights on its average feed intake (g/bird) by broiler chickens during different growth periods.

Days	Photoperiod	Incandescent	White	Blue	Green	Red
14	16 h	806.18 ^{ab} ±16.27	817.94 ^{ab} ±16.61	748.94 ^a ±21.62	795.74 ^{ab} ±22.97	843.13 ^b ±23.34
	23 h	813.53 ^{abc} ±16.48	851.76 ^{bc} ±23.59	766.8 ^a ±22.14	789.56 ^{ab} ±22.79	872.19 ^c ±25.18
21	16 h	1745.88 ^a ±50.40	1933.23 ^b ±55.81	1734.23 ^a ±50.06	1848.15 ^{ab} ±53.35	1987.88 ^b ±57.38
	23 h	1667.35±48.13	1827.06±52.74	1660.91±47.95	1796.47±51.86	1816.25±52.43
28	16 h	2663.16 ^a ±76.88	3047.64 ^b ±87.98	2791.66 ^{ab} ±80.59	3059.02 ^b ±88.31	3061.68 ^b ±88.38
	23 h	2642.94±76.29	2765±79.82	2644.55±76.34	2781.12±80.28	2630±75.92
35	16 h	3725.06 ^a ±107.53	4303.16 ^{bc} ±123.22	3952.5 ^{ab} ±114.09	4168.88 ^{bc} ±122.36	4355.12 ^c ±125.72
	23 h	3815.88 ^{ab} ±110.15	3807.74 ^{ab} ±110	3681.97 ^{ab} ±106.29	4023 ^b ±116.63	3554.37 ^a ±102.60
42	16 h	4581.27 ^a ±132.25	5379.67 ^b ±155.30	5403.36 ^b ±155.98	5654.54 ^b ±163.16	5454.81 ^b ±157.47
	23 h	4717.90 ^{ab} ±136.19	4932.02 ^{bc} ±142.37	5221.63 ^{cd} ±150.73	5607.59 ^d ±161.88	4459.22 ^a ±128.73

Table III. Effect of treatment with different colour lights on its average water intake (ml/bird) by broiler chickens during different growth periods.

Days	Photoperiod	Incandescent	White	Blue	Green	Red
14	16 h	887.35 ^a ±20.56	959.59 ^b ±22.23	870.16 ^a ±20.17	1011.11 ^b ± 16.43	909.78 ^{ab} ±21.09
	23 h	939.75±21.78	880.83±20.42	932.08±21.60	869.14±20.14	917.95±21.28
21	16 h	1634.87 ^{ab} ±54.12	2396.83 ^b ±57.87	2286.66 ^a ±53	2831.84 ^c ±65.64	1653.08 ^{ab} ±54.54
	23 h	1629.55±54.00	2310.08±55.86	1648.22±54.43	2321.92±56.14	2294.57±53.18
28	16 h	3431.76 ^a ±99.07	3667 ^{ab} ±105.86	3466.62 ^a ±100.07	3937.94 ^b ±113.68	3353.13 ^a ±96.80
	23 h	3316.76±95.75	3461.47±99.92	3416.61±98.63	3471.62±100.22	3228.44±93.20
35	16 h	5341.47 ^a ±154.19	5561.12 ^{ab} ±160.53	5650.56 ^{ab} ±163.12	5975.88 ^b ±172.51	5186.25 ^a ±149.71
	23 h	5120 ^{ab} ±147.80	5220.59 ^{ab} ±150.70	5330.55 ^b ±153.87	5306.62 ^b ±153.18	4770.19 ^a ±137.70
42	16 h	6388.97 ^a ±184.43	6897.37 ^a ±199.11	8268.06 ^b ± 168.67	8477.76 ^b ±234.73	6431.88 ^a ±185.67
	23 h	6135 ^a ±18.20	6467.46 ^a ±186.70	7860.55 ^b ±226.91	7951.62 ^b ±229.54	5908.31 ^a ±170.56

Table IV. Effect of treatment with different colour lights on feed conversion ratio (FCR) during different growth periods.

Days	Photoperiod	Incandescent	White	Blue	Green	Red
14	16 h	1.21 ^{bc} ±0.03	1.16 ^b ±0.03	1.05 ^a ±0.03	1.26 ^c ±0.03	1.22 ^{bc} ±0.03
	23 h	1.25 ^{bc} ±0.03	1.17 ^{ab} ±0.035	1.14 ^a ±0.03	1.10 ^a ±0.03	1.29 ^c ±0.03
21	16 h	1.65 ^a ±0.04	1.85 ^b ±0.04	1.67 ^a ±0.04	1.67 ^a ±0.04	1.93 ^b ±0.05
	23 h	1.65 ^{ab} ±0.04	1.75 ^{bc} ±0.04	1.59 ^a ±0.04	1.67 ^{ab} ±0.04	1.85 ^c ±0.04
28	16 h	1.83 ^a ±0.03	2.03 ^b ±0.03	1.86 ^a ±0.03	2.00 ^b ±0.03	1.99 ^b ±0.03
	23 h	1.84 ^{abc} ±0.03	1.91 ^c ±0.03	1.76 ^a ±0.03	1.87 ^{bc} ±0.03	1.89 ^{bc} ±0.03
35	16 h	1.75 ^a ±0.03	1.96 ^{cd} ±0.03	1.85 ^{ab} ±0.03	1.95 ^{cd} ±0.03	2.01 ^d ±0.03
	23 h	1.84 ^{bc} ±0.03	1.82 ^{abc} ±0.03	1.73 ^a ±0.03	1.94 ^d ±0.03	1.74 ^{ab} ±0.03
42	16 h	1.98 ^a ±0.03	2.02 ^a ±0.03	2.15 ^b ±0.04	2.19 ^b ±0.04	2.19 ^b ±0.04
	23 h	2.08 ^b ±0.04	1.87 ^a ±0.03	2.32 ^d ±0.04	2.17 ^c ±0.04	1.84 ^a ±0.03

comparison to green light post day 21. Among birds reared with 23-h lighting Non-significant ($P>0.05$) difference was found between the water intake of birds in all the groups up to day 28. However, significantly ($P<0.05$) lower water intake was observed in birds reared under red light in comparison to other treatment groups post 28 days of observations. On day 42 water intake in birds reared under incandescent, white and red light were similar and were significantly ($P<0.05$) lower than other four groups.

