Exposure of albino rats (*Rattus norvegicus*) to lights of varying wavelengths; effect on haematological profile, plasma electrolytes and weight gain

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Abstract

The study investigated the effect of exposure to monochromatic lights (ambient – control, blue, red, yellow, white) on haematology, plasma electrolytes, growth hormone and weight changes in albino rat (*Rattus norvegicus*). Albino rats were exposed to lights of varying wavelengths (15 watts energy saving fluorescents) from suckling age to 63 days; the light intensity was regulated to 300 lux. An Inverter was used to ensure uninterrupted power throughout the study-period. At the end of 63 days of exposure, blood samples were collected from anaesthetized rats through cardiac puncture into plain bottles (plasma electrolytes analysis) and EDTA-bottles (haematological parameter analysis). Rats were weighed to the nearest gram on a top-loading Metler electronic balance. Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC) counts, Haemoglobin (Hgb) concentration, Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Heamoglobin Concentration (MCHC), leukocyte differential and platelet counts were estimated by standard haematological methods. Spectrophotometry method was used to analyse plasma electrolyte and Growth Hormone (GH). Data obtained were expressed as means and standard deviation; means were compared using ANOVA and separated using Duncan's multiple range test at $p<0.05$. Rat weights were not significantly ($p>0.05$) affected by exposure to the lights. PCV (%) was significantly different ($p<0.05$); rats exposed to ambient, blue and red lights recorded significantly ($p<0.05$) higher values of 42.35, 40.00 and 36.33 respectively; no significant difference ($p>0.05$) was recorded in the other blood parameters; plasma electrolyte concentrations showed no significant difference ($p>0.05$) among the rats. The growth hormone concentration was significantly different ($p<0.05$) with rats exposed to red light having significantly ($p<0.05$) higher (14.80 mg/ml) concentration. Exposure of albino rats to monochromatic lights of varying wavelengths for 63 days had significant impact on PCV and growth hormone of albino rats.

Keywords: Light wavelengths; monochromatic; haematology; growth hormone; plasma electrolytes.

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Introduction

Light is electromagnetic radiation within a certain portion of the electromagnetic spectrum (Vandergriff, 1991). Rea *et al* (2010) described light as optical radiation with a spectral power distribution anywhere within the visible region of the electromagnetic spectrum (approximately 380nm to 730nm). Light is one of the environmental factors that strongly influences the physiology and behaviour of animals, and a major ecological factor that affects the distribution and abundance of animals in an ecosystem. It regulates the temporal pattern of animal behaviour and physiology, regulates circadian rhythms, and stimulates and synchronizes breeding cycles (Clough, 1982). The effects of light on animals can be related to three aspects such as its; intensity, wavelength and duration (photoperiod) (Castelhano-Carlos and Baumans, 2009; Kim *et al* 2013 and Olanrewaju *et al* 2013). Responses to light in animals include activation of vitamin D (Matt, 2007) stimulation of hormone production (Walton *et al* 2011) and the fine-tuning of cyclical changes in animals (RCEP, 2009).

Different authors have extensively explored the ecological consequences of artificial light. Rich and Longcore (2006) had reported several ecological consequences of artificial light on both vertebrate and invertebrates. Davies *et al* (2012) reported on how Street lighting changes the composition of invertebrate communities. Charlotte and Matt (2011) reviewed the impact of artificial light on invertebrates. Elena and Paolo (2010) reported that some animals were indirectly attracted and sometimes for predation like some reptiles, bats and birds.