Average weekly body weight gain

Among the broiler reared under in 16 h lighting regimen the incandescent bulb had showed significant ($P<0.05$) reduced body weight gain with respect to all LED groups in 6th week of study. In general body weight gain was lower in incandescent bulb group than white LED group.

Similarly, among the broiler reared under in 23

h lighting regimen the incandescent bulb had showed significant ($P<0.05$) reduced body weight gain with respect to coloured LED groups in 5th and 6th week of study. In general body weight gain was lower in incandescent bulb group than white LED group.

Feed conversion ratio

Data pertaining to the feed conversion ratio during different growth periods are presented in Table IV. Trend in FCR was in-consistent among treatment groups. Up to day 1 to 28, significant lower ($P<0.05$) FCR was observed in blue light, incandescent light as compared to other groups. Trend for lower FCR in incandescent light was observed up to day 35 of study. Overall FCR up to day 42 was significantly ($P<0.05$) lower in Incandescent16 and White 16 groups than Blue16, Green16 and Red16 groups.

Table V. Effect of treatment with different colour lights for 16 h and 23 h on carcass characteristics of broilers.

Carcass characteristics	Incandescent	White	Blue	Green	Red
16 h regimen					
Live weight (g)	2607.5 ^a ±71.14	2790.33 ^{ab} ± 94.67	2960.67 ^b ± 133.31	2984 ^b ± 157.02	2764.67 ^{ab} ± 45.56
Hot carcass weight (g)	1712.5 ^a ± 48.08	1922.5 ^{ab} ± 73.65	2063 ^b ± 89.71	2077.67 ^b ± 98.33	1762.67 ^a ± 119.12
Dressing (%)	65.74 ± 1.29	68.85 ± 0.46	69.71 ± 0.4	69.78 ± 0.9	63.71 ± 4.07
Head weight (g)	65.33 ^a ± 1.28	67.17 ^a ± 5.21	82 ^b ± 5.21	86.83 ^b ± 3.5	76.5 ^{ab} ± 2.43
Legs weight (g)	73.17 ^a ± 1.74	80.67 ^{ab} ± 4.45	105.17 ^d ± 6.49	99.17 ^{cd} ± 3.84	82.5 ^{ab} ± 4.71
Skin+Feather weight (g)	345.83 ^a ± 12.3	385.33 ^{ab} ± 9.81 ^{ab}	363.17 ^{ab} ± 20.46	380.17 ^{ab} ± 16.87	374.5 ^{ab} ± 21.94
Liver weight (g)	46± 3.44	44.83 ± 2.64	48.67 ± 3.96	52 ± 2.03	45.83 ± 2.55
Filled Gizzard weight (g)	67.17 ^{ab} ± 4.85	65.83 ^a ± 5.08	65.17 ^a ± 5	69.17 ^{ab} ± 7.9	81.67 ^b ± 2.91
Heart weight (g)	12.67 ^a ± 0.21 ^a	15.67 ^b ± 0.84	19.33 ^{cd} ± 1.33	20.5 ^d ± 0.99	16 ^b ± 0.37
Offal weight (g)	125.83 ± 6.42	126.33 ± 5.67	133.17 ± 8.11	141.67 ± 9.27	143.5 ± 4.79
Intestine length (cm)	199.5 ^a ± 8.34	221.17 ^{ab} ± 10.28	233.5 ^b ± 11.46	230 ^b ± 12.68	214 ^{ab} ± 5.2
Muscle bone ratio	3.65 ± 0.05	3.58 ± 0.1	3.69 ± 0.09	3.72 ± 0.08	3.58 ± 0.07
Muscle fibre diameter (µm)	36.48 ^a ±1.44	44.8 ^b ±3.76	44.96 ^b ±1.12	45.84 ^b ±1.76	42.08 ^{ab} ±2.64
23 h regimen					
Live weight (g)	2560.67 ^{ab} ± 125.95	2515 ^a ± 115.69	2953 ^c ± 79.97	2836 ^{abc} ± 84.36	2741.33 ^{abc} ± 111.96
Hot carcass weight (g)	1679.33 ^a ± 99.04	1710.67 ^a ± 101.63	2000 ^b ± 47.84	1992.83 ^b ± 49.72	1790 ^{ab} ± 52.5
Dressing (%)	65.51 ^a ± 1.48	67.83 ^{ab} ± 1.39	67.77 ^{ab} ± 0.54	70.36 ^b ± 1.06	65.46 ^a ± 0.99
Head weight (g)	60.83 ^a ± 4.14	64 ^{ab} ± 1.98	81.17 ^c ± 4.23	73.5 ^{bc} ± 2.09	72 ^{bc} ± 2.37
Legs weight (g)	75.5 ± 2.91	84.17 ± 3.91	91.67 ± 6.64	80.83 ± 5.17	82.17± 7.42
Skin+Feather weight (g)	315.5 ^a ± 15.29	359.5 ^b ± 19.61	386 ^b ± 11.49	377.17 ^b ± 10	383 ^b ± 5.67
Liver weight (g)	58± 2.94	42.5 ^a ± 2.7	53.17 ^{bc} ± 2.06	50.83 ^{abc} ± 4.26	44.33 ^{ab} ± 3.12
Filled Gizzard weight (g)	63.5 ^a ± 7.86	68 ^{abc} ± 3.56	81.5 ^{bc} ± 1.48	84.17 ^{bc} ± 6.72	66.83 ^{ab} ± 6.49
Heart weight (g)	14.67 ± 0.84	13.33 ± 0.84	16 ± 1.1	16 ± 0.73	16.33 ± 1.52
Offal weight (g)	136.17 ^{abc} ± 8.21	116.83 ^a ± 3.75	150.67 ^c ± 2.39	151 ^c ± 9.11	127.5 ^{ab} ± 6.71
Intestine length (cm)	204.33 ^{ab} ± 8.25	196 ^a ± 7.84	232.67 ^b ± 7.99	213.5 ^{ab} ± 10	212.33 ^{ab} ± 11.18
Muscle bone ratio	3.68 ^{ab} ± 0.08	3.67 ^{ab} ± 0.08	3.62 ^{ab} ± 0.09	3.74 ^{ab} ± 0.08	3.51 ^a ± 0.07
Muscle fibre diameter (µm)	39.64 ^a ±3.36	38.56 ^a ±1.84	47.28 ^b ±1.12	47.84 ^b ±1.76	43.44 ^{ab} ±1.12

FCR trend in 23-h lighting regime was inconsistent among all groups in different days of study. FCR of Blue 23 was significantly ($P<0.05$) higher from other treatment groups and FCR of Red 23 and White 23 were significantly ($P<0.05$) lower from other treatment groups up to day 42.

Carcass characteristics

Data pertaining to the carcass characteristics of broiler chicken are presented in Table V. Broilers kept in Green16, and Blue16 showed significantly ($P<0.05$) higher pre slaughter live body weight as compared to broilers kept in Incandescent16. The mean values of hot carcass had showed a statistically significant ($P<0.05$) difference in broilers reared under 16 h regimen of monochromatic LEDs. The broilers hot carcass weight in blue and green light were significantly ($P<0.05$) higher than those reared

under incandescent and red lighting.

The mean values of calculated dressing percentage have showed a statistically non-significant ($P>0.05$) difference among all the groups under the study. The mean values of head, legs and skin revealed that treatment group kept in Blue16 and Green16 had significantly ($p<0.05$) higher head weight (g) than the broilers reared in Incandescent16 and White16. Treatment group kept in Blue16 was also recorded significantly ($p<0.05$) higher leg weight (g) than the broilers kept under Incandescent16. The results of the study revealed that the differences among all the groups for liver and offal's weight were statistically non-significant ($P>0.05$). However, significantly lower weight of heart was recorded in incandescent light group. The broiler reared under Green16 had significantly ($p<0.05$) higher heart weight (g) than other groups. Treatment groups

kept in Green16 was recorded significantly ($p < 0.05$) higher Intestine length (cm) than the broilers which are kept in Incandescent16 group. The results of the study revealed that the differences among all the groups for Muscle bone ratio were statistically non-significant ($P > 0.05$).

Broilers kept in Blue23 showed significantly ($P < 0.05$) heavier pre slaughter live body weight (g) as compared to broiler kept in White23 and Incandescent23. The treatment group kept in Blue23 and Green23 showed significant ($P < 0.05$) heavier hot carcass weight (g) than the broilers kept in Incandescent23 and White23 groups. Broilers kept in Green23 showed significantly ($P < 0.05$) higher dressing percentage as compared to those kept in Red23 and Incandescent23. The results of the study revealed that treatment group kept in Blue23 had significantly ($p < 0.05$) higher head weight than the broilers which kept in Incandescent23. legs weight showed the statistically non-significant variation. While skin with feather weight (g) was significantly ($p < 0.05$) lower in Incandescent23 birds than other treatment groups. The results of the study revealed that broilers kept in Incandescent23 had significantly ($p < 0.05$) higher liver weight than the broilers kept in White16. The heart weight did not show any significant differences among all the groups of study. Offal weight of Incandescent 23 group broiler had no significant difference with other groups. Treatment group kept in blue light was recorded with significantly ($p < 0.05$) higher intestine length (cm) than the broilers which were kept in white light. No significant ($p > 0.05$) difference was observed in Incandescent23 group broilers with other groups under observation for intestine length (cm). Similar observations were recorded for muscle bone ratio. Treatment groups Blue23 and Green23 were recorded with significantly ($p < 0.05$) higher thigh muscle fibre diameter (μm) than the white23 groups.

Haematological parameters

Data pertaining to the haematological parameters on day 14 are presented in Table VI. The results of the study revealed that different colour treatment have no effect on the WBC count of broilers. While in case of RBC count and PCV%, Red16 group showed significantly ($p < 0.05$) higher value than the broilers kept in Incandescent16. The mean values of haemoglobin in Red16 was recorded with significantly ($p < 0.05$) higher haemoglobin than the broilers in Green16 and White16 groups. The mean values of WBC count revealed that 23-h regimen on day 14 did not have any significant effect on the WBC count of broilers. While in case of RBC count and PCV% treatment group Red 23 showed significantly ($p < 0.05$) higher RBC number than the broilers of White23 and Blue23 groups. The mean values of haemoglobin (g/dl) 23-h lighting treatment groups. Birds under different group have not shown any significant variation for haemoglobin on day 14.