The physiological implications of artificial light have been traced largely to varying wavelength of the light. Each wavelength has its different and significant effects on several physiological responses in animal. Wise (2007) reported that tadpole did not undergo metamorphoses when exposed to light, which was an indication of

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suppression of melatonin, which enhances the metamorphosis hormone thereby retarding the growth of the tadpole. Extensive studies have been conducted on the effects of light on avian physiology in the areas of growth performance, egg production, immune response, haematology, blood electrolytes and hormones (Olanrewaju *et al* 2008, 2010, 2013, 2014, 2015, Hassan *et al* 2013, 2017, Kim *et al* 2013 and Chang *et al* 2016). Not much has been done on the effects of light on the physiology of mammals most importantly the haematological parameters, growth response and growth hormone profile. From some of the researches conducted, the spectral quality of the light was found to elicit greater physiological responses in animals such as suppression of melatonin (Fabio *et al* 2011). Therefore, this research is aimed to determine the effect of light of varying wavelength on the haematology, blood electrolytes and growth performance of albino rat.

Materials and methods

Study area

The study was carried out at the Federal University of Agriculture Abeokuta, Ogun State, Nigeria (FUNAAB) Zoo Park.

Study design

The study was performed on newly born male albino rats. Immediately after birth, albino rat pups were exposed to artificial light for 12 hours daily (6.00 pm-6.00 am) till 63 days of age. The rats were divided into 5 groups of 5 rats each. Each group was exposed to different light treatments, which are Red light (RL) (700-635 nm), Blue light (BL) (490-450 nm), Yellow light (YL) (590-560 nm), White light (WL) (730-380 nm) and Ambient light (CL). Three rats were randomly selected for analysis at the end of 63 days from each light treatment. The rats fed on their mother's milk for the first 30 days and were fed 10% of their body weight daily until they were sacrificed.

The lights were generated from 15 watts energy-saving fluorescence light bulbs and the light intensity was regulated to 300 lux and monitored by a light meter. Inverter was used to ensure uninterrupted power supply throughout the study period.

Blood sample collection

The rats were anaesthetized using diethyl ether and blood sample was collected through cardiac puncture and part was collected into EDTA-containing sample tubes for haematological assay and the rest of blood samples were centrifuged at 4,000 rpm for 15 minutes and the blood serum were transferred into cryo-bottles and stored at -80^{o}C for the analysis blood electrolytes and growth hormone.

Haematological assessment

Total red blood cell counts (TRBC) were determined by diluting blood samples with Grower's solution, and then red blood cell were counted in 5 red blood cells square of haemocytometer. Total white blood cell counts (TWBC) were counted in Neubauer's haemocytometer after the blood samples were diluted (1:20) in Turk's solution. The Packed Cell Volume (PCV) was measured by filling blood into a heparinised capillary tube and centrifuged at 11,000 rpm for 5 minutes. The PCV values were measured with haematocrit reader. Haemoglobin (HGB) concentration was done by adding 20 ìl of the blood sample into 5 ml of Drabkin's solution. The optical density was measured in a spectrophotometer at 540 nm and was calculated from HGB standard curve. Some haematological parameters such as mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelets were also calculated. For determining the percentage of leukocytes (white blood cells), the blood smear was made and stained with Wright's dye. Finally, percentage of lymphocyte, monocyte, neutrophil, eosinophil and basophil were counted under light microscope.

Measurement of weight

The weight of the rat was taken at 7 days interval starting from the first day of delivery using top loading electric balance and recorded into the nearest 0.01 g. From the weight data collected, the following weight indices were calculated:

Average initial body weight = $(Wo)n^{-1}$.
Average final weight gain = $(Wt)n^{-1}$.
Average Daily Weight Gain (*ADWg*) (g/day) = Wg^{-1}.
Average Weight Gain (*AWg)* (g) = $(Wg)n^{-1}$.
Percentage Weight Gain (*PWg*) (%) = $Wt - Wo/Wo$ x 100.
W_t = Final weight at time *t*, W_o = Initial weight at time *t,* *t* = time and *n* = number of rats.

Growth hormone analysis

Serum samples were analyzed for growth hormone concentration. 1 µl of blood sample was pipetted by means of a micropipette into a 15 ml test-tube, 10 ml of 0.05 M Sodium citrate buffer and mixed on a Vortex mixer. 0.1 ml of orthophenilenediamine was added, followed by the addition of 5 ml of 0.05 M Sodium Carbonate buffer, mixed thoroughly and incubated for 1 hour to develop a bluish colour. 0-10 ng/ml of somatotrophin working standard solutions was prepared from 50ng/ml of stock somatotrophin. The working standard solutions were treated like the samples as above. The absorbance of sample as well as working standard solutions were read on a Cecil 2486 UV-V Spectrophotometer at a wavelength of 425 nm:

Somatotrophin in ng/ml = (absorbance of sample x gradient factor x Dilution factor) Volume of sample $taken^{-1}$.

Analysis of blood electrolytes

The blood electrolytes (potassium, sodium, calcium, chloride and phosphate) were analyzed by standard methods of AOAC (1990).

Statistical analysis

Means were compared for significant differences by Analysis of Variance (ANOVA) at $p<0.05$. The Means were separated into homogenous subsets by Duncan's Multiple Range Test (DMRT) at $p<0.05$.

Results

Table 1 shows the weight gain of the rat exposed to the monochromatic lights. There was no significant difference ($p>0.05$) between the rats. The highest weight gain and average weight gain (104.48 ± 8.31 and 1.66 ± 0.13 g) respectively was recorded under yellow light followed by blue (100.21 ± 21.16 and 1.59 ± 0.33 g) and the least was in control rats (76.78 ± 17.07 and 1.22 ± 0.27 g) respectively. The percentage weight gain was significantly ($p<0.05$) higher in rats exposed to light of varying wavelength as compared to the control. The highest percentage weight gain was recorded in the rats under yellow light (2308.88±243.83%) followed by those under red (2048.71±63.67%).

The weekly variation in body weight changes (Figure 1) revealed that rats exposed to blue and red lights had better growth performance between 0 and 57 days followed by those exposed to white light. Rats exposed to yellow light recorded an increase in weight between 57 and 63 days than those under blue, red and white light. The Control (ambient light) rats showed a slight decrease in weight between 35 and 42 days which remained consistently lower till the end of the study at the 63 days.

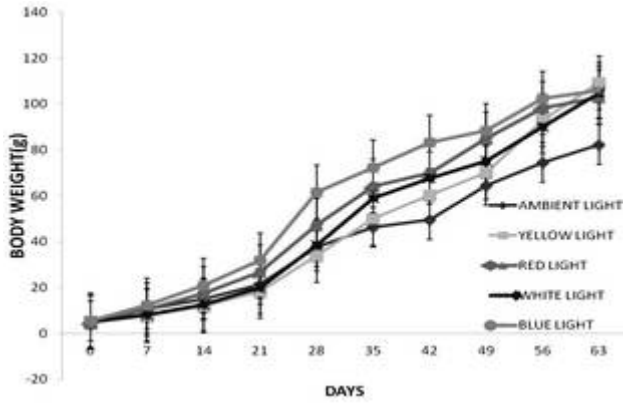

Figure 1. Daily variation in the body weights of albino rats. exposed to light of varying colour.

Table 2 shows the haematological parameters of albino rats exposed to varying colours of light. The PCV (%) of the rats was significantly different ($p<0.05$) with those exposed to ambient (42.35%), blue (40.00%) and red (36.33%) lights recording significantly higher values while those exposed to white light had significantly ($p<0.05$) lower (26.00%) value. Also, the eosinophil of the rats was higher significantly ($p<0.05$) under yellow light (2.00 ± 0.00%) and significantly reduced under control (0.00±0.00%), white light (0.33 ± 0.58%), blue light and red light (0.67 ± 0.58%). There were no significant differences ($p>0.05$) between the other haematological parameters. The Hgb and RBC of the rats exposed to ambient (13.90 g/dl; 6.43×10^{12}/L), blue (13.17 g/dl; 6.80×10^{12}/L) and red (11.83 g/dl; 6.17×10^{12}/L) lights respectively were higher than those exposed to yellow (11.03±1.45 g/dl and $5.80\pm1.18\times10^{12}$/L) and white lights (11.63±1.17 g/dl and $5.87\pm0.90\times10^{12}$/L). On the other hand, rats exposed to yellow and white lights had higher WBC, 5.63×10^{9}/L and 5.60×10^{9}/L respectively than those exposed to other lights. Platelet count was highest under white light (213.67± 152.05×10^{3}) followed by red light (149.00 ± 19.67 × 10^{3}) and least in blue light (127.33 ±8.74 × 10^{3}). MCV recorded the highest value in control (65.83±7.19 fl) followed by white light 61.50±2.17 fl) and least in blue light (58.67±1.79 fl). MCH recorded the highest value in white light (35.40± 6.61 pg) and least in control 21.63± 2.44 pg). MCHC recorded the highest value in blue light (33.20 ±0.10 g/dl) and least in yellow light (32.03±2.02 g/dl).