Biochemical parameters

The recorded data on total protein (g/dl), glucose (mg/dl), cholesterol (mg/dl), triglyceride (mg/dl), serum glutamic-oxaloacetic transaminase (SGOT) (IU/l) and serum glutamic pyruvic transaminase (SGPT) (IU/l) of broilers at the 35th day of experiment in 16-h regimen have been tabulated in Table VI. There were no significant changes were observed in the plasma total protein, glucose, triglyceride and SGOT level at 35th day of experiment. The plasma cholesterol and plasma SGPT levels were observed to differ non-significantly ($P > 0.05$) when compared Incandescent16 group bird with birds reared under different color lighting groups. There were no significant changes in the serum total protein, SGOT and SGPT level on 35th day of experiment. Plasma glucose, cholesterol and triglyceride levels were observed to differ non-significantly ($P > 0.05$) when compared Incandescent23 group bird with birds reared under different color lighting group.

Plasma hormonal parameters

The recorded data of triiodothyronine T_3 (ng/ml), T_4 ($\mu\text{g/dl}$) and growth hormone (ng/ml) of broilers at the 35th day of experiment have been tabulated in Table VI. The results of the study revealed that in 23 h lighting regimen there were significantly ($P < 0.05$) lower T_3 in the group Blue16 as compared to Red16 and Incandescent16 groups. T_4 level was significantly ($P < 0.05$) higher in Incandescent16 and Blue16 groups as compared to Red16 group. Non-significant ($P > 0.05$) variation in growth hormone was observed when compared between incandescent, red, blue and green light reared birds. In 23 h lighting regimen no significant variations were observed in birds reared under incandescent and white light for T_3 and T_4 . There were no significant changes in the plasma growth hormone level.

Behaviour observations

Day 14

The recorded data for various behaviour observations on the 14th day of experiment have been tabulated in Table VII. There were no significant changes in standing time among all the treatment groups. Whereas, walking time was significantly ($P < 0.05$) lower in the group kept in Blue16 as compared to Incandescent16 and White16 groups. Sleeping time was significantly ($P < 0.05$) higher in the Red16 and significantly ($P < 0.05$) lower in the Incandescent16 as compared to all other groups under the study. Feather pecking (frequency/h) and other aggressions (frequency/h) behaviour showed non-significant ($P > 0.05$) variation but were observed to be on higher side in Red16 group. Human approach test showed non-significant ($P > 0.05$) variation. Distributions of broilers in the pen were also affected by light colour. Birds kept in blue and

green light exhibit uniform distribution throughout the farm. Clustering around the specific area like near feeder, waterer or near corners were observed in red light.

The recorded data for various behaviour parameters on the 14th day of experiment under 23 h shows that the feeding time was significantly ($P<0.05$) lower in Red16 group and was significantly ($P<0.05$) higher in Incandescent23 and White23 group as compared to other treatment groups. Whereas no significant ($P>0.05$) difference was observed for drinking time among all group under the study. In mobility parameters at day 14 standing time was significantly ($P<0.05$) lower in White23

and Red23 as compared to other groups. Walking time was significantly ($P<0.05$) lower in the group kept in Blue23 as compared to other treatment groups. Whereas, sleeping time was significantly ($P<0.05$) higher in the in Red23 and Green 23 as compared to other groups. Feather pecking (frequency/h) and other aggressions (frequency/h) behaviour showed non-significant ($P>0.05$) variation but were relatively higher in Red23 group. Human approach test also showed non-significant ($P>0.05$) variation among all group under study. Same distribution pattern as seen in 16 h regimen is also seen in 23 h regimen also.

Table VI. Effect of treatment with different colour light for 16 h and 23 h on haematological, biochemical and hormonal parameters of broilers.

Parameters	Photoperiod	Incandescent	White	Blue	Green	Red
Haematological parameters						
WBC (10^3 cells/ μ l)	16 h	13.09 $\pm$ 0.75	13.25 $\pm$ 0.62	12.87 $\pm$ 0.7	12.55 $\pm$ 0.72	13.53 $\pm$ 0.54
	23 h	13.12 $\pm$ 0.4	13.38 $\pm$ 0.74	12.99 $\pm$ 0.97	11.88 $\pm$ 0.61	13.55 $\pm$ 0.7
RBC (10^6 cells/ μ l)	16 h	2.03 ^{ab} $\pm$ 0.01	2.02 ^{ab} $\pm$ 0.04	1.96 ^a $\pm$ 0.03	2.03 ^{ab} $\pm$ 0.03	2.18 ^b $\pm$ 0.12
	23 h	2.19 ^d $\pm$ 0.1	2.06 ^{bcd} $\pm$ 0.07	1.99 ^{bc} $\pm$ 0.05	1.88 ^{ab} $\pm$ 0.02	2.16 ^{cd} $\pm$ 0.08
PCV (%)	16 h	36.95 ^c $\pm$ 0.54	30.67 ^a $\pm$ 1.78	34.35 ^{bc} $\pm$ 0.72	35.5 ^{bc} $\pm$ 0.73	32.83 ^{ab} $\pm$ 1.08
	23 h	37.35 ^d $\pm$ 1.16	29.5 ^a $\pm$ 0.9	33.6 ^{bc} $\pm$ 0.85	33.75 ^{bc} $\pm$ 0.46	32 ^{ab} $\pm$ 1.15
Hb (g/dl)	16 h	10.77 ^{ab} $\pm$ 0.18	11.03 ^{ab} $\pm$ 0.36	10.98 ^{ab} $\pm$ 0.46	11.18 ^{ab} $\pm$ 0.26	11.7 ^b $\pm$ 0.22
	23 h	10.1 ^a $\pm$ 0.3	11.47 ^{bc} $\pm$ 0.56	10.77 ^{ab} $\pm$ 0.34	10.42 ^{ab} $\pm$ 0.3	12.17 ^c $\pm$ 0.3
Biochemical parameters						
Total protein (g/dl)	16 h	3.58 $\pm$ 0.18	4.53 $\pm$ 0.39	3.58 $\pm$ 0.39	3.65 $\pm$ 0.34	4.31 $\pm$ 0.42
	23 h	3.63 $\pm$ 0.37	3.57 $\pm$ 0.39	3.83 $\pm$ 0.38	3.48 $\pm$ 0.25	3.37 $\pm$ 0.48
Glucose (mg/dl)	16 h	191.49 $\pm$ 8.95	184.3 $\pm$ 9.17	175 $\pm$ 10.57	180.7 $\pm$ 12.75	180.88 $\pm$ 9.06
	23 h	174.91 ^{abc} $\pm$ 5.29	171.05 ^{ab} $\pm$ 6.25	171.58 ^{ab} $\pm$ 9.58	176.4 ^{abc} $\pm$ 8.77	154.74 ^a $\pm$ 7.01
Cholesterol (mg/dl)	16 h	213.87 ^{ab} $\pm$ 16.68	222.27 ^{ab} $\pm$ 13.76	217.2 ^{ab} $\pm$ 10.73	230.93 ^b $\pm$ 10.74	212.27 ^{ab} $\pm$ 14.49
	23 h	188.4 ^{ab} $\pm$ 12.16	177.87 ^a $\pm$ 11.2	216.27 ^{ab} $\pm$ 11.98	202.8 ^{ab} $\pm$ 14.86	188.9 ^{ab} $\pm$ 10.32
Triglyceride (mg/dl)	16 h	54.8 $\pm$ 9.64	69.47 $\pm$ 5.39	64.53 $\pm$ 7.47	55.07 $\pm$ 9.51	62.13 $\pm$ 13.09
	23 h	64.67 ^{ab} $\pm$ 7.27	60.8 ^{ab} $\pm$ 8.28	82.26 ^b $\pm$ 3.81	55.33 ^a $\pm$ 11.32	65.73 ^{ab} $\pm$ 6.91
GOT (IU/l)	16 h	53.57 $\pm$ 6.89	50.23 $\pm$ 10.63	43.32 $\pm$ 5.02	43.32 $\pm$ 7.09	38.63 $\pm$ 3.68
	23 h	48.57 $\pm$ 9.26	41.2 $\pm$ 6.48	33.94 $\pm$ 2.32	46.56 $\pm$ 10.87	42.76 $\pm$ 9.51
GPT (IU/l)	16 h	13.68 ^{ab} $\pm$ 1.27	23.2 ^{ab} $\pm$ 2.4	15.86 ^{ab} $\pm$ 2.07	26.08 ^{ab} $\pm$ 6.77	19.91 ^{ab} $\pm$ 3.07
	23 h	18.96 $\pm$ 4.4	12.51 $\pm$ 2.5	18.58 $\pm$ 1.63	18.32 $\pm$ 2.3	20.29 $\pm$ 4.28
Hormonal parameters						
T3 (ng/ml)	16 h	1.85 ^b $\pm$ 0.18	1.49 ^{ab} $\pm$ 0.03	1.32 ^a $\pm$ 0.16	1.69 ^{ab} $\pm$ 0.17	2.59 ^c $\pm$ 0.21
	23 h	1.9 ^{bc} $\pm$ 0.12	2.23 ^c $\pm$ 0.18	1.94 ^{bc} $\pm$ 0.12	1.66 ^{ab} $\pm$ 0.1	1.77 ^{ab} $\pm$ 0.08
T4 (μ g/dl)	16 h	1.47 ^b $\pm$ 0.25	1.23 ^{ab} $\pm$ 0.06	1.38 ^b $\pm$ 0.07	1.3 ^{ab} $\pm$ 0.14	0.95 ^a $\pm$ 0.03
	23 h	1.21 ^{ab} $\pm$ 0.06	1.07 ^a $\pm$ 0.01	1.13 ^a $\pm$ 0.05	1.18 ^{ab} $\pm$ 0.07	1.18 ^{ab} $\pm$ 0.01
GH (ng/ml)	16 h	8.74 ^a $\pm$ 1.26	13.65 ^b $\pm$ 3.02	8.91 ^a $\pm$ 0.33	8.18 ^a $\pm$ 0.33	8.74 ^a $\pm$ 0.79
	23 h	9.6 $\pm$ 0.51	10.34 $\pm$ 1.31	7.99 $\pm$ 0.35	9.15 $\pm$ 0.43	9.15 $\pm$ 1.39

Table VII. Effect of treatment with different colour light on behaviour parameters on day 14th and 35th of experiment.