Table 3 shows the plasma electrolyte concentrations of albino rats exposed to varying colours of light. There were no significant differences ($p>0.05$) in the mean values of the rats exposed to different light treatments. Calcium recorded the highest value under blue light (8.37 ± 0.90 meq/l) and least under red light (5.50 ± 1.14 meq/l). Sodium recorded the highest value under white

Table 1. Body weights of albino rats exposed to light of varying colours.

Body Weight Parameters (g)	Light Treatments (n=3)				
	Ambient Light(CL)	White Light (WL) 730-380 nm	Yellow Light (YL) 590-560 nm	Blue Light (BL) 490-450 nm	Red Light (RL) 700-635 nm
Initial Body Wt (g)	5.55 ± 0.10	5.20 ± 0.09	4.73 ± 0.29	5.72 ± 0.15	5.03 ± 0.02
Final Body Wt(g)	82.33 ± 17.39[a]	104.91 ± 14.02[a]	109.21 ± 8.25[a]	105.93 ± 20.99[a]	103.05 ± 8.44[a]
Av. Wt gain(g)	76.78 ± 17.07[a]	99.71 ± 13.90[a]	104.48 ± 8.31[a]	100.21 ± 21.16[a]	98.02 ± 8.52[a]
Percentage Wt Gain (%)	1483.42±133.33[a]	2017.50±135.80[b]	2308.88±243.83[b]	1851.92±399.20[b]	2048.71±63.67[b]
Av. Daily Wt gain(g)	1.22 ± 0.27[a]	1.58 ± 0.23[a]	1.66 ± 0.13[a]	1.59 ± 0.33[a]	1.55 ± 0.13[a]

Means with the same superscript in a column are not significantly different ($p>0.05$).

Table 2. Haematological parameters of albino rats exposed to varying light colours.

Blood Parameters	Light treatments				
	Ambient light	Blue light 490-450nm	Yellow light 590-560nm	Red light 700-635nm	White light 730-380nm
PCV (%)	42.33 ± 4.62[b]	40.00 ± 6.00[b]	34.67 ± 6.66[ab]	36.33 ± 1.53[b]	26.00 ± 5.57[a]
HGB (g/dl)	13.90 ± 1.40[a]	13.17 ± 2.20[a]	11.03 ± 1.45[a]	11.87 ± 0.51[a]	11.63 ± 1.17[a]
RBC ($10^{12/L}$)	6.43 ± 0.49[a]	6.80 ± 0.85[a]	5.80 ± 1.18[a]	6.17 ± 0.61[a]	5.87 ± 0.90[a]
WBC ($10^{9/L}$)	3.87 ± 0.47[a]	4.73 ± 0.72[a]	5.63 ± 1.95[a]	5.60 ± 0.87[a]	4.93 ± 2.54[a]
Neutrophil (%)	33.00 ± 1.00[a]	29.67 ± 6.81[a]	31.33 ± 0.58[a]	28.00 ± 5.20[a]	27.67 ± 5.51[a]
Lymphocyte (%)	65.67 ± 1.53[a]	67.67 ± 6.43[a]	63.67 ± 0.58[a]	69.33 ± 5.03[a]	69.33 ± 5.13[a]
Eosinophil (%)	0.00 ± 0.00[a]	0.67 ± 0.58[a]	2.00 ± 0.00[b]	0.67 ± 0.58[a]	0.33 ± 0.58[a]
Basophil (%)	0.67 ± 0.58[a]	1.00 ± 1.00[a]	0.67 ± 0.58[a]	0.00 ± 0.00[a]	0.67 ± 0.58[a]
Monocyte (%)	0.67 ± 0.58[a]	1.67 ± 0.58[a]	1.33 ± 1.15[a]	2.00 ± 1.73[a]	1.67 ± 0.58[a]
Platelet ($\times 10^3$)	137.33 ± 11.24[a]	127.33 ± 8.74[a]	131.33 ± 5.86[a]	149.00 ± 19.67[a]	213.67± 152.05[a]
MCV (fl)	65.83 ± 7.19[a]	58.67 ± 1.79[a]	59.83 ± 0.67[a]	59.20 ± 5.04[a]	61.50 ± 2.17[a]
MCH (pg)	21.63 ± 2.44[a]	19.27 ± 1.01[a]	19.20 ± 1.39[a]	19.27 ± 1.12[a]	35.40 ± 26.61[a]
MCHC (g/dl)	33.17 ± 0.06[a]	33.20 ± 0.10[a]	32.03 ± 2.02[a]	3 2.60 ± 1.35[a]	32.33 ± 0.86[a]