Parameters	Photoperiod	Incandescent	White	Blue	Green	Red
Day 14th						
Feeding time (sec/h)	16 h	920 ^c ± 19.56	810 ^b ± 12.85	799.17 ^b ± 15.35	789.67 ^b ± 32.7	709.33 ^a ± 33.54
	23 h	905.5 ^c ± 41.12	903.5 ^c ± 17.91	810.5 ^{bc} ± 18.82	769.5 ^b ± 53.11	644.5 ^a ± 20.13
Drinking time (sec/h)	16 h	251.67 ^{ab} ± 20.1	259.67 ^{ab} ± 20.15	274.83 ^b ± 13.01	290 ^b ± 13.65	209.83 ^a ± 22.99
	23 h	298.67 ± 11.46	279.83 ± 23.43	250.83 ± 17.01	255 ± 22.22	168.67 ± 18.53
Standing time (sec/h)	16 h	571.83 ± 17.49	551.5 ± 17.31	562 ± 20.03	571.17 ± 12.88	536.33 ± 21.77
	23 h	636.83 ^b ± 91.07	451 ^a ± 22.46	657.67 ^b ± 25.44	788.67 ^b ± 35.2	469.67 ^a ± 61.81
Walking time (sec/h)	16 h	357.67 ^b ± 29.62	360.83 ^b ± 19.7	268.83 ^a ± 20.81	301 ^{ab} ± 17.39	310 ^{ab} ± 21.01
	23 h	373 ^b ± 49.38	369.83 ^b ± 17.84	251.67 ^a ± 17.89	298 ^{ab} ± 26.6	360 ^b ± 14.3
Sleeping time (sec/h)	16 h	727 ^a ± 21.29	783.5 ^{ab} ± 8.29	739.33 ^b ± 36.63	808 ^{bc} ± 32.75	866 ^c ± 18.77
	23 h	684.67 ^a ± 29.97	812 ^{ab} ± 20.94	731.17 ^{ab} ± 46.35	853.5 ^c ± 27.03	867 ^c ± 54.88
Feather pecking (no. per h)	16 h	1.83 ± 0.31	1.67 ± 0.56	1.17 ± 0.17	1.83 ± 0.75	2.5 ± 0.43
	23 h	2 ± 0.45	2.17 ± 0.4	1.83 ± 0.31	2.33 ± 0.56	3 ± 0.73
Aggression (no. per h)	16 h	2 ± 0.73	1.67 ± 0.33	1.83 ± 0.48	1.67 ± 0.56	2.17 ± 0.31
	23 h	2.17 ± 0.65	2 ± 0.52	1.67 ± 0.42	1.5 ± 0.72	2.5 ± 0.43
HA test (sec)	16 h	148.5 ± 23.72	165.67 ± 11.35	138.83 ± 15.81	112.83 ± 17.84	152.5 ± 19.46
	23 h	146.83 ± 28.65	125.83 ± 29.09	126.33 ± 21.22	96.5 ± 15.19	157.17 ± 26.08
Day 35th						
Feeding time (sec/h)	16 h	920 ^c ± 19.22	860 ^{bc} ± 17.13	779.5 ^{ab} ± 32.7	785 ^{ab} ± 23.13	735.83 ^a ± 47.73
	23 h	998 ^d ± 27.81	964 ^{cd} ± 16.53	817.67 ^b ± 39.08	861 ^b ± 18.19	649.33 ^a ± 42.69
Drinking time (sec/h)	16 h	250.83 ± 45.17	260 ± 46.99	259.67 ± 20.15	239.83 ± 13.8	219.67 ± 40.03
	23 h	293 ± 29.84	279.83 ± 57.17	250.67 ± 39.71	256.5 ± 51.33	237.67 ± 43.89
Standing time (sec/h)	16 h	552.33 ± 39.06	561.67 ± 18.75	562.5 ± 13.73	602.33 ± 31.62	575.17 ± 28.65
	23 h	563.17 ^{ab} ± 49.22	498 ^a ± 52.97	653 ^b ± 52.96	669.17 ^b ± 58.29	550.17 ^{ab} ± 34.25
Walking time (sec/h)	16 h	365.67 ± 16.69	329 ± 22.22	268.33 ± 49.02	271.17 ± 22.32	326.67 ± 40.79
	23 h	354.33 ± 37.23	369.67 ± 40.96	251.83 ± 40.98	298 ± 49.99	355.17 ± 40.51
Sleeping time (sec/h)	16 h	836.83 ^b ± 32.21	760.67 ^{ab} ± 15.26	780.17 ^{ab} ± 45.14	850.67 ^{bc} ± 26.8	933.33 ^c ± 13.55
	23 h	821 ± 33.09	832 ± 14.42	742.67 ± 46.9	853.67 ± 10.21	873 ± 26.32
Feather pecking (no. per h)	16 h	1.83 ^a ± 0.31	2.33 ^{ab} ± 0.49	2.33 ^{ab} ± 0.33	2.33 ^{ab} ± 0.61	3.33 ^b ± 0.49
	23 h	2.17 ^{ab} ± 0.48	2 ^{ab} ± 0.58	1.5 ^a ± 0.43	2 ^{ab} ± 0.73	3.33 ^b ± 0.42
Aggression (no. per h)	16 h	2.67 ± 0.42	2 ± 0.58	1.67 ± 0.33	1.83 ± 0.48	2.83 ± 0.6
	23 h	2.67 ± 0.42	2.17 ± 0.7	1.67 ± 0.56	2.5 ± 0.62	3.5 ± 0.56
HA test (sec)	16 h	142.17 ± 32.97	103.67 ± 14.97	114.17 ± 22.62	116.17 ± 23.81	125.33 ± 21.11
	23 h	115.67 ± 25.55	143.67 ± 19.41	113.33 ± 23.14	125.83 ± 17.25	157.17 ± 22.29

Day 35

The recorded data for various behaviour parameters on the 35th day of experiment under 16 h of lighting regimen have been tabulated in Table VII. Feeding time was significantly ($P < 0.05$) higher in Incandescent16 as compared to other treatment groups except White16. Drinking time showed non-significantly ($P > 0.05$) variations in all groups. No significant difference was observed for standing time and walking time among all group under the study. Sleeping time was significantly ($P < 0.05$) higher in Red16. as compared to Incandescent16 group. Among aggression

behaviour analysis, feather pecking (frequency/h) was significantly ($P < 0.05$) higher in Red16 as compared to Incandescent16 group. Other aggression (frequency/h) behaviours and human approach test showed non-significant variation.