Means with the same superscript in a column are not significantly different (p>0.05).

Table 3. Plasma electrolytes of albino rats exposed to varying light colours.

Light treatments	Plasma electrolytes				
	Calcium (meq/l)	Sodium (meq/l)	Potassium (meq/l)	Chloride (meq/l)	Phospate (meq/l)
Ambient light	6.50 ± 1.28[a]	109.67 ± 10.60[a]	3.77 ± 0.93[a]	103.00 ± 9.00[a]	8.27 ± 2.51[a]
Blue light	8.37 ± 0.90[a]	111.33 ± 6.43[a]	4.23 ± 0.49[a]	101.00 ± 3.61[a]	15.27 ± 9.64[a]
Yellow light	7.03 ± 1.62[a]	107.63 ± 12.58[a]	4.10 ± 0.44[a]	95.00 ± 13.45[a]	9.77 ± 1.75[a]
Red light	5.50 ± 1.14[a]	106.67 ± 14.05[a]	4.60 ± 0.89[a]	98.33 ± 12.50[a]	10.37 ± 2.40[a]
White light	6.67 ± 2.06[a]	119.00 ± 15.52[a]	3.97 ± 0.80[a]	106.67 ± 8.08[a]	9.00 ± 1.93[a]

Means with the same superscript in a column are not significantly different (p>0.05).

light (119.00 ± 15.52 meq/l) and least in red light. Potassium was highest under red light (4.60.00 ±0.89 meq/l) and least under white light (3.97 ± 0.80 meq/l). Chloride recorded the highest value under white light and least under yellow light and phosphate was highest in rat exposed to blue light (15.27 ± 9.64 meq/l) and least in rats exposed to white light (9.00 ± 1.93 meq/l).

Table 4 presents the growth hormone (GH) of the albino rats exposed to varying colours of light. The growth hormone concentration of the rats was significantly different (p<0.05). Rats exposed to the monochromatic lights elicited elevated levels of growth hormone compared to those exposed to the ambient (control) light. Rats exposed to red light had highest value (14.80 ng/ml) followed by the yellow and blue light groups with 14.08 ± 0.20 ng/ml and 14.08 ± 0.06 ng/ml respectively, and white light (12.93 ± 0.33 ng/ml) and significantly reduced by ambient (control) light (12.22 ± 0.08 ng/ml).

Table 4. Growth hormone (GH) of albino rats exposed to light of varying colours.