Under 23-h lighting regimen feeding time was significantly ($P < 0.05$) lower in Red16 as compared to other groups. Whereas, drinking time was non-significantly ($P > 0.05$) varied in all group under study. Standing time was significantly ($P < 0.05$) lower in the birds kept under white light. No significant difference was observed for walking

time and sleeping time among all group under the study. With respect to coloured group under study, no significant ($P>0.05$) difference in feather pecking (frequency/h), other aggressions (frequency/h) behaviour and human approach test were observed in bird reared under incandescent and white light.

Keeping that in mind this research was designed to find out the effect of monochromatic blue, green and red LED light with white LED light and traditionally used yellow incandescent light for Indian agroclimatic conditions.

In the current study bird reared under different lighting color have showed varying effect on recorded behaviors in both 16 and 23-h photoperiod group. Recording of data on day 14 and 35 for 16- and 23-h lighting regimen for behaviour study was done. The red group has showed significantly higher sleeping time among all groups at all days in both photoperiod treatment times. Overall activity time was also lowest in blue light group and highest in incandescent light group in both 16 and 23-h lighting schedule. The walking time in bird reared under white and incandescent light groups was on higher side with respect to other group of studies in both photoperiod regimen. Similar trend was observed in feeding time where time spent in incandescent light group was highest and significant lower time was spent for feeding by birds reared under the red light. However, the study of data further revealed that there was higher pecking and other aggressive behavior was observed in red group. Overall, there were distinct behavioral changes observed in birds reared under different lighting color.

We also observed that in white-reared birds displaying increased walking activity, while red-reared birds showed more floor pecking, wing stretching, and aggression. In current study bird reared under blue had shown reduced pecking and other aggressive behavior than red and white light under most of observations time.

Such behavioral changes observed may be due to the hypothalamically stimulating effects of red light may enhance interactive behavior, with a stronger impact observed in the red-light treatment due to greater exposure of long-wavelength light to the hypothalamus compared to the white treatment. The heightened muscular activity observed in birds under red and white lights seems to have resulted in increased sleep requirements for these birds, whereas those under green and blue lights spent relatively more time sitting or dozing as observed in our study. The change in behavioral pattern may be helpful in bird rearing by several way as can affect overall metabolizable energy and hence growth of broiler chicken.

Morbidity and mortality

No morbidity was observed in any of group in 16

h as well as in 23-h lighting regimen in any color group throughout the experimentation period. However, 1.8% mortality was observed during brooding phase. After this no mortality was observed in any of color groups.

DISCUSSION

To understand the growth of broiler chicken in our study data of feed intake, FCR, weight and carcass weight were also observed and recorded. In the present study it was observed that overall feed and water intake was lower in incandescent bulb than blue, green light groups in all photoperiods. There was difference in feed intake observed in significant manner post day 35 of our study. This may be due to higher body weight in birds of the groups showing more feed intake. In general, the feed intake agreed with the trend of body weight of birds under different group may be to meet the BMR of birds.

The birds reared under the blue and green light were in general heavier than those reared under white and incandescent light on day 42 in both the photoperiod group. Its reason may be that green light accelerates muscle growth and stimulates early-age growth, possibly due to increased satellite cell proliferation during the initial days of growth (Halevy *et al.*, 1998; Cao *et al.*, 2008), whereas blue light stimulates growth in older birds (Rozenboim *et al.*, 1999; Rozenboim *et al.*, 2004; Cao *et al.*, 2008). Wavelengths between 435 and 600 nm (blue, green, and yellow) had positive effects, while higher wavelengths like orange and red had negative effects on broiler performance as observed in our study also.

Shorter wavelengths (blue and green) enhance body growth and feed efficiency (North and Bell, 1993; Prayitno *et al.*, 1997) which may be due to higher penetration of longer wavelengths in avian skull compare to short wavelength leads to increased bird activity by stimulating hypothalamus and this increase activity had negative impact on body weight gain and FCR. Also, changes in androgen production also can be a potential factor contributing to differences in body weight, as blue and green light have been shown to impact the synthesis of testosterone, thereby promoting myofiber growth (Cao *et al.*, 2008).

The FCR on day 42, was significantly higher in blue group in comparisons to white and incandescent group. This may be due to higher body weight gain at higher base level need more feed intake. The trend is in comparisons to studies of Fan *et al.* (2008) where faster growth leads more FCR. For other colour the FCR was on higher side than incandescent mimicking the trend of body weight. In Red group under 23-h lighting has better FCR than blue and green group but mimicking the trend of body weight.

A higher hot carcass weight was observed in blue and green lights as compared to incandescent light in both photoperiods at the end of experiment on day 42. In 16-h, photoperiod non-significant but higher dressing percentage was observed in green and blue light while in 23-h photoperiod green light showed significant higher dressing %. In both the regimen LED light groups shows the better dressing % than traditionally used Incandescent light. These findings are in agreement with [Kumar *et al.* \(2017\)](#) as they also found that light spectra do not have any significant effect on carcass weight. Our results were also in agreement with [Ke *et al.* \(2011\)](#) as they also reported that carcass weight was heavier under blue light as compared to white and red light. In our study, head, leg and skin with feather weight was in relation to corresponding pre slaughter body weight. Other than body weight dependent change of carcass parameters, no specific effect of light treatment as such was observed on these parameters in our study. [Soliman and Hassan \(2019\)](#) in their study also found that blue light birds yield a significant higher live body weight, carcass weight, spleen, and heart weights compared to other lighting colours and regimens.