Light Treatments	Wavelength (nm)	Frequency (THz)	Growth Hormone Concentration (ng/ml) Mean±SD
Ambient	–	–	12.22± 0.08[a]
White	730-380	435-670	12.93± 0.33[b]
Yellow	590-560	515-560	14.08± 0.20[c]
Blue	490-450	610-670	14.08± 0.06[c]
Red	700-635	435-495	14.80± 0.09[d]

Means with the same superscript in the column are not significantly different (p>0.05).

Discussion

Haematological parameter is a useful tool for determining the health status of animals as it signals largely the state of the internal environment of animals (Al-Samarai and Al-Jbory, 2017). Various studies had reported the effect of light on haematological parameters of some avian and mammals. Olanrewaju *et al* (2013) reported that long day exposure to light significantly reduced haematocrit and hemoglobin in broiler. The study of Pecinova *et al* (2015) revealed that light intensity (400 lux) significantly reduced the haemoglobin and leucocytes of brown rat, while erythrocytes and haematocrit were not significantly affected. Hassan *et al* (2017) on the other hand reported that monochromatic lights have no effect on the haematocrit of duck but did not state the duration of exposure to light.

The PCV of rats reared under ambient, blue and red lights in this study, shared similar significantly higher values than those under other light spectral with white light triggering a significantly lower value suggesting that white light may be inducing anaemia in the rats exposed to it. In addition, the platelet concentration was higher in rats

exposed to white light than those under other lights. This finding agrees with Castelhano-Carlos and Baumans (2009) who reported that artificial light caused stress in rats. Furthermore, the effect of white light on the rats could be explained by the suggestion of Ji *et al* (2014) who stated that white (polychromatic) light exhibited a combination of several wavelengths, of which certain energy in it may have strong biological effect on animals.

Rats under yellow light recorded significantly higher eosinophil concentration than those under other light conditions. This elevated level may be as a result of stress induction by the yellow light (Dauchy *et al* 2010) which might have compromised the immune system of the rat. Eosinophil is an important component of the immune system of mammals as a first-responder to allergic inflammation and helps to remove damaged cells. As shown by Ear and McDonald (2008), neutrophils are highly mobile and quickly congregate at a focus of infection, attracted by cytokines released by activated endothelium of inflamed tissues.

The study suggested that light significantly reduced plasma potassium and chloride levels at the juvenile stage, but had no significant effects on calcium and sodium. In this study, there was no significant effect of the light treatment on the blood electrolytes of the rats. Olanrewaju *et al* (2013) discovered from their study on broiler that short day photoperiod significantly reduced Na^+, K^+, Ca^{2+}, Cl^- compared with long day photoperiod and also discovered that light intensity had no significant effect on these electrolytes. In a study conducted by Olanrewaju *et al* (2008) on broilers, the age of the broiler was found to play a role in relation to light affecting blood electrolytes.

Although the weight changes in the rats under all treatments were not significantly different, monochromatic lights (blue, red, yellow) and the polychromatic white light generally enhanced their weight changes compared with the control (ambient light). Firouzi *et al* (2014) reported that birds reared under yellow light had higher body weight, whereas Rozenboim (1999) and Hassan *et al* (2013) also reported higher body weight in birds reared under blue light.

Generally, the light treatments enhanced production of growth hormone in the rats with those reared under red light producing a significantly higher level followed by those under blue light. The higher concentration of growth hormone under red light may probably be as a result of enhanced melatonin level in the rats. Melatonin was reported to enhance growth hormone secretion (Zeman *et al* 1999; Díaz *et al* 2001; Fideleff, *et al* 2010). In addition, the activities of animals were reported to increase on exposure to red light, and exercise has also been implicated in increasing growth hormone (Meeking, *et al* 1999).

In conclusion, exposure to lights of varying wavelengths had significant effects on the rats; blue, red and yellow monochromatic lights may serve as growth enhancers during the juvenile age, in addition to boosting their immunity through the increased inflammatory response as seen under yellow light. White (polychromatic) light on the other hand may act as a physiological stressor which triggered the low haematocrit and anaemia and thereby mimicking the likely effects on human, in the event of exposure to these light wavelengths.

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