A significant higher heart weight was observed in green light while lower heart weight was observed in incandescent light under 16 h photoperiod. Similar trend was observed in 23-h photoperiod. The higher heart weight in green light is may be due to higher body weight and BMR which required more blood pumping. Various studies have revealed that heart weight increases with body weight ([Kumar *et al.*, 2014](#); [Jin, 2013](#); [Lansford and Rugonyi, 2020](#)). However, liver weight was significantly higher in incandescent light than most of the LED treatment groups. This may be because of higher liver activity in incandescent group. A higher Filled gizzard weight was observed in blue, green group under 23 h photoperiod groups. This may be due to heavier gut content in these groups ([Prayitno *et al.*, 1997](#)). A longer intestinal length was observed in green light in both photoperiods rearing system. In our study birds reared under green light had more water intake and comparatively lesser activity. This may be the factor responsible for longer intestinal length. This may be due to higher water intake by green birds, as demand of water absorption increases the total intestinal length may increase ([Duque-Correa *et al.*, 2022](#)). Furthermore, birds showing lesser activity have also higher intestinal length ([Soliman and Hassan, 2019](#)).

The muscle bone ration had not differed significantly between incandescent and blue or green light bulb groups. Muscle diameter was significantly higher in birds reared under the blue and green light in comparisons with incandescent light. The finding was in agreement with the [Cao *et al.* \(2008\)](#) where greater carcass, breast and thigh

yield were observed in broiler reared in blue LED light. [Liu *et al.* \(2010\)](#) also found that birds reared under green light had higher breast muscle weight than those reared under blue, red and white light. [Sultana *et al.* \(2013\)](#) also found that better breast and thigh muscle fibre diameter was also observed in birds under green and blue light. [Fernandes *et al.* \(2018\)](#) also found that in early phase that is up to day 17, green light was found better for the breast and thigh muscle development. [Halevy *et al.* \(1998\)](#) reported that the body weight was greater in chicks exposed to green and blue light which may be due to proliferation of satellite cells in muscle due to stimulation of light of shorter wavelength (green and blue) that resulted in heavier body weights and higher muscle bone ratio.

In haematological indices, in general all parameter were under normal range indicate no adverse effect of any of light treatment on the physiological wellbeing of the bird. In general, there was an increase in RBC, PCV%, and haemoglobin concentration was observed in birds reared under red light group. The result was in agreement of study of [Rozenboim *et al.* \(2004\)](#) on 640 birds that red light exposure may lead to an increase in red blood cell count and haemoglobin concentration, potentially indicating enhanced erythropoiesis. Similar results were found by [Kim *et al.* \(2013\)](#) in their study. But contradictory results had also been observed by [Gharahveysi *et al.* \(2020\)](#) in their study; found that birds reared in red light had lower haemoglobin and haematocrit compared to those reared in green light. The higher RBC and haemoglobin may be related to higher activities in red light as compared to blue, green and incandescent light was observed by [Prayitno *et al.* \(1997\)](#).

Biochemical parameters like total protein, glucose, cholesterol, triglyceride, SGOT and SGPT were under normal physiological limit in all groups. The levels of serum glucose, cholesterol, and other biochemical parameters serve as markers for stress, with birds experiencing stress showing values differ from normal physiological level ([Puvadolpirod and Thaxton, 2000](#)). These findings may be attributed to the improvements in growth performance and health condition ([Kim *et al.*, 2013](#); [Yang *et al.*, 2016](#)). Furthermore, all these parameters were influenced by light colour with better findings in broilers reared under coloured light compared to those experienced white light. A relatively higher level of cholesterol and triglycerides in blue and green light was observed as compared to incandescent light. This finding is supported by [Soliman and Hassan \(2019\)](#) in their study as they reported that higher cholesterol and triglycerides level in blue and green light is due to less activity in birds of these lighting groups. Similarly, plasma T_3 , T_4 and growth hormone were also within normal physiological limit in all groups under both

photoperiods. Hormonal parameters are affected by light spectra as reported by Soliman and Hassan (2019) that GH, T₃ and T₄ serum level was relatively higher in birds reared in blue light than white and red light reared birds, but no such trend was observed in our study.

Higher body weight gain is one of the prime aims in the poultry production system. If, done in an economical way, will be beneficial to the farmer or the organization involved in the rearing of the poultry and also in the food security system of the country. The data of present study indicates that broiler reared under blue and green lights have higher body weight with respect to bird reared under white Led and incandescent light. This indicates that higher production can be achieved if such colour bulb is introduced in spite of current general practice of incandescent bulb. Our findings of present study are in agreement with various other studies done worldwide in different strains (Halevy *et al.*, 1998; Rozenboimet *et al.*, 2004; Cao *et al.*, 2008; Ke *et al.*, 2011; Sultana *et al.*, 2013; Fernandes *et al.*, 2018; Abdelazeem, 2019).

The increase in the body weight in the present study can be interpreted through the fact that most of the behavioural changes are favourable in the blue and green lights both in 16 and 23-h regime period of the study. The 16-h regime period of the study have more favourable body weight gain depending upon the requirement of the meat type in the Indian system either as a wholesome meat or a meat of nearly 1.5 to 1.8 kg used for the production of tandoori chicken.

In a condition, where pricing of feed will be significantly higher, red light for 42 days in 23-h system can have better economical outcome. Most of the other physiological parameter including the haematological, biochemical and hormonal parameters are well within the physiological limits and hence indicate that welfare does not differ between the treatment groups. So, depending upon the requirement, colour lighting has favourable effect. Furthermore, the carcass parameter in the study have also showed similar trend to the body weight where the carcass dressing percentage have slight favourable advantage in blue colour lighting system.

DECLARATIONS

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Ethical statement

The animal experiment was carried out in compliance with the approved guidelines set forth by the Institutional Animal Ethics Committee, as per regulation 12/CPCSEA with number IAEC/LUVAS/27/10, dated June 12, 2023.

Generative AI and AI-assisted technology statement

The authors declare that no generative artificial intelligence (AI) or AI-assisted technologies were used in this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